

# Role of Biomarkers in the Diagnosis, Risk Assessment, and Management of Pulmonary Hypertension

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**ABSTRACT:** Pulmonary hypertension is a severe and debilitating disease with no definite cure, and the domain of targeted therapies is a promising field for better management of this severe condition. The disease comprises pulmonary arterial remodeling, hypoxia, endothelial dysfunction, and inflammation, with subsequent organ damage including right heart and liver dysfunction. Biomarkers have a valuable role at different levels of the disease, from diagnosis to risk assessment and management, in order to decrease the burden of the disease in terms of both morbidity and mortality.

**KEYWORDS:** pulmonary, hypertension, arterial, biomarkers

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## Introduction

Pulmonary hypertension (PH) is still a clinical dilemma with regard to accurate risk assessment and efficient management. PH is defined as an increase in mean pulmonary arterial pressure  $>25$  mmHg at rest as assessed by right heart catheterization.<sup>1</sup> PH is a complex condition, and despite significant progress in understanding its pathophysiology, PH patients continue to suffer significant morbidity and mortality; in addition, PH prevalence continues to rise and significant gaps remain in our understanding of the disease.<sup>2</sup> PH is characterized by pulmonary vascular remodeling consisting of medial hypertrophy, intimal proliferative changes, adventitial thickening, and fibrosis. Patients with PH require a comprehensive assessment, as no single clinical or paraclinical variable provides sufficient information to guide clinical decisions. Biomarkers are considered of crucial importance and may serve as a useful noninvasive tool in the clinical armamentarium for a better diagnosis, along with accurate risk assessment and appropriate management. A biomarker is defined as “a characteristic that is objectively measured and evaluated as an indicator of normal biologic processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention.”<sup>3</sup>

Biomarkers in the context of PH may reflect different levels of dysfunction or stages of PH, including vascular dysfunction and inflammation, myocardial stress, tissue hypoxia, and secondary organ damage.<sup>1</sup> In this study, we review and discuss the value and usefulness of biomarkers for diagnosis, risk assessment, and management of PH.

## Methodology

Through a MEDLINE/PubMed search for articles published from 2000, and using the terms “pulmonary hypertension, markers, biomarkers, prognosis, and management,” 116 articles were analyzed. From this initial group, 38 articles found to be relevant to the studied topic were selected.

## Background

PH is a rare disease that is associated with significant morbidity and mortality. PH is characterized by pulmonary endothelial cell dysfunction, oxidative stress, imbalance in the vasoactive and vasoproliferative mediators, vascular inflammation, and fibrosis.<sup>4</sup> Ultimately, these changes lead to vascular media hypertrophy with vasoconstriction and a subsequent increase in pulmonary arterial pressure.<sup>5</sup> Based on clinical and hemodynamic findings, PH can range from mild to severe, and this spectrum of severity raises several clinical questions in terms of distinctive management in each of the severity stages.<sup>6</sup>

The term pulmonary arterial hypertension (PAH) describes a category of PH characterized by the presence of precapillary PH, in the absence of other causes such as chronic thromboembolic PH or lung diseases. Precapillary PH is considered present when PH is associated with a pulmonary artery wedge pressure  $\leq 15$  mmHg and a pulmonary vascular resistance  $>3$  Wood units.<sup>7</sup>

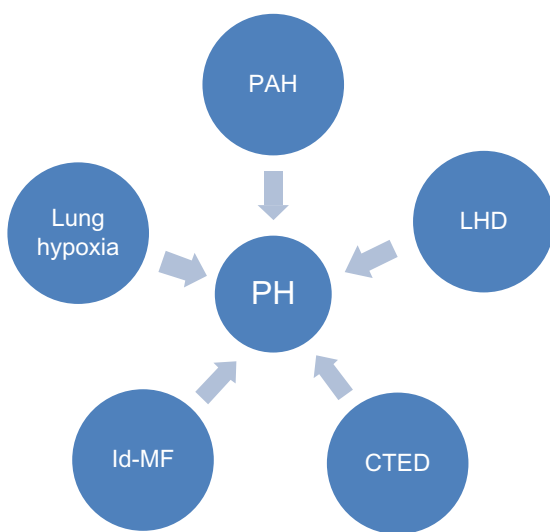
PH inflicts a range of symptoms, including impaired exercise capacity, and ultimately leads to right ventricular failure, renal and hepatic dysfunction.<sup>1</sup> A number of pathological



abnormalities have been identified in PH, whether directly correlated to PH or as a consecutive process, including ischemia and hibernation of the right ventricle, autonomic imbalance due to desensitization of  $\beta$ -adrenergic receptors, metabolic abnormalities (notably increased glycolysis), and increased fibrotic processes.<sup>8</sup>

