Pulmonary Arterial Hypertension
Special Issues and Current Therapeutic Approaches:
Part One

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The term pulmonary arterial hypertension (PAH) refers to conditions that share common isolated elevations in pulmonary arterial pressure defined as at resting mean pulmonary arterial pressure $> 25$ mmHg with a normal pulmonary capillary or left atrial pressure ($<15$ mmHg)$^1$. PAH remains a challenging condition to diagnose and manage. In 1991, estimates from the US Department of Health and Human Services National institutes of Health Registry painted a grim portrait of survival in patients with pulmonary hypertension, establishing primary pulmonary hypertension with one, three, and five year survival rates of 68%, 48% and 34%, respectively $^2$. Significant advances in the treatment, including combination therapy, have occurred over the last 10 years and long-term follow-up studies have provided better information on prognosis and expected outcomes. This review
describes specific insights in pathogenesis, biomarkers, pregnancy and treatment of PAH patients.

**Pathogenesis**

Endothelin (ET), a peptide produced by vascular endothelial cells, has been characterized as a powerful vasoconstrictor and mitogen for smooth muscle. Activation of the ET system has been shown in both plasma and lung tissue of PAH patients, as well as in animal models supporting a prominent role of ET in the pathogenesis of this condition $^{3-4}$.

**Endothelial Progenitor cells**

Endothelial dysfunction, resistance to apoptosis, and proliferation of pulmonary vasculature endothelial cells are implicated in the mechanisms of PAH. Endothelial progenitor cells (EPCs) are mobilized from the bone marrow and contribute to the vascular homeostasis, neovascularization, and endothelial function. Evidence suggests that EPCs in pulmonary vasculature could induce restoration of the plexogenic lesions, proliferative vessels and regeneration of pulmonary perfusion, improving pulmonary hemodynamics and survival in PAH patients $^{5-7}$. In idiopathic PAH, intravenous infusion of cultured autologous cells improved six-minute walk tests (6minWT) and pulmonary hemodynamics $^8$. Endothelial progenitor cells are reduced in Eisenmenger and IPAH and rising inflammatory mediators indexes of nitric oxide (NO) synthesis have been directly correlated with EPC levels $^9$. Considering that restoration of the normal endothelial function is the main target of new available treatments, sildenafil could increase EPCs levels in IPAH patients. Whether this drug stimulates an intrinsic NO-cGMP pathway and thereby augments EPCs is an unanswered question. Strong and current evidence is mandatory to understand therapeutic implications of circulating EPCs in PAH.

**Endothelial cell dysfunction and cellular changes.**

Endothelial, smooth muscle, and fibroblast in the pulmonary vascular wall play a specific role in the response to injury. A common denominator of many of the
molecular mechanisms underpinning PAH is dysfunction of endothelial processes provoked by various possible sources of injury: shear stress, inflammation, toxins, hypoxia, and other causes yet to be discovered. The epitome of endothelial dysfunction is reflected by the plexiform lesion, a disordered proliferation of endothelial cells that in IPAH appears to be monoclonal in origin, which have been characterized as “tumorlets” 10. Disorganized endothelial cell proliferation leading to formation of plexiform lesions is described in many cases of PAH 11-13. Inflammatory mechanisms appear to play a significant role in some types of PAH including monocrotaline- induced cases in rats and PAH of various origins in humans including connective tissue diseases and human immunodeficiency virus infection 14.

Inflammation and coagulation
Several observations suggest that inflammation may be involved as a mechanism in some forms of PAH. Autoantibodies, cytokines, and inflammatory infiltrates have been observed 15. A wide array of procoagulant abnormalities have been identified in PAH patients, in part related to endothelial dysfunction and to abnormalities of the coagulation cascade and disordered platelet function 14, 15.

