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Review Article

BIOMARKERS IN PULMONARY ARTERIAL HYPERTENSION

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Abstract: Pulmonary hypertension (PH) is a heterogeneous disorder likely to be comprised of overlapping syndromes with varying origins and pathobiologies that presents with many phenotypes. Pulmonary arterial hypertension (PAH) is progressive and results in the deterioration of cardiopulmonary function and premature death. The mean age at diagnosis of IPAH is 50 years with a higher female to male ratio. Often, PAH is misdiagnosed due to the lack of clinical knowledge of the disease. Presently, clinical, echocardiographic, and hemodynamic parameters are the most decisive methods for evaluation, prognosis, and diagnosis of PAH. These patients routinely require supportive evidence for the diagnosis of PAH. Therefore, additional methods such as non-invasive biomarkers are necessary help to assess patient prognosis, clinical outcomes, and overall quality of life for patients with PAH. Over the years, researchers have uncovered new biomarkers important for our understanding of the pathophysiology of PAH. In this review, we discuss PAH diagnosis and progression as well as common and potentially novel biological markers used in the prognosis, diagnosis, and mortality prediction of PAH.

Key words: pulmonary arterial hypertension, biomarkers, clinical outcomes, and diagnosis

Introduction*:* Pulmonary Arterial Hypertension (PAH) is a rare syndrome with poor prognosis. Right ventricular heart failure develops in patients at advanced periods of this disease, and mortality is frequently observed within a year for approximately 15% of these patients [1-4].

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The resting average pulmonary artery pressure is known to be higher than 25mmHg [2,5,6] in PAH patients. The most recent updated World Health Organization (WHO) classification of PAH is demonstrated in table 1. PAH is often confused and misdiagnosed with other diseases due to the lack of clinical knowledge of this devastating disease. In addition to routine hematological, biochemical, and immunological tests, patients require supporting evidence for the diagnosis of PAH and are typically

symptomatic approaches. Echocardiography, computed tomography, and scintigraphy can be used to make preliminary diagnosis of PAH. However, right heart catheterization and 6-minute walk test (6MWT) should be routinely done in order to confirm the diagnosis and determine the severity of the disease.

In recent years, many researchers have demonstrated the presence of new biomarkers that are significant in the pathophysiology of PAH. In this review, we discuss PAH diagnosis and progression as well as potentially novel and common biological markers used in the mortality prediction of PAH (the list of biomarkers and isolated cells are given in table 2).

Natriuretic Peptides: Secreted by cardiac myocytes, brain natriuretic peptide (BNP), and atrial natriuretic peptide (ANP) are 2 of the basic hormones in the natriuretic peptide family. While BNP is secreted from ventricular tissue in response to cardiac pressure and volume overload, ANP is released from storage granules in atrial tissue [7]. BNP is synthesized as a precursor molecule (pro-BNP) and is generated through enzymatic cleavage [8] resulting in the active BNP and the inactive N-terminal portion of the prohormone.

Studies have shown that PAH patients with high plasma BNP levels correlate with poor outcomes and disease severity. These data are based on the statistical analysis of plasma BNP levels with WHO-class, New York Heart Association (NYHA) functional class, exercise capacity, mean pulmonary arterial pressure (mPAP), mean right arterial pressure (mRAP), and pulmonary vascular resistance (PVR). In addition, negative correlations were shown with other clinical parameters, including cardiac output (CO), 6MWT, peak oxygen consumption, and the cardiac index (CI) [9,10].

In a previous report, Leuchte and colleagues demonstrated that BNP was increased multiple forms of PAH [11-14]. Consistent with this, BNP level that were greater than 150pg/ml were shown to be associated with low survival [9]. Therefore, the measurement of BNP is one of the most important biomarkers for prognosis of PAH disease and follow-up after treatment.

N-terminal pro-brain natriuretic peptide: Interestingly, results have shown that plasma levels of the N-terminal pro-brain natriuretic peptide (NT-proBNP) hormone may be a useful prognostic indicator of heart failure [15]. Recently, several studies related to the inactive N-terminal fragment of BNP underscores its importance as a biomarker of PAH. Elevated NT-proBNP levels, particularly in systemic sclerosis associated with secondary PAH and idiopathic pulmonary arterial hypertension, have been reported [14,16]. Nevertheless, screening for plasma levels of BNP seems to be more advantageous because of stability and the precision of measurement compared to NT-proBNP [16,17]. Studies aimed at measuring the levels of NT-proBNP in PAH should proceed with caution since diseases including renal dysfunction and patients with left heart disease can interfere with the exact indication of its levels. Further studies are needed to determine if NT-proBNP is a bona fide biomarker for PAH.

