Current Cardiology Reports (2021) 23: 141
https://doi.org/10.1007/s11886-021-01572-6

HEART FAILURE (HJ EISEN, SECTION EDITOR)

# Chronic Thromboembolic Pulmonary Hypertension: the Bench

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Accepted: 27 April 2021 / Published online: 19 August 2021


## Abstract

**Purpose of Review** Chronic thromboembolic pulmonary hypertension (CTEPH) is an uncommon complication of acute pulmonary embolism (PE), in which the red, platelet-rich thrombus does not resolve but forms into an organized yellow, fibrotic scar-like obstruction in the pulmonary vasculature. Here we review the pathobiology of CTEPH.

**Recent Findings** Our current knowledge has predominantly been informed by studies of human samples and animal models that are inherently limited in their ability to recapitulate all aspects of the disease. These studies have identified alterations in platelet biology and inflammation in the formation of a scar-like thrombus that comprised endothelial cells, myofibroblasts, and immune cells, along with a small vessel pulmonary arterial hypertension-like vasculopathy.

**Summary** The development of CTEPH-specific therapies is currently hindered by a limited knowledge of its pathobiology. The development of new CTEPH medical therapies will require new insights into its pathobiology that bridge the gap from bench to bedside.

**Keywords** Chronic thromboembolic pulmonary hypertension · Pulmonary embolism · Pulmonary hypertension · Inflammation · Platelet · Myofibroblast

## Introduction

Multiple axes contribute to the pathobiology of CTEPH, including abnormalities in platelet and endothelial function and dysregulated inflammation. This results in the lack of resolution of an acute PE and promotes both large vessel remodeling that results in the yellow "thrombus" material and small vessel vasculopathy that are characteristic of the disease.

## The Role of Inflammation and Fibrosis in CTEPH

To maintain homeostasis, the vascular endothelium suppresses coagulation, platelet activation, and leukocyte adhesion. Pathologic inflammation disrupts these inhibitory mechanisms resulting in endothelial dysfunction and adverse pulmonary vascular remodeling. Inflammation and fibrosis are known to be important mediators of vascular dysfunction in

This article is part of the Topical Collection on *Heart Failure*

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the pathogenesis of pulmonary arterial hypertension (PAH) [1]. Similarly, inflammation is considered to be relevant in the development of CTEPH, largely by impairing angiogenesis and thrombus resolution and promoting in situ thrombosis. There has been a long-standing association between CTEPH and patients with underlying inflammatory conditions and infections, including infected pacemakers, ventriculo-atrial shunts, and chronic osteomyelitis [2, 3]. For example, in pulmonary endarterectomy (PEA) specimens from CTEPH patients with ventriculo-atrial shunts, *Staphylococcus aureus* DNA was isolated in 6 out of 7 patient samples [4]. Subsequently, staphylococcal infection was found to delay thrombus resolution in vivo in parallel with upregulation of transforming growth factor (TGF)-β and connective tissue growth factor [4].

Moreover, it has been observed that circulating inflammatory markers are elevated in numerous cross-sectional studies involving CTEPH patients (Table 1). For example, higher levels of C-reactive protein (CRP) and tumor necrosis factor (TNF)-α are detected in patients with CTEPH compared with healthy controls, and levels of both biomarkers decrease following PEA [5, 6]. Some inflammatory markers correlate with adverse clinical profiles: in patients with CTEPH, levels of IP- 10 (CXCL-10), a chemokine that induces fibroblast activation and migration, inversely correlate with cardiac index and exercise capacity, while IL-6 levels positively correlate with pulmonary vascular resistance (PVR) [7]. Further, elevations in circulating inflammatory markers parallel local inflammatory cell infiltration in the pulmonary arteries of patients with CTEPH. Histopathologic analyses of the surgically resected thromboemboli demonstrate an inflammatory infiltrate consisting of neutrophils, macrophages, and lymphocytes. Elevated CRP and circulating matrix metalloproteinase (MMP)-9 have been correlated with neutrophil and macrophage accumulation, respectively [9].

