

Can biomarker predict development and progression of pulmonary artery hypertension?

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Abstract

Pulmonary Arterial Hypertension (PAH) is a heterogenic group of devastating disorders that characterizes higher rates of mortality and morbidity worldwide. Recent clinical studies have shown that clinical features, hemodynamic parameters, echocardiography patten, multi-spiral computer tomography findings, exercise capacity, and anti-nuclear antibody profiles could be used as predictors of clinical severity and outcomes in PAH patients. However, the reliability, sensitivity, specificity and predictive value are derived from PAH patients with different comorbidities and specific complications associated with CTD, congenital heart disease and respiratory disease might be unacceptable. The aim of the review: to summarize the knowledge regarding predictive value of several biomarkers in PAH individuals. The review is argued the perspective to utilize single sample and serial measurements of biomarkers as a regulatory peptide contributed in PAH pathogenesis aimed to improve prediction of PAH development and progression.

Keywords: Pulmonary Artery Hypertension; Biomarkers; Prognostication

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Introduction

Pulmonary Arterial Hypertension (PAH) is a heterogenic group of distinct disorders that includes Idiopathic PAH (IPAH), familial PAH, Chronic Thromboembolic Pulmonary Hypertension (CTEPH), and PAH associated with other

conditions (APAH) such as Connective Tissue Disease (CTD-APAH), respiratory disease, congenital or acquired left-heart inflow/outflow obstructive lesions or congenital cardiomyopathies etc. [1, 2]. PAH is characterized by pulmonary capillary endothelial metabolic dysfunction directly related to microvascular inflammation that leads to increased pulmonary arterial pressure and pulmonary vascular resistance [3-5]. Despite histological findings that are suitable for the pulmonary vascular lesions in PAH complicating CTD are similar to those observed in IPAH, familial PAH and APAH, morbidity and mortality rates between these patient cohorts sufficiently distinguish. Compared with IPAH patients and familial PAH individuals, patients with PAH associated with CTD have an older age of onset and exhibited worse prognosis in survival [6] Survival of patients with respiratory disease-related or congenital heart disease-related PAH in the modern treatment era is better than CTD-APAH [6, 7]. Although clinical features, hemodynamic parameters, echocardiography patten, multi-spiral computer tomography findings, exercise capacity, and anti-nuclear antibody profiles were found as powerful factors predicted a development of PAH due to several diseases [8], the reliability, sensitivity, specificity and predictive value are derived from PAH patients with different comorbidities and specific complications associated with CTD, congenital heart disease and respiratory disease might be unacceptable [9]. In this context, taken into consideration pathophysiological heterogeneity of PAH to risk stratification based on biological markers reflected several faces of nature evolution of the disease might be useful and appears to be attractive [10-16]. The aim of the review: to summarize the knowledge regarding predictive value of several biomarkers in PAH individuals.

Challenging in Biomarker Discovery in PAH

Because pathogenesis of PAH as a devastating disease characterized by a progressive increase in pulmonary vascular resistance resulting in micro vascular inflammation / immunological disturbances, vasospasm / obliterative pulmonary vasculopathy, endothelial dysfunction, thrombosis / hyper coagulation, development of ventilation-perfusion mismatch, right ventricle acute / chronic heart failure, and premature death, biomarkers might reflect various faces of pathogenesis, several stages of disease development, risk of complications or disease progression, risk of clinical outcomes / death, clinical response to the treatment (Table 1).

Natriuretic Peptides

Natriuretic Peptides (NPs) are useful indicator of cardiac wall stretching due to overload or injury [17]. Current clinical guidelines recommend to measure single sample peak and serial concentrations of NPs for identifying patients with HF at high risk of outcomes, death and re-admission, as well as probably to adjust daily doses of remedies that are used for treatment of HF patients [18]. Taken into consideration that age, renal failure, obesity, and some comorbid conditions, i.e. diabetes, anemia, chronic obstructive pulmonary disease, cardiac hypertrophy, required to be considered when interpreting the results due to higher biological variability and worsening of NP' clearance [19, 20].