Pathologic Evidence
Pulmonary vascular thrombosis and thrombotic arteriopathy have been observed as frequent pathologic findings in PAH. Thrombotic lesions are most commonly recognized as nonlaminar, eccentric intimal fibrotic lesions, suggesting chronic organization of a previous thrombotic event. These are believed to be either the result of unrecognized pulmonary vascular emboli from distant sources or active in situ pulmonary vascular thrombosis. Occasionally, complete vascular obliteration by thrombosis may be seen, with evidence of organization as well as recanalization of the organized thrombus.
When such thrombotic lesions are the predominant pathologic finding in the pulmonary vasculature, the term *thrombotic arteriopathy* may be applied. The prevalence rates of isolated thrombotic arteriopathy are 40% to 57%. Although, there is not any relationship among, age, presentation, specific symptoms, functional class, or family history of IPAH with thrombotic lesions, a significant correlation between histologic classification and severity of PAH has been identified. In addition, patients with a predominant thrombotic arteriopathy had a significantly better survival rate than other histologic forms of PAH.

Chronic pulmonary vascular thrombotic lesions have also been described in pathologic samples from patients with PAH associated with exogenous toxins (aminorex) and portal hypertension. Although, thrombotic lesions may not be specific to IPAH, this could be a complication related with severity and duration of PAH. These kind of pathologic findings are common in adults, but unusual in children with IPAH. Evidence shows a close relationship among the presence of thrombotic lesions with age (p < 0.002) and duration of PAH (p < 0.007). Taken together, these observational studies indicate a relatively high prevalence of thrombotic lesions. Furthermore, these studies suggest the presence of thrombosis is related to age and disease duration, but is not specific for the etiology.

**Blood coagulation and fibrinolysis abnormalities**

Coagulation is a complex process characterized by the interaction of endothelial cells with both soluble elements (plasma coagulation proteins) and cellular elements of blood (platelets). In the healthy state, a balance exists between ongoing thrombosis and prevention of significant clot formation by antithrombotic and fibrinolytic mechanisms. The endothelium is central in the regulation of this thrombotic-antithrombotic balance and has active participation in the process of coagulation, activating factor X, facilitating formation of the thrombin-activating prothrombinase complex, and activating the extrinsic pathway of coagulation via release of tissue factor. In addition, endothelial cells produce and release von
Willebrand factor (vWF), which functions as an adhesive protein in the interaction of platelets with the vessel wall, as well as a carrier for factor VIII\textsuperscript{16}.

The endothelium not only facilitates the thrombotic process but also actively inhibits thrombosis and promotes fibrinolysis. Endothelial cell production and release of NO and prostacyclin, two potent inhibitors of platelet aggregation, are important mechanisms in the prevention of thrombosis. In addition, thrombomodulin expression, a high affinity receptor for thrombin, on the surface of endothelial cells prevents the cleavage of fibrinogen to fibrin. Endothelial cells are also a source of tissue plasminogen activator (t-PA), a key activator of plasminogen (factor XIII) in the fibrinolytic cascade. Of note, endothelial cells also synthesize and release plasminogen activator inhibitor-1 (PAI-1), an inhibitor of t-PA, highlighting the role of the endothelium in regulating the fine balance of prothrombotic and antithrombotic mediators and cascades\textsuperscript{16}.

The recognition of the critical role of the endothelium in this balance between prothrombotic and antithrombotic mechanisms suggested that endothelial dysfunction might contribute to the pathophysiology of PAH through abnormalities of the blood coagulation and fibrinolytic systems. Thus, active intravascular thrombosis may be present in PAH\textsuperscript{16}. These include increasing levels of plasma fibrinopeptide A, fibrinogen, vWF, plasminogen activator inhibitor - 1, serotonin, and thromboxane and decreasing levels of tissue plasminogen activator, thrombomodulin, NO, and prostaglandins I2.

**Fibrinopeptide A**

Plasma levels of fibrinopeptide A, a byproduct and a marker of fibrin generation, were found to be elevated in IPAH patients (100% to 61%). Recently, an actual gradient of fibrinopeptide A was found across the lung in a patient with IPAH, suggestive of pulmonary vascular-specific fibrin formation rather than a generalized vascular prothrombotic state. In several members of a family with familial PAH, elevated fibrinopeptide A levels and lung histologic evidence of thrombotic
pulmonary arteriopathy were observed. Abnormalities in the plasma levels of fibrinogen and fibrinogen metabolism have also been described in IPAH patients.