Troponin T: Cardiac troponins are regulatory proteins of the thin actin filaments of cardiac myocytes. One of the family members, cardiac troponin T (cTnT), is a very sensitive and specific biomarker used for the determination of myocardial damages, such as acute myocardial ischemia and infarction. Torbicki et al evaluated the prognostic significance of this marker in 56 patients with severe PAH for their study. They reported a significant increase in cTnT levels for 8 of 56 patients. Similarly, cardiac hemodynamics confirmed that the 8 patients had significantly worse survival than the rest of the cohort. Using a multivariate analysis, it was determined that mortality rates were independently predicted together with cTnT levels, 6MWT, and PVR [18].

It is known that high-sensitive troponin T (hsTnT) increases in response to high exercise in patients with PAH. Völkers et al (2013) measured hsTnT levels in 21 patients with PAH before exercise and showed that hsTnT correlated with NT-proBNP. Interestingly, measurement of hsTnT levels in PAH patients after 300 minutes of exercise showed a significant increase. The elevation in hsTnT levels increased on average from 7.5ng/L to

14.62ng/L. Consistent with previous reports, NT-proBNP levels did not change over time [19]. The investigation of other thrombins such as troponin-I have not been characterized in PAH disease, and the relationship remains unknown.

Endothelins: Endothelin-1 (ET-1) is a 21 amino acid peptide known for its proliferative function and vasoconstriction properties (Figure 1). The source of ET-1 is derived from endothelial cells and secreted into the pulmonary circulation [20]. ET_A and ET_B are the receptors for ET-1 and their levels vary between cell types. ET_A is predominantly found in vascular smooth muscle cells while, ET_B is more prominent in endothelial cells. Vasoconstriction, along with the activation of these receptors and downstream signaling effectors, facilitates the increased cell proliferation noted in smooth muscle cells. Depending on age or gender, Beta-blockers, vasodilators, and angiotensin converting enzyme inhibitors, can be used to decrease the expression of the ET-1 receptors [21,22]. A previously published clinical evaluation of ET-1 levels correlated strongly with 6MWD, PVR, and CI suggesting the importance of this marker in PAH [23]. ET-1 has become a select biomarker for PAH disease severity [22], indicating the prognostic significance of ET-1 levels.

Other members of the endothelin family have not been studied for their role in PAH. In fact, ET-3 and its role in lung endothelium are largely unknown and remain to be determined [24]. Montani et al suggested that the ET-1/ET-3 rate might have prognostic significance in PAH disease, yet no information on this axis has been documented [22]. Future work is need to develop the causal relationship between ET-1 and ET-3 in PAH and their combined importance in clinical practice.

HbA1c: Glycated hemoglobin or hemoglobin A1c (HbA1c) is a minor form of hemoglobin that contains bound glucose and is used as a measurement of blood glucose levels. HbA1c is reflective of the average blood glucose levels over a period of 6 to 8 weeks and is an indicator of high blood sugar and predisposition to insulin resistance or diabetes. Past reports have documented insulin resistance in PAH patients suggesting a relationship between PAH and glucose intolerance [25-27]. In addition, Hemnes and colleagues noted an increase in glycated hemoglobin in PAH patients further providing evidence for HbA1c as potential indicator of disease [28]. Collectively, these reports demonstrate HbA1c as an indicator of PAH and highlight the metabolic dysregulation noted in the disease.

Nitric Oxide: Nitric Oxide (NO) is known as the physiological regulator of blood vessel tone [29-31]. NO functions to induce vascular smooth muscle relaxation in the lungs and has other capabilities, including a regulator of inflammation and proliferation [32,33]. The biosynthesis of NO has been well characterized [34]. In particular, nitric oxide synthase (NOS) enzymes release NO while converting L-arginine to L-citruline. It diffuses in to the vascular smooth muscle cells and binds to soluble guanylate cyclase (sGC) (Figure 2). Cyclic guanosine monophosphate (cGMP) is produced through the stimulation of sGC and muscle relaxation by the NO molecules [35,36]. Bogdan et al reported increased levels of cGMP in the urine of patients with PAH compared to asthma patients and control groups [37]. Another report demonstrated that cGMP levels are inversely proportional to the vein oxygen saturation and CI clinical parameters [37].

NO deficiency is a known hallmark of PAH. Furthermore, NO and its reaction products is lower in patients with PAH [3] compared to controls, and is also known to reflect the degree of PAH. A recent report was published on breath (exhaled) NO [3] and 24-hour urinary NO metabolites (nitrate and nitrite) comparing the levels in PAH patients and controls [38]. Low NO values, within 3 months of Bosentan treatment, were shown to return to levels that were comparable to the control groups. There are potential caveats to this experiment. Measurement of nitrate/nitrite levels from urine is very difficult and impractical. Patients must adhere to a very strict low nitrate diet. Even still, the levels are very low and difficult to measure, and would benefit from a method to increase the sensitivity of the assay. Conversely, breath-NO measurement is a better alternative

and works well to track the advancement of PAH as well as response to therapy.