By now, B-type NPs (BNP) and N-Terminal pro-BNP (NT pro-BNP) are widely used to determine the severity and progression of Right Ventricular (RV) heart failure, as well as outcomes from both these conditions [21]. However, the advantages of BNP or NT pro-BNP single sample measurement compared two-dimensional / three-dimensional echocardiography (including speckle tracking analysis) and Doppler-echocardiography in assessing PAH severity and predicting outcome is insufficiently defined [22]. Therefore, there are evidence regarding right ventricular dysfunction based on ventricle dilation and / or ejection fraction declining measured by cardiac magnetic resonance imaging or CT scan are correlate well with serum peak BNP or NT pro-BNP levels [23]. Although BNP and NT pro-BNP had prognostic value in patients with symptomatic PAH, the prognostication of clinical evolution among asymptomatic PAH individuals or stratification in population at higher risk PAH development are not fully clear and

require more investigations [24]. There are insufficient data regarding head-to-head comparison between other echocardiographic parameters and biomarkers. Furthermore, there is needed to determine confirmation whether the combination of echocardiography / exercise capacity and the NPs is sufficiently accurate to rule out PAH when testing symptomatic patients [25, 26].

Galectin-3

Gal-3 is an adhesion-modulated and growth-regulatory β -galactoside-binding lectin that secreted by activated mononuclears / macrophages and contributed to various pathophysiological processes affected cardiac / vascular remodeling, cardiac fibrosis, and inflammation [27]. As a multi factorial regulator of cell function Gal-3 is involved in cell proliferation / differentiation, angiogenesis, neovascularization, as well as malignancy, tumor cell adhesion and metastasis [28]. The main regulators of Gal-3 secretion are pro-inflammatory cytokines, i.e. interferon- γ , tumor necrosis factor alpha, and interleukin-17, components of oxidative stress, free radicals, aldosterone, and transforming growth factor-beta [29]. Recent studies have shown that Gal-3 exhibits a pro-inflammatory response by recruiting and activating lymphocytes, mononuclears / macrophages and other antigen-presenting cells [30, 31]. Indeed, Gal-3 galectin-3 exerts cytokine-like regulatory actions on production of inflammasome via activation of the JAK-STAT cascade [32]. Vergaro G et al. (2016) [33] reported that Gal-3 may interact with aldosterone in promoting macrophage infiltration and vascular fibrosis. Therefore, Gal-3 contributes to cardio renal remodeling and endothelial dysfunction induced by aldosterone [34]. Interestingly, in animal models Gal-3 not only causes cardiac hypertrophy, but directly induces cardiac dysfunction through involvement of the Transforming Growth Factor (TGF)-beta/Smad3 signaling pathway [35-37]. Moreover, inhibition of Gal-3 was associated with a down-regulation in collagen I and III production, collagen processing, cleavage, cross-linking, and deposition [38]. Taken together these data have suggested that Gal-3 could be a novel therapeutic target in cardiac fibrosis and inflammation, while clinical relevance of these findings requires more investigations.

Recent study has shown that Gal-3 appears to be localized on the osteoclast cell surface, and its suppression sufficiently inhibited osteoclast differentiation and reduced the

number of mature osteoclasts [39]. These findings elucidate the role of Gal-3 in modulation of bone mineralization / osteoporosis and link the process with inflammation. Finally, Gal-3 signaling is argued as an important mechanism of cardiac / vascular remodeling development based on inducing of hypertrophy / fibrosis and inflammation.

PAH is characterized by abnormal elaboration of vasoactive substance (endothelin-1, serotonin, bone morphogenetic proteins, Rho kinase, and hypoxia-inducible factor 1), endothelial cell dysfunction, vascular remodeling, and inflammation, which collectively contribute to its pathogenesis [40]. Overall, pathogenic transformation of the pulmonary vascular bed is argued a main cause of PAH development with and without associated various clinical phenotypes. Indeed, despite the pathogenesis of PAH is a complex and multifactorial process, inflammation could be a trigger of endothelial injury, pulmonary artery smooth muscle cell proliferation, chemoattractant over-production. There is evidence regarding the role of pulmonary arterial smooth muscle and endothelial cells in vasoconstriction, development of vaso-occlusive lesions and ventilation-perfusion mismatch [41]. Therefore, PAH might occur due to several molecular mechanisms resulting in heterogeneous genetic defects affecting defects of the transforming growth factor beta pathway that could be regulated with Gal-3 [42]. At this pathway, Gal-3 may play a pivotal role in establishment and progression of PAH via inducing of endothelial dysfunction and hyperplasia of the underlying medial layer potentially through direct activation of (TGF)-beta/Smad3 signaling.

