Circulating Biomarkers in Pulmonary Arterial Hypertension

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Pulmonary arterial hypertension (PAH) is a progressive disease of the pulmonary vasculature that leads to exercise limitation, right heart failure, and death. There is a need for biomarkers that can aid in early detection, disease surveillance, and treatment monitoring in PAH. Several potential molecules have been investigated; however, only brain natriuretic peptide is currently recommended at diagnosis and for follow-up of PAH patients. This review will focus on potential biomarkers in PAH and will discuss their pathophysiology, prognostic significance, as well as their limitations.

Pulmonary arterial hypertension (PAH) is a debilitating vascular disease of the pulmonary circulation that leads to elevation in the pulmonary artery pressure and pulmonary vascular resistance with resultant right ventricular failure and death.[1,2] Despite expanding therapeutic options, PAH continues to have unacceptably high morbidity and mortality.[3] Endothelial cell dysfunction, impaired eicosanoid balance, inflammation, oxidative stress, thrombosis, vascular proliferation, and metabolic dysregulation have all been implicated in the pathogenesis of PAH.[1,4] Early detection, risk assessment, and follow-up for disease progression are essential components of the clinical management of PAH. Currently, treatment decisions are based on assessment of disease severity determined by symptoms and exercise capacity.[5] Unfortunately there is a significant amount of subjectivity and imprecision in these assessments. As a result, there has been increasing interest and research for the identification of useful biomarkers in PAH. The US Food and Drug Administration (FDA) defines a biomarker broadly as a "characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention."[6] A valuable biomarker is reproducible, inexpensive, and easy to measure and interpret. While several biomarkers have been proposed for use in PAH, only brain natriuretic peptide (BNP) and N-terminal fragment of proBNP (NT-proBNP) level measurement are currently recommended in clinical guidelines for risk assessment and longitudinal follow-up of PAH patients.[7,8]

This review focuses on the current state of knowledge of not only BNP and NT-proBNP, but also other potential biomarkers for PAH, and will detail the available data supporting their use as well as known limitations. Table 1 provides a summary.

Key Words—right heart failure, endothelial dysfunction, exercise capacity
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BIOMARKERS OF CARDIAC DYSFUNCTION OR OVERLOAD

Brain natriuretic peptide is a peptide hormone produced from cardiac myocytes in response to ventricular stretch due to volume or pressure overload.[9] Previously used as a marker and prognosticator of left ventricular dysfunction, BNP and NT-proBNP have emerged as recommended biomarkers of dysfunction of the right ventricle (RV) in PAH, given their stability and relatively long half-life (20 minutes and 1–2 hours, respectively).[10,11] The prognostic significance of BNP was first demonstrated in a study of PAH patients initiated on prostacyclin therapy. In that study, patients with supramedian BNP at baseline (≥150 pg/mL) or after prostacyclin therapy (≥180 pg/mL) had significantly increased mortality.[12] BNP >180 pg/mL was confirmed to be independently associated with mortality in a large US-based registry.[13] BNP obtained at or near the time of right heart catheterization (RHC), or at initiation of therapy for PAH reflects invasively determined hemodynamic parameters and markers of exercise capacity including positive correlation with mean pulmonary artery pressure (mPAP), pulmonary vascular resistance (PVR), right atrial

Table 1.