PH is classified into five groups: group 1, PAH (comprising idiopathic, heritable, drug-induced, associated PAH, and persistent PH of the newborn); group 2, PH consecutive to left heart disease; group 3, PH consecutive to lung diseases and/or hypoxia; group 4, PH consecutive to chronic thromboembolic disease; and group 5, PH with unclear and/or multifactorial mechanisms (Fig. 1).<sup>9</sup> According to the REVEAL study,<sup>10</sup> variables independently associated with increased mortality in PH include the following: high pulmonary vascular resistance ( $>32$  Wood units), high mean right atrial pressure ( $>20$  mmHg), resting systolic blood pressure  $<110$  mmHg, heart rate  $>92$  bpm, right ventricular dysfunction, portal hypertension, men  $>60$  years old, family history of PH, presence of renal insufficiency, PH associated with connective tissue disease, New York Heart Association (NYHA) functional class  $>2$ , brain natriuretic peptide (BNP)  $>180$  pg/mL, percent predicted carbon monoxide diffusing capacity  $<32\%$ , and presence of pericardial effusion (Table 1).

Evaluation of patients with PH must comprise—for accurate diagnosis, risk estimation, and appropriate management—assessment of functional class and exercise tolerance, hemodynamic and echocardiographic studies, and the study of relevant biomarkers.<sup>11</sup> Biomarkers have different sensitivities and specificities, and this variability yields different robustness, accuracy, and reproducibility, regarding diagnosis and risk assessment in the setting of PH.



**Figure 1.** Schematic figure representing the etiologies of PH.

**Abbreviations:** PH, pulmonary hypertension; PAH, pulmonary arterial hypertension related to idiopathic, heritable, drug-induced, or associated PH; LHD, left heart disease; CTED, chronic thromboembolic disease; Id-MF, idiopathic, multifactorial etiology.

**Table 1.** Variables independently associated with increased mortality in PH.

PARAMETER	VALUE
Sex	Men
Age	$>60$ years
Mean right atrial pressure	$>20$ mm Hg
Resting systolic blood pressure	$<110$ mm Hg
Resting heart rate	$>92$ bpm
Right ventricle dysfunction	(QV)
Portal hypertension	(QV)
Pulmonary vascular resistance	$>32$ wood units
Family history of PH	(QV)
NYHA	$>2$
BNP	$>180$ pg/ml
DLCO	$<32\%$
Pericardial effusion	(QV)
Renal insufficiency	(QV)
Associated condition	Connective tissue disease

**Abbreviations:** PH, pulmonary hypertension; NYHA, New York Heart Association; BNP, brain natriuretic peptide; DLCO, percent predicted carbon monoxide diffusing capacity; bpm, beat per minute; QV, qualitative value.

### Use of Biomarkers in PH

In the context of PH, circulating biomarkers are useful for diagnosis, risk assessment, and management; in this regard, biomarkers may reflect vascular dysfunction (eg, asymmetric dimethylarginine [ADMA]), myocardial stress (eg, BNP), inflammation (eg, C-reactive protein), tissue hypoxia (eg,  $p\text{CO}_2$ , osteopontin), and secondary organ damage (eg, creatinine, bilirubin).<sup>1</sup> However, none of these biomarkers shows all the characteristics of the ideal biomarker and a multimarker strategy is often required, along with other clinical or paraclinical tests such as analysis of symptoms and echocardiography.<sup>12</sup>

**Use of biomarkers in the diagnosis of PH.** PH diagnosis is mainly based on right heart catheterization, and echocardiography is also useful in this context. Accordingly, biomarkers are rarely needed for diagnosis, given their low specificity, and they are rather more useful for risk assessment and for guiding therapy.<sup>1</sup>

