The role of thrombosis in severe pulmonary hypertension

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ABSTRACT: Considering the important surface in pulmonary circulation where blood can interact with the endothelium, the maintenance of blood fluidity through the lung, by antithrombotic pathways and products of the endothelium, is essential. This function appears to be ineffective in primary pulmonary hypertension and in severe secondary pulmonary hypertension. Thrombotic lesions are frequently found in pulmonary arteries in these diseases. Thrombin activity appears to be increased in severe pulmonary hypertension. Antithrombotic pathway disorders may account for this abnormality, particularly in chronic thromboembolic pulmonary hypertension and primary pulmonary hypertension. Injured endothelium, a constant feature in severe pulmonary hypertension, either primary or secondary, enhances thrombus formation in pulmonary vessels. This is probably related to thrombomodulin and tissue factor imbalance, impairment of prostacyclin and nitric oxide release, as well as inefficiency of fibrinolysis. Moreover, platelets appear to be activated in the pulmonary circulation of these patients. They release several mediators acting on vascular tone and as mitogenic agents, and may also contribute to thrombin and clot generation. Long-term oral anticoagulant and continuous infusion of prostacyclin, treatments which impede thrombosis, are known to improve the survival rate in patients with primary pulmonary hypertension.

These are the strongest arguments, so far, in favour of the role of thrombosis in severe pulmonary hypertension. However, we do not know whether these abnormalities result from a previous vascular injury or represent the primary disturbance.

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A small number of such lesions is found in lungs of people without cardiac or pulmonary disease who are over the age of 40 yrs [10]. By contrast, they are found much more commonly in all forms of severe PH. They affect approximately 75% of patients with CTEPH [11], and 50% of patients with PPH or PH caused by congenital cardiac defects [12]. In the American National Registry of PPH, 28 out of 58 (48%) patients had thrombotic lesions in pulmonary arteries and 19 out of 58 (33%) had TPA [13]. In other studies devoted to PPH, the frequency of TPA has ranged 20–56% [9, 12, 14]. Furthermore, extensive central thrombi has recently been described in three PPH patients [15]. Although the occurrence of thromboemboli is excluded by definition in PPH, thrombotic lesions and TPA are among the most frequent histopathological appearance in this disease, and, consequently, they are probably the result of local thrombosis.

Although, thrombotic lesions in pulmonary arteries and TPA have never been correlated with the severity of PH, the studies quoted above have shown that in all forms of severe PH, thrombotic lesions could be found in pulmonary arteries. These observations provide a starting point in the discussion of the role of thrombosis in severe PH.

The mechanism and consequence of increased thrombin activity

The complex process involved in blood coagulation can be simplified into a series of linked reactions in which a number of enzymes, zymogens, are activated. These reactions take place on the surface of cell membranes, e.g. platelets, endothelial cells and inflammatory cells. Transformations are accelerated by nonenzyme protein co-factors, which either cause conformational changes or bind the zymogens to cell surfaces. There are two coagulation pathways (fig. 1), intrinsic and extrinsic [1]. The latter is initiated by tissue factor (TF) and circulating factors VII and VIIa (α=activated) [16]. Both pathways converge with the binding of activated factors X and V to the cell surface endothelium or platelets to form the prothrombinase complex (Xa+Va). This enzyme converts circulating prothrombin into thrombin. Finally, thrombin is responsible for converting fibrinogen to fibrin and releasing fibrinopeptide A (FpA).

There are a series of circulating inhibitors which prevent blood coagulation. They inhibit intravascular coagulation and normally limit any coagulation to a local site of injury. The first deficiency of these factors described, and probably the most important one, is the deficiency of antithrombin (AT). This factor inactivates thrombin, and also inhibits the other serine proteinases, including factors XIIa, XIa, IXa and Xa [2]. AT causes inhibition by the formation of a stable 1:1 molar complex between the enzyme active site and AT at its arginine amino acid residue in position 385, or at the serine residue in position 386. Heparin binds at different sites on AT. It is thought to produce marked conformational change in AT. This enhances the ability of AT to inhibit thrombin and Xa. Heparin co-factor II is also a heparin-dependent plasma inhibitor of thrombin. It acts independently of AT, being less effective, and may also be deficient in certain individuals [17, 18].