Biomarker	Cellular Pathways	Associations
Related to cardiac dysfunction and overload		
Brain natriuretic peptide	Marker of myocardial stretch	Mortality, Hemodynamic measurements (RAP, mPAP, PVR), functional class, 6MWD
Cardiac troponin isofroms	Marker of cardiac injury	Mortality, BNP/NT pro-BNP and 6MWD
Related to vascular endothelial dysfunction		
Endothelin-1 and related molecules	Potent vasoconstrictor	Mortality, hemodynamic measurements (mPAP, PVR, CI), 6MWD
von Willebrand factor	Plays a key role in homeostasis and is a marker of endothelial dysfunction	Mortality
Angiopoeitin	Angiogenic factor involved in neovascularization	Hemodynamic measurements (CI, PVR)
Endostatin	Anti-angiogenic factor	Disease severity, Right ventricular dysfunction, mortality
Circulating endothelial cells	Marker of vascular damage	Clinical response to treatment
Exhaled nitric oxide	Marker of eicosanoid imbalance	Unknown
Cyclic GMP	Downstream player in the nitric oxide pathway responsible for vasodilation	Hemodynamic measurements (CI, SvO2)
Pentraxin	Regulates angiogenesis, inflammation and cell proliferation	Unknown
Related to collagen metabolism		
Procollagen, collagen and related molecules	Extracellular matrix deposition & remodeling	Mortality, hemodynamic measurements (RAP), functional class, quality of life
Related to systemic inflammation or oxidative injury		
Interleukins, especially interleukin-6	Inflammatory mediators	Mortality, quality of life
Osteopontin	Mediates cell migration, adhesion, remodeling and survival of vascular cells	Hemodynamic measurements (RAP), functional class, 6MWD
Isoprostanes	Marker of lipid peroxidation of arachidonic acid	Mortality
Related to non-cardiac dysfunction		
Serum creatinine	Marker of renal function	Mortality, hemodynamic measurements (RAP, CI)
Serum sodium	Reflects water imbalance	Mortality
Red cell distribution of width	Unclear significance	Mortality
Related to other processes		
Circulating fibrocytes	Contribute to organ fibrosis and extracellular matrix deposition	Unknown
MicroRNAs	Regulate gene-expression	Functional class, 6MWD, mortality
Hemoglobin A1c	Marker of metabolic dysfunction	Mortality
High density lipoprotein	Marker of vascular health	Mortality, hemodynamic measurement (PVR, CI)

RAP = Right atrial pressure; mPAP = Mean pulmonary artery pressure; PVR = Pulmonary vascular resistance; 6MWD = Six-minute walk distance; CI = Cardiac index; SvO2 = Mixed venous saturation.

pressure (RAP), and World Health Organization (WHO) functional classification; and negative correlation with cardiac index (CI), maximal oxygen consumption (VO_2 max), and 6-minute walk distance (6MWD).[11,14] Similar to BNP, NT-proBNP correlates with contemporaneously measured hemodynamics and mortality in PAH.[15-19] Serum NT-proBNP $\geq$ 1400 ng/mL was associated with reduced survival and observed in 10 of 32 patients with PAH (31%) in one study.[16] BNP and NT-proBNP are also useful as a serially obtained marker of response to therapy and progression of disease. Change in BNP during the course of therapy has been shown to correlate with change in WHO functional classification, 6MWD, and pulmonary hemodynamics.[20] More compelling are several studies demonstrating that, compared to an increase, a decrease in BNP or NT-proBNP during therapy is associated with improved survival.[12] These data support the use of BNP or NT-proBNP as a biomarker of hemodynamic and clinical severity of disease at therapy initiation, as well as serial change in BNP in assessment of risk of progression despite PAH-directed therapy. However, measurement and interpretation of natriuretic peptide levels have limitations. For example, BNP and NT-proBNP are affected by impaired renal function, obesity, age, and gender.[21-24]

Detectable plasma levels of cardiac-specific troponin proteins are an indicator of myocardial injury, and the use of this biomarker—both its presence and degree of elevation—is well established in the evaluation of acute coronary syndromes and left-sided heart failure. In PAH, detectable circulating cardiac troponin T is associated with RV dysfunction and lower mixed venous oxygen saturation (MVO_2), 6MWD, and survival.[25-27] There are far fewer studies of cardiac troponin T than the natriuretic peptides for risk stratification in PAH, but the studies available demonstrate close association of the two markers. One recent study supports utilizing highly sensitive cardiac troponin T in addition to BNP, although the incremental improvement in predictive ability was small and the number of patients studied was low. The wide-

spread use of troponin T has been limited due to its lack of specificity, as troponin T levels are elevated in other conditions including acute coronary syndrome, left ventricular dysfunction, renal insufficiency, and pulmonary embolism.[28] In addition, different cardiac troponin isoforms[26] and assays[27] may have differing predictive ability, as has been previously demonstrated. Clinical availability of different troponin isoform assays (including standard- and high-sensitivity assays) may be a limitation in widespread clinical use.