Pentraxin 3 (PTX3): The pentraxin family includes the C-reactive protein (CRP), however; CRP, contrary PTX3 macrophages, is expressed predominantly in the atherosclerotic lesions with neutrophils, dendritic cells, and smooth muscle cells [39]. Tamura et al (2012) showed that PTX3 is a specific biomarker for PAH patients. In this report, 50 patients with PAH were evaluated for PTX3, BNP, and CRP concentrations in plasma. As a result, the plasma concentration of PTX3 was determined to be a better biomarker than the other markers tested for PAH, especially in patients associated with connective tissue disorders [40]. Therefore, further studies are needed to address the role of PTX3 in PAH and validate it as a biomarker for PAH.

Serotonin: Serotonin is a vasoactive agent found in platelets and vascular endothelial cells [41]. Increases in plasma serotonin levels are an indicator of endothelial dysfunction or aberrant platelet activation. Based on this, it is not surprising that high plasma levels of serotonin have been identified in patients with PAH [42,43]. Interestingly, however, the degree of PAH disease was not reflected by the changes in serotonin [43]. Because epoprostenol therapy does not normalize the increased serotonin levels, platelet activation does not to contribute to the increasing amounts of serotonin. Therefore, the measurement of serotonin levels to monitor treatment of PAH patients is not recommended.

Von Willebrand factor: Plasma von Willebrand factor (vWF) is a large glycoprotein that is synthesized by endothelial cells. vWF plays a crucial role in the activation of platelets and in binding to vascular damaged areas [44]. High level of vWF is a biological marker for endothelial cell damage, a known phenomenon in PAH. Increased vWF antigen levels have been observed in patients with PAH [45-49]. Even basal levels of vWF antigen correlate with the risk of death [46]. Leary et al (2012) found that vWF activity was associated with PAH right ventricle (RV) function. However, the relationship between the structure and function of the RV is unknown in individuals without vWF activity and cardiovascular disease. High vWF activity was associated with lower RV mass, RV end-diastolic volume, and RV stroke volume in 1,976 patients, suggesting a relationship between vWF activity and the RV structure and function [50]. For these reasons, it can be stated that vWF measurements in pulmonary hypertensive patients may be an indicator of disease severity and help determine the disease state after treatment and follow-up.

D-dimer: D-dimer is a specific reagent that shows microvascular thrombosis and cross-linked fibrin (Figure 3). *In situ* thrombosis is a known phenotype of PAH disease [5]. Shitrit et al reported higher levels of D-dimer in IPAH patients compared to controls [51,52]. The same group reported that D-dimer levels correlated with PAH patient strength, oxygen saturation, and PAP [51]. This study was performed on a small patient cohort and requires a larger patient population to determine the validity of D-dimer in PAH as a disease indicator.

Uric acid: Uric acid levels have been shown to correlate with congestive heart failure and chronic obstructive pulmonary disease (COPD) severity [53]. Gotsman et al researched the relationship between congestive heart failure and uric acid amount by evaluating patients entering the hospital and after death. They demonstrated elevated uric acid levels by regression analysis [54]. Eduardo et al observed elevated serum uric acid levels in COPD patients [55]. In addition, Nagaya et al reported increased serum uric acid levels in PAH patients compared to controls. This increase inversely correlated with the CI but was directly proportional to pulmonary strength as well as the mortality of these patients [56]. Later, other researchers found a connection between serum uric acid levels and mRAP [57,58]. These studies indicate that the uric acid measurement correlates with hemodynamic parameters and is a valid marker to test in PAH.

Asymmetric dimethylarginine: Asymmetric dimethylarginine (ADMA) is a competitive inhibitor of the endogenous NO synthase. ADMA is generated in the kidneys from the catabolism of proteins, which contain arginine methyl residues, and any renal impairment will

result in accumulation of ADMA levels [59]. In pulmonary vascular disease, it is a marker of endothelial dysfunction [60,61]. Plasma levels were shown to be higher in patients with hypercholesterolemia disorders and associated endothelium-dependent vasodilatation [62]. Some studies demonstrated high plasma ADMA concentrations in PAH patients when compared to a control group [63-65]. In the same study, high ADMA levels were found to correlate with ven oxygen saturation, mRAP, and CI. These studies demonstrate a strong need to advance studies on ADMA, which may be a good indicator of PAH.

Discussion and conclusion: In this review, we summarized the literature on the current and potential biomarkers for PAH. Increasing our understanding of these biomarkers using medical research will help us shed light on the pathogenesis and pathophysiology of PAH disease.

