Eur Respir J 2013; 41: 1200–1206
DOI: 10.1183/09031936.00101612


**SERIES: "CHRONIC THROMBOEMBOLIC PULMONARY HYPERTENSION"**
**Edited by J. Pepke-Zaba, M.M. Hoeper and M. Humbert**
**Number 5 in this Series**

# Chronic thromboembolic pulmonary hypertension: animal models

**Olaf Mercier and Elie Fadel**

**ABSTRACT: Chronic thromboembolic pulmonary hypertension (CTEPH) is a life-threatening disease due to pulmonary artery obstruction by persistent organised clots related to one or more episodes of acute pulmonary embolism. To date, the pathogenesis of CTEPH remains unexplained. Pulmonary endarterectomy removes obstruction from pulmonary vessels and can cure patients. However, some unreachable distal pulmonary obstruction and/or associated distal pulmonary vasculopathy could induce persistent pulmonary hypertension, the main postoperative complication.**

**The pathophysiology of CTEPH is not fully understood and improving knowledge of this disease could improve our future surgical and medical management. Many attempts, conducted over several decades, have failed to reproduce this chronic disease in animals. However, several animal models have provided insights into the pathophysiology and pathogenesis of CTEPH. Here, we review all the animal models that have improved the comprehension of CTEPH and hold promise for further investigations. This short review analyses strengths and weaknesses of all animal models available to study the pathophysiology of CTEPH.**

**KEYWORDS: Chronic thromboembolic pulmonary hypertension, pulmonary circulation, remodelling, right ventricular function**

Regarding the Dana Point 2009 clinical classification [1], chronic thromboembolic pulmonary hypertension (CTEPH) is a type-4 subtype of pulmonary hypertension (PH), in which pulmonary endarterectomy (PEA) [2] is effective in preventing death by right ventricle (RV) failure. CTEPH is due to obstruction of pulmonary arteries by persistent organised clots formed during one or more episodes of acute pulmonary embolism. The reason for clot persistence is unknown and the pathogenesis of CTEPH remains unexplained. To date, the lack of risk factors predicting the evolution from acute pulmonary embolism to CTEPH does not allow the development of preventive care and/or screening programmes.

The increase in pulmonary vascular resistance is believed to result from a combination of proximal pulmonary artery obstruction and distal pulmonary vasculopathy [3, 4], which have to be quantified before PEA to estimate risks and predict surgical success. However, the mechanisms underlying lesion development in the obstructed and unobstructed peripheral vascular beds remain unknown. Hence, medical management of inoperable CTEPH or operable CTEPH with an important distal vasculopathy remains troublesome because of a lack of efficient therapy.

To elucidate the pathophysiology of CTEPH, considerable effort has been expended in attempting to develop reliable animal models. Acute pulmonary embolism is easily produced in several animal species. In contrast, the induction of a disease replicating all the components of human CTEPH has proved challenging. These

AFFILIATION
Laboratory of Surgical Research and INSERM U999, Hôpital Marie-Lannelongue and Paris-Sud University, Le Plessis Robinson, France.

CORRESPONDENCE
O. Mercier
Dept of Thoracic and Vascular Surgery and Heart–Lung Transplantation
Hôpital Marie-Lannelongue (Paris-Sud University)
133 Avenue de la Résistance
Le Plessis Robinson
France
E-mail: o.mercier@ccml.fr

Received:
July 02 2012
Accepted after revision:
Dec 08 2012
First published online:
Jan 11 2013

**Previous articles in this Series. No. 1:** Delcroix M, Vonk-Noordegraaf A, Fadel E, *et al.* Vascular and right ventricular remodelling in chronic thromboembolic pulmonary hypertension. *Eur Respir J* 2013; 41: 224–232. **No. 2:** Lang IM, Pesavento R, Bonderman D, *et al.* Risk factors and basic mechanisms of chronic thromboembolic pulmonary hypertension: a current understanding. *Eur Respir J* 2013; 41: 462–468. **No. 3:** Jenkins DP, Madani M, Mayer E, *et al.* Surgical treatment of chronic thromboembolic pulmonary hypertension. *Eur Respir J* 2013; 41: 735–742. **No. 4:** Pepke-Zaba J, Jansa P, Kim NH, *et al.* Chronic thromboembolic pulmonary hypertension: role of medical therapy. *Eur Respir J* 2013; 41: 985–990.

European Respiratory Journal
Print ISSN 0903-1936
Online ISSN 1399-3003

components consist of clot persistence and organisation, pulmonary hypertension, chronic pulmonary-artery obstruction by unresolved intraluminal material, the development of a systemic blood supply to ischemic lung regions, pulmonary vasculopathy in unobstructed territories, and RV remodelling. Here, we review all the animal models described in the international literature that have been used to study the pathogenesis and pathophysiology of CTEPH (table 1).