BIOMARKERS OF VASCULAR AND ENDOTHELIAL DYSFUNCTION

Endothelin-1 (ET-1) is a peptide found in abundance in the human lung and, through action of endothelin receptors (ET_A and ET_B) on vascular smooth muscle cells, is implicated in the pathogenesis of PAH.[29] Endothelin receptor antagonists are approved for the treatment of PAH. Levels of circulating ET-1 and related molecules are logical biomarkers of interest in PAH. ET-1 is elevated in PAH compared to controls, and correlates with pulmonary hemodynamic parameters.[30-33] In addition, higher ET-1 levels are associated with increased mortality in patients treated for PAH.[34] ET-1's precursor, big-ET-1, has a longer half-life and hence is more stable than ET-1.[35] In a small study of PAH patients, big ET-1 was strongly correlated with pulmonary hemodynamics including PVR, mPAP, and CI, as well as 6MWD.[36] Carboxy-terminal pro-endothelin-1 (CT-pro-ET-1) is derived from the ET-1 propeptide in equal amounts as ET-1, and has recently been investigated as a potential biomarker in PAH.[37] In a small study of PAH patients, patients in whom the composite endpoint of clinical worsening, lung transplantation, or death occurred had higher levels of CT-pro-ET-1 after multivariate adjustment for other biomarker levels (NT-proBNP, troponin I, and others).[37] Despite promising associations between these potential mediators and outcomes in PAH, several limitations have prevented them from adoption into mainstream clinical practice. None of these associations have been validated longitudinally, and the effects of PAH-approved therapies are unpredictable. While some of the studies above included patients on various PAH-approved agents, other studies show ET-1 levels rise,[38,39] are unchanged,[40] or are lower[41,42] depending on the agent used. Moreover, ET-1 level varies with race, age, gender, and certain medications including statins and beta-blockers, which makes it difficult to determine meaningful cutoff values.[43]

Von Willebrand factor (vWF) is a large glycoprotein produced in endothelial cells and megakaryocytes that plays a significant role in clot formation and platelet recruitment. Elevated plasma levels of vWF are another indicator of endothelial dysfunction and are found in multiple cardiovascular diseases, particularly valvular disease. Higher vWF level has been reported in patients with PAH compared to other forms of pulmonary hypertension (PH) and is associated with increased mortality.[44,45]

Angiopoietin-1 (Ang-1) is an angiogenic factor that binds receptor tyrosine kinase TIE2 on endothelial cells and is necessary for vascular formation. Ang-1 and its competitive inhibitor for TIE2 binding, Angiopoietin-2 (Ang-2), have been implicated in the pathogenesis of PAH.[46] In a study of patients with idiopathic PAH, plasma levels of Ang-1 and Ang-2 were higher in PAH patients as compared to healthy controls.[47] Moreover, higher plasma levels of Ang-2 were associated with lower CI and mixed venous oxygen saturation (SvO_2) and higher PVR, and, with therapy initiation, changes in Ang-2 correlated with changes in hemodynamics. Further studies of Ang-2 as a possible biomarker of PAH are warranted.

Like Ang-2, endostatin is an antiangiogenic peptide. It is synthesized by myocardium, is detectable in the peripheral circulation of patients with decompensated heart failure, and predicts mortality.[48] In PAH, reduced RV myocardial oxygen delivery is felt to contribute to a transition from RV adaptation to failure. Elevated levels of endostatin have been documented in the serum of patients with PAH and correlate with disease severity, RV dysfunction, and mortality.[49] Whether the level changes with response to therapy or provides additional prognostic or physiologic insight beyond BNP is uncertain.

Free circulating endothelial cells (CECs) have been detected in states of vascular damage, remodeling, and dysfunction.[50] The number of CECs measured by flow cytometry has been shown to be higher in patients with PAH as compared to healthy controls or those with chronic thromboembolic pulmonary hypertension (CTEPH) and correlated with pulmonary artery pressure.[51,52] In a separate study of children with PAH, the number of CECs decreased after PAH-targeted therapy initiation, suggesting a correlation with response to treatment. In fact, an increase in the number of CECs preceded clinical deterioration.[53] These results are promising; however, they need to be validated longitudinally and in larger cohorts. In addition, the wide differences in the methodology and the availability of techniques to measure CECs may not be ready for clinical use.

Impaired nitric oxide production has long been implicated in the pathogenesis of PAH and is another marker of endothelial dysfunction.[54] While more difficult to measure in the serum, levels of exhaled nitric oxide (eNO) have been explored in patients with PAH, but results have been mixed.[55,56] Treatment with prostacyclin therapy or bosentan has been demonstrated to increase the level of eNO, but whether the presence or magnitude of an increase translates to beneficial outcomes is uncertain.[57,58]

Cyclic guanosine monophosphate (cGMP) is an intracellular second messenger of nitric oxide and an indirect marker of natriuretic peptide production.[59] Urinary[60] and plasma[61] cGMP have been shown to be elevated in PAH (compared to controls), and urinary cGMP correlates inversely with CI and SvO_2. The response of plasma cGMP to PAH-specific therapy has been variable. One study of PAH patients showed that cGMP levels decreased by 30% following inhaled iloprost administration.[61] Another study

examined the response of cGMP levels to the administration of oral sildenafil and inhaled nitric oxide, and found that combination treatment yielded the greatest increase in blood cGMP levels in patients with PAH.[62]

Human pentraxin 3 (PTX3) is a protein synthesized by vascular cells that regulates angiogenesis, inflammation, and cell proliferation.[63] In a study of PAH patients (idiopathic and connective-tissue disease-related), PTX3 level was significantly elevated in PAH patients compared to controls and more prominently in the connective-tissue disease-related PAH.[64] Confirmation of these findings in other cohorts of patients with PAH and determination of PTX3 levels in patients with connective tissue disease without PAH are needed.

BIOMARKERS RELATED TO COLLAGEN METABOLISM

The main feature of vascular remodeling seen in PAH is collagen deposition in the remodeled pulmonary vessels. The best way to quantify collagen deposition in the pulmonary vasculature is by tissue analysis at autopsy or of explanted lungs. Antemortem assessment of collagen in the pulmonary vasculature is not possible with current imaging techniques, nor is lung biopsy considered safe. Several studies suggest that ongoing collagen metabolism in the pulmonary vasculature can be assessed by measuring circulating levels of collagen metabolites. A recently published study by Safdar et al showed that circulating levels of N-terminal propeptide of procollagen III (PIIINP), carboxy-terminal telopeptide of collagen I (CITP), matrix metalloproteinase-9 (MMP-9), and tissue inhibitor of metalloproteinase I (TIMP-1) were elevated in PAH patients as compared to age- and gender-matched healthy controls.[65] In particular, PIIINP levels increased with PAH disease severity, and there was a trend toward worse outcome in terms of mortality and lung transplantation in patients with higher PIIINP tertiles. In another study, elevated PIIINP levels further associated with worse indices of quality of life domains such as the Cambridge Pulmonary Hypertension Outcome Review (CAMPHOR), Minnesota Living With Heart Failure (MLWHF) physical, and EuroQOL 5 dimensions questionnaire (EQ-5D).[66] In addition, PIIINP showed good predictive capabilities with respect to the levels of RAP, and worsening WHO functional class and 6MWD, all 3 of which are widely accepted parameters of PAH severity.[100]

BIOMARKERS RELATED TO SYSTEMIC INFLAMMATION AND/OR OXIDATIVE INJURY

Inflammation is thought to play a key role in the pathogenesis of PAH, and several inflammatory markers have been found to be elevated in plasma of PAH patients including multiple interleukins: IL-1β, IL-2, IL-4, IL-6, IL-8, IL-10, and IL-12p70.[67,68] IL-6 in particular has been shown to correlate with mortality,[68,69] and a recent study demonstrated an association with quality of life domains in patients with PAH.[70] Not all studies replicate these findings.[71]

Osteopontin (OPN) is a matricellular protein that mediates cell migration, adhesion, remodeling, and survival of the vascular and inflammatory cells and has been investigated as a biomarker in experimental PH and in human disease. In a study of idiopathic PAH patients, OPN levels measured at the time of the diagnostic RHC were elevated as compared to controls and OPN levels correlated with RAP, WHO functional classification, and 6MWD. Moreover, in multivariate analysis, OPN was found to be an independent predictor of mortality in idiopathic PAH patients.[72]

F2-isoprostane is a marker of lipid peroxidation of arachidonic acid, which stimulates endothelial cell proliferation and ET-1 synthesis and may play a role in the pathogenesis of PAH.[73] Urinary F2-isoprostane, when measured at the time of diagnosis, has been shown to be an independent predictor of mortality in a cohort of incident PAH patients.[74] In patients with idiopathic PAH, plasma concentration of 15-F_{2t}-isoprostane is elevated as compared to controls and is independently associated with mortality.[75]

BIOMARKERS RELATED TO NON-CARDIOPULMONARY ORGAN DYSFUNCTION

The presence of comorbidities increases morbidity and mortality in PAH. The most consistently demonstrated effect has been that of renal failure. As a possible prognostic biomarker, elevated serum creatinine has been associated with higher RAP and lower CI and is an independent predictor of mortality in patients with PAH.[76,77] A large prospective observational registry reported that renal dysfunction was associated with mortality in PAH, but did not utilize quantifiable markers of kidney function.[13]

A marker of combined cardiac and renal dysfunction, hyponatremia has been demonstrated to be present in patients with left-sided heart failure. Hyponatremia as defined by a decreased serum sodium concentration $\leq$136 mEq/L, and is associated with RV dysfunction and hemodynamics in patients with PAH.[78] Hyponatremia has also been independently associated with increased mortality in patients with PAH.[78,79]

The red cell distribution of width (RDW) is routinely measured via the complete blood count and reflects the variability in the size of circulating red blood cells. Increased RDW was found to be independently associated with increased mortality in a cohort of mixed PH cases, including PAH patients.[80] In another study of idiopathic PAH patients, RDW was again found to be prognostically significant and added significant value to the measurement of NT-proBNP and exercise capacity.[81] Several mechanisms have been proposed linking pulmonary vascular disease and increased RDW; however, none has been definitively demonstrated.

BIOMARKERS RELATED TO OTHER NOTABLE PROCESSES

Circulating fibrocytes are bone marrow-derived cells ($CD45^+$/collagen I^+) that contribute to organ fibrosis and extracellular matrix deposition.[82] Experimental hypoxia-induced PH models have suggested a role for circulating fibrocytes in the development of vascular remodeling. In a study of mice, administration of continuous treprostinil infusion

significantly inhibited the recruitment of these cells into the remodeled pulmonary vessel walls and reduced RV systolic pressure.[83] In a study of children and young adults with PAH, patients with PAH were found to have higher number and percentage of circulating fibrocytes as compared to controls and the number of fibrocytes correlated with mPAP.[84] Like CECs, broad application of an analytical method that relies on flow sorting of live cells is unlikely due to multiple limitations including issues with specimen handling, required technical expertise and equipment, and cost.

MicroRNAs (miR) are a group of noncoding RNAs 18 to 25 nucleotides in length that regulate about 30% to 60% of the human genome.[85,86] Several miRs have been implicated in the pathogenesis of PAH including miR-150, miR 17-92, miR-21, and miR-204.[87-91] Circulating miRs are highly stable and protected from RNase digestion; therefore, they may have a potential role as biomarkers for PAH.[92] The levels of multiple circulating miRs have been shown to be differentially expressed in patients with PAH compared to healthy controls. miR-26a,[93] miR-204,[87] and miR-150[94] are all known to be reduced in patients with PAH. Reduced levels of miR-150 were associated with 6MWD and WHO functional classification, and have been shown to be an independent risk factor for mortality in PAH.[94] Nucleic acid amplification techniques are now more readily available for clinical testing. Unfortunately, the level of various miRs in a healthy population, effects of other related and unrelated disease states, and effects of therapy are uncertain at this time. Further study and validation may allow miRs to be a promising biomarker in multiple cardiovascular diseases including PAH.

There are new data suggesting a connection between metabolic dysfunction and PAH.[95] In a cohort of incident PAH patients, mean hemoglobin A1c was elevated in patients with PAH as compared to age-matched nondiabetic controls and was an independent predictor of mortality.[96] In addition, high-density lipoprotein (HDL) cholesterol is a marker of vascular health and is affected by insulin resistance. In a study of PAH patients, HDL levels were lower in PAH patients as compared to controls, and higher HDL levels were associated with decreased mortality.[97,98] Serum HDL levels were positively correlated with CI and negatively correlated with PVR.[98] These findings could not be confirmed in a separate study of incident PAH patients.[99]

CONCLUSION

Pulmonary arterial hypertension is associated with increased morbidity and mortality. Early detection and treatment have improved outcomes. Biomarkers are needed to help identify patients early, risk classify them, and evaluate their response to treatment. Presently, natriuretic peptides have demonstrated utility in assessing disease severity at diagnosis and during the course of the disease, as well as a marker of treatment response. Despite many other substances being investigated as potential biomarkers in PAH, more research is needed to validate the results of small studies and assess their clinical utility. Widespread clinical use of current investigational biomarkers will require validated clinical laboratory techniques and increased knowledge of levels in the healthy population as well as other disease states. Nonetheless, biomarkers are a promising means of improving our ability to differentiate PAH from other related diseases, assess treatment response, and identify patients at high risk of disease progression or death from PAH, and thus ongoing studies are warranted.

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