References:

1. Farber HW, Loscalzo J, Pulmonary arterial hypertension. *N Engl J Med.* 2004, 351: 1655-65.
2. Ghamra ZW, Dweik RA, Primary pulmonary hypertension: an overview of epidemiology and pathogenesis. *Cleve Clin J Med.* 2003, 70 Suppl 1: S2-8.
3. Kaneko FT, Arroliga AC, Dweik RA, Comhair SA, Laskowski D, Oppedisano R, Thomassen MJ, Erzurum SC, Biochemical reaction products of nitric oxide as quantitative markers of primary pulmonary hypertension. *Am J Respir Crit Care Med.* 1998, 158: 917-23.
4. Humbert M, Morrell NW, Archer SL, Stenmark KR, MacLean MR, Lang IM, Christman BW, Weir EK, Eickelberg O, Voelkel NF, Rabinovitch M, Cellular and molecular pathobiology of pulmonary arterial hypertension. *J Am Coll Cardiol.* 2004, 43: 13s-24s.
5. Rubin LJ, Primary pulmonary hypertension. *N Engl J Med.* 1997, 336: 111-7.
6. The International Primary Pulmonary Hypertension Study (IPPHS). *Chest.* 1994, 105: 37S-41S.
7. Yap LB, Mukerjee D, Timms PM, Ashrafian H, Coghlan JG, Natriuretic peptides, respiratory disease, and the right heart. *Chest.* 2004, 126: 1330-6.
8. Vuolteenaho O, Ala-Kopsala M, Ruskoaho H, BNP as a biomarker in heart disease. *Advances in clinical chemistry.* 2005, 40: 1-36.
9. Nagaya N, Ando M, Oya H, Ohkita Y, Kyotani S, Sakamaki F, Nakanishi N, Plasma brain natriuretic peptide as a noninvasive marker for efficacy of pulmonary thromboendarterectomy. *The Annals of thoracic surgery.* 2002, 74: 180-4; discussion 4.
10. Leuchte HH, Holzapfel M, Baumgartner RA, Neurohr C, Vogeser M, Behr J, Characterization of brain natriuretic peptide in long-term follow-up of pulmonary arterial hypertension. *Chest.* 2005, 128: 2368-74.
11. Leuchte HH, Holzapfel M, Baumgartner RA, Ding I, Neurohr C, Vogeser M, Kolbe T, Schwaiblmair M, Behr J, Clinical significance of brain natriuretic peptide in primary pulmonary hypertension. *J Am Coll Cardiol.* 2004, 43: 764-70.
12. Nagaya N, Nishikimi T, Uematsu M, Kyotani S, Satoh T, Nakanishi N, Matsuo H, Kangawa K, Secretion patterns of brain natriuretic peptide and atrial natriuretic peptide in patients with or without pulmonary hypertension complicating atrial septal defect. *American heart journal.* 1998, 136: 297-301.
13. Nagaya N, Nishikimi T, Uematsu M, Satoh T, Kyotani S, Sakamaki F, Kakishita M, Fukushima K, Okano Y, Nakanishi N, Miyatake K, Kangawa K, Plasma brain natriuretic peptide as a prognostic indicator in patients with primary pulmonary hypertension. *Circulation.* 2000, 102: 865-70.
14. Goetze JP, Videbaek R, Boesgaard S, Aldershvile J, Rehfeld JF, Carlsen J, Pro-brain natriuretic peptide as marker of cardiovascular or pulmonary causes of dyspnea in patients with terminal parenchymal lung disease. *J Heart Lung Transplant.* 2004, 23: 80-7.
15. Richards M, Troughton RW, NT-proBNP in heart failure: therapy decisions and monitoring. *European journal of heart failure.* 2004, 6: 351-4.
16. Allanore Y, Borderie D, Meune C, Cabanes L, Weber S, Ekindjian OG, Kahan A,

