Online supplement: Material and Methods

Histology of human samples

Lung samples from all patients were retrospectively analyzed by conventional light microscopy. Lung tissue was systematically sampled after lung transplantation (n = 16) or during autopsy (n = 1). For each case, 10 HE or HES-stained slides from peripheral lung areas were randomly chosen and analyzed; in addition, all available slides from central lung areas (3 to 8) were submitted to microscopy (table 2 and 3). Analysis corresponded to a semi-quantitative score-based evaluation using a prospectively designed evaluation sheet with several histologic items. Scores were defined as: 0 = none or exceptional, 1 = moderate, 2 = numerous, 3 = abundant. After completing the evaluation, most relevant items were retained (at least ‘moderate’ item-presence on at least 1 slide in at least 3 cases). Scores of each item for all slides of one case were summed and divided by the number of analyzed slides. The resulting item-scores were summed (all cases) and the overall item-score was not categorized, but displayed as an absolute value. Since assessment of bronchial artery hypertrophy (BAH) was only possible on few, central lung samples, just one global score was displayed reflecting the prevailing primary semi-quantitative evaluation (scores 0-3, roman numbers).

Ink injection procedure of one human CTEPH case and of the porcine CTEPH model

Lungs of 1 human CTEPH case, 2 CTEPH animals and 1 sham animal underwent an *ex vivo* ink-injection procedure in order to trace the origin of remodeled microvessels and

to reveal possible shunting between systemic and pulmonary vasculature. The right lung from 1 patient of the inPEA group (patient DA) was injected during transplantation: first, one bronchial artery was identified and explored with a 0.7 mm thick Jelco® intravenous catheter (Smiths Medical International, Lancashire, UK) at the lung hilus. After catheterization, a 2 ml syringe filled with blue ink was manually injected into the vessel which eventually was clamped. Then, the right pulmonary vein was injected directly at hilus level with a syringe containing 5 ml of green ink and clamped hereafter. The lung was then formalin-fixed through airway injection. Dissection was performed after 24 hours of fixation. Of note, for animals the blue ink was injected from the dissected aorta into the ostia of bronchial arteries. Injection of green ink into pulmonary veins and fixation was similar to the procedure in the human case.

Porcine CTEPH model

We studied 10 Large White piglets weighing 21±3 kg. The study complied with the Principles of Laboratory Animal Care developed by the National Society for Medical Research and was approved by our local ethics committee on animal experiments. The piglets were randomly allocated to two groups of 5 animals each. Post-embolic pulmonary hypertension was induced during five weeks in the experimental CTEPH group as previously described, sham-operations were performed in the control group [1, 2]. All procedures were performed under general anesthesia.

Briefly, experimental CTEPH was induced by ligation of the left pulmonary artery (PA) through a midline sternotomy, followed by weekly embolization of enbucrilate tissue adhesive (Histoacryl®; B. Braun, Melsungen, Germany) into the right lower lobe arteries

for 5 weeks [10]. Sham piglets underwent left PA dissection without ligation through a median sternotomy followed by weekly saline solution injections for 5 weeks. Pulmonary hemodynamic variables were measured before and after each experiment. right heart catheterization including measurements of CO and TPR were performed using the Seldinger technique with an 8F sheath inserted via the jugular vein. The right heart catheter was a 7F, two-lumen, thermodilution pressure-measuring tipped catheter (Edwards Lifesciences, Irvine, CA, USA). Sham-operations were performed in the control group. All animals were studied 10 weeks after the first procedure. Before animal sacrifice, right heart catheterization including measurements of CO and TPR were performed.

Histology / morphometry of porcine samples

All animals were studied 10 weeks after the first procedure. After sacrifice, lungs of all animals were collected, submitted to gross dissection and histology (HES-staining), and eventually analyzed by light-microscopy. In order to confine the morphometric analysis to CTEPH-mimicking proximal (= complete), distal (= partial) post-obstructive pulmonary vasculopathy, as well as to non-obstructed areas of compensatory overflow, the left lung, the right lower lobe and the right upper lobe were defined and differentiated as histological areas of interest. Results were compared to the left lung of sham operated controls. Morphometric measurements were performed on 1 slide per area of interest from every CTEPH animal and on 1 slide of the left lung from all sham animals. Measurements were done with the Nikon NIS elements software (Nikon, France). Three items of interest, evolving from the human CTEPH histology analysis, were chosen:

degree of bronchial arterial remodeling, degree of pulmonary vein remodeling and degree of microvessel remodeling (arterioles / venules). Bronchial arterial remodeling was assessed by measuring the surface of bronchial arteries (including arterial wall and lumen) and relating it to the surface of the associated bronchus (in bronchi with a diameter between 3000 and 4000 µm, including the bronchial wall with cartilage and comprising the lumen). Pulmonary vein remodeling was assessed by measuring the surface of all morphologically remodeled veins (including walls and lumen) within all lobular septa of one slide and relating it to the surface of the whole lung section of the same slide. Microvessel remodeling was assessed by counting all muscularized microvessels (< 50 µm in diameter) of 10 consecutive microscopic fields with magnification x 100 (objective 10), starting from the first encountered muscularized microvessel on.

References

1. Mercier O, Fadel E. Chronic thromboembolic pulmonary hypertension: animal models. *Eur Respir J* 2013.

2. Mercier O, Dorfmuller P, de Perrot M, Raoux F, Decantes B, Eddahibi S, Dartevelle P, Fadel E. Piglet model of chronic pulmonary hypertension. 2013: Pulm Circ 2013;3(4) :908-915 DOI: 10.1086/674757: 908-915.