Elevated levels of circulating Gal-3 has found in Cardiovascular (CV) disease (coronary artery disease, hypertension, heart failure, atrial fibrillation / flutter), renal and metabolic (diabetes mellitus, metabolic syndrome, obesity) disease and were strongly associated with asymptomatic atherosclerosis, premature CV events including myocardial infarction, acute / acutely decompensated heart failure, and sudden death [43-47]. In general population increased Gal-3 concentrations were found as predictor of CV disease and diabetes development [48, 49]. Furthermore, Gal-3 has potential relations with CV mortality and all-cause mortality beyond classical and new markers of CV risk [50, 51]. However, the predictive role of Gal-3 in PAH individuals are still not fully clear.

The heterogeneity of nature evolution, clinical features, prognosis, and treatment response supports the need for identifying PAH patients at risk with various methods. However, lack of universal predictive model mediate to discovery of novel biomarkers and / or utilize a multi biomarker approaches. In this context, predictive model based on Gal-3 single sample measurements could be useful for risk stratification of PAH individuals. Moreover, serial measurements of Gal-3 within treatment are argued a perspective tool for assay the positive response in long-term period affecting mortality rate in PAH. Indeed, Calvier L et al. (2016) [15] reported that elevated level of Gal-3 was closely associated with severity of PAH independently etiology. Fenster BE et al. (2015) [16] presented evidence regarding sufficient relation between circulating level of Gal-3 and morphologic changes in PAH including estimate RV systolic pressure and measure RV strain. Authors found that Gal-3 positively correlated with the extracellular matrix markers, i.e. Tissue Inhibitor of Metalloproteinase-1 (TIMP-1), and hyaluronic acid. However, the evidence regarding multi maker strategy to identify patients at higher risk of PAH development and complications are very limited and requires a confirmation in the large clinical trials.

Chemokine CXC ligand 13

Chemokine CXC ligand 13 (CXCL13) known also as C-X-C motif chemokine 13, B-Lymphocyte-Chemoattractant (BLC), B-cell-attracting chemokine-1 (BAC-1) is member of the chemokine super family [52]. CXCL13 is constitutively expressed in lymphoid organs and plays a pivotal role in B-cell receptor affinity maturation, organizing of B-cell follicles and will be a critical factor to neutralizing of broadly spectrum of antibodies [53-55].

Recent studies have shown that CXCL13 has been implicated in perivascular inflammation and pulmonary vascular remodeling in IPAH affecting plexiform lesion development [56, 57]. Moreover, morphologically and immunohistochemically plexogenic IPAH is concerned as the concentric obliterative arteriopathy associated with represent abnormal growth of factor VIII-related antigen-positive endothelial cells [57]. CXCL13 mediates the presence of perivascular inflammatory cells resulting in cytokines and growth factors and thereby cooperates in lymphoid neogenesis [58]. This mechanism is essential for worsening endothelial function and perivascular fibrosis development [59].

Based on evidence concerning over-expressed CXCL13 might contribute to vascular remodeling and mediate the plexiform phenotype of PAH, CXCL13 is discussed as biomarker of severity of IPAH. Indeed, Olsson KM et al. (2016) [60] have found an increased serum concentrations of CXCL13 in patients with IPAH and CTEPH. Authors reported that CXCL13 probably reflects pulmonary vascular lesions. Unfortunately, serum level of CXCL13 showed weak and inconsistent correlations with markers of inflammation or PAH severity based on clinical findings. However, taken into consideration a serious limitation of investigation number with respect to the predictive role of CXCL13 in PAH more clinical trials are required.

Cystatin C

Cystatin C is low molecular protein belonging to a group of cysteine proteases inhibitors that is produced in the hematopoietic nucleated cells in a constant amount and is regulated at both transcriptional and post-translational levels [61]. Serum concentration of Cystatin C does not depend on gender, age, and muscle mass and protein intake [61]. Because Cystatin C exhibits tightly renal clearance and renal metabolism it is an alternative biomarker used to estimate glomerular filtration rate, while there are some factors (thyroid dysfunction, glucocorticoids use, malignancies etc.) that can affect serum cystatin C level [62].