N-terminal pro-brain natriuretic peptide as a diagnostic marker of early pulmonary artery hypertension in patients with systemic sclerosis and effects of calcium-channel blockers. *Arthritis Rheum.* 2003, 48: 3503-8.
17. Mukerjee D, Yap LB, Holmes AM, Nair D, Ayrton P, Black CM, Coghlan JG, Significance of plasma N-terminal pro-brain natriuretic peptide in patients with systemic sclerosis-related pulmonary arterial hypertension. *Respiratory medicine.* 2003, 97: 1230-6.
18. Torbicki A, Kurzyna M, Kuca P, Fijalkowska A, Sikora J, Florczyk M, Pruszczyk P, Burakowski J, Wawrzynska L, Detectable serum cardiac troponin T as a marker of poor prognosis among patients with chronic precapillary pulmonary hypertension. *Circulation.* 2003, 108: 844-8.
19. Volkers M, Rohde D, Zelniker T, Weiss CS, Giannitsis E, Katus HA, Meyer FJ, High-sensitive Troponin T increase after exercise in patients with pulmonary arterial hypertension. *BMC pulmonary medicine.* 2013, 13: 28.
20. Giaid A, Yanagisawa M, Langleben D, Michel RP, Levy R, Shennib H, Kimura S, Masaki T, Duguid WP, Stewart DJ, Expression of endothelin-1 in the lungs of patients with pulmonary hypertension. *N Engl J Med.* 1993, 328: 1732-9.
21. Shah R, Endothelins in health and disease. *Eur J Intern Med.* 2007, 18: 272-82.
22. Montani D, Souza R, Binkert C, Fischli W, Simonneau G, Clozel M, Humbert M, Endothelin-1/endothelin-3 ratio: a potential prognostic factor of pulmonary arterial hypertension. *Chest.* 2007, 131: 101-8.
23. Rubens C, Ewert R, Halank M, Wensel R, Orzechowski HD, Schultheiss HP, Hoeffken G, Big endothelin-1 and endothelin-1 plasma levels are correlated with the severity of primary pulmonary hypertension. *Chest.* 2001, 120: 1562-9.
24. Nagaya N, Nishikimi T, Goto Y, Miyao Y, Kobayashi Y, Morii I, Daikoku S, Matsumoto T, Miyazaki S, Matsuoka H, Takishita S, Kangawa K, Matsuo H, Nonogi H, Plasma brain natriuretic peptide is a biochemical marker for the prediction of progressive ventricular remodeling after acute myocardial infarction. *American heart journal.* 1998, 135: 21-8.
25. Zamanian RT, Hansmann G, Snook S, Lilienfeld D, Rappaport KM, Reaven GM, Rabinovitch M, Doyle RL, Insulin resistance in pulmonary arterial hypertension. *The European respiratory journal.* 2009, 33: 318-24.
26. Brunner NW, Skhiri M, Fortenko O, Hsi A, Haddad F, Khazeni N, Zamanian RT, Impact of insulin resistance on ventricular function in pulmonary arterial hypertension. *J Heart Lung Transplant.* 2014, 33: 721-6.
27. Hansmann G, Wagner RA, Schellong S, Perez VA, Urashima T, Wang L, Sheikh AY, Suen RS, Stewart DJ, Rabinovitch M, Pulmonary arterial hypertension is linked to insulin resistance and reversed by peroxisome proliferator-activated receptor-gamma activation. *Circulation.* 2007, 115: 1275-84.
28. Pugh ME, Robbins IM, Rice TW, West J, Newman JH, Hemnes AR, Unrecognized glucose intolerance is common in pulmonary arterial hypertension. *J Heart Lung Transplant.* 2011, 30: 904-11.
29. Palmer RM, Ferrige AG, Moncada S, Nitric oxide release accounts for the biological activity of endothelium-derived relaxing factor. *Nature.* 1987, 327: 524-6.
30. Ignarro LJ, Buga GM, Wood KS, Byrns RE, Chaudhuri G, Endothelium-derived relaxing factor produced and released from artery and vein is nitric oxide. *Proc Natl Acad Sci U S A.* 1987, 84: 9265-9.
31. Nathan C, Xie QW, Nitric oxide synthases: roles, tolls, and controls. *Cell.* 1994, 78: 915-8.
32. Dweik RA, Nitric oxide production in the lung and its regulation by oxygen., in Disease Markers in Exhaled Breath: basic mechanisms and clinical applications (NATO Science Series). 2002.
33. Dweik RA, Comhair SA, Gaston B, Thunnissen FB, Farver C, Thomassen MJ, Kavuru M, Hammel J, Abu-Soud HM, Erzurum SC, NO chemical events in the human airway during the immediate and late antigen-induced asthmatic response. *Proc Natl Acad Sci U S A.* 2001, 98: 2622-7.