Activated protein C (APC) is another natural anticoagulant [3] (fig. 2). When circulating, thrombin binds to a protein expressed on the surface of endothelial cells, called thrombomodulin. The product is a powerful activator of protein C. APC shears factor Va at arginine amino acid residue in position 506, causing inactivation. By so doing, it blunts both the extrinsic and intrinsic coagulation pathways at the crucial prothrombinase complex. Protein S is a cofactor required for the factor Va and VIIIa inactivation by APC and may also be deficient.

There is evidence that intravascular coagulation in the pulmonary circulation may be a continuous process in patients with severe PH. The level of FpA is a measure of thrombin activity. EISENBERG et al. [19] reported that FpA was abnormal in all PPH patients and was markedly elevated in 61%. Moreover, in one PPH patient a gradient of FpA level was found across the lung [20]. In a family of PPH patients with abnormal haemoglobin, elevated FpA was also observed [21]. This was associated with evidence of TPA on lung histology [22]. Although, FpA can be raised in both secondary PH and PPH, we do not know if these changes are a result rather than the cause of the pulmonary vascular disease.
It can be questioned whether a deficiency of antithrombotic pathways could contribute to the pathogenesis in patients with severe PH. These deficiencies are now well-described and their genetics are known. Indeed, deficiencies of AT, heparin co-factor II, or of protein C and protein S can be expected in about 5% of patients with symptomatic thrombotic disease (deep vein thrombosis or pulmonary emboli) [7]. Furthermore, a new mechanism of familial thrombosis has recently been described [23]. It involves APC resistance due to a mutation in the factor V gene. This mutation results in replacement of arginine in position 506 with a glutamine amino acid residue. Since APC cleaves factor Va at arginine in position 506, the mutated factor Va has normal procoagulant activity, but is resistant to APC [24].

In a consecutive series of patients with venous thrombosis or thromboembolic disease, approximately 40% had APC resistance [25]. This is much higher than all other deficiencies of the antithrombotic pathways [7]. The prevalence in a control population is 3–7% [26], suggesting that the factor V mutation is quite common. Familial studies have suggested an autosomal dominant mode of inheritance for these antithrombotic pathway deficiencies [27]. However, the high frequency of heterozygous protein C deficiency and heterozygous APC resistance in an asymptomatic general population is not in agreement with a dominant mode of inheritance, unless external factors are important. It is possible that these antithrombotic pathway deficiencies in themselves are relatively weak risk factors for thrombosis, and that the risk is enhanced only if they are associated with other genetic or acquired risk factors, such as a surgical procedure or major injury. The pathogenesis of PPH is supposed to be quite similar and can be characterized by the occurrence of various stimuli which may initiate the development of vascular lesions in predisposed patients [28]. One can suppose that this susceptibility may be an anti-thrombotic pathway disorder in some patients with PPH (fig. 3).

Therefore, it is well-known that patients with AT, protein C, or protein S deficiencies, or resistance to APC have a high risk of developing deep venous thrombosis and pulmonary embolism. We must await further studies in order to establish the prevalence of such deficiencies in patients with severe PH and whether these inherited deficiencies of coagulation contribute either to PPH or to CTEPH.

Injured endothelium

Endothelium participates actively in the process of coagulation. Indeed, STERN et al. [29] showed that the endothelium is capable of sustaining the activation of factor X. Moreover, the binding on the endothelial surface of the enzyme IXa is augmented by the addition of co-factor (VIII) and the substrate (X). Notably, factors IX and X act at the junction of the intrinsic and extrinsic pathways. Another important step in coagulation is the formation of the prothrombinase complex (Xa+Va). It is demonstrated that this complex is not only bound on membrane of platelets but also on the surface of endothelial cells (fig. 4) [30]. The endothelium can also act at the level of TF expression, which is the exclusive point of control of the extrinsic pathway of the coagulation. It is known that cytokines increase TF expression by endothelial cells, which increase thrombotic activity by initiating the extrinsic pathway of the coagulation [31, 32]. As endothelial cells are injured in severe PH, these functions must be affected; however, little is known about them in patients with PH. This must represent a further important area for investigation.

Von Willebrand Factor (vWF) is a glycoprotein which plays an important role in primary haemostasis. It
functions as an adhesive protein for platelets to the vessel wall, as well as a carrier for factor VIII. The release of vWF from endothelial cells is carried out by the formation of storage granules termed Weibel-Palade bodies, which contain multimers of vWF, or directly as pro-vWF dimers [33]. In PH secondary to congenital heart disease and in PPH, the expression of vWF is increased [34, 35]. These patients also exhibit abnormal vWF multimer pattern [36], possibly due to degradation of vWF main subunit [37]. These abnormalities of vWF may be related to endothelial cell injury and may cause a decrease of platelet aggregation to ristocetin, since these multimers of vWF are defective. This suggests that platelet adhesion to vessel walls, involving vWF, is decreased in pulmonary hypertensive patients. However, vWF binding is only one aspect of platelet adhesion to subendothelium in pulmonary hypertensive patients. For instance, platelet-activating factor is released by injured endothelial cells and can itself activate platelets and cause an adhesion to the vascular wall [38].