Measurement of serum cystatin C levels widely incorporates into routine clinical practice and needing of both single sample and serial measurements of the biomarker are supported by recent kidney disease guidelines [63, 64]. Prospective clinical studies have shown that patients from general population with increased cystatin C exhibited a higher risk of developing both CV disease and chronic renal disease [65-67]. Moreover, recent clinical trials have shown that higher cystatin C levels in subjects with various clinical scenarios were directly associated with a higher risk of death from all causes, CV disease, infections and renal disease in a dose-response manner [68-72]. Interestingly cystatin C as elastolytic protease has been shown to be directly involved in the atherosclerotic process and inflammation that could explain its predictive value in CV disease [73]. Indeed, local cystatin C deficiency has been demonstrated in atherosclerotic and aneurismal lesions, suggesting a protective role of cystatin C in the vessel wall, possibly in concert with TGF- β 1 [73, 74].

Serum level of cystatin C correlated with RV dimensions in IPAH patients giving it serious potential to determine prognosis [75, 76]. Fenster BE et al. (2014) [77] reported that cystatin C levels has not only elevated in PAH individuals, but it exhibited accurately correlation with RV pressure, RV ejection fraction, RV strain and strain rate, and morphology of RV. Kaiser R et al. (2014) [78] have revealed that elevated level of cystatin C has associated with both haemodynamic parameters and long-term survival in patients with PAH. In contrast, in patients with chronic respiratory diseases without previous history of symptoms related to PAH NT-proBNP has demonstrated a predictive value in terms of mortality, whereas cystatin C did not [79]. Taken together it is needed to conclude that serum cystatin C level was not a good surrogate marker to predict mortality in asymptomatic subjects suspected PAH. Finally, single measurement of serum cystatin C level might improve early risk stratification of symptomatic PAH individuals compared with haemodynamic parameters, whereas evaluation of precision in predicting mortality in this patient population beyond critical state remains uncertain and requires more investigations.

Endothelin-1

Endothelin-1 is neurohormon, which is produced by the endothelium and mediates vasoconstriction, as well as it exhibits pro-coagulation and proliferative effects directly and via aldosterone realize affecting vascular smooth muscle, the kidneys, and cardiomyocytes [80]. The biological effects of endothelin-1 translate through two types of receptors: ETA and ETB [80]. The wide pleiotropic capability of endothelin-1 may include contribute to production of reactive oxygen species, fibrosis, ectopic calcification, inflammation, cell growth and differentiation, insulin resistance, visceral adipose tissue accumulation, and immunity [81-85]. Although both secretion and expression of endothelin-1 is sensitive to inflammatory stimuli (interleukin-1, interleukin-6, tumor necrosis factor- α), the link between development and progression of CV disease and endothelin-1 is still uncertain [86]. Probably, endothelin-1 might regulate the function of the endothelium and underlying smooth muscle cell by extracellular vesicles exchange and mobbing of endothelial progenitor cells [87, 88].

The serious limitation concerning endothelin-1 use in routine clinical practice is a very short half-life of this hormone (40-70 s) that may be cause of underestimation of its circulating level [89]. Elevated level of circulation endothelin-1 was found in several CV diseases including hypertension, heart failure, arrhythmias, myocardial infarction, cardiomyopathies, valvular heart disease, pulmonary thromboembolism, PAH. By now, endothelin-1 is of interest as an emerging biomarker of CV dysfunction and endothelial damage with high mortality predictive value in subjects with heart failure [90, 91].

Over-expressed level of endothelin-1 in lung was found in animals with Chronic Thromboembolic Pulmonary Hypertension (CTEPH) [92]. In clinical setting it has been defined that endothelin-1 might act not only on the distal vasculopathy in the unobstructed vessels but could stimulate smooth muscle cell proliferation within chronic clot in CTEPH [93]. Parikh RV (2016) [94] have shown that higher levels of endothelin-1 predict disease severity and mortality in other forms of PAH. However, needing to measure serum endothelin-1 aimed to stratify individuals suspected PAH or patients with known PAM is still debating.