34. Andrew PJ, Mayer B, Enzymatic function of nitric oxide synthases. *Cardiovascular research.* 1999, 43: 521-31.
35. Barnes PJ, Kharitonov SA, Exhaled nitric oxide: a new lung function test. *Thorax.* 1996, 51: 233-7.
36. Barnes PJ, Belvisi MG, Nitric oxide and lung disease. *Thorax.* 1993, 48: 1034-43.
37. Bogdan M, Humbert M, Francoual J, Claise C, Duroux P, Simonneau G, Lindenbaum A, Urinary cGMP concentrations in severe primary pulmonary hypertension. *Thorax.* 1998, 53: 1059-62.
38. Girgis RE, Champion HC, Diette GB, Johns RA, Permutt S, Sylvester JT, Decreased exhaled nitric oxide in pulmonary arterial hypertension: response to bosentan therapy. *Am J Respir Crit Care Med.* 2005, 172: 352-7.
39. Inoue K, Kodama T, Daida H, Pentraxin 3: a novel biomarker for inflammatory cardiovascular disease. *International journal of vascular medicine.* 2012, 2012: 657025.
40. Tamura Y, Ono T, Kuwana M, Inoue K, Takei M, Yamamoto T, Kawakami T, Fujita J, Kataoka M, Kimura K, Sano M, Daida H, Satoh T, Fukuda K, Human pentraxin 3 (PTX3) as a novel biomarker for the diagnosis of pulmonary arterial hypertension. *PloS one.* 2012, 7: e45834.
41. Lee SL, Wang WW, Fanburg BL, Superoxide as an intermediate signal for serotonin-induced mitogenesis. *Free Radic Biol Med.* 1998, 24: 855-8.
42. Herve P, Launay JM, Scrobohaci ML, Brenot F, Simonneau G, Petitpretz P, Poubeau P, Cerrina J, Duroux P, Drouet L, Increased plasma serotonin in primary pulmonary hypertension. *Am J Med.* 1995, 99: 249-54.
43. Kereveur A, Callebert J, Humbert M, Herve P, Simonneau G, Launay JM, Drouet L, High plasma serotonin levels in primary pulmonary hypertension. Effect of long-term epoprostenol (prostacyclin) therapy. *Arterioscler Thromb Vasc Biol.* 2000, 20: 2233-9.
44. Cronberg S, Nilsson IM, Silwer J, Studies on the platelet adhesiveness in von Willebrand's disease. *Acta Med Scand.* 1966, 180: 43-54.
45. Collados MT, Sandoval J, Lopez S, Masso FA, Paez A, Borbolla JR, Montano LF, Characterization of von Willebrand factor in primary pulmonary hypertension. *Heart Vessels.* 1999, 14: 246-52.
46. Lopes AA, Maeda NY, Almeida A, Jaeger R, Ebaid M, Chamone DF, Circulating platelet aggregates indicative of in vivo platelet activation in pulmonary hypertension. *Angiology.* 1993, 44: 701-6.
47. Lopes AA, Maeda NY, Bydlowski SP, Abnormalities in circulating von Willebrand factor and survival in pulmonary hypertension. *Am J Med.* 1998, 105: 21-6.
48. Kawut SM, Horn EM, Berekashvili KK, Widlitz AC, Rosenzweig EB, Barst RJ, von Willebrand factor independently predicts long-term survival in patients with pulmonary arterial hypertension. *Chest.* 2005, 128: 2355-62.
49. Friedman R, Mears JG, Barst RJ, Continuous infusion of prostacyclin normalizes plasma markers of endothelial cell injury and platelet aggregation in primary pulmonary hypertension. *Circulation.* 1997, 96: 2782-4.
50. Leary PJ, Barr RG, Bluemke DA, Bristow MR, Hough CL, Kronmal RA, Lima JA, McClelland RL, Tracy RP, Kawut SM, Von Willebrand factor and the right ventricle (the MESA-Right Ventricle Study). *The American journal of cardiology.* 2012, 110: 1846-51.
51. Shitrit D, Bendayan D, Bar-Gil-Shitrit A, Huerta M, Rudensky B, Fink G, Kramer MR, Significance of a plasma D-dimer test in patients with primary pulmonary hypertension. *Chest.* 2002, 122: 1674-8.
52. Shitrit D, Bendayan D, Rudensky B, Izbicki G, Huerta M, Fink G, Kramer MR, Elevation of ELISA d-dimer levels in patients with primary pulmonary hypertension. *Respiration.* 2002, 69: 327-9.
53. Fishel ML, Kelley MR, The DNA base excision repair protein Ape1/Ref-1 as a therapeutic and chemopreventive target. *Molecular aspects of medicine.* 2007, 28: 375-95.
54. Gotsman I, Keren A, Lotan C, Zwas DR, Changes in uric acid levels and allopurinol use in chronic heart failure: association with