## ANIMAL MODELS OF PERSISTENT INTRAVASCULAR THROMBOSIS

The mechanisms by which the pulmonary emboli or thrombi fail to undergo lysis and instead organise into occluding fibrotic material remain unknown. Hypotheses include predisposing pulmonary endothelial cell abnormalities [42] and impairments in the vascular repair process [43]. A role for *in situ* thrombosis related to endothelial cell dysfunction may explain why up to 63% of patients with CTEPH have no documented history of acute pulmonary embolism [3]. This hypothesis has not been studied in animals, as no endothelial cell abnormalities have been identified to date in humans with CTEPH. In contrast, the process of thrombus organisation has been studied in reliable animal models of low flow-induced inferior vena cava thrombosis (table 2). Most of these animal models were developed in rodents, with the goal of investigating venous thrombus resolution [5–22]. Thrombosis can be induced by venous stasis alone or combined with induced blood hypercoagulability or mechanical endothelial damage. KANG *et al.* [13] studied a piglet model of jugular vein thrombosis after stenosis and mechanical endothelial damage [15]. Inferior vena cava occlusion has been also studied in monkeys [15]. However, mice and rats remain the most widely used animals due their cost-effectiveness and efficiency [7]. Low-flow rodent models are characterised by a laminar thrombus that resolves within 3–4 weeks *via* a process of recanalisation that requires inflammatory cell recruitment and angiogenic signals [5–11, 17–22].

In studies of clinical risk factors, CTEPH was associated neither with the classical plasma-factor abnormalities associated with venous thromboembolism nor with impairments in fibrinolysis. However, having a ventriculo-atrial shunt or history of pacemaker infection increased the risk of CTEPH [44, 45]. This finding prompted studies in animal models. Thus, BONDERMAN *et al.*[23] used a mouse model of low-flow venous thrombosis induced by inferior vena cava stenosis and endothelial damage to study the role for staphylococcal infection in delaying thrombus resolution and promoting the expression of profibrotic molecules.

## DUAL VASCULAR COMPARTMENT THEORY

MOSER *et al.* [4] were the first to describe two compartments in the pulmonary vascular bed of CTEPH patients: an obstructed compartment subjected to chronic ischemia and an unobstructed compartment subjected to increased flow and shear stress (fig. 1). Thus, in addition to the obstruction of large pulmonary arteries, patients with CTEPH have vascular lesions in distal unobstructed territories. These distal lesions are similar to those found in patients with other forms of PH.

Many observations are consistent with a role for both territories in increasing pulmonary-vascular resistance. First, for the same degree of pulmonary artery obstruction as measured by lung scanning, CTEPH is associated with higher pulmonary resistance values compared to acute pulmonary embolism [46]. Secondly, the onset of CTEPH disease is characterised by an asymptomatic honeymoon period during which pulmonary vascular resistance increases gradually without evidence of recurrent embolism. These two vascular

**TABLE 1** Insights into thrombus resolution and organisation learned from stagnant flow induced inferior vena cava thrombus animal models

| Animal models | Species | Strengths | Weaknesses |
|---|---|---|---|
| **IVC ligation or stenosis models (0–28 days) [5–23]** | Rodents, pig, primate | Reproduce thrombus resolution and organisation | Studies on vein (not PA)<br>No PH<br>No additional vasculopathy<br>No pathogenesis |
| **PA ligation models (5 weeks) [24–35]** | Rodents, pig | Reproduce obstructed territories with post-obstructive vasculopathy (bronchial circulation, distal pulmonary vasculopathy) | No PH<br>No RV remodelling and dysfunction<br>No distal vasculopathy in non-obstructed lung |
| **Pulmonary overflow models (aorto-pulmonary shunt, 5-weeks overflow) [36–40]** | Pig | Reproduce non-obstructed territories with distal pulmonary vasculopathy with media thickness | No pathogenesis<br>No post-obstructive vasculopathy<br>No pulmonary obstruction |
| **PA ligation+glue embolisation (5 weeks) [41]** | Pig | Reproduce obstructed and non-obstructed territories<br>Reproduce RV remodelling and dysfunction<br>Reproduce sustained PH | No pathogenesis |

IVC: inferior vena cava; PA: pulmonary artery; PH: pulmonary hypertension; RV: right ventricle.

**TABLE 2** Current animal models used to study pathogenesis and pathobiology of chronic thromboembolic pulmonary hypertension