Micro-RNAs

MicroRNAs (miRNAs) are small, endogenous, conserved, single-stranded, non-coding RNAs involved in the regulation of posttranscriptional gene expression affected target cell function [95]. It has been defined that several pathogenic processes that are essential for PAH development and progression, such as pulmonary inflammation, vascular remodeling, angiogenesis, and right heart hypertrophy, might be regulated via miRNAs' pathways [96, 97]. The impairment of miRNA's expression has been involved in vascular cell remodeling processes, i.e. adventitial fibroblast migration; pulmonary arterial smooth muscle cell proliferation and pulmonary arterial endothelial cell dysfunction observed in PAH [96]. Indeed, increased mRNA expression of genes of endothelin-1 (ET-1), the transforming growth factor beta ligands, and their receptors, hypoxia inducible factor-1 were found [98, 99]. In animal model it has found that miRNAs-17, -21, and -223 were consistently upregulated in lung, whereas miRNAs-126, -145, -150, -204, -424, and -503 were downregulated in PAH [100]. Interestingly, the human prostacyclin synthase overexpression restored miRNAs to levels in PAH to levels measured in naive controls. It is supposed to mean that impaired signature miRNAs

might be secondary to pulmonary arterial smooth muscle cell / endothelial cell dysfunction. Deng B et al. (2016) [99] reported that loss of suppression on hypoxia inducible factor-1 by miR-103/107 might contribute to excess proliferation of pulmonary arterial smooth muscle cells and vascular remodeling in PAH individuals. Moreover, there is evidence that the dysregulation of miRNAs appears to be specifically occurred depending on etiology of PAH [101]. Unfortunately, there is no evidence supporting the use of mRNA signature to stratify patients with PAH, whereas miRNAs might provide new possibilities of diagnosis, prognosis and treatment choices for disease [102, 103].

Other Biomarkers

The role of metabolomics' biomarkers, soluble vascular endothelial growth factor receptor-1, growth differentiation factor-15, C-reactive protein, and interleukin-6 is currently investigating, while the clinical relevance of practical use of these molecules in PAH is not still defined.

Conclusion

There are no significant evidence regarding both single sample and serial measurements of circulating biomarkers aimed to improve prediction of PAH development and progression based on conventional methods including clinical findings, echocardiography / Doppler echocardiography parameters, exercise capacity, and NT-proBNP concentrations. More clinical trials are needed to explain the predictive role of novel biomarkers in PAH individuals with several etiology.

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Table 1: Circulating biomarkers in diagnosis and prediction of PAH

Name of biomarkers	Relation to pathogenic staging	Relation to etiology of PAH	Evidence for clinical practice use
NPs	RV dysfunction	any	Higher prediction for symptomatic PAH
Galectin-3	Microvascular inflammation, fibrosis	any	Risk of PAH in general population, risk of clinical outcomes in patients with known PAH
ET-1	Neurohumoral activation, endothelial dysfunction,	any	Emerging biomarker of CV dysfunction and endothelial damage. Predictor of PAH-related mortality
Cystatin C	Microvascular inflammation, RV dysfunction, lowered renal function	any	Predictor of higher risk of death from all causes and PAH-associated causes
Chemokine CXC ligand 13	perivascular inflammation, fibrosis	idiopathic PAH	Predictor of endothelial dysfunction
MicroRNAs	Cardiac and vascular remodeling	any (?)	not defined
sVEGFR-1	Vascular remodeling	any (?)	not defined
C-reactive protein	Microvascular inflammation	any (?)	not defined
soluble endoglin	Cardiac and vascular remodeling	not defined	not defined
Pentraxin 3	Cardiac and vascular remodeling	not defined	not defined
GDF-15	Cardiac and vascular remodeling, fibrosis, inflammation	not defined	not defined
IL-6	Microvascular inflammation	any	Predictor of RV remodeling, severity of PAH, low exercise capacity

Abbreviations: NPs, Natriuretic Peptides; PAH, Pulmonary Arterial Hypertension; ET-1, Endothelin-1; Svegfr-1, Soluble Vascular Endothelial Growth Factor Receptor-1; GDF-15, Growth Differentiation Factor-15; IL-6, Interleukin-6; RV, Right Ventricle