improved survival. *Journal of cardiac failure.* 2012, 18: 694-701.
55. Garcia Pachon E, Padilla Navas I, Shum C, [Uric acid and its relationship to creatinine levels and hypoxia]. *Archivos de bronconeumologia.* 2007, 43: 523.
56. Nagaya N, Uematsu M, Satoh T, Kyotani S, Sakamaki F, Nakanishi N, Yamagishi M, Kunieda T, Miyatake K, Serum uric acid levels correlate with the severity and the mortality of primary pulmonary hypertension. *Am J Respir Crit Care Med.* 1999, 160: 487-92.
57. Voelkel MA, Wynne KM, Badesch DB, Groves BM, Voelkel NF, Hyperuricemia in severe pulmonary hypertension. *Chest.* 2000, 117: 19-24.
58. Hoeper MM, Hohlfeld JM, Fabel H, Hyperuricaemia in patients with right or left heart failure. *The European respiratory journal.* 1999, 13: 682-5.
59. Stuhlinger MC, Tsao PS, Her JH, Kimoto M, Balint RF, Cooke JP, Homocysteine impairs the nitric oxide synthase pathway: role of asymmetric dimethylarginine. *Circulation.* 2001, 104: 2569-75.
60. Boger RH, Bode-Boger SM, Szuba A, Tsao PS, Chan JR, Tangphao O, Blaschke TF, Cooke JP, Asymmetric dimethylarginine (ADMA): a novel risk factor for endothelial dysfunction: its role in hypercholesterolemia. *Circulation.* 1998, 98: 1842-7.
61. Cooke JP, A novel mechanism for pulmonary arterial hypertension? *Circulation.* 2003, 108: 1420-1.
62. Ito A, Tsao PS, Adimoolam S, Kimoto M, Ogawa T, Cooke JP, Novel mechanism for endothelial dysfunction: dysregulation of dimethylarginine dimethylaminohydrolase. *Circulation.* 1999, 99: 3092-5.
63. Kielstein JT, Zoccali C, Asymmetric dimethylarginine: a cardiovascular risk factor and a uremic toxin coming of age? *Am J Kidney Dis.* 2005, 46: 186-202.
64. Gorenflo M, Zheng C, Werle E, Fiehn W, Ulmer HE, Plasma levels of asymmetrical dimethyl-L-arginine in patients with congenital heart disease and pulmonary hypertension. *J Cardiovasc Pharmacol.* 2001, 37: 489-92.
65. Skoro-Sajer N, Mittermayer F, Panzenboeck A, Bonderman D, Sadushi R, Hitsch R, Jakowitsch J, Klepetko W, Kneussl MP, Wolzt M, Lang IM, Asymmetric dimethylarginine is increased in chronic thromboembolic pulmonary hypertension. *Am J Respir Crit Care Med.* 2007, 176: 1154-60.
66. Simonneau G, Robbins IM, Beghetti M, Channick RN, Delcroix M, Denton CP, Elliott CG, Gaine SP, Gladwin MT, Jing ZC, Krowka MJ, Langleben D, Nakanishi N, Souza R, Updated clinical classification of pulmonary hypertension. *J Am Coll Cardiol.* 2009, 54: S43-54.
67. Leuchte HH, Neurohr C, Baumgartner R, Holzapfel M, Giehrl W, Vogeser M, Behr J, Brain natriuretic peptide and exercise capacity in lung fibrosis and pulmonary hypertension. *Am J Respir Crit Care Med.* 2004, 170: 360-5.
68. Mopidevi B, Ponnala M, Kumar A, Human Angiotensinogen +11525 C/A Polymorphism Modulates Its Gene Expression Through MicroRNA Binding. *Physiological genomics.* 2013.
69. Sprenger JU, Nikolaev VO, Biophysical Techniques for Detection of cAMP and cGMP in Living Cells. *International journal of molecular sciences.* 2013, 14: 8025-46.
70. Adam SS, Key NS, Greenberg CS, D-dimer antigen: current concepts and future prospects. *Blood.* 2009, 113: 2878-87.

Table 1: An updated clinical classification of pulmonary hypertension (Dana Point, 2008) [66]			
1.	**Pulmonary arterial hypertension (PAH)**		
	1.1.	Idiopathic PAH	
	1.2.	Heritable	
		1.2.1.	BMPR2
		1.2.2.	ALK1, endoglin (with or without hereditary hemorrhagic telangiectasia)
		1.2.3.	Unknown
	1.3.	Drug- and toxin-induced	
	1.4.	Associated with	
		1.4.1	Connective tissue diseases
		1.4.2.	HIV infection
		1.4.3.	Portal hypertension
		1.4.4.	Congenital heart diseases
		1.4.5.	Schistosomiasis
		1.4.6.	Chronic hemolytic anemia
	1.5.	Persistent pulmonary hypertension of the newborn	
1`.	Pulmonary veno-occlusive disease (PVOD) and/or pulmonary capillary hemangiomatosis (PCH)		
2.	Pulmonary hypertension owing to left heart disease		
	2.1.	Systolic dysfunction	
	2.2.	Diastolic dysfunction	
	2.3.	Valvular disease	
3.	**Pulmonary hypertension owing to lung diseases and/or hypoxia**		
	3.1.	Chronic obstructive pulmonary disease	
	3.2.	Interstitial lung disease	
	3.3.	Other pulmonary diseases with mixed restrictive and obstructive pattern	
	3.4.	Sleep-disordered breathing	
	3.5.	Alveolar hypoventilation disorders	
	3.6.	Chronic exposure to high altitude	
	3.7.	Developmental abnormalities	
4.	**Chronic thromboembolic pulmonary hypertension (CTEPH)**		
5.	**Pulmonary hypertension with unclear multifactorial mechanisms**		
	5.1	Hematologic disorders: myeloproliferative disorders, splenectomy	
	5.2	Systemic disorders: sarcoidosis, pulmonary Langerhans cell histiocytosis: lymphangioleiomyomatosis, neurofibromatosis, vasculitis	
	5.3	Metabolic disorders: glycogen storage disease, Gaucher disease, thyroid disorder	
	5.4	Others: tumoral obstruction, fibrosingmediastinitis, chronic renal failure on dialysis	
ALK1 = activin receptor-like kinase type 1; BMPR2 = bone morphogenetic protein receptor type 2; HIV = human immunodeficiency virus			