The endothelium has not only procoagulant functions. Endothelial cells actively contribute to prevention of thrombosis by inhibiting platelet aggregation. Two powerful vasodilators, PGI2 and NO, are synthesized and released by endothelial cells. They both inhibit platelet aggregation and can also reverse the process [4, 5]. There is now evidence of impaired release of PGI2 [39] and NO [40] by endothelial cells of pulmonary arteries in patients with primary and secondary PH (fig. 5). This can be considered as a further mechanism by which thrombosis can be enhanced in pulmonary circulation.

Thrombomodulin is produced by endothelial cells. It is a membrane-bound co-factor with a high affinity receptor for thrombin, rendering it incapable of cleaving fibrinogen or activating platelets. Furthermore, it rapidly converts protein C to APC (fig. 2). This is another method by which endothelial cells are able to avoid clot formation. Thrombomodulin production is reduced when endothelial cells are exposed to cytokines [41, 42]. Thus, cytokine exposure creates an imbalance between thrombomodulin and TF production by endothelial cells, and may promote coagulation in pulmonary vessels. Recently, two studies have shown, firstly, an increase in the serum levels of two cytokines, interleukin 1 and 6 (IL-1 and IL-6) [43], and, secondly, a significant decrease of thrombomodulin [44] in patients with PPH.

The endothelium also has an important role at the level of fibrinolysis, since endothelial cells are a source of t-PA [6]. The endothelium releases t-PA both continuously and acutely in response to triggering factors. Endothelial cells of pulmonary arteries play a part in the continuous production of t-PA. Since t-PA has only limited activity on a pre-existing fibrin clot, acute and local production of t-PA plays a major role in fibrinolysis and, thus, maintains a thromboresistant state in the pulmonary circulation. Notably, endothelial cells also synthesise and release plasminogen activator inhibitor type-1 (PAI-1), which inhibits t-PA by rapid formation of enzyme inhibitor complex. In fact, plasminogen activator inhibitors are rapidly converted to an inactive latent form [45]. However, the mechanism regulating the conversion remains unknown.

EISENBERG et al. [19] found an increase of PAI-1 in plasma of 17 out of 29 PPH patients. Although the plasma concentration of t-PA was not decreased, the t-PA level in pulmonary circulation may be insufficient to induce fibrinolysis in the face of increased PAI-1 activity. These authors suggested that, in patients with PPH, thrombosis may be exacerbated by inadequate fibrinolytic activity. In CTEPH, it is known that a failure to resolve thrombi leads to development of PH [46]. A recent study [47] showed a significant increase of t-PA and PAI-1 antigen in "resting" plasma (before venous occlusion) of 32 patients with CTEPH compared to age-matched control subjects, and a greater increase in plasma t-PA antigen and activity in response to venous occlusion. Therefore, in spite of fibrinolytic abnormalities in these patients, the authors did not find a blunted response of t-PA or plasminogen activator activity to venous occlusion to explain the failure to resolve thrombi in pulmonary arteries. Similarly, SCHULMAN et al. [48] did not find abnormalities in lytic mechanisms in 10 patients with PPH and seven patients with secondary PH compared to nine normal volunteers. Conversely, HUBER et al. [49] found that patients with PPH and CTEPH exhibited a decreased t-PA level and an increased PAI-1 level after venous occlusion compared with healthy subjects (fig. 4).

In experimental models of endothelial injury, the role of fibrinolysis is also debated. SCHULTZE and ROTH [50] found no change in fibrinolytic properties of cultured pulmonary endothelial cells exposed to monocrotaline; however, the same authors [51] reported a decrease of fibrinolytic activity of rat lung tissue after exposure to monocrotaline.
Whilst several arguments lead us to suppose that coagulation is activated in the pulmonary circulation of patients with severe PH, a blunted fibrinolytic response has not yet been convincingly demonstrated.