Although PH is a relatively common complication of many rheumatic heart diseases, this etiology is sometimes ignored and PH is classified as idiopathic in such cases.<sup>13</sup> In fact, the causal relationship is often overlooked given the long interval between the occurrence of rheumatic disease and the development of PH, or because of the lack of a specific paraclinical test to assess such a causal relationship. MicroRNAs (miRNAs) are a class of single-stranded endogenous noncoding RNA molecules, involved in the regulation of cell proliferation and apoptosis. PH is characterized by enhanced proliferation and reduced apoptosis of pulmonary artery smooth muscle cells; miR-1183 and miR-1299 play a specific role in the



pathogenesis of PH associated with rheumatic heart diseases, and therefore, they may be used as biomarkers to diagnose PH etiology in such cases.<sup>13</sup>

Caveolin-1 is a biomarker of value for screening, diagnosis, and follow-up of patients with PH. Caveolin-1 is a protein that is highly expressed in type I pneumocytes. However, its secretion in vascular endothelial cells is decreased in patients with PH, and a serum cutoff of 17.17 pg/mL is highly suggestive of idiopathic PH.<sup>14</sup>

Elastin is a structural constituent of blood vessels, and its synthesis requires cross-linking of monomers by two amino acids, desmosine and isodesmosine. In patients with PH, elastin turnover is elevated, and consequently, plasma and urine levels of desmosine and isodesmosine are high. In view of this, desmosine and isodesmosine are regarded as potential screening biomarkers for patients with PH.<sup>15</sup>

Seyfarth et al.<sup>16</sup> reported that patients with PH have a change in their vascular architecture, and in this regard, they observed higher levels of angiogenic markers (angiogenin, tumor necrosis factor- $\alpha$  [TNF- $\alpha$ ]) in the breath condensate of patients with PH in comparison to healthy controls.

**Use of biomarkers for risk assessment and prognosis of PH.** The use of biomarkers as a stand-alone test for diagnosis of PH is rare in clinical practice, given the availability of other paraclinical tests that are more accurate and reproducible, such as echocardiography and right heart catheterization. However, biomarkers are typically used for risk assessment of the disease and for guiding management.<sup>1</sup>

Özpelit et al showed that many cytokines are correlated with low survival in PH, namely, interleukin-6, TNF- $\alpha$ , and transforming growth factor-beta (TGF- $\beta$ ).<sup>17</sup> Moreover, osteopontin is an endogenous modulator of pulmonary adventitial fibroblasts and promotes several cellular activities, including growth and migration of the pulmonary adventitial fibroblasts. It has been shown to improve risk stratification in PH.<sup>18</sup> In PH, endothelial dysfunction is associated with impaired apoptotic signaling, leading to intimal proliferation and increased circulating endothelial cells (CECs). In this regard, CECs are considered valuable biomarkers signaling poor prognosis in PH and allowing discrimination between reversible and irreversible forms of PH, especially when correlated with congenital heart diseases.<sup>19,20</sup> Endothelial dysfunction manifests as increased oxidative stress with elevated reactive oxygen species and decreased nitric oxide (NO). Of note, ADMA is an endogenous NO synthase inhibitor, and high ADMA plasma levels in patients with PH are associated with unfavorable prognosis.<sup>21</sup> Similarly, miRNAs are involved in the pathophysiological mechanisms of endothelial dysfunction, leading to progression of PH, and high levels of miRNAs reflect the severity of PH.<sup>22</sup>

Plasma levels of BNP may be difficult to interpret owing to the presence of different confounding factors affecting BNP concentrations, such as age, sex, and NYHA functional class. However, high baseline BNP concentration and its serial changes

seem to predict survival and/or hospitalization in PH patients, by reflecting the degree of increased right ventricular wall stress and associated deterioration in right ventricular function.<sup>23,24</sup>

PH involves variable physiopathological mechanisms, including endothelial dysfunction, and it leads to variable organ damage, including right ventricular failure. In this regard, plasma levels of relevant biomarkers reflect the extents of such dysfunctions: natriuretic peptides (BNP and pro-BNP) for right heart dysfunction, ADMA for endothelial dysfunction, and vascular endothelial growth factor (VEGF) for endothelial proliferation.<sup>24</sup> Moreover, vasopressin is a key regulator of body fluid homeostasis and the cosecreted protein, copeptin, serves as surrogate for plasma vasopressin levels. Accordingly, elevated plasma levels of copeptin reflect an unfavorable hemodynamic profile and a poor outcome in patients with PH.<sup>25</sup>

Kolditz et al reported that in patients with PH, mid-regional pro-adrenomedullin (MR-proADM), a marker of neuroendocrine activation, strongly correlates with exercise capacity as measured by six-minute walk distance (6MWD) and  $VO_2$  max.<sup>26</sup> Moreover, the authors found that MR-proADM may predict survival in patients with PH, independent of age.