**Fibrinogen and plasminogen activator inhibitor - 1**
Plasma fibrinogen levels are inconsistent. In one study a decreased half-life was found in IPAH. In contrast, another study showed that fibrinogen levels were higher (p < 0.01) in IPAH and CTPAH than in either control patients or patients with PAH associated to congenital heart disease (CHD). Furthermore, upper-extremity venous occlusion-stimulated fibrinolysis, assessed by the increase in t-PA activity, was blunted in patients with IPAH or CTEPH. Increasing PAI-1 levels have been reported in IPAH compared to control subjects. In one study increased PAI-1 levels in IPAH were associated with decreased plasma soluble thrombomodulin and a prolonged euglobulin lysis time, a global *in vitro* measure of fibrinolytic activity. Lower fibrinolytic activity correlated with a higher mean PAP (r = 0.41, p < 0.003). Finally, 10% of patients with IPAH had antibodies to fibrin-bound t-PA, suggesting another possible mechanism for an impaired fibrinolytic state.

**Von Willebrand factor**
A protein synthesized and stored in endothelial cells, megakaryocytes, and platelets is essential in the interaction of platelets with endothelial cells. Abnormalities have been described in vWF levels and activity in PAH. Patients with IPAH have elevated *in vitro* vWF activity relative to immunologically measured vWF antigen levels, with milder increases seen in CHD patients with or without associated PAH. Enhanced endothelial secretion of vWF can be stimulated by thrombin, fibrin, several cytokines, complement, and increased shear stress. In IPAH, abnormalities of vWF are likely a marker of endothelial injury or dysfunction rather than platelet defects, since *in vitro* assessment of plasma vWF activity is done with normal platelets from healthy blood donors.

**Inherited Thrombophilic States**
Deficiencies of the classical inhibitors of coagulation, (antithrombin III) and abnormal procoagulant factors (factor V Leiden) are well-recognized risk factors for pulmonary thromboembolic disease. Overall, there is no evidence to suggest an increased tendency to PAH, based upon the frequency of antithrombin III, protein C, protein S, factor V, and factor II mutations between PAH patients and control subjects\textsuperscript{16}.

**Chronic thromboembolic pulmonary arterial hypertension**

In patients with CTPAH, the reasons for incomplete resolution of pulmonary emboli have not been identified. The normal pulmonary vascular bed carries a high fibrinolytic potential, but alterations in the fibrinolytic system have not yet been identified. Secretion by pulmonary vascular endothelial cells of tissue plasminogen activator and plasminogen activator inhibitor-1 is not different between lungs from CTEPH patients and donor lungs. Remarkably, thrombophilia resulting from mutations in protein C, protein S, antithrombin, prothrombin, or factor V has not been associated with CTEPH. The only factors that have been linked to CTEPH thus far are anticardiolipin antibodies, which are found in 10% to 20% of these patients, and elevated levels of factor VIII, but similar findings have been reported in patients with other forms of PAH\textsuperscript{15}.

Several risk factors for the development of CTEPH have been identified, including chronic inflammatory disorders, myeloproliferative syndromes, the presence of a ventriculoatrial shunt, and splenectomy. The association with these conditions suggests that chronic infection and/or chronic inflammatory processes are involved in the pathogenesis of CTEPH. This hypothesis is supported by numerous experimental findings showing that inflammation may cause a prothrombotic state and impair resolution of pulmonary thrombemboli. The high proportion of splenectomized patients in the CTEPH population has gained considerable attention. The interval between splenectomy and diagnosis of CTEPH ranges between 2 and 34 years, and the pathogenetic link between these conditions remains unclear. Current hypotheses include prothrombotic activity of abnormal
erythrocytes, interactions between abnormal erythrocyte membranes and the pulmonary vasculature, or abnormal platelet activation\textsuperscript{15}.

