ONLINE SUPPLEMENT

Detailed methods of morphometry

Morphometric item "patent lumen fraction of pulmonary arteries": The measurements of patent (unobliterated) lumen fraction were assessed for each encountered and transversally oriented distal muscular-type artery (within the limits of a cross-sectional diameter between 70 and 300 µm, measured from one external elastic lamina to its opposite counterpart) on seven slides of the lung periphery. The surfaces of the whole artery and of the patent lumen area were measured with NIS-Br Nikon morphometry software and expressed in square-micrometers. Eventually, the ratio was calculated as follows: (patent lumen surface) / (whole artery surface) X 100, the patent lumen being delimited by the endothelial cell layer, and the whole artery being delimited by the external elastic lamina.

Morphometric item: "plexiform lesion density": Plexiform lesions density was obtained by counting plexiform lesions on seven slides of the lung periphery and by calculating the ratio with the analyzed lung surface (expressed in cm^2).

Morphometric item "vascular inflammation": The inflammation score was calculated by applying the method previously described by Stacher and co-workers to assess vascular inflammation in PAH transplanted lung (1). The inflammatory score resulted of [0xn vessels score 0 + 1xn vessels score 1 + 2xn vessels score 2 + 3xn vessels score 3]/number of analyzed vessels. This arithmetical analysis was carried out on seven slides of the lung periphery.

Morphometric item "vein remodeling": To identify muscular hyperplasia of pulmonary venous walls we first measured the "normal" muscular thickness in lungs from 7 control patients (on actin-stained slides): 20 cross-sectional septal veins per

case (septal veins size varied between 30 and 150 µm in diameter) were assessed with the following formula: (mean of thickest and thinnest muscular diameter) / (mean of major and minor cross-sectional diameter) x 100. We then calculated the mean value, which we considered the “baseline” muscular thickness of unremodelled veins, in our case this value was 9 per cent. Eventually, we compared venous muscular thickness in controls with PAH lungs (on actin-stained slides). Septal veins were considered to show muscular remodelling / smooth muscle cell hyperplasia, when displaying at least the twofold value of the baseline value. 7 slides per patient were used to retrieve 3 well-oriented, vein-rich interlobular septa, starting from the visceral pleura. We then determined the density of septal veins displaying smooth muscle cell hyperplasia (2 times the baseline value or more), by counting remodeled veins in these three interlobular septa for each case: the result was expressed as n remodeled veins per septum (mean of 3 septa).

Morphometric item “bronchial artery wall thickening and dilatation”: Bronchial arteries are found within the bronchial wall in contrast to pulmonary artery branches. To determine bronchial artery remodeling we measured the bronchial artery surface (including the arterial wall and the lumen and hence taking into account muscular hypertrophy and/or dilatation) in two transversally oriented bronchi on 2 central lung slides, when available. The size of the bronchus varied between 4 to 5 mm in diameter. NIS-Br Nikon morphometry software was used to measure the bronchial artery surface in square-µm and the ratio between bronchial artery surface and bronchial surface was eventually calculated.

Morphometric item “bronchial microvascularization”: We also studied the bronchial microvascularization on 1 central slide, when available, with 2 transversally sectioned bronchi. We therefore used an antibody directed against CD31 highlighting

endothelial cells. The staining was quantified on three fields at 10x magnification in two bronchial sections using NIS-Br Nikon software. The three fields were chosen as follows: for all cases, we first selected a field with maximal CD31-staining; eventually we added the adjacent fields to each side to the analysis, as it is usually performed for mitotic counts in malignant disease.

References

1. Stacher E, Graham BB, Hunt JM, Gandjeva A, Groshong SD, McLaughlin VV, et al. Modern age pathology of pulmonary arterial hypertension. Am J Respir Crit Care Med. 2012;186(3):261-72.