It is proposed that vascular inflammation impairs angiogenesis and thrombus resolution. The adaptive response to thrombosis involves dissolution of the thrombus through fibrinolysis and angiogenesis, a process that is dependent on leukocyte migration into the affected compartment. Platelet endothelial cell adhesion molecule (PECAM)-1 is a glycopeptide receptor expressed on endothelial cells that responds to inflammatory stimuli and participates in leukocyte transmigration [11]. In a mouse model of venous thrombosis, PECAM-1 deficiency resulted in significantly larger thrombi and misguided thrombus resolution [12]. Investigators also showed accumulation of a cleaved form of PECAM-1 in unresolved human DVTs with parallel increased plasma levels of soluble cleaved PECAM-1, compared to patients whose DVTs had resolved [12]. In PEA specimens from patients with CTEPH, relative decreased endothelial PECAM-1 expression has been observed [13]. Collectively, the evidence suggests vascular inflammation and the ensuing endothelial dysfunction impair angiogenesis and thrombus resolution, thereby promoting persistent platelet-endothelial interactions.

Fibrosis is also closely linked to both inflammation and angiogenesis in CTEPH. PEA samples display areas of fibrosis accompanied by inflammation and angiogenesis [9, 14, 15]. Notably, the majority of cells that comprise CTEPH thrombus (see below) are myofibroblast-like, with expression of markers such as collagens and a loss of other markers such as α-smooth muscle cell actin [15, 16]. In a study of human PEA material and a mouse model of venous thromboembolism, endothelial TGF-β1 signaling and endothelin-1 (ET-1) signaling were shown to contribute to endothelial-to-mesenchymal transition (endoMT) and thrombofibrosis [17•]. Together, a pro-inflammatory and pro-fibrotic state contributes to vascular remodeling and impaired thrombus resolution in CTEPH.

**Table 1** Elevated inflammatory markers in patients with CTEPH

| Inflammatory marker(s) | Finding | Reference |
|---|---|---|
| C-reactive protein (CRP) | Elevated in plasma of patients with CTEPH and decreases following PEA. Correlates positively with neutrophil accumulation in PEA specimens | Quarck et al. (2009) [5] |
| Tumor necrosis factor (TNF)-α | Elevated in plasma of patients with CTEPH and decreases following PEA | Langer et al. (2004) [6] |
| Interleukin (IL)-1β, IL-2, IL-4, IL-6, IL-8, IL-10 | Elevated in serum of patients with CTEPH. IL-6 levels correlate positively with PVR | Zabini et al. (2014) [7]<br>Soon et al. (2011) [8] |
| Interferon-γ-induced protein (IP)-10 (CXCL-10) | Monokine that induces fibroblast activation and migration found to negatively correlate with cardiac index and exercise capacity | Zabini et al. (2014) [7] |
| Macrophage inflammatory protein (MIP)-1α | Produced by macrophages and monocytes and found to be elevated in PEA specimens | Zabini et al. (2014) [7] |
| Monocyte chemotactic protein (MCP)-1 | Chemokine that attracts monocytes. Elevated in patients with CTEPH and is positively correlated with PVR | Quarck et al. (2015) [9]<br>Kimura et al. (2001) [10] |
| Matrix metalloproteinase (MMP)-9 | Correlates positively with macrophage accumulation in PEA specimens | Quarck et al. (2015) [9] |
| Chemokine ligand 5 (RANTES) | Recruits leukocytes to sites of inflammation and is found to be elevated in PEA specimens | Zabini et al. (2014) [7] |

*PEA* pulmonary artery endarterectomy, *PVR* pulmonary vascular resistance, *CXC* C-x-C chemokine ligand, *RANTES* regulated upon activation, normal T cell expressed and presumably secreted.