**Platelets**

In the normal state, platelets circulate through the lungs in an inactive form and display little inclination to interact with pulmonary microcirculation. However, when platelets are activated they become a catalytic surface for coagulation and release numerous mediators [52, 53]. These mediators interact particularly with endothelial cells, neutrophils, fibroblasts and smooth muscle cells. Once activated, there is a complex co-operative interaction between these cells, which can result in either maintenance of normal lung function or a contribution to structural alteration of the lung by means of procoagulant, vasoactive and mitogenic mediators [54, 55].

Arguments for the role of activated platelets in PH in creating thrombi in the pulmonary vasculature have come from experimental models. Vascular thrombi are usually found in the pulmonary vessels after using monocrotaline to injure the lung and cause PH [56]. The presence of platelets in the thrombi and the fact that experimentally induced thrombocytopenia can reduce the development of PH [57] suggests that platelets play a role in the occurrence of PH. Perhaps the thrombosis itself contributes to PH. Clinical studies also reveal conflicting results. Two studies [20, 39] have shown an increase in amounts of the markers of platelet activation; whereas, another study [48] reported no difference in these markers between patients with primary or severe secondary PH and normal volunteers.

In addition to their role in coagulation, platelets appear to play an important part in the pathogenesis of severe PH. When activated, they are known to release the powerful vasoconstrictor thromboxane A$_2$ (TXA$_2$). It is of importance that in patients with PPH or severe secondary PH, TXA$_2$ production is elevated; this is probably platelet derived [39]. In addition to TXA$_2$, platelets also release many other substances, including serotonin (5-HT), platelet-derived growth factor, epidermal growth factor and transforming growth factor-β [55]. These have a powerful mitogenic effect on vascular smooth muscle cells [58, 59], fibroblasts and endothelial cells [60]. It is reasonable to suspect that they induce the remodelling of pulmonary vasculature, which occurs in severe secondary or primary PH. Serotonin which is stored in platelets is a mitogenic factor and a weak pulmonary vasoconstrictor [61]. Serotonin also potentiates the mitogenic effect of growth factors [62]. HERVE et al. [63] described a patient suffering from a familial platelet storage pool disease associated with PPH. Since the platelets of this patient exhibited a deficiency in ability to store 5-HT in dense granules, the free level of 5-HT was high. In a further study, they demonstrated elevated 5-HT levels in the plasma of patients with PPH, which remained after lung transplantation suggesting a primary abnormality [64]. These data assume special importance, as the 5-HT uptake inhibitors, fenfluramine and dexfenfluramine, have been associated with the development of PPH [65, 66].

Whilst the evidence for a contribution by platelets in local pulmonary thrombosis to the development of severe PH is weak, there is an emerging role of platelet-selective release of mediators, such as 5-HT, TXA$_2$ and growth factors, occurring in patients with severe PH (fig. 5). Future work needs to be devoted to the relationship of these mediators and local thrombosis in the pulmonary circulation.

**Antiphospholipid antibodies**

The presence in plasma of APL antibodies is one of the more common causes of acquired thrombophilia [67]. These antibodies can be found either in patients suffering from systemic lupus erythematosus (SLE) or mainly in patients without biological and clinical criteria for SLE but associated with a history of venous and/or arterial thrombosis. The latter patients may not have accompanying thrombocytopenia [68]. Various potential mechanisms have been proposed to explain the increased risk of thrombosis in SLE and APL syndrome. These include a decrease in the plasma level of free protein S [69], and the occurrence of a plasma inhibitor of endothelial activation of protein C [70]. A plasma inhibitor of factor Va degradation [71], increased plasma level of PAI-1 [72], and a plasma factor which inhibits endothelial cell release and/or production of PGI$_2$ [73] have also been reported. A recent in vitro study has shown an increase of platelet aggregation and adherence to the subendothelium, as well as an increase in immunoglobulin binding to platelets in the presence of APL [74]. These observations remain doubtful when considering a mechanism which is active in vivo, particularly the potential inhibition of PGI$_2$ production by endothelial cells [75].

PPH and CTEPH have been well described in APL syndrome [76, 77]. However, it is an uncommon complication of APL syndrome. In a multi-centre study of 70 patients suffering from APL syndrome [68], 18 patients had pulmonary embolism but only two had PH (one related to pulmonary embolism and the other resembling the PPH). Moreover, ASHERON et al. [78] reported 24 patients with PH, 22 had SLE, one primary APL syndrome, and one SLE/progressive systemic sclerosis "overlap syndrome". It is interesting to note that 17 out of 24 patients (67%) had APL antibodies, which is higher than expected in SLE patients without PH. In the same study, the prevalence of PH in these patients was about 5%. Conversely, OLMAN et al. [47], in 32 consecutive patients with surgically documented CTEPH, found antiphospholipid antibodies in only two patients.