**Platelets**

Thrombotic lesions and platelet dysfunction are potentially important processes in PAH. In situ pulmonary artery thrombosis may be initiated or aggravated by abnormalities in the clotting cascade, the endothelial cells, or the platelets. Thrombin activity appears to be increased in severe PAH. Antithrombotic pathway disorders may account for this abnormality, particularly in chronic thromboembolic CTPAH and IPAH. Injured endothelium, a constant feature in severe pulmonary hypertension, either IPAH or associated, enhances thrombus formation in pulmonary vessels\textsuperscript{14, 15}.

**Risk markers**

**Hyperuricemia**

High levels were found to correlate with survival as an independent prognostic indicator in IPAH patients\textsuperscript{17, 18}. Hyperuricemia is frequently associated with myeloproliferative or lymphoproliferative diseases such as leukemia or lymphoma or with cyanotic congenital heart disease. Increased nucleic acid metabolism is the mechanism for the hyperuricemia observed in the proliferative and myeloproliferative disorders, and polycythemia-related enhancement of purine metabolism is the mechanism in patients with CHD.

Hyperuricemia has been reported in patients with ischemic heart disease. Uric acid can be released from ischemic tissues, including the heart, during angina, and has been suggested as a risk factor for the development of atherothrombotic diseases. A possible explanation for this relates to the inhibition of adenosine diphosphate degradation by uric acid and the subsequent stabilization of platelet aggregates. In addition, hyperuricemia increases platelet adhesiveness, and patients with chronic severe PAH are at a high risk to develop in situ pulmonary vascular thrombosis\textsuperscript{17}.
In PAH patients under right heart catheterization, 43% patients with IPAH and associated had measurement of the serum uric acid close to the hemodynamic evaluation. All patients had severe PAH and a positive correlation between the natural logarithm of the serum uric acid and the mean right atrial pressure ($r = 0.47$; $p < 0.001$) was identified. The stronger correlation was between uric acid and right atrial pressure in IPAH patients ($r = 0.642$; $p < 0.001$). This relationship cannot be explained by diuretic use or impaired hepatocellular function. Neither mean pulmonary artery pressure nor cardiac output was as well correlated with the right atrial pressure when compared with the uric acid. In IPH patients, serum uric acid measurements repeated during treatment with chronic intravenous prostacyclin infusion were obtained. In 61% a decrease in serum uric acid levels was observed. These findings suggesting a positive correlation between the right atrial pressure and the serum uric acid levels in IPAH patients. However, serum uric acid levels drop in some, but not all in IPAH patients during chronic prostacyclin infusion therapy.

**Brain natriuretic peptide**

Theoretically, this biomarker (BNP) and NT-proBNP should have important utility in screening patients with right ventricular dysfunction. In PAH and scleroderma patients, NT-proBNP (cut-off level of 395 pg/mL) had sensitivity (69%), and specificity (100%) to cardiovascular adverse events $^{19}$. Plasma BNP correlated significantly with right-sided hemodynamic variables including mean pulmonary artery pressure (mPAP), total pulmonary resistance (TPR), mRAP, RVEDP, and cardiac output$^{19-21}$. According to multivariate analysis in a follow-up of 24 months, baseline BNP > 150 pg/mL was found to be an independent predictor of mortality $^{22}$. Recently, similar results were observed in a pediatric population $^{23}$. Brain natriuretic peptide identifies patients with higher activity of the neurohormonal system; its levels could be used to identify high risk patients to adverse events in acute phase and in the follow-up, in-hospital or outpatient treatment, and could possibly be used to determine the best time for pregnancy interruption in PAH patients. Evidence coming from randomized control trials is mandatory.
**Troponin**
It is a contractile regulatory protein complex of striated muscles consisting of three components C, I, and T. Cardiac troponin T (cTnT) and troponin I (cTnI) are established specific markers of myocardial damage and prognostic indicators in acute coronary syndromes and left ventricular heart failure. In the setting of PAH patients, there are only few reports concerning importance of elevated levels of troponin in patients with chronic right ventricular dysfunction. As in pulmonary embolism, an explanation for abnormal levels of this biomarker is ongoing subtle myocardial ischemia.