## The Role of Platelets in CTEPH

Platelets are critical regulators of hemostasis, thrombosis, and inflammation. The lung is a primary site of terminal platelet synthesis, accounting for approximately 50% of total platelet production [18]. Under physiological conditions, the pulmonary circulation is protected from atherothrombosis due to the iterative branching of the pulmonary arterioles in series, which renders it a low resistance, low pressure, and low shear stress vascular circuit [19]. Nonetheless, platelets have been implicated in the pathogenesis of various pulmonary disorders, including pulmonary fibrosis, serotonin-mediated pulmonary hypertension, and CTEPH [19]. The exact role platelets play in the transition from an acute PE to chronic thromboembolism is unclear but the focus of increased attention.

While acute PE specimens contain predominantly fibrin, erythrocytes, and inflammatory cells, surgically resected thromboemboli from patients with CTEPH are characterized by organized thrombi with areas of recanalization, neoangiogenesis, chronic inflammation, and fresh thrombus in situ [14]. Since approximately 25% and 44% of patients diagnosed with CTEPH lack a documented history of PE or deep vein thrombosis (DVT), respectively [3], it is hypothesized that in situ thrombosis contributes to the development and progression of CTEPH in these cases. While clinically occult PE is likely an inciting factor, pulmonary arterial microthrombosis is also known to arise from local endothelial dysfunction [20, 21] suggesting local platelet-endothelial interactions are relevant to thrombus persistence.

Platelet-activating conditions, such as thyroid hormone replacement therapy and splenectomy, are established risk factors for CTEPH, further implicating persistent platelet activation and aggregation in the pulmonary circulation as a potential mechanism of disease progression [3, 4]. In a mouse model of stagnant flow venous thrombosis, splenectomy increased thrombus volume attributable to platelet activation as well as the number of circulating platelet-derived microparticles [22]. An increase in platelet-derived microparticles was also observed in PEA specimens from asplenic patients with CTEPH [22]. Platelet abnormalities are also observed in unselected CTEPH populations: in one study, PEA specimens expressed increased levels of platelet factor 4, a protein released by platelets at sites of vascular injury [23]. Compared with controls, patients with CTEPH demonstrate both increased platelet activation as measured by cell surface expression of P-Selectin [24] and spontaneous platelet aggregation [25]. However, increased platelet activation is also seen in patients with PAH [24] suggesting an overlapping pathobiology between these otherwise distinct clinical entities that may be due to secondary pulmonary vascular remodeling.

The role of platelet-endothelial interactions in CTEPH remains understudied; however, there is evidence that platelets contribute directly to pulmonary vascular dysfunction. In sickle cell disease, neutrophil-platelet aggregates have been demonstrated to accumulate in lung arterioles and contribute to endothelial dysfunction, a process that could be abrogated by inhibiting platelet P-Selectin [26]. Recently, the scaffolding protein NEDD9 was shown to regulate pathologic collagen deposition in the pulmonary vascular endothelium. NEDD9 is a malignancy protein that is regulated by hypoxia through HIF-1$\alpha$ and has been implicated in thrombotic pathophenotypes [27]. Recent work has suggested that pulmonary endothelial NEDD9 binds directly to platelet P-Selectin to promote platelet-endothelial adhesion. In patients with CTEPH, NEDD9 is upregulated in both plasma and pulmonary artery endothelial cells, suggesting that chronic upregulation of NEDD9 by local hypoxia following pulmonary embolism may serve as a potential mechanism for persistence and organization of luminal thrombus [28••].