The neutrophil-to-lymphocyte ratio (NLR) in patients with PH has also been found to offer significant prognostic value. Özpelit et al reported that high NLR values reported on admission of patients with PH correlated significantly with important prognostic markers in PH, such as NYHA functional class and BNP, and therefore, NLR was found to be useful for the assessment of disease severity.<sup>17</sup> Similarly, other authors reported that markers of systemic inflammation (ie, hs-CRP), when measured in patients with PH in stable medical condition, are significantly correlated with the severity of the disease.<sup>27</sup>

Liver dysfunction in PH is usually consecutive to right heart failure, and elevated serum bilirubin has been reported as a predictor of poor prognosis and higher mortality.<sup>28</sup> Similarly, low von Willebrand factor (vWF) activity together with low cholesterol levels are associated with increased risk of death and increased frequency of transplantation in patients with PH.<sup>29</sup>

Osteoprotegerin (OPG) is a soluble member of the TNF that blocks the binding of TNF and therefore prevents apoptosis. Condliffe et al reported that OPG levels are associated with pulmonary vascular remodeling and that these levels are significantly elevated in patients with idiopathic PH.<sup>30</sup> The authors concluded that elevated OPG values represent a marker of poor prognosis in PH.

C-terminal pro-endothelin-1 (CT-proET-1), a precursor of endothelin-1, has been investigated as part of a panel of biomarkers in patients with PH; elevated values of CT-proET-1 were found to be independently associated with poor outcome and all-cause mortality.<sup>31</sup> Yanagisawa et al studied the value of the circulating amino acid profile in patients with PH and



determined the Fischer ratio (branched-chain amino acids/aromatic amino acids); the authors found that this ratio is correlated with venous oxygen saturation and 6MWD and that the ratio decreases in proportion to the severity of PH.<sup>32</sup>

Vascular remodeling and fibrosis represent significant physiopathological features in PH. Circulating collagen biomarkers reflect disease severity, and notably, N-terminal propeptide (type III procollagen), a marker of collagen metabolism, is elevated in severe cases of PH.<sup>3</sup>

**Use of biomarkers in the management of PH.** PH is a severe, debilitating, and progressive disease, and there is no cure. Disease progression is inevitable in the majority of cases, and the long-term prognosis remains poor. Currently, there are three classes of drugs approved for the treatment of PH: prostacyclin analogs, endothelin receptor antagonists, and NO phosphodiesterase type 5 inhibitors.<sup>1</sup> In view of this, there is a clear and urgent need for additional therapeutic options, and the availability of targeted therapies may lead to major advances in this regard.

Appropriate management starts with an accurate and early diagnosis, risk stratification, and judicious use of therapy. Many treatment options are feasible, according to the clinical scenario, including initial monotherapy, initial combination therapy, or sequential combination therapy.<sup>1</sup> In general, the current goals of therapy in PH comprise improvement in NYHA functional class, increasing the 6MWD to more than 380 m, and improvement of right ventricular size and function, decreasing or normalization of BNP, decreasing right atrial pressure below 8 mmHg, and increasing cardiac index, with the ultimate objective of reducing the need for hospitalization and improving survival.<sup>33</sup> In this regard, current PH-specific therapies must target one or many of these goals to improve clinical outcome.<sup>12,34</sup> Serum level of natriuretic peptides is an effective tool that may be used for determining timing of therapeutic interventions in PH.<sup>4,24</sup> Interleukin-33 (IL-33) and suppression of tumorigenicity 2-ligand (ST2 L) interact to decrease inflammatory response; when soluble ST2 (sST2) binds IL-33, it suppresses the interaction with ST2 L; sST2, by acting as a decoy receptor, could prevent the beneficial effects of IL-33/ST2 L interaction. Therefore, sST2 measurements in blood samples could be a clinical biomarker useful in risk stratification and management of patients suffering from myocardial infarction, apnea, chronic obstructive pulmonary disease, asthma, pulmonary embolism, and PH.<sup>35</sup>