| | Year | Animals | Models | Hypothesis | Conclusions |
|---|---|---|---|---|---|
| **Animal model of venous thrombosis** | | | | | |
| MILLET [16] | 1996 | Rat | Wessler stasis model (injection of serum+IVC temporary ligation) | Comparison of injection of homologous *versus* heterologous serum to obtain thrombus model | Homologous serum induced the same thrombus than heterologous serum |
| FOWLKES [15] | 1998 | Rat and primate | IVC thrombosis by ligation or balloon occlusion | Thrombus ultrasound echogenicity could predict the age of clots in two different animal species | Thrombus echogenicity could predict the age of the thrombus in rat and baboon and was correlated to the intrathrombus fibrin concentration |
| KANG [13] | 2003 | Pig | De-endothelialisation with 50% or 80% stenosis of jugular vein, replacement of a venous segment by a Gore-Tex vascular prosthesis, ±thromboplastin infusion | Description of six new animal models of venous thrombosis mimicking human disease | |
| XIE [21] | 2004 | Rat | IVC thrombosis by ligation | Thrombus ultrasound elasticity could predict clots age | Spontaneous resolution of IVC thrombus induced progressive hardening with age that could be determined by duplex ultrasound |
| **Inflammation and thrombus resolution** | | | | | |
| WAKEFIELD [22] | 1995 | rat | IVC thrombosis by ligation | Inflammation into the vein wall during thrombosis, extravasation of neutrophil and monocyte/macrophage: role of cytokines | Neutrophil attractants (ENA-78, TNF-α, IL-6, MCP-1) and monocyte/macrophage attractants (MIP-1α, ICAM-1, TNF-α) increased during thrombus formation |
| WAKEFIELD [17] | 1999 | Rat | IVC thrombosis by ligation | Role of IL-8 in thrombus neovascularisation | IL8 augmented thrombus neovascularisation |
| LONDY [14] | 1999 | Rat | IVC thrombosis by ligation | Role of IL-10 in reducing perivenous inflammation quantified by MRI | MRI could quantify perivenous inflammation which is modified by IL-10 treatment |
| HENKE [20] | 2004 | Mice and CXCR2-/- mice | IVC thrombosis by ligation | Inflammation and thrombus resolution: role of neutrophil and its receptor CXCR2 | Thrombus resolution involved CXCR2 pathway |
| VARMA [19] | 2003 | Rat | IVC thrombosis by ligation | Role of neutrophil in thrombus resolution | Neutropenia altered fibrinolytic activity slowing down thrombus resolution and increased intrathrombus collagen |
| HENKE [18] | 2001 | Rat | IVC thrombosis by ligation | Role of IL-8 (pro-inflammatory and pro-angiogenic cytokine) in thrombus resolution | IL-8 enhanced thrombus resolution and increased fibrosis by improving thrombus neovascularisation and increasing neutrophil |
| SINGH [12] | 2003 | Mice, tPA-/- mice, and uPA-/- mice | IVC thrombosis by ligation and endothelial damage | Role of uPA and tPA in thrombus resolution | Unlike tPA, uPA induced monocytes recruitment into the thrombus and enhanced thrombus resolution |
| HUMPHRIES [11] | 1999 | Rat | IVC thrombosis by 80–90% external stenosis | Role of MCP-1 in thrombus resolution | Injection of MCP-1 into the thrombus enhanced its organisation and resolution |
| MCGUINNESS [9] | 2001 | Rat | IVC thrombosis by 80–90% external stenosis | Role of the monocytes in thrombus resolution | Monocytes migrated into the thrombus during resolution |
| BONDERMAN [23] | 2008 | Mice | IVC thrombosis by 80–90% external stenosis ±*S. aureus* injection | Role of *S. aureus* infection in misguiding thrombus resolution | *S. aureus* thrombus infection enhanced fibrotic vascular remodelling after thrombosis misguiding natural resolution |
| **Angiogenesis and thrombus resolution and organisation** | | | | | |
| WALTHAM [10] | 2000 | Rat | IVC thrombosis by 80–90% external stenosis | Role of VEGF and bFGF in thrombus resolution | VEGF and bFGF had temporal expression patterns during thrombus resolution and organisation |
| WALTHAM [8] | 2003 | Rat | IVC thrombosis by 80–90% external stenosis | Role of VEGF in thrombus resolution | Injection of VEGF in to the thrombus enhanced its resolution and organisation |
| WALTHAM [6] | 2005 | Rat | IVC thrombosis by 80–90% external stenosis | Effect of VEGF gene transfection on thrombus resolution | Human VEGF gene plasmid transfection into the thrombus enhanced resolution and recanalisation |
| MODARAI [7] | 2008 | Rat and mice | IVC thrombosis by 80–90% external stenosis | Effect of adenovirus-mediated VEGF gene therapy on thrombus resolution | Injection of adenovirus-mediated VEGF gene therapy enhanced thrombus resolution and recanalisation with macrophage recruitment |

IVC: inferior vena cava; ENA: extractable nuclear antigen; TNF-α: tumour necrosis factor-α; IL: interleukin, MCP: monocyte-chemoattractant protein; MIP: macrophage inflammatory protein; ICAM: intercellular adhesion molecular; MRI: magnetic resonance imaging; tPA: tissue plasminogen activator; uPA: urokinase plasminogen activator; VEGF: vascular endothelial growth factor; bFGF: basic fibroblast growth factor; *S. aureus*: *Staphylococcus aureus*.

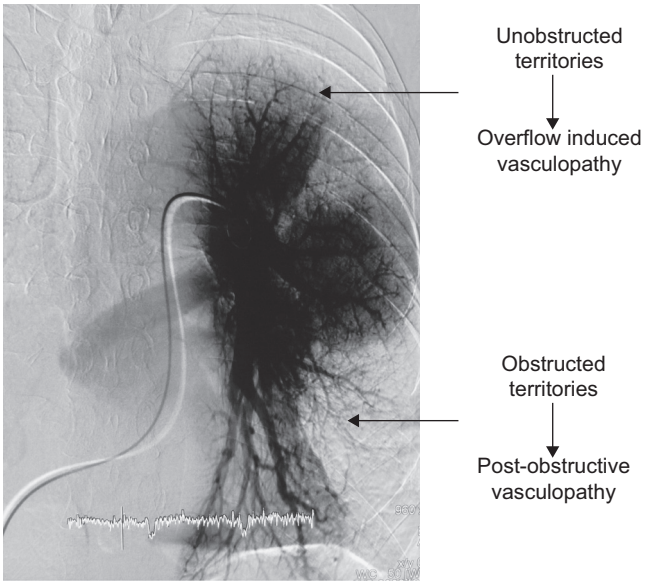


**FIGURE 1.** Anteroposterior pulmonary angiogram showing the two vascular territories in a patient with chronic thromboembolic pulmonary hypertension. The obstructed territory is subjected to chronic ischaemia and the unobstructed territory to an increase in blood flow.

compartments have been reproduced in animals separately and, more recently, simultaneously in a piglet model.