## Cell Populations that Contribute to CTEPH Thrombus

As noted above, thrombus in CTEPH differs significantly from acute PE. In acute PE, the fresh clots are red, easily detached from the pulmonary artery wall, and consist mainly of red cells and platelets in a fibrin mesh. This is a major contrast from CTEPH, where the chronic clots are yellow, incorporated in the pulmonary vascular wall, and contain collagen, elastin, and inflammatory cells [29, 30]. This "thrombus" is better described as a chronic scar of the pulmonary vasculature. In addition to these chronic clots, there is a small vessel disease with remodeling throughout the pulmonary vascular bed [30–32]. Our current understanding of CTEPH thrombus is largely based on studies that focused on identification of specific cell types within CTEPH thrombus using either histopathologic analyses or in vitro culture systems and animal models primarily based on embolization of foreign material. Inherited and acquired thrombophilias may play a role in some cases of CTEPH, but usually a combination of different mechanisms (such as prothrombotic tendency, impaired fibrinolysis, and chronic inflammation) along with other clinical factors contribute to thrombus nonresolution and subsequent formation of CTEPH thrombus [33]. Although many studies have identified defects in coagulation, fibrinolysis, and inflammatory pathways in CTEPH, it is largely unclear how this contributes to the abnormal cellular proliferation of CTEPH thrombus (reviewed in 33, 34), which is the hallmark of progressive disease.

Numerous studies have focused on identifying potential pathogenic cells in CTEPH thrombus. These studies, while providing important insights into disease pathophysiology, have only provided limited insights into the heterogeneous cell populations that compose CTEPH thrombus (Figure 1) [35]. These studies have largely relied on obtained tissue from

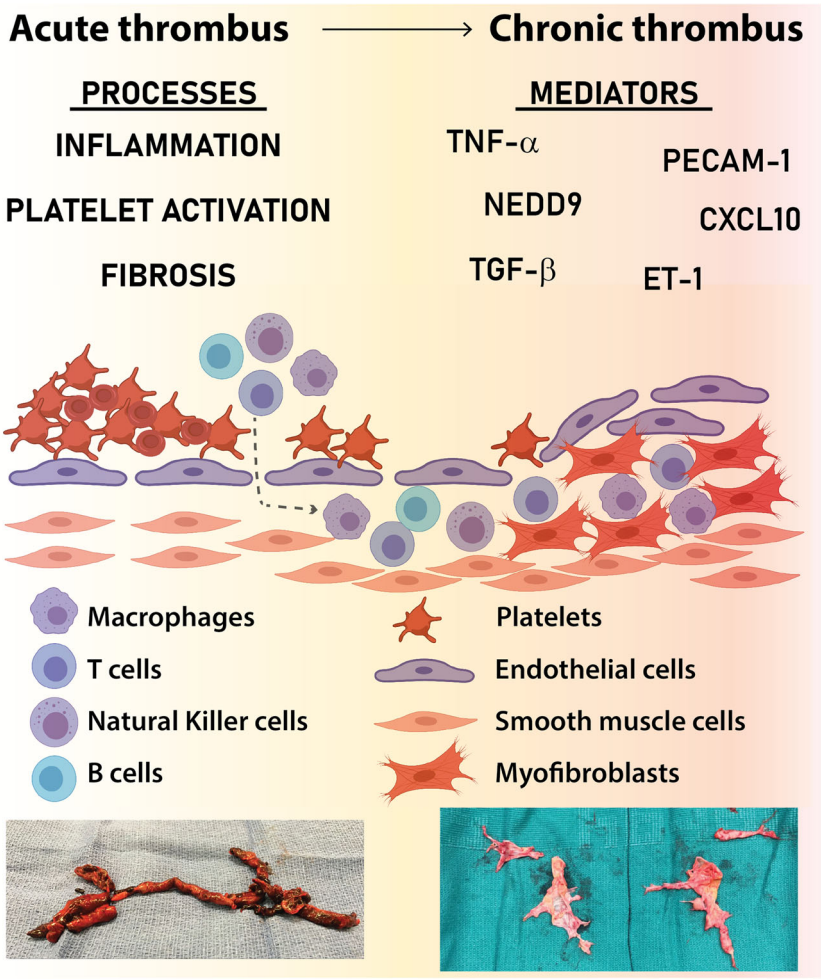


**Fig. 1** Cellular changes from acute to chronic thrombus. The change from acute (*red*, platelet rich, bottom left) to chronic thrombus (*yellow*, fibrotic, bottom right) is associated with specific processes (inflammation, fibrosis, platelet activation) and mediators that promote the infiltration of inflammatory cells, proliferation of smooth muscle, and endothelial cells with loss of platelets and red blood cells. A population of myofibroblasts has also been noted in CTEPH thrombus, along with cells that appear to have a stem cell-like phenotype.

