

**Quantitative CT assessment of bronchial and vascular alterations in severe precapillary pulmonary hypertension**

**Supplemental methods**

*Study population*

This study was observational, retrospective and conducted according to the principles of the Declaration of Helsinki, French law and approved by our institutional review board. Every patient was informed and agreed that their data can be used for further research studies anonymously. From a prior study <sup>1</sup>, 20 COPD patients were also included in the current study (out of 24 patients) and none in the 2 other groups of severe PH. However, these 20 patients were completely re-analysed by another radiologist. Data were anonymized and they complied with the requirements of the “commission nationale informatique et libertés” (CNIL), that approved the methods, this organisation is responsible for ensuring the ethical use of data collected for scientific purposes in France (approval number: 1909530 v 0). Severe PH patients were consecutively referred between January 2008 and January 2017 to our institution, a tertiary medical center for complete examination of PH, before initiation of any treatment. Each patient underwent within 1 week: medical questioning, physical examination, arterial blood gases, 6-minutes walk tests (6-MWT), blood tests (C-reactive protein (CRP), brain natriuretic peptide (BNP), antinuclear antibodies and HIV serology), trans-thoracic echocardiography, ventilation/perfusion scintigraphy (V/Q scan), pulmonary function testing (PFT), right heart catheterisation (RHC), and unenhanced computed tomography (CT) within a minimal period of 1 month of disease stability. Final statement about disease aetiology, for every patient, was

made by agreement between experienced cardiologists, pneumologists and radiologists, after careful review of all information available.

Only patients with no treatment undertaken, fully screened and with mPAP value equal to or higher than 35 mmHg<sup>2</sup> were included in the study. In addition, we only evaluated pre-capillary PH from chronic obstructive pulmonary disease (COPD), idiopathic pulmonary arterial hypertension (iPAH) and chronic thromboembolic pulmonary hypertension (CTEPH), in adults with a pulmonary capillary wedge pressure (PCWP) values less than 15 mmHg and with no other condition to explain PH in each group.

From the 189 consecutive screened patients, 103 patients had both mPAP value equal or higher than 35 mmHg at RHC<sup>2</sup> and a diagnosis of COPD, iPAH and CTEPH. However, 16 patients were not fully screened and 19 patients could not be included because of related associated diseases. Thus, from the remaining 68 patients included, we focused on 56 adult patients with severe precapillary PH from either COPD, iPAH, or CTEPH, without any other condition to explain PH and with an adequate CT without any other abnormality possibly altering CT measurements. The 19 non-included patients presented concomitant: left heart disease as defined by history of coronaropathy and a left heart function on echocardiography less than 40% (n = 12), sleep apnea syndrome (n = 6), or sarcoidosis (n = 1). No patient appeared to have other concomitant cause of PH, all were precapillary only. Twelve patients were then excluded because of prominent motion artifacts on CT examination (n = 6), presence of lung micronodules (n = 3), pleural plaques (n = 1), pleural effusion (n = 1) and consolidation (n = 1). Among the 56 severe PH patients, 24 patients demonstrated severe PH associated to COPD, diagnosed on PFT (post-bronchodilator FEV<sub>1</sub>/FVC < 70%) and exposure to tobacco; 16 had with severe iPAH, and 16 had severe CTEPH.

#### *Pulmonary function tests*

Body plethysmography (BodyBox, Medisoft, Belgium) were used to assess lung mechanics. Functional parameters were recorded in litres and expressed as percentages of predicted values: forced expiratory volume in 1 second (FEV<sub>1</sub>), forced vital capacity (FVC), total lung capacity (TLC), residual volume (RV). Transfert lung capacity of carbon monoxide (TLCO) were recorded, using HypAir Compact (Medisoft), and expressed as percentage of predicted value. European Respiratory Society and American Thoracic Society (ATS) guidelines were chosen for reference values<sup>3,4</sup>. Arterial blood, at room air, was drawn in order to record arterial blood gases PaO<sub>2</sub>, PaCO<sub>2</sub> (mmHg). ATS recommendation were followed to perform 6-MWT and expressed in meters.

#### *CT protocol*

Chest CT scans were acquired with a 64-section multidetector CT scanner (Somatom Definition; Siemens, Erlangen, Germany) by using the following parameters: 110-kV tube voltage, 50-mAs tube current and 75-msec rotation time, 0.75-mm collimation, no contrast injection. Data were acquired in the supine position at full inspiration and reconstructed with both high-spatial-frequency and standard algorithm, with a 1-mm reconstruction section thickness, 1-mm reconstruction interval, 320x320-mm field-of-view, pixel size (0.625mm)<sup>2</sup>, and 512x512 matrix. CT scans were anonymized in a blinded fashion before automatic quantitative analysis.

#### *Quantitative CT analysis of bronchial wall thickness and emphysema*

High-spatial-frequency algorithm reconstