Abstract Pulmonary hypertension prevalence continues to rise and remains a clinical dilemma with regards to patient recognition and management. Despite advances in our understanding of the pathophysiology and pathogenesis behind pulmonary hypertension (PH), this heterogeneous cohort continues to demonstrate significant morbidity and mortality. Biomarkers serve as a dynamic, noninvasive tool in a physician’s clinical armamentarium. Their role is to impact clinical decision-making and to facilitate patient education with respect to diagnosis, prognosis, and therapeutic intervention. This review will elucidate the relationship between PH and serum biomarkers related to inflammation, myocardial dysfunction or stress, and endothelial dysfunction. Over the last two decades, the utilization and incorporation of biomarkers into the evaluation and management of pulmonary hypertension has exploded. Consequently, current guidelines and consensus documents have adopted their use. The additive roles of both established and innovative biomarkers in individuals with pulmonary arterial hypertension (PAH) will be discussed.

Keywords Biomarkers · Pulmonary hypertension · Pulmonary arterial hypertension · Inflammation · Endothelial injury · Myocyte injury · Treatment · Prognosis · Screening · Natriuretic peptide · Troponin · ST2 · Cystatin C · Uric acid · Osteopontin

Introduction

Awareness and diagnosis of pulmonary arterial hypertension (PAH) continues to grow and is no longer considered a rare entity [1]. PAH is defined by a mean systolic artery pressure of at least 25 mmHg at rest with a pulmonary capillary wedge pressure of <15 mmHg and can be further categorized based on a pulmonary vascular resistance greater than 3 Wood units [1–3]. The World Health Organization (WHO) first developed a classification system for pulmonary hypertension (PH) in 1998, which subsequently has been modified, and today, there are five main groups [4, 5]. WHO group I is PAH. It is a heterogeneous cohort comprised of individuals with idiopathic PAH (IPAH), familial PAH (FPAH), or entities associated with PAH (APAH). APAH includes a diverse group of individuals with connective tissue disorders, congenital disease, portal hypertension, HIV, drugs, and toxins [1, 2]. It is important to account for the distinction within the WHO class I as the morbidity and mortality within each of these populations is variable [6, 7]. Physicians should be aware of symptoms and consider screening high-risk populations, like scleroderma. These individuals may warrant early modification of medical management.

Recent registry data from REVEAL (Registry to Evaluate Early and Long-Term Pulmonary Arterial Hypertension Disease Management), a prospective database comprised of 3515 patients from 55 US centers, reported the incidence of PAH to be 2 per million adult individuals with a prevalence of 10.6 per million [8••, 9]. Despite growing PAH prevalence, registry data demonstrates that with the advancements in therapeutic intervention and recognition, the overall morbidity and mortality while still high is on the decline [1, 2, 8••]. Initial National Institutes of Health (NIH) registry data from over 20 years ago reported 1-year survival at 68 and 34 % at 5 years within IPAH patients [8••, 10]. Most recently, these numbers have improved to 91 %, with 1-year survival in IPAH patient population and 85 % in PAH and 5-year survival at 65 and
57% [8••]. In addition, previous median survival from NIH registry was 2.8 years compared to more than 7 years based on REVEAL [10, 11]. These numbers should be interpreted with caution as they do not account for lead time bias. Based on PH registry data, the mean time from symptom onset to definitive diagnosis ranges from 2.3 to 2.8 years [12]. Despite modern advances, pulmonary arterial hypertension remains a clinical dilemma and the advent of serum biomarkers may serve to assist physicians in making critical decisions.

A clinically relevant biomarker should fulfill three principle criteria: provide new information, be cost-effective and readily reproducible, and provide objective support in medical management [13, 14]. Biomarkers demonstrate an additional tool to facilitate diagnosis and preclinical screening, to objectively monitor therapeutic interventions, and to provide important prognostic information to patients. Hochholzer et al. described a multi-marker method which elucidates the additive effect of biomarker classes based on pathophysiologic mechanisms in patients presenting with acute myocardial infarction [15]. To our knowledge, the scientific community has yet to identify a serum biomarker that is unique to PAH, like hemoglobin A1c for diabetes. However, the utilization of biomarkers in PAH is increasingly becoming in vogue. Current guidelines have adopted the clinical use of brain natriuretic peptide (BNP) and N-terminal pro-brain natriuretic peptide (NT-proBNP). The evolution of histopathology and pathophysiology within PAH has been translated to biomarker-targeted treatment pathways [16, 17]. Clinical applications of both recognized and potential PAH serum biomarkers will be reviewed.

**Detection and Screening**

Early development of pulmonary arterial hypertension may present with nonspecific findings which requires physicians and providers to have a low threshold for further evaluation. Initial signs and symptoms may include but are not limited to dyspnea, chest pain, edema, and syncope [6]. Aside from invasive pressure quantification, there is no single test to determine a diagnosis of PH. PAH evaluation begins with a noninvasive assessment, but for definitive diagnosis, hemodynamic assessment by right heart catheterization is required. Initial evaluation includes a standard history and physical, which is further supported by a combination of imaging modalities, laboratory data, pulmonary function testing, and an EKG. Echocardiography is considered a vital screening tool in this stepwise approach by estimation of right ventricular systolic pressure (RVSP) and assessment of right ventricular size and function. Tricuspid annular plane excursion (TAPSE), Tei index, and presence of pericardial effusion are additional echocardiographic measurements and findings that have been validated as prognostic markers [2, 18–20]. An RVSP greater than 40 mmHg should raise suspicion but does not confirm a diagnosis of PAH [1, 19]. Echocardiography has the potential to over- and underestimate pulmonary pressures secondary to individual’s anatomy, loading conditions, and operator’s ability to acquire images [2, 21, 22, 23••]. The potential variability in imaging assessment prompts a need for additional supporting data.

To date, the use of both serum biomarkers—NT-proBNP and BNP—have been supported by small, single-center studies, registry data, and current European Society of Cardiology (ESC)/American Heart Association (AHA)/American College of Cardiology Foundation (ACCF) guidelines to serve as dynamic markers with regard to prognostication and therapeutic intervention. These markers reflect myocyte injury, inflammation, and ventricular remodeling [24]. They are secreted in response to an increase in ventricular wall stress and consequently promote vasodilatation and natriuresis [25]. Initially described as a marker of left ventricular dysfunction, BNP levels correlated to a level of right ventricular (RV) dysfunction as illustrated by Nagaya et al. It is also dynamic with regard to changes in pulmonary vascular resistance (PVR) and RV end-diastolic pressure in patients with primary pulmonary hypertension [26]. NT-proBNP is the molecular precursor to BNP. It has a longer, storage half-life and has been found to be a more stable compound both in vivo and ex vivo. Like BNP, it is a dynamic marker with response to changes in hemodynamics [1, 2, 25]. The guidelines have not incorporated the use of BNP nor NT-proBNP with regards to PAH identification, but collection is recommended to assess prognosis and guide therapy (to be discussed in subsequent sections).

Screening for PAH is recommended in a subset of patients, which includes individuals with known BMPR2 mutation, strong family history of PAH, systemic sclerosis (SSc), and liver transplant candidates to exclude portal hypertension [1, 2]. Biomarker collection of NT-proBNP has recently been incorporated into the screening algorithm for patients with systemic sclerosis. The prevalence of PAH within this population remains high with reports between 7 and 12%. It is the leading cause of death in this cohort with up to 50% survival at 3 years, emphasizing the need for early detection [27–29]. The DETECT trial was an international, multicenter cross-sectional study designed to identify PAH in SSc [30]. Of the 466 SSc patients with DLCO <60%, 31% demonstrated PH by RHC. This was further delineated by WHO classification: 19% class I (PAH), 6% class II (left heart disease), and 6% class III (lung disease). Over 100 variables were captured, and through logistic regression analyses, six non-imaging variables were chosen for step 1 of the screening algorithm, including NT-proBNP. Overall, this novel screening algorithm demonstrated a detection sensitivity and specificity of 96 and 48% [3, 30]. Using conventional biomarkers as part of a diagnostic armamentarium poses question. In a small, prospective single-center study, Mathai et al. compared features of individuals with known PAH-SSc to IPAH [31]. Their group captured
significant elevations of NT-proBNP in the SSc group compared to IPAH despite better hemodynamic profile in the PAH-SSc cohort [31]. Individuals with SSc continue to have a worse prognosis than IPAH [1, 7]. This observation challenges the community to find a single marker for this heterogeneous population. It also signifies the difference and value of both a molecular and hemodynamic assessment.

**Prognosis**

The backbone of PH pathobiology and pathophysiology is based on cellular proliferation and vascular remodeling, thrombosis, inflammation, and vasoconstriction [32, 33]. Subsequently, these pathways set the stage for development of serum biomarkers and ultimately therapeutic intervention (Fig. 1). Myocardial dysfunction in PAH is observed objectively by echocardiography with septal flattening in both diastole and systole. This is further confounded by poor RV contractility and remodeling of the myocardium and pulmonary vasculature. It is subjectively demonstrated by individual symptoms and clinical manifestations of RV dysfunction. At the cellular level, specific biomarkers within PAH have been quantified and categorized. This includes but is not limited to markers of myocyte injury, inflammation, and endothelial dysfunction (Table 1). The latter includes biomarkers that serve as our current therapeutic target pathways: nitric oxide, endothelin-1, and cyclic AMP and GMP [17].

**Myocyte Injury**

*Natriuretic Peptides*

Current guidelines support the use of NT-proBNP and BNP with regard to prognostic impact [1, 2]. It is important to understand that biomarkers, like natriuretic peptides, are used in conjunction with additional subjective and objective data points including RV dysfunction, symptoms, WHO functional class, 6-min walk distance (6MWD), cardiopulmonary exercise testing (CPET), and hemodynamic data. The guidelines do not provide strict cutoff points with regard to natriuretic peptide level but simply state low risk with minimal elevation and high risk with significant elevation [1, 2]. One small, prospective single-center trial in PH patients demonstrated an NT-proBNP level greater than 1400 pg/ml related to worse outcomes and correlated with functional, hemodynamic, and imaging assessments [34]. The REVEAL registry demonstrated increased survival in individuals with a BNP level less than 50 pg/ml or an NT-proBNP level less than 300 pg/ml and increased risk of morbidity and mortality with respective levels greater than 180 and 1500 pg/ml [35]. The REVEAL risk score is an additional, clinical tool which prognosticates 1-year survival. The risk score is comprised of

![Fig. 1](Targets for current therapies in pulmonary arterial hypertension. Reprinted from Benza et al. [17], with permission from Elsevier)
nine variables and includes BNP or NT-proBNP. One point is allocated for a BNP level greater than 180 pg/ml or an NT-proBNP level greater than 1500 pg/ml, and two points are subtracted if BNP is less than 50 pg/ml or NT-proBNP is less than 300 pg/ml. The score ranges from 1 to 15, and groups are broken down by score into predicted 1-year survival: low (95–100 %), average (90 to <95 %), moderately high (85 to <90 %), high (70 to <85 %), or high risk (less than 70 %) [36]. The REVEAL algorithm provides a c-index of 0.726 with a 95 % confidence interval (CI) of 0.678–0.775 which compares and is perhaps stronger than previous registry-derived risk score algorithms, like NIH with a c-index of 0.588 and French 0.57. However, when compared head to head and adjusted for time of diagnosis or matched patients, prognostic power is similar across registry data sets [8••, 36, 37]. The interpretation of natriuretic peptide data may be confounded by renal dysfunction, age, increase in body habitus, and lack of specificity to PAH as its value may be overtly over- or underestimated [38].

**Troponins**

Cardiac troponins T and I are well-established biomarkers in the realm of cardiovascular medicine and particularly in acute coronary syndrome (ACS). They are detected in 13–20 % of patients with PAH [39]. These are regulatory proteins within the tropomyosin complex that are released in the setting of acute myocyte injury from ischemia and/or myocardial dysfunction secondary to pressure overload, as in PH or volume overload. Torbicki et al. first described cardiac troponin T (cTnT) as an independent predictor of increased mortality in a PH cohort. This small, single-center study demonstrated a positive cTnT in 14 % or eight of 56 patients with WHO class I PH. This was done using a commercially available third-generation, high-sensitivity assay by Roche with a lower detection limit of 0.01 ng/ml. The mean level in this cohort was 0.034±0.022. Two-year survival in the cTnT-positive population was significantly less at 29 versus 81 % (p=0.001) [40]. In addition, cTnT positivity correlated with other markers of worse outcomes including NT-ProBNP, 6MWD, higher heart rate, and decreased pulmonary arterial saturation [40]. A subsequent study by Filusch et al. utilized a novel, not commercially available high-sensitivity cTnT (hs-cTnT) assay by Roche with a lower detection limit of 2 pg. The novel hs-cTnT was positive in 27.3 % of patients compared to 10.9 % using the commercial assay. This troponin T assays again demonstrated increased morbidity and mortality in individuals with positive biomarker. The positive predictive value of NT-proBNP in this cohort for death at 12 months was 33.4 compared to 100 with both cTnT and hs-cTnT. Both troponin assays were 100 % sensitive and 100 % specific with regard to predicting mortality at 12 months in comparison to NT-proBNP which was 100 % sensitive but only 82 % specific. Again, this was a small, prospective single-center study looking at a heterogeneous group known as WHO class I PH patients [41].

The utility of cardiac troponin I (cTnI) has also been evaluated in PAH patients. Detectable cTnI has been correlated with more advanced disease and 4.7-fold increased risk of morbidity and mortality [42]. As opposed to an undetectable cTnI, this yielded an 84.7 % transplant-free survival. This small, single-center study with 36-month follow-up of individuals receiving contemporary medical therapy validated current predictors of right ventricular failure and/or death with BNP univariable hazard ratio (HR) 2.01 and 95 % CI (1.39–2.9) with a p value less than 0.001, 6MWD 0.60 (0.37–0.97) with a p value equal to 0.038, and detectable cTnI 4.74 (1.89–11.89) with a p value less than 0.001. A recent moderately sized single-center PH study including 255 patients using a novel, high-sensitivity cardiac troponin I (hs-cTnI) assay demonstrated its ability to identify high-risk PH patients and to

---

**Table 1** PH Biomarkers

<table>
<thead>
<tr>
<th>Category</th>
<th>Biomarkers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Myocyte insult (injury/stress)</td>
<td>NT-proBNP&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>BNP&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Troponin I and T</td>
</tr>
<tr>
<td></td>
<td>ANP</td>
</tr>
<tr>
<td></td>
<td>ST2</td>
</tr>
<tr>
<td>Endothelial dysfunction</td>
<td>Nitric oxide&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Endothelin I&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>cGMP&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Asymmetric dimethylarginine (ADMA)</td>
</tr>
<tr>
<td>Inflammation and oxidative stress</td>
<td>Osteopontin</td>
</tr>
<tr>
<td></td>
<td>Galectin-3</td>
</tr>
<tr>
<td></td>
<td>RDW</td>
</tr>
<tr>
<td></td>
<td>Interleukins (1B, 6, 8, 12p70)</td>
</tr>
<tr>
<td></td>
<td>C-reactive protein</td>
</tr>
<tr>
<td></td>
<td>Growth differentiation factor 15</td>
</tr>
<tr>
<td></td>
<td>Soluble CD40 ligand</td>
</tr>
<tr>
<td></td>
<td>Tumor necrosis factor alpha</td>
</tr>
<tr>
<td></td>
<td>High-density lipoprotein cholesterol</td>
</tr>
<tr>
<td></td>
<td>CXC chemokine ligand 10</td>
</tr>
<tr>
<td></td>
<td>Platelet-derived growth factor</td>
</tr>
<tr>
<td>End-organ dysfunction</td>
<td>Hyponatremia</td>
</tr>
<tr>
<td></td>
<td>Total bilirubin</td>
</tr>
<tr>
<td></td>
<td>Creatinine</td>
</tr>
<tr>
<td></td>
<td>PaCO&lt;sub&gt;2&lt;/sub&gt;</td>
</tr>
<tr>
<td></td>
<td>Cystatin C</td>
</tr>
</tbody>
</table>

Adapted from Braunwald [13]

<sup>a</sup> Supported by current ACCF/AHA/ESC guidelines for clinical monitoring and prognostication

<sup>b</sup> Current therapeutic targets
prognosticate mortality within this cohort over a 3.5-year follow-up period [43]. Vélez-Martínez et al. also showed a direct relationship with elevated BNP levels and an inverse relationship with 6MWD, hemodynamic indices, and abnormalities on cardiac magnetic resonance imaging. Limitations to the use of troponin in PH, like other established biomarkers, include a lack of specificity for PH. These proteins can be elevated in both cardiac and noncardiac clinical syndromes and is reliant on adequate renal function for clearance [44].

Uric Acid

Serum uric acid (UA) concentrations are elevated in patient populations with high left and right atrial filling pressures and are known to be a marker of morbidity and mortality in heart failure [45–47]. Exact mechanisms are unclear but may be secondary to increased production as a result of tissue hypoxia versus decreased excretion in the setting of renal dysfunction. Tissue hypoxia occurs as a result of low cardiac output which leads to the depletion of adenosine triphosphate (ATP), yielding production of uric acid, a terminal by-product of purine metabolism, and stimulates production of xanthine oxidase [45, 48]. UA levels within PH cohorts have been evaluated and are known to directly correlate with degree of morbidity and mortality, pulmonary vascular resistance, and right atrial pressure and to negatively correlate with cardiac indices [45, 47–49]. Studies have also shown a dynamic response to UA levels with the addition of vasodilator therapy [47, 48]. However, the long-term effect of lowering UA levels within the PH population remains unknown. Large-scale therapeutic intervention targets of uric acid reduction are needed to demonstrate an impact on cardiovascular disease and PH [50]. Possible therapeutic interventions include the use of urosuric agents versus xanthine oxidase inhibitors or drugs promoting the breakdown of UA. Like other PH biomarkers, UA also lacks specificity [51].

Inflammation

Markers of inflammation have also been linked to increased morbidity and mortality and cardiovascular risk within PH patients. A number of markers have been described and include but are not limited to osteopontin, red cell distribution width (RDW), C-reactive protein (CRP), and interleukins [39, 51, 56, 57]. Elevation is a consequence of the pathophysiologic changes secondary to neurohormonal activation, pressure-volume status, and fluctuation in cardiac indices.

Osteopontin

Osteopontin (OPN) is a glycoprotein that has been previously described as being upregulated in individuals with heart failure and has been defined as playing a role in ventricular remodeling [58, 59]. It is found in both cardiomyocytes and fibroblasts. It functions as a cytokine, mediating interactions between cardiac-specific integrins, like β1-integrin [60]. In addition to individuals with chronic heart failure, Rosenberg et al. found a significant elevation in OPN within a small PAH cohort. This small, prospective single-center study demonstrated a significant increase in OPN compared to a control group with a mean of 396 versus 849 ng/ml with a p value less than 0.0001. The OPN value directly correlated with functional class and NT-proBNP. Both NT-proBNP and OPN were found to predict mortality [61]. Elevated levels also predict and correlate with degree of RV remodeling and dysfunction [62]. While being not specific to the heart, OPN appears to serve as a potential prognostic marker as its concentrations are not affected by renal dysfunction and body habitus like natriuretic peptides and troponins. However, levels can be elevated in certain malignancies, like mesothelioma [62]. Further studies are needed to demonstrate whether this will be a dynamic marker that can support therapeutic monitoring in addition to providing prognostic information.

End-organ Dysfunction

Cystatin C

Cystatin C (CysC) is a low molecular weight protein that is an early marker of renal dysfunction [63]. In addition to renal dysfunction, elevated levels have been seen in individuals with heart failure [64]. Recently, its role in PAH has been evaluated with regards to serum concentration and RV anatomy and physiology. A small, prospective study by Fenster et al. demonstrated significantly elevated levels of CysC in individuals with PAH compared to a control group, p = 0.001. In addition, they showed a significant positive correlation between RVSP (p = 0.002), RV end-diastolic (p = 0.01), and end-systolic volumes (p = 0.003) [65]. Unlike natriuretic peptides, CysC concentration is independent of age and muscle mass. However, it is affected by increased cell turnover states, like
hyperthyroidism [65]. This pilot study suggests a potential role of cystatin C but, again, is limited by small sample size, single sample collections, and lack of hemodynamic data.