Table 2: A list of PAH biomarkers studied and their respective origin

Biomarkers	Origin	References
Brain natriuretic peptide (BNP)	Cardiac myocytes	7,9,13,67
Pro-Brain natriuretic peptide (Pro-BNP)	Cardiac myocytes	14,16,17
Troponin T	Cardiac myocytes	18,19
Uric Acid	Purine	54,56
Endothelin-1	Endothelial cells	20,21,23
Serotonin	Platelets	41-43
Angiotensin I-III	Angiotensinogen	68
Nitric Oxide (NO)	Endothelial cells	3,32,33,38
cGMP	Vascular smooth muscle	35-37,69
D-dimers	Fibrinogen	70

Table 3: Biomarkers and their significance for diagnosis and prognosis in PAH

	Measurement	Diagnosis	Prognosis
Troponin	High after exercise	XX	XXX
Endothelin	Vasoconstriction and cell proliferation	XX (ET-1)	XX (ET-1/ET-3)
BNP	High in plasma	XX	XX
NT-proBNP	High in plasma	XXX	XX
HbA1c	High in blood sugar	XX	XX
Nitric Oxide	Lower in lung	XX	XXX
Pentraxin 3	High plasma concentration	XXX	_
Serotonin	High in plasma	XX	X
Von Willebrand Factor	High vWF antigen levels	XX	XX
D-dimer	High levels of D-dimer	X!	X!
Uric Acid	High levels of serum UA	XX*	XX
ADMA	High plasma concentration	X!	X!

XXX Very good diagnostic
XX Good diagnostic
X Poor (not recommended for use as a determinant)
X! Insufficient studies
***** In patients with renal impairment it is not recommended for use as a determining factor for PAH

Figure 1

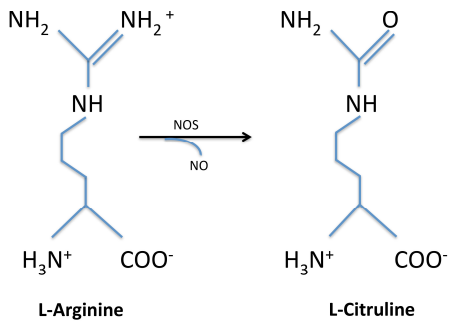

Figure 1: Nitric oxide is a significant factor for PAH. Nitric oxide synthase (NOS) enzymes release NO through the conversion of L-arginine to L-citruline.

Figure 2

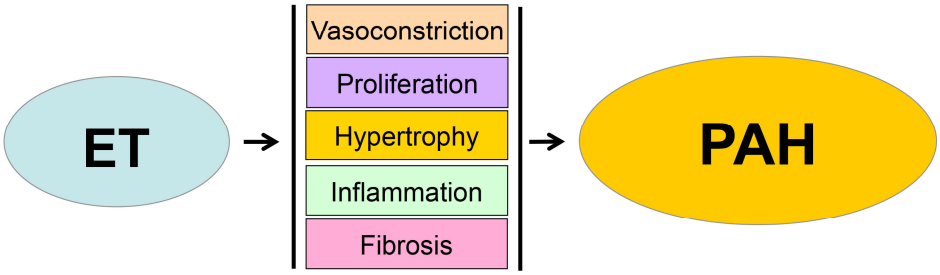

Figure 2: The vasoconstrictor ET is a potent factor involved in PAH. Increased ET levels results in several disease processes that result in PAH.

Figure 3

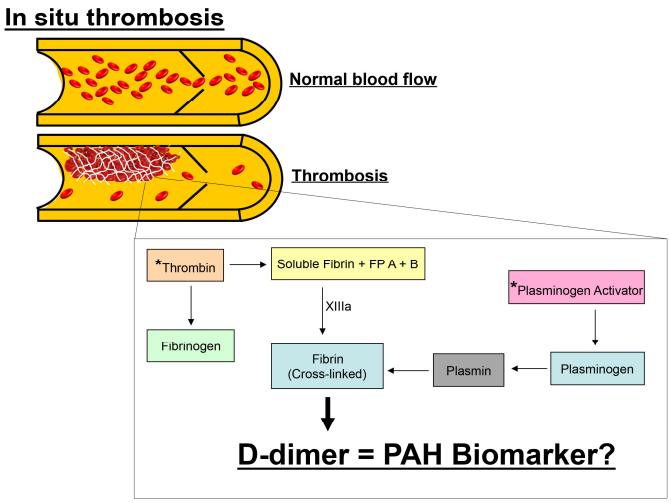

Figure 3: The generation of D-dimer cross-linked fragments in PAH. During *in situ* thrombosis, a hallmark of PAH, D-dimer fragments are generated by the thrombin activation cascade or by plasmin activation and fibrin processing. Abbrv. FP A + B = Fibrinopeptide A or B;