PEA. Two major limitations apply to the majority of these studies [16, 23, 36–38]: first, they rely on histopathology based on a set of limited markers that may have limited specificity or may be silent to relevant cell populations, and second, they culture cells from thrombus, thereby enriching for cells that grow under culture conditions rather than those cells that may be biologically relevant to the pathogenesis of CTEPH. This approach may lead to the selection of cells that grow under cell culture conditions and/or phenotypic modulation of cells under culture conditions. Approaches are sorely needed to determine the cellular heterogeneity and specific cell types that directly contribute to CTEPH pathophysiology.

## Endothelial Cells

The role of the endothelium in CTEPH differs markedly from its role in other diseases of the pulmonary vasculature, such as PAH. Although not considered to be a primary effector of the disease state, the endothelium does not appear to be a mere bystander in CTEPH. Even in patients with compensated hemodynamics, endothelial dysfunction has been observed with invasive measures in studies of hemodynamic vasoreactivity [39]. Endothelial-dysfunction related thrombosis has been hypothesized as one reason why many patients with CTEPH have no documented history of pulmonary embolism [40]. Endothelial cells isolated from CTEPH thrombus showed significantly different calcium homeostasis as compared to control endothelial cells, with an increase in angiostatic factors like platelet factor 4 (CXCL4), collagen type I, and CXCL10 that likely contribute to endothelial dysfunction [23]. Despite these insights, the role of the endothelium in CTEPH is incompletely understood due to a lack of animal models that fully recapitulate the disease state.

Multiple experimental studies have been conducted in vitro on cultured endothelial cells from surgical specimens after PEA. Endothelial cells cultured from PEA specimens have increased proliferation relative to cultured endothelial cells from patients with other disease states [38, 41]. A transcriptomic microarray study of endothelial cells isolated from CTEPH patients further demonstrated increased expression of genes necessary for proliferation, as well as genes in pathways of inflammation, thrombosis, and fibrosis with notable upregulation of TGF-β pathway genes [15, 42]. In light of the association of CTEPH with

chronic inflammatory states, it has been observed that CRP stimulation of pulmonary artery endothelial cells results in upregulated EDN1, ICAM1, and VCAM1 in an NFkB-dependent pathway [43, 44]. Circulating endothelial cells (CECs) have been implicated in numerous types of pulmonary vascular diseases, including PAH and CTEPH. CECs are considered to be noninvasive markers of vascular damage, remodeling, and dysfunction. A study of a small cohort of patients with PAH and CTEPH evaluated the number of CECs in PAH and CTEPH and found that PAH patients, but not CTEPH patients, had significantly increased number of CECs [45]. Interestingly, another study demonstrated that CTEPH patients had significantly increased levels of endothelial microparticles in CTEPH plasma compared to the plasma of patients with PE and healthy controls [46]. The CTEPH endothelial microparticles were able to enhance cell survival and angiogenesis (as measured by tube formation) in vitro. While these findings may suggest that these microparticles may be protective of endothelial cells in CTEPH, whether they may also contribute to the disease pathogenesis via enhancing aberrant proliferation of pulmonary vascular endothelial cells remains to be determined.

Finally, how the endothelial cell populations respond to therapeutic interventions in CTEPH is an important topic for future studies. Comparison of endothelial progenitor cells (EPCs) from subjects treated with riociguat and those who were treatment-naïve found that circulating EPC number was modestly higher in the riociguat group compared to the control group [47]. EPCs from riociguat-treated patients demonstrated an increase in their ability to induce tube formation. While these are intriguing findings, further studies are needed to determine how therapies such as riociguat, and others under investigation, may impact the behavior of endothelial cells and ultimately how the endothelial cell response to these therapies may dictate clinical outcomes in CTEPH.