In addition, elevated troponin levels were an independent predictor of fatal outcome in a two year follow-up. The high incidence of tachycardia in these patients suggests that excessive sympathetic activation with secondary systemic hypotension could induce right ventricular myocardial injury. Currently, it is not clear whether a troponin leak detected with a high-sensitive test is merely a prognostic marker or also indicates ongoing clinically relevant necrosis of the myocytes contributing to right ventricular failure. The role of monitoring troponin levels in patients with PAH still requires confirmation in future studies.

**D-dimer**
D-dimer assay, a specific marker for cross-linked fibrin, is often used to exclude pulmonary embolism (negative D-dimer) and for disseminated intravascular coagulation. In patients without evidence of coagulopathy, the D-dimer may represent microvascular thrombosis. In two studies, D-dimer has been associated with the severity of the disease, as it serves as a prognostic marker and highlights thrombosis mechanism in PAH pathophysiology. Recently in idiopathic pulmonary embolism, it identified high-risk patients to recurrence after three or six-month oral anticoagulation. Currently, whether D-dimer could be used to stratify PAH patients to receive oral anticoagulation is an unmet question and further
research is required to identify the role of this maker of active fibrin in the setting of PAH patients.

**C Reactive protein**

It is a well-known marker of inflammation and tissue damage, widely recognized as a risk predictor of cardiovascular and coronary heart diseases. Recently, plasma levels of C reactive protein (CRP) have been measured in PAH and CTPAH patients, at the time of right heart catheterization; CRP levels were increased in all groups with PAH compared with those in control subjects (p < 0.0001). In addition, CRP levels correlated with New York Heart Association (NYHA) functional class (r = 0.23), right atrial pressure (r = 0.25), and 6MWT (r = 0.19) and were significantly higher in non-survivors than in survivors (p < 0.003). All PAH, IPAH, and patients naive for disease-specific medication with CRP levels > 5.0 mg/liter had a significantly lower survival rate (p < 0.02, p < 0.009, and p < 0.05, respectively)\(^28\).

In CTPAH patients, CRP levels significantly decreased in the 12 months after pulmonary endarterectomy (p < 0.004). PAH patients normalizing their CRP levels under treatment (n = 29), assigned as responders, had a significantly higher survival rate (p < 0.05). The proportion of patients treated with a parenteral prostacyclin-analogue was significantly higher among the responders than the non-responders (55% vs. 17%, p < 0.002). This is the first evidence of the role of an inflammatory marker in predicting outcome and response to therapy in PAH. These results showed a role of local and systemic inflammation in PAH\(^28\). This evidence is another link among regional and systemic inflammation, endothelial dysfunction and PAH. More evidence is required to establish the CRP role in PAH.

**The six minute walk test**

It is a simple test used globally to evaluate exercise capacity. Hemoglobin desaturation, as measured by pulse oximetry during a 6-MWT, is predictive of mortality in patients with IPAH, and the distance walked has been increasingly used as a primary outcome in clinical trials of new drugs indicated in the treatment of PAH\(^29\). This test was chosen as a surrogate end point for PAH trials because
the regulatory authorities acknowledged that the patients are symptomatic because of poor exercise tolerance and that improving the symptom that is attributable to the disease is valid. However 6-MWT could be affected by several factors, including age, gender, height, weight and osteoarthritis, making it difficult to know what the normal value would be in any given patient.

However, it is easy to perform, inexpensive, standardized, and reproducible. It would seem meaningful if the improvement in the distance walked during the 6-MWT translated into improved functional class, since improved functional class reflects successful therapy in patients with heart failure. However, in the PAH clinical trials, even though changes in functional class trended toward an improvement, in most of the trials the majority of patients remained in functional classes III and IV, which are associated with a poor prognosis, while receiving active therapy.²⁹