## MODELS REPLICATING PULMONARY ARTERY OBSTRUCTION

The first attempt to replicate chronic pulmonary artery obstruction in an animal model was conducted in piglets by FADEL *et al.* [24] who used proximal embolisation of coils and tissue adhesive into the left pulmonary artery. Chronic obstruction (for 5 weeks) of the left pulmonary artery resulted in chronic lung ischemia without PH. The result was an increase in the systemic blood supply to the lung *via* bronchial, mediastinal, and intercostal arteries, as well as distal post-obstructive pulmonary vasculopathy. These lesions were similar to those seen after chronic pulmonary artery ligation [25].

The left pulmonary artery was chosen for ligation, because it could be easily re-implanted into the main pulmonary artery to replicate reperfusion injuries after PEA. The systemic vascular response to chronic-pulmonary vascular obstruction differs across species, with proliferation of bronchial arteries into the intraparenchymal airways in large animals (dogs and piglets) and rats or of intercostal arteries into the pleural space in mice [26]. Although CTEPH studies have been conducted chiefly in piglets, a few studies of bronchial circulation have been performed in a rat model of pulmonary artery ligation [27]. Occlusion of one of the main pulmonary arteries stimulates angiogenesis in the bronchial vessels of the ipsilateral lung [28]. Bronchial arteries begin to enlarge as soon as 2–3 days after pulmonary artery ligation and supply the pulmonary circulation *via* precapillary anastomoses. These anastomoses may maintain airway epithelium oxygenation, thus explaining the lesser degree of reperfusion injury after chronic lung ischemia than after acute lung ischemia [29].

In addition to bronchial circulation hypertrophy, post-obstructive vasculopathy is characterised by pulmonary artery abnormalities including increased media thickness [30], impaired vasoreactivity [31], impaired endothelial nitric-oxide-synthase function [30], and increased reactivity to endothelin (ET)-1 [32] leading to increased resistance after reperfusion [33].

Lung reperfusion after chronic ischemia was followed by gradual reversal of the post-obstructive vasculopathy [25, 34], after an early phase of ischemia-reperfusion injury with endothelial cell damage [35].

Closure of the aorto-pulmonary shunt replicates the hemodynamic conditions in unobstructed territories after PEA. Shunt closure induced normalisation of ET-1 and ETA expression, followed by media hypertrophy reversal in the distal pulmonary arteries. These findings were consistent with the gradual improvement in pulmonary vascular resistance observed 3–6 months after PEA.

## MODELS REPLICATING LESIONS OF UNOBSTRUCTED PULMONARY ARTERIES

During the early phase of CTEPH, the unobstructed territories are subjected to a chronic blood flow increase due to cardiac output redistribution. Therefore, systemic-to-pulmonary shunts have been used in animals to replicate the lesions seen in unobstructed territories in patients with CETPH [36]. In an animal model initially developed by RENDAS *et al.* [37] to replicate congenital heart disease, an aorto-pulmonary shunt is induced by implanting a short prosthesis between the ascending aorta and main pulmonary artery. This model has been used to induce high-flow pulmonary vascular lesions similar to those seen in unobstructed territories in CTEPH (fig. 2). As with the left pulmonary artery ligation models, the aorto-pulmonary shunt was achieved through a median sternotomy to avoid pleural opening and a subsequent inflammatory response. High-flow pulmonary vasculopathy induced by 5 weeks of aorto-pulmonary shunting was characterised by increased media thickness of the distal pulmonary arteries (fig. 3) related to smooth muscle cell proliferation (as shown by proliferating cell nuclear antigen labelling) and by elevated levels of ET-1 and its receptor endothelin receptor A (ETA) in lung tissue. ET-1 is a potent vasoconstrictor and mitotic peptide for vascular smooth muscle cells. ET-1 overexpression has been found in other animal models of high pulmonary flow [38–40]. ET-1 overproduction is probably a response to stimuli such as shear stress resulting from arterial pressure elevation [47].

## ANIMAL MODEL REPLICATING ALL FEATURES OF CTEPH

Although the animal models described earlier provided useful information on impaired thrombus resolution and on the pathophysiology of lesions in obstructed and unobstructed territories, they failed to replicate important features of human CTEPH including pulmonary hypertension, interactions between the two pulmonary vascular compartments and, above all, RV remodelling and dysfunction.

Since the 1990s, several attempts to develop animal models of CTEPH [48–52] failed because of clot lysis by the very efficient endogenous fibrinolytic system [53] and of the remarkable

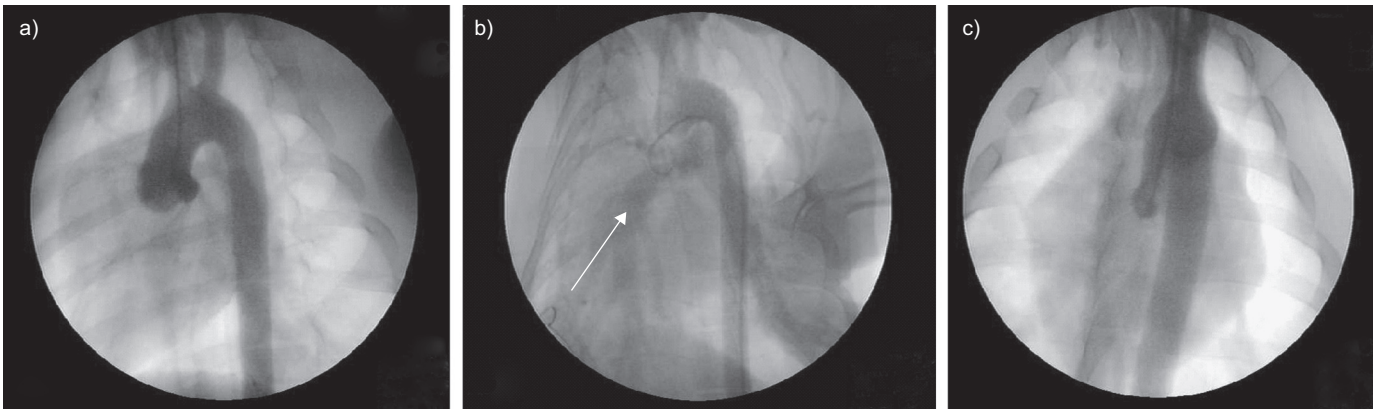


**FIGURE 2.** Aortography showing in a sham-group piglet. a) The aortic arch with the two supra-aortic arteries. b) Visualisation of the pulmonary vascular bed (white arrow) at the same time as the aortic arch after creation of an aorto-pulmonary shunt. c) No opacification of the pulmonary vascular bed after shunt closure.

adaptive capabilities of the pulmonary circulation. Thus, 3 h after acute pulmonary embolism in dogs [49], only 30% of the initial injected thrombus volume remained inside the pulmonary artery. Adding tranexamic acid [49] or plasminogen activator inhibitor-1 [50] to delay thrombus resorption between injections failed to solve this problem. The second difficulty was the large pulmonary circulation reserve, which required obstruction of more than half the pulmonary vasculature to achieve an increase in pulmonary vascular resistance. The adaptive capabilities of the pulmonary vasculature explain why repeated injections of small, inert, non-absorbable materials failed to replicate CTEPH. Thus, in a dog model of chronic pulmonary-artery injections of ceramic beads, 3 mm in diameter, pulmonary pressures and resistances returned to normal within 1 week after each injection [51]. With 100–300 μm microspheres, 60 days of repeated embolisation were required to increase the pulmonary-artery pressure [52]. After 60 days, signs of PH started to develop, but there was no bronchial artery hypertrophy, post-obstructive or high-flow vasculopathy, or proximal vascular obstruction [52]. A third challenge lies in replicating RV remodelling. Extensive acute obstruction of the pulmonary arterial tree is usually lethal by heart failure in the absence of previous RV training and hypertrophy. To tolerate a persistent increase in pulmonary arterial pressure, the RV must undergo remodelling, which consists in gradual ventricular wall hypertrophy followed by right-heart-chamber enlargement with a paradoxical septal motion. In the event of persistent pulmonary hypertension, RV failure develops.

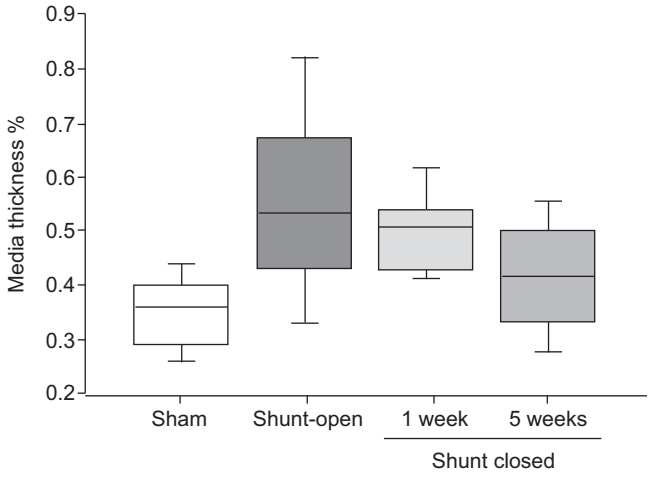


**FIGURE 3.** Box plot of media thickness as a percentage of small pulmonary arteries (<150 μm) calculated in: the sham (35.9±0.8%), shunt-open (55.6±1.2%), 1-week shunt-closed (48.7±1%), and 5-weeks shunt-closed (40.9±1%) groups [36]. Media thickness percentage was calculated as follows: external diameter minus internal diameter and divided by the external diameter. Aorto-pulmonary shunting was created by a graft interposition between the ascending aorta and the pulmonary trunk in piglets. Closure of the shunt was obtained by dividing the graft.

We recently developed a CTEPH piglet model [41], consisting of a primary left pulmonary artery ligation *via* a sternotomy that was followed by weekly transcatheter embolisations, under fluoroscopic control, of the tissue adhesive enbucrilate (Histoacryl) into the right lower lobe for 5 weeks. Pulmonary artery ligation overwhelmed the pulmonary circulation reserve and led to PH within a few weeks, with progressive obstruction of the remaining lung vasculature. The progressive nature of the obstruction achieved *via* weekly embolisation allowed the RV to adapt to the pressure increase, thus preventing death by acute RV failure. The tissue adhesive enbucrilate solidifies immediately after contact with blood and adheres to the arterial wall. The result was proximal obstruction by unresolved material in the right lower-lobe artery. Thus, the right upper-lobe arteries remained patent and exhibited lesions replicating those seen in unobstructed territories in CTEPH. After 5 weeks, this piglet model replicated all the features of human CTEPH: increased pulmonary vascular resistance, increased mean pulmonary artery pressure, increased media thickness of distal pulmonary arteries in both obstructed and unobstructed territories, increased systemic blood supply through the bronchial arteries in the obstructed territories, RV hypertrophy, RV enlargement, and paradoxical septal motion (fig. 4). Interestingly, although the embolisations were stopped after 5 weeks, the increase in pulmonary vascular resistance persisted for up to 1 month later after the last embolisation (unpublished data). Overexpression of ET-1 and its receptors ETA and ETB was documented in the remodelled distal arteries of the unobstructed territories, in keeping with previous findings in

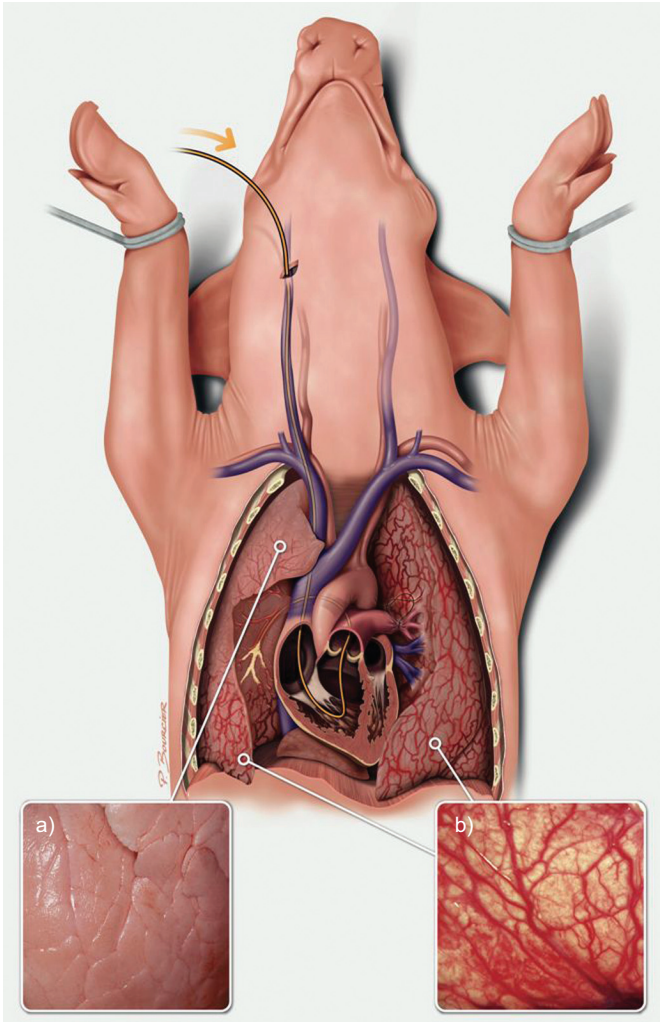


**FIGURE 4.** Chronic thromboembolic pulmonary hypertension piglet model consisting of left pulmonary artery ligation and repeated embolisation in the right lower lobe: bronchial artery hypertrophy in the ischaemic lung territory (b), apparently normal right upper lobe (a), unresolved intravascular material made of tissue adhesive and fibrin moulding the arterial tree, and right ventricle remodelling.

piglets with high-flow vasculopathy [36, 38]. In addition, ETA overexpression in the obstructed territories was consistent with the results of previous studies of post-obstructive vasculopathy [33]. Further experiments are needed to monitor pulmonary resistance at a distance from the last pulmonary embolisation, to investigate RV remodelling and failure, and to assess interactions between the obstructed and unobstructed territories. However, one should bear in mind that this model does not replicate the impaired thrombus resolution seen in human CTEPH.

## CONCLUSIONS

Animal models developed over several decades have provided valuable information on the pathophysiology of CTEPH. Thrombus resolution and obstructed and unobstructed territories have been studied separately in several animal models. Recently, a model replicating all the major aspects of human CTEPH was developed. This model should prove useful for investigating RV dysfunction and distal lung vessel abnormalities. However, additional models are still needed to elucidate the pathobiology of thrombus persistence.

## STATEMENT OF INTEREST

None declared.

## REFERENCES

1 Simonneau G, Robbins IM, Beghetti M, *et al.* Updated clinical classification of pulmonary hypertension. *J Am Coll Cardiol* 2009; 54: Suppl. 1, S43–S54.
2 Dartevelle P, Fadel E, Mussot S, *et al.* Chronic thromboembolic pulmonary hypertension. *Eur Respir J* 2004; 23: 637–648.
3 Hoeper MM, Mayer E, Simonneau G, *et al.* Chronic thromboembolic pulmonary hypertension. *Circulation* 2006; 113: 2011–2020.
4 Moser KM, Bloor CM. Pulmonary vascular lesions occurring in patients with chronic major vessel thromboembolic pulmonary hypertension. *Chest* 1993; 103: 685–692.
5 Modarai B, Humphries J, Burnand KG, *et al.* Adenovirus-mediated VEGF gene therapy enhances venous thrombus recanalization and resolution. *Arterioscler Thromb Vasc Biol* 2008; 28: 1753–1759.
6 Waltham M, Burnand K, Fenske C, *et al.* Vascular endothelial growth factor naked DNA gene transfer enhances thrombus recanalization and resolution. *J Vasc Surg* 2005; 42: 1183–1189.
7 Modarai B, Burnand KG, Humphries J, *et al.* The role of neovascularisation in the resolution of venous thrombus. *Thromb Haemost* 2005; 93: 801–809.
8 Waltham M, Burnand KG, Collins M, *et al.* Vascular endothelial growth factor enhances venous thrombus recanalisation and organisation. *Thromb Haemost* 2003; 89: 169–176.
9 McGuinness CL, Humphries J, Waltham M, *et al.* Recruitment of labelled monocytes by experimental venous thrombi. *Thromb Haemost* 2001; 85: 1018–1024.
10 Waltham M, Burnand KG, Collins M, *et al.* Vascular endothelial growth factor and basic fibroblast growth factor are found in resolving venous thrombi. *J Vasc Surg* 2000; 32: 988–996.
11 Humphries J, McGuinness CL, Smith A, *et al.* Monocyte chemotactic protein-1 (MCP-1) accelerates the organization and resolution of venous thrombi. *J Vasc Surg* 1999; 30: 894–899.
12 Singh I, Burnand KG, Collins M, *et al.* Failure of thrombus to resolve in urokinase-type plasminogen activator gene-knockout mice: rescue by normal bone marrow-derived cells. *Circulation* 2003; 107: 869–875.
13 Kang C, Bonneau M, Brouland JP, *et al. In vivo* pig models of venous thrombosis mimicking human disease. *Thromb Haemost* 2003; 89: 256–263.
14 Londy FJ, Kadell AM, Wrobleski SK, *et al.* Detection of perivenous inflammation in a rat model of venous thrombosis using MRV. *J Invest Surg* 1999; 12: 151–156.
15 Fowlkes JB, Strieter RM, Downing LJ, *et al.* Ultrasound echogenicity in experimental venous thrombosis. *Ultrasound Med Biol* 1998; 24: 1175–1182.
16 Millet J, Vaillot M, Theveniaux J, *et al.* Experimental venous thrombosis induced by homologous serum in the rat. *Thromb Res* 1996; 81: 497–502.
17 Wakefield TW, Linn MJ, Henke PK, *et al.* Neovascularization during venous thrombosis organization: a preliminary study. *J Vasc Surg* 1999; 30: 885–892.
18 Henke PK, Wakefield TW, Kadell AM, *et al.* Interleukin-8 administration enhances venous thrombosis resolution in a rat model. *J Surg Res* 2001; 99: 84–91.
19 Varma MR, Varga AJ, Knipp BS, *et al.* Neutropenia impairs venous thrombosis resolution in the rat. *J Vasc Surg* 2003; 38: 1090–1098.
20 Henke PK, Varga A, De S, *et al.* Deep vein thrombosis resolution is modulated by monocyte CXCR2-mediated activity in a mouse model. *Arterioscler Thromb Vasc Biol* 2004; 24: 1130–1137.

21 Xie H, Kim K, Aglyamov SR, *et al.* Staging deep venous thrombosis using ultrasound elasticity imaging: animal model. *Ultrasound Med Biol* 2004; 30: 1385–1396.
22 Wakefield TW, Strieter RM, Wilke CA, *et al.* Venous thrombosis-associated inflammation and attenuation with neutralizing antibodies to cytokines and adhesion molecules. *Arterioscler Thromb Vasc Biol* 1995; 15: 258–268.
23 Bonderman D, Jakowitsch J, Redwan B, *et al.* Role for staphylococci in misguided thrombus resolution of chronic thromboembolic pulmonary hypertension. *Arterioscler Thromb Vasc Biol* 2008; 28: 678–684.
24 Fadel E, Riou JY, Mazmanian M, *et al.* Pulmonary thromboendarterectomy for chronic thromboembolic obstruction of the pulmonary artery in piglets. *J Thorac Cardiovasc Surg* 1999; 117: 787–793.
25 Fadel E, Michel RP, Eddahibi S, *et al.* Regression of postobstructive vasculopathy after revascularization of chronically obstructed pulmonary artery. *J Thorac Cardiovasc Surg* 2004; 127: 1009–1017.
26 Mitzner W, Wagner EM. Vascular remodeling in the circulations of the lung. *J Appl Physiol* 2004; 97: 1999–2004.
27 Wagner EM, Jenkins J, Perino MG, *et al.* Lung and vascular function during chronic severe pulmonary ischemia. *J Appl Physiol* 2011; 110: 538–544.
28 Charan NB, Carvalho P. Angiogenesis in bronchial circulatory system after unilateral pulmonary artery obstruction. *J Appl Physiol* 1997; 82: 284–291.
29 Fadel E, Mazmanian GM, Chapelier A, *et al.* Lung reperfusion injury after chronic or acute unilateral pulmonary artery occlusion. *Am J Respir Crit Care Med* 1998; 157: 1294–1300.
30 Fadel E, Mazmanian GM, Baudet B, *et al.* Endothelial nitric oxide synthase function in pig lung after chronic pulmonary artery obstruction. *Am J Respir Crit Care Med* 2000; 162: 1429–1434.
31 Shi W, Hu F, Kassouf W, *et al.* Altered reactivity of pulmonary vessels in postobstructive pulmonary vasculopathy. *J Appl Physiol* 2000; 88: 17–25.
32 Shi W, Giaid A, Hu F, *et al.* Increased reactivity to endothelin of pulmonary arteries in long-term post-obstructive pulmonary vasculopathy in rats. *Pulm Pharmacol Ther* 1998; 11: 189–196.
33 Michel RP, Hakim TS. Increased resistance in postobstructive pulmonary vasculopathy: structure-function relationships. *J Appl Physiol* 1991; 71: 601–610.
34 Fadel E, Wijtenburg E, Michel R, *et al.* Regression of the systemic vasculature to the lung after removal of pulmonary artery obstruction. *Am J Respir Crit Care Med* 2006; 173: 345–349.
35 Sage E, Mercier O, Van den Eyden F, *et al.* Endothelial cell apoptosis in chronically obstructed and reperfused pulmonary artery. *Respir Res* 2008; 9: 19.
36 Mercier O, Sage E, de Perrot M, *et al.* Regression of flow-induced pulmonary arterial vasculopathy after flow correction in piglets. *J Thorac Cardiovasc Surg* 2009; 137: 1538–1546.
37 Rendas A, Lennox S, Reid L. Aorta-pulmonary shunts in growing pigs. Functional and structural assessment of the changes in the pulmonary circulation. *J Thorac Cardiovasc Surg* 1979; 77: 109–18.
38 Rondelet B, Kerbaul F, Motte S, *et al.* Bosentan for the prevention of overcirculation-induced experimental pulmonary arterial hypertension. *Circulation* 2003; 107: 1329–1335.
39 Reddy VM, Wong J, Liddicoat JR, *et al.* Altered endothelium-dependent responses in lambs with pulmonary hypertension and increased pulmonary blood flow. *Am J Physiol* 1996; 271: H562–H570.
40 Ovadia B, Reinhartz O, Fitzgerald R, *et al.* Alterations in ET-1, not nitric oxide, in 1-week-old lambs with increased pulmonary blood flow. *Am J Physiol Heart Circ Physiol* 2003; 284: H480–H490.
41 Mercier O, Tivane A, Raoux F, *et al.* A reliable piglet model of chronic thrombo-embolic pulmonary hypertension. *Am J Respir Crit Care Med* 2011; 183: A2415.
42 Egermayer P, Peacock AJ. Is pulmonary embolism a common cause of chronic pulmonary hypertension? Limitations of the embolic hypothesis. *Eur Respir J* 2000; 15: 440–448.
43 Lang I. Advances in understanding the pathogenesis of chronic thromboembolic pulmonary hypertension. *Br J Haematol* 2010; 149: 478–483.
44 Bonderman D, Wilkens H, Wakounig S, *et al.* Risk factors for chronic thromboembolic pulmonary hypertension. *Eur Respir J* 2009; 33: 325–331.
45 Bonderman D, Jakowitsch J, Adlbrecht C, *et al.* Medical conditions increasing the risk of chronic thromboembolic pulmonary hypertension. *Thromb Haemost* 2005; 93: 512–516.
46 Azarian R, Wartski M, Collignon MA, *et al.* Lung perfusion scans and hemodynamics in acute and chronic pulmonary embolism. *J Nucl Med* 1997; 38: 980–983.
47 Galié N, Manes A, Branzi A. The endothelin system in pulmonary arterial hypertension. *Cardiovasc Res* 2004; 61: 227–237.
48 Moser KM, Guisan M, Bartimmo EE, *et al. In vivo* and *post mortem* dissolution rates of pulmonary emboli and venous thrombi in the dog. *Circulation* 1973; 48: 170–178.
49 Moser KM, Cantor JP, Olman M, *et al.* Chronic pulmonary thromboembolism in dogs treated with tranexamic acid. *Circulation* 1991; 83: 1371–1379.
50 Marsh JJ, Konopka RG, Lang IM, *et al.* Suppression of thrombolysis in a canine model of pulmonary embolism. *Circulation* 1994; 90: 3091–3097.
51 Kim H, Yung GL, Marsh JJ, *et al.* Endothelin mediates pulmonary vascular remodelling in a canine model of chronic embolic pulmonary hypertension. *Eur Respir J* 2000; 15: 640–648.
52 Sato H, Hall CM, Griffith GW, *et al.* Large animal model of chronic pulmonary hypertension. *ASAIO J* 2008; 54: 396–400.
53 Lang IM, Marsh JJ, Konopka RG, *et al.* Factors contributing to increased vascular fibrinolytic activity in mongrel dogs. *Circulation* 1993; 87: 1990–2000.