### Myofibroblasts and Other Smooth Muscle Cell Populations

Smooth muscle and endothelial cells that contribute to CTEPH proximal vascular remodeling display modified proliferative and migratory responses consistent with a proliferative phenotype [38]. The predominant cell type in endarterectomized tissue appears to be myofibroblast-like [48], which demonstrate a hyperproliferative, anchorage-independent, invasive, and serum-independent phenotype [16]. In cell culture, these myofibroblast-like cells promote a microenvironment which results in endothelial cell dysfunction and transition to a mesenchymal phenotype [36]. Other cell types with a proliferative phenotype have been observed in CTEPH thrombus. For example, some cells within chronic thrombus that contribute to neointimal formation have been described as multipotent mesenchymal progenitor cells capable of adipogenic and osteogenic differentiation [48]. Others have been described as "sarcoma-like" cells that when injected into mice subcutaneously developed into solid, undifferentiated tumors at the site of injection and when injected intravenously via tail vein developed tumors which grew along the intimal surface of the pulmonary vessels [37]. Notably, this growth could be inhibited by matrix metalloproteinase inhibitors [49], which suggests that these sarcoma-like cells may be similar to myofibroblasts observed in other studies [50]. Moreover, due to the similar methodologies used in these studies, primarily relying on culturing of cells from endarterectomized CTEPH thrombus, it is unclear if the cells identified in these studies describe a single cell type or a heterogeneous cell population. For example, "myofibroblasts" observed in these studies may be a unique cell population or a mixture of smooth muscle-like and fibroblast-like cells.

### Inflammatory Cells

Other studies have described the marked presence of inflammatory cells (B and T lymphocytes, macrophages, and neutrophils) in CTEPH thrombus [7, 9]. Using immunohistochemistry of CTEPH thrombus, different types of lesions, such as neointima, thrombotic, recanalized, and atherosclerotic lesions, were associated with different patterns of inflammatory cells [9]. Specifically, accumulation of macrophages, T lymphocytes, and neutrophils was found mainly in atherosclerotic and thrombotic lesions, while angiogenesis was observed in all lesions. Notably, enhanced systemic inflammation paralleled local inflammatory cell infiltration in the pulmonary arteries in advanced stages of CTEPH, which suggests that inflammation and impaired angiogenesis contribute to CTEPH progression [9]. Consistent with this, PEA specimens have been shown to have high levels of interleukin (IL)-6, monocyte chemoattractant protein-1 (CCL2), CXCL10, macrophage inflammatory protein (MIP)1$\alpha$ (CCL3), and RANTES (CCL5) compared to healthy lung tissue [7]. Notably, CXCL10 levels were associated with severity of pulmonary hemodynamics and physical capacity, suggesting its involvement in CTEPH pathology [7].

## Small Vessel Disease

In addition to the blockage of the proximal and distal pulmonary vasculature by CTEPH thrombus, there is also evidence for a small vessel vasculopathy similar to PAH in CTEPH [30–32]. This microvascular or "small vessel" disease mirrors that seen in PAH, involving arterioles that are less than 300 μm in diameter [51]. The full range of pulmonary hypertensive lesions in the small arteries can be observed in CTEPH

and imply that pulmonary arterial hypertension cannot be separated from potentially correctable CTEPH based on histopathologic findings in the small pulmonary arteries [31]. The histologic pattern observed in CTEPH is remodeling of small pulmonary arteries/arterioles, septal veins and pre-septal venules, and pronounced hypertrophy and enlargement of bronchial systemic vessels. This remodeling of the small pulmonary arteries and septal veins is accompanied by pronounced hypertrophy of the bronchial systemic vessels [32]. Notably, similar venular involvement in microvascular disease and post-capillary bronchopulmonary shunting are observed in patients and a piglet model of CTEPH [32] where small vessel disease developed in both occluded and non-occluded territories and improved in both territories after lung reperfusion [52]. Notably, this was associated with a decrease in ET-1 and IL-6 gene expression in non-occluded territories, suggesting that vasoactive, proliferative, and inflammatory signaling contributes to the small vessel disease that develops in CTEPH. This remodeling of the small vessels likely explains some of the benefit of PAH medical therapies in CTEPH [53–55] as well as some of the benefit of reperfusion seen with balloon pulmonary angioplasty (BPA).

## Animal Models of CTEPH

Another critical limitation to the study of CTEPH is the lack of suitable cellular or animal models for CTEPH that take into account the important role of the pulmonary vascular remodeling over months that is seen in the human disease. A major difficulty in using animal models for thrombosis are inherent differences between human and animal systems. For example, while swine have similar platelet counts and coagulation profile to humans [56], their fibrinogen is more highly crosslinked, leading to plasmin resistance [56]. Models in rodents, monkeys, and piglets for persistent intravascular thrombosis have all been developed, but rodent models have been more commonly used since they are cheaper and more efficient [40]. Mouse models of venous thrombosis include the ferric chloride model, the inferior vena cava (IVC) ligation model, the IVC stenosis model, and the electrolytic IVC injury model [57•]. These studies have demonstrated an important role for inflammation in thrombosis, thrombus resolution, and vascular remodeling. Inflammation, in the setting of staphylococcal infection [4] or splenectomy [22], can limit thrombus resolution. Fibrosis also plays a central role, as a mouse IVC ligation model recapitulated findings from human CTEPH with elevated ET-1 signaling mediated by TGF-β1/TGFβRI signaling (with impaired TGFβRII signaling) and is associated with endoMT [17•]. Angiogenesis is also important for thrombus recanalization and resolution, as thrombus nonresolution occurs with treatment with antiangiogenic therapy [58] or in endothelial cell-specific deletion of vascular endothelial growth factor receptor 2 (VEGFR2) followed by vein ablation [13]. Notably, this last study also found that human CTEPH thrombi demonstrated low expression of genes important in angiogenesis, such as VEGFR2, VE-cadherin, and podoplanin. Conversely, promoting angiogenesis in these thrombosis models has been shown to enhance thrombus recanalization and resolution [59, 60].

A major difficulty in animal models is modeling the chronicity of disease required for transition from acute to chronic PE. Some models have largely relied on repeated microsphere embolization into the pulmonary circulation, sometimes in combination with drugs that promote endothelial dysfunction. In a canine model, repeated embolization of microspheres through an indwelling catheter over several months led to the development of CTEPH but with only moderate RV dysfunction and tricuspid valve endocarditis in all animals [61]. Combining microsphere embolization with eNOS inhibitor L-NAME treatment in swine resulted in pulmonary microvascular remodeling with a decreased activity of PDE5 and Rho-kinase [62]. Using microsphere embolization along with VEGFR1/2 inhibition (SU-5416) in a rodent model resulted in PH with right heart failure, with significant pulmonary vascular remodeling and cellular proliferation [63]. However, while embolization models can mirror the transition from acute PE to CTEPH seen in humans, these approaches typically require multiple embolizations with non-native particles that result in microvascular obstruction.

Pulmonary artery (PA) ligation models alone can reproduce the hemodynamics of obstructed lesions but do not result in thrombus formation or distal vasculopathy by themselves [40]. However, combining PA ligation with embolization results in the development of PH with increased bronchial collaterals and small vessel disease of the distal pulmonary arteries throughout the lungs [64]. In this piglet model, chronic PH was induced in piglets by left PA ligation followed by weekly embolization of the right lower lobe arteries with enbucrilate tissue adhesive resulting in a model of chronic PH related to the development of post obstructive and overflow vasculopathy. Overflow vasculopathy refers to the increased blood flow of the non-obstructed portions of the pulmonary vasculature that are subjected to high shear stress on the endothelium, which is known to modulate endothelial phenotype and barrier function [65] and results in severe pulmonary vascular injury and remodeling in large animal models of disease [66]. Notably, subsequent left PA reperfusion resulted in a regression of pulmonary vascular remodeling and a decrease in pulmonary pressures [52]. Development of CTEPH in a swine model required both proximal PA coiling along with distal embolization with dextran, with distal embolization alone being insufficient to result in the development of PH [67]. Together, these findings suggest that both proximal disease (either induced by ligation or coiling) in combination with small vessel disease (by distal embolization) are required for the development of CTEPH.