While a number of inflammatory markers have been defined and associated with worse outcomes in PH, it remains unclear whether or not intervention leading to change in concentration will have a meaningful clinical impact. In the treatment of some cardiovascular diseases, reduction in a biomarker does not necessarily correlate to an improvement in clinical outcomes. Recently, a recent prospective single-center, double-blinded randomized control trial examining the impact of colchicine in patients with chronic, symptomatic reduced heart failure failed to demonstrate a significant impact on morbidity and mortality despite of a significant reduction in inflammatory markers (IL-6 and hs-CRP) [66].

Treatment

Over the last two decades, the field of PH has been revolutionized with the recognition and development of therapeutic interventions as our understanding of its pathophysiology and biology has evolved. The ESC and ACCF/AHA have developed guidelines with regard to treatment and management algorithm. To date, objective measures are used in conjunction with subjective metrics to assist physicians in monitoring a patient’s response to treatment at baseline and follow-up. These measures include WHO functional class, physical signs of right heart failure, echocardiography, hemodynamics from right heart catheterization, 6-min walk distance or cardiopulmonary exercise testing, and BNP or NT-proBNP [1, 2]. With regard to cardiac biomarkers, precise values for NT-proBNP or BNP are not delineated, but the guidelines equate minimal elevation with better prognosis versus significant elevation with poor prognosis. The role of serial biomarker measurements has not been clearly delineated; however, it could be reasonable to obtain as an objective data point during a follow-up visit [23••]. ACCF/AHA guidelines have not incorporated serial measurement into their guidelines, whereas ESC recommends (class I, level of evidence C) a baseline BNP/NT-proBNP collection, along with serial measurements at each follow-up and when there is an acute change in clinical status. It is recommended that stable patients be evaluated every 3 to 6 months, and unstable patients should be seen in 1- to 3-month intervals or sooner for clinical worsening [1, 2].

Aside from day-to-day clinical management, biomarker serum measurements serve as secondary endpoints in pharmacological trials. The theory is to demonstrate a dynamic value with inverse correlation to morbidity and mortality. For example, the clinical trial looking at Riociguat, a soluble guanylate cyclase stimulator, demonstrated a significant decrease in NT-proBNP levels after 3 months which also correlated with primary outcome of improved exercise capacity in PAH patients [67]. Epoprostenol is the sole PAH drug shown to have a mortality benefit [68]. This trial did not specifically bank biomarker data, but small trials and registries have shown that treatment with prostacyclin or other agents have a dynamic, positive effect on BNP/NT-proBNP values and other markers described in this review [2, 7, 26, 35, 69]. Initiation of treatment can lead to a dynamic impact on biomarker values, and while initial value is an indicator or predictor of prognosis, the overall impact on mortality remains a question.

Conclusion

Despite advances in our clinical and biologic understanding of PH, this clinical syndrome remains complex and challenging with regard to patient management and decision-making. As described, PH biomarkers serve a critical role from a diagnostic, prognostic, and therapeutic standpoint. Routine patient evaluation is based on clinical, functional, and hemodynamic assessments. The addition of biomarkers complements the clinical evaluation and provides a predictive and prognostic assessment. Biomarkers may be the first or only evidence of PH or disease progression. The question remains as to whether or not there is a PH biomarker that can assist in characterizing the development of a preventative, predictive model within this heterogeneous and initially, clinically silent cohort.

Compliance with Ethics Guidelines

Conflict of Interest  Julie L. Rosenthal and Miriam S. Jacob declare that they have no conflict of interest.

Human and Animal Rights and Informed Consent  This article does not contain any studies with human or animal subjects performed by any of the authors.

References

Papers of particular interest, published recently, have been highlighted as:
• Of importance
•• Of major importance

53. Kakkar R, Lee RT. The IL-33/ST2 pathway: therapeutic target and novel biomarker. Nat Rev Drug Discov. 2008;7(10):827–40. This is a review of IL-33/ST2 pathway which elucidates the biology of ST2 biomarker and potential role in PH.