Thus, an association between APL antibodies and PH, though uncommon, is well-established. In some patients, there is a link between thrombosis in pulmonary arteries and the occurrence of PH [78, 79]. We must emphasize that in this disease the presence of APL antibodies and, subsequently, thrombosis in pulmonary arteries may be the primary cause of PH. These data justify the screening of patients with PPH and CTEPH for APL antibodies.
Anticoagulant therapy

Since several studies have shown an increase of thrombin activity [19, 21] and an activation of platelets in severe PH [39], as well as a decrease of the thromboresistant nature of injured endothelium [44, 49], anticoagulant therapy may be justified in these patients. A further major reason to prescribe an anticoagulant therapy in severe PH is not related to the pathogenesis of PH but to its consequences on haemodynamic status. It is well-known that patients with severe PH have a low cardiac output, and low flow states are a major risk factor in development of lethal thromboembolism disease [8].

Long-term oral anticoagulant therapy has been assessed in PPH with respect to survival. Fuster et al. [80] first observed an improvement in survival of PPH patients on warfarin or coumadin. Ricci et al. [81] compared PPH patients on warfarin with those on no long-term anticoagulant therapy. They reported enhanced survival in the treated patients. A retrospective study on the survival of pulmonary hypertensive patients who had taken the dietary suppressant aminorex [82] also showed that anticoagulant therapy increased survival rates. This effect was greater when the treatment was initiated early after the first symptoms. Furthermore, 2 out of 3 of these patients treated with warfarin experienced restoration of functional and haemodynamic status.

An alternative to formal anticoagulation is the use of antiplatelet treatments. PG12 is a powerful vasodilator but also inhibits platelet aggregation and inhibits smooth muscle proliferation [4]. The latter effect has been suggested to be the consequence of inhibition of platelet aggregation on vessel walls [62]. Long-term infusion of PG12 was first tested in the UK [83, 84]. This treatment improved survival rates of PPH patients [85]. It should be emphasized that PG12 had its greatest effect on survival in those patients with the poorest vasodilatory capacity. Long-term heparin has never been evaluated in these patients. Survival improvement under long-term anticoagulant therapy is not necessarily due to prevention of local thrombosis in pulmonary vessels. Warfarin also prevents fatal thromboembolism, and PG12 is also a powerful vasodilator and inhibits smooth muscle cell proliferation. These separate roles of the two agents would also account for their efficiency in improvement of survival. Whatever the mechanism involved, anticoagulant therapy may be warranted in all patients with PPH [28, 81]. PG12 is reserved for those with the worst chance of survival [86]. CTEPH and PH associated with APL antibodies also requires long-term anticoagulant therapy. As regards PH secondary to cardiac defect, anticoagulant therapy must not be prescribed, as these patients have a greater risk for haemoptysis.

In conclusion, the pulmonary vascular endothelium plays an important role in maintaining blood fluidity through the lung. Studies have shown an impairment of this function in patients with PPH or severe secondary PH. Local thrombosis can result, which has been confirmed by the appearance of increased thrombin activity in these patients, as well as platelet activation. Several mechanisms have been involved. There is an imbalance between thrombomodulin and TF when pulmonary endothelial cells are stimulated by cytokines. An increased blood level of TxA2 has also been well-demonstrated in these patients. The elevated level of TxA2 is probably platelet-derived. Furthermore, there is now evidence of impaired release of PGI2 and NO by endothelial cells of pulmonary arteries in patients with primary and secondary PH. These mediators do not act only on the vascular tone but also increase local thrombosis in the pulmonary circulation when their release by endothelial cells are impaired. Finally, the presence of APL antibodies, a common cause of thrombophilia, appears to be implied in the occurrence of PH. These are the strongest arguments, so far, for the role of thrombosis in severe PH.

With the exception of PH secondary to cardiac defect and PH associated with APL antibodies, in which these abnormalities of coagulation represent, respectively, a consequence or the cause of PH, it is unknown whether they characterize causal mechanisms or simply epiphenomena. Further work must be performed, in particular to establish the relationship between severe PH and antithrombotic pathway disorders and the exact roles of fibrinolysis and platelets in these diseases.

The isolation of risk factor of PPH or CTEPH related to coagulation should lead to a better understanding of the pathophysiology. This would make it possible to treat these coagulation abnormalities in order to avoid further damage to the lung.

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