These animal models are limited by a reliance on the embolization of foreign materials, leading other groups to embolize autologous blood clots. Dogs have a brisk thrombolytic response that prevents the development of acute embolism, but if given a fibrinolytic inhibitor (tranexamic acid) along with venous thrombi, they can develop CTEPH, although with a rather limited increase in mPAP and PVR [68, 69]. A more recent study used a similar approach in rats by combining tranexamic acid along with autologous clots and resulted in some obstruction in the large PAs with a limited increase in mPAP and PVR [70]. Notably, a recently described model from Quarck and colleagues used repeated clot embolization combined with SU5416 treatment in rabbits to generate chronic thrombus with severe pulmonary hypertension [71]. In this model, they observed blockages in the large proximal PAs by fibro-thrombotic lesions and the remodeling of the pulmonary microvasculature similar to CTEPH human pathology. At this time, studying human tissue and samples provides insights into CTEPH pathophysiology that are lacking from animal models, but only an animal model can allow a full dissection of the pathophysiological mechanisms that contribute to the development of CTEPH. Hopefully, this and other animal models that result in chronic thrombus and pulmonary hypertension can be further developed in parallel with studies using CTEPH thrombus from patients to yield novel pathophysiological insights into CTEPH.

## Conclusions

CTEPH has both a complex approach to its diagnosis and treatment and a complex pathobiology. While there are a number of therapeutic options for patients, there is still a fundamental knowledge gap in our understanding of CTEPH pathobiology. The development of new CTEPH medical treatments will require the identification of specific cell populations, such as endothelial, smooth muscle and immune cells, and pathways, such as inflammation and proliferation, that underlie its pathogenesis. The identification and testing of these therapies will require both human samples and animal models of disease, which can then be tested back at the bedside in clinical trials.

**Abbreviations** CEC, Circulating endothelial cells; CRP, C-reactive protein; CTEPH, Chronic thromboembolic pulmonary hypertension; DVT, Deep venous thrombosis; endoMT, Endothelial-to-mesenchymal transition; EPC, Endothelial progenitor cells; ET-1, Endothelin-1; IVC, Inferior Vena Cava; mPAP, Mean pulmonary arterial pressure; MMP-9, Matrix metalloproteinase-9; PA, Pulmonary artery; PAH, Pulmonary arterial hypertension; PE, Pulmonary embolism; PEA, Pulmonary endarterectomy; PECAM-1, Platelet endothelial cell adhesion molecule-1; PH, Pulmonary hypertension; PVR, Pulmonary vascular resistance; RV, Right ventricle; TGF-β, Transforming growth factor β; TNF-α, Tumor necrosis factor α

## Declarations

**Conflict of Interest** Dr. Tapson reports grants from Bayer, and grants and personal fees from Janssen and Actelion, outside the submitted work.

Dr. Maron reports personal fees from Actelion, outside the submitted work. In addition, Dr. Maron has a patent US patent 9,605,047 issued, a patent US pending patent PCT/US2019/059890 pending, and patent applications 62475955 and 029672 pending.

Dr. Rajagopal reports grants and personal fees from Janssen and United Therapeutics, and personal fees from Altavant, Apie Therapeutics, Bayer, Insmed, and Liquidia Technologies, outside the submitted work. In addition, Dr. Rajagopal has a patent US Patent 62/673,175. "Dynamic 129Xe Gas Exchange Spectroscopy" licensed to Polarean Corporation.

The other authors declare that they have no conflict of interest.

**Human and Animal Rights and Informed Consent** This article does not contain any studies with human or animal subjects performed by any of the authors.

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Papers of particular interest, published recently, have been highlighted as:
- • Of importance
- •• Of major importance


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