The etiology of PAH is incompletely understood, and the genetics of PAH are also complex due to incomplete penetrance and genetic heterogeneity. However, genes play an important role in idiopathic and heritable form of PAH: mutations in bone morphogenetic protein receptor 2 (*BMPR2*) gene have been identified in more than 70% of cases of familial PAH, as well as in 10%–40% of idiopathic PAH cases.<sup>36</sup> Of note, *BMPR2* is a member of the *TGF-β* superfamily of receptors, and mutations in genes of the *TGFβ* family members (*ALK-1*,

*ENG*, *SMAD4*, and *SMAD8*) are additional rare causes of PAH. Moreover, caveolin-1 gene (*CAV1*) regulates *SMAD2/3* phosphorylation, and mutations in *CAV1* are also a rare cause of PAH.<sup>36</sup> An enhanced understanding of the pathophysiology of PH, namely, endothelial dysfunction, is one of the pathways that must be explored further and targeted for more effective management of PH. Biomarkers of endothelial dysfunction may serve as indices of efficacy of related therapy. Similarly, the realization that many components of PH have a genetic basis must allow new therapeutic fields to be targeted, such as cell therapy or organ transplantation.<sup>37,38</sup> In this regard, genetic target-based therapy is an interesting pathway to be explored in order to improve the outcome of patients with PH (Table 2).<sup>22</sup>

### Clinical Implications and Limitations

Currently, there is no biomarker with high sensitivity and specificity in PH, whether for diagnosis, risk assessment, or management; this is due to the complex physiopathology of PH along with the complex interplay of many processes that determine prognosis and survival.<sup>1</sup> Many challenges remain to be overcome, including the identification of specific genetic markers,<sup>22</sup> improvement of screening and early diagnosis, improvement of reproducibility of biomarkers, identification of appropriate use criteria of biomarkers, improvement of treatment adherence, improvement of coordination among practitioners, and advancement in research regarding morbidity and mortality data.

The armamentarium of treatment of PH is expanding, and management of PH requires coordinated teamwork among the primary care physician, rheumatologist, pneumologist, cardiologist, and internist. Moreover, the formation of PH specialty centers, with specialized staff and suitable equipment, is valuable for better management of patients with PH.<sup>1</sup>

**Table 2.** Classification of markers according to their diagnostic, prognostic, and managerial value.

Markers of diagnosis	Echocardiography parameters, right heart catheterization parameters, miRNA, Cav1, Desmosin, Angiogenin, TNF- $\alpha$
Markers for risk assessment	Interleukin-6, TNF- $\alpha$ , TGF- $\beta$ , CEC, osteopontin, ADMA, miRNA, BNP, VEGF, MR-proADM, 6MWD, NLR, vWF, low cholesterol, OPG, circulating AA profile, type III procollagen, CT-proET-1
Markers with managerial value	NYHA, 6MWD, RVD and RVF, BNP, RAP, CI

**Abbreviations:** miRNA, microRNA; Cav1, caveolin-1; TNF- $\alpha$ , tumor necrosis factor-alpha; TGF- $\beta$ , transforming growth factor-beta; CECs, circulating endothelial cells; ADMA, asymmetric dimethylarginine; BNP, brain natriuretic peptide; VEGF, vascular endothelial growth factor; MR-proADM, mid-regional pro-adrenomedullin; 6MWD, six-minute walk distance; NLR, neutrophil-to-lymphocyte ratio; vWF, von Willebrand factor; OPG, osteoprotegerin; AA, amino acid; CT-proET-1, C-terminal pro-endothelin-1; NYHA, New York Heart Association; RVD, right ventricular dimensions; RVF, right ventricular function; RAP, right atrial pressure; CI, cardiac index.





## Conclusion

Currently, there is no single biomarker in PH that shows superiority in predicting prognosis or patient response, and therefore, an integrative approach is necessary, using a combination of biomarkers along with other clinical and paraclinical tests like NYHA functional class and echocardiography. Moreover, each patient with PH represents an individual clinical scenario, and management should be tailored accordingly.

## Author Contributions

Conceived and designed the experiments: VR, AK. Analyzed the data: VR, AK. Wrote the first draft of the manuscript: VR, AK. Contributed to the writing of the manuscript: VR, AK. Agree with manuscript results and conclusions: VR, AK. Jointly developed the structure and arguments for the paper: VR, AK. Made critical revisions and approved final version: VR, AK. All authors reviewed and approved of the final manuscript.

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