**Pregnancy**

Pregnancy in women with PAH has a high maternal mortality (30% - 56%). The physiological changes that occur during pregnancy and the peri-partum seem to be poorly tolerated in these patients. Recent practice guidelines from the European Society of Cardiology and the American College of Cardiology / American Heart Association strongly discourage pregnancy in these patients and advise termination should pregnancy occur. During the past decade, new advance therapies for PAH have emerged and progress in high-risk pregnancy management has been made. In IPAH, CHD-PAH or associated PAH patients receiving advanced therapies (1997 – 2007), a mortality reduction was observed (25% vs 38%, p < 0.04) when compared to statistics from 1978 to 1996. Seventy-eight per cent of deaths occurred within the first month after delivery. Primigravidae and parturients who received general anesthesia were at higher risk of death (p < 0.03 and p < 0.02). Maternal mortality in parturients with PAH remains prohibitively high, despite lower death rates than previous decades.³⁰
In a recent review, the majority of deaths in the last and in previous decades occurred in the post-partum period, mainly within the first month from delivery. Pregnancy-induced systemic vasodilatation and the increase in cardiac output may enhance right-to-left shunting and exacerbate pre-existing hypoxia in patients with CHD-PAH, leading to further pulmonary vasoconstriction. Further hemodynamic stress occurs during labor and delivery, when hypercarbia and acidosis may increase PAH acutely, leading to refractory right heart failure, the main cause of peri-partum death. The effects of pregnancy on the cardiovascular system persist for several months after delivery.\textsuperscript{30}

Maternal mortality in IPAH was lower than in CHD-PAH and associated PAH patients, although this failed to reach statistical significance possibly due to the small number of patients in individual groups. It is possible that the availability and more liberal use of advanced therapies in those patients resulted in improved outcomes. The optimal mode of delivery (vaginal vs. caesarean section) in patients with PAH and/or CHD-PAH remains a matter of debate.

Vaginal delivery, however, is associated with smaller shifts in blood volume, fewer clotting or bleeding complications, and a lower risk of infection. Caesarean section may become necessary in cases of maternal hemodynamic deterioration or fetal distress requiring urgent delivery. Compared with previous era, fewer patients had vaginal delivery, and the proportion of premature deliveries was higher. Closer surveillance and a lower threshold for intervention with early signs of maternal or fetal distress may have resulted in higher rates of caesarean section and premature delivery compared with previous era.\textsuperscript{30}

**Anesthesia in pregnancy PAH patients**

The higher maternal mortality observed with general anesthesia could reflect the higher risk of general anesthetics in PAH patients. It could depress cardiac contractility, increase pulmonary vascular resistance (positive pressure ventilation), and pulmonary arterial pressure (laryngoscopy and intubation). General or epidural
anesthesia should be performed by an experienced anesthetist early during labor to avoid an increase in cardiac output associated with contraction and pain.

Careful monitoring of arterial oxygen saturation, cardiac rhythm, and blood pressure are recommended in the peri-partum period. Although we do not have any evidence, arterial line and a central venous catheter for the monitoring of right atrial pressure should be considered. Invasive pulmonary arterial pressure monitoring remains controversial, however, owing to associated complications such as pulmonary artery rupture and the lack of evidence that it improves outcomes in these patients. The use of direct pulmonary arterial pressure monitoring in more than half of the patients should be questioned. Whether BNP monitoring could identify patients to early pregnancy interruption remains unknown.

**Thromboprophylaxis**

There is no standard thromboprophylaxis for pregnant women with PAH, especially for Eisenmenger patients with thrombotic and bleeding diatheses. Low-dose subcutaneous heparin prophylaxis is generally recommended in pregnant women with PAH, but patients with a history of thrombo-embolic events or atrial fibrillation require higher levels of anticoagulation. Even though not reported as the primary cause of death, almost half of the CHD-PAH patients who died had peri-partum bleeding or a significant drop in hemoglobin concentration, which leads to hemodynamic instability and compromises oxygen carrying capacity.

Pulmonary embolism was responsible for the death in 25% of CHD-PAH without thromboprophylaxis. In addition, pulmonary embolism was also responsible for 50% deaths despite prophylactic anticoagulation in high-risk thromboembolic patients (protein C deficiency and antiphospholipid syndrome) and no deaths due to secondary pulmonary embolisms were observed in IPAH, when 50% received thromboprophylaxis. Although it was not possible to specify the type and extent of thromboprophylaxis required for pregnancy and PAH, its timely consideration
needs to be discussed within the multidisciplinary team and agreed to with the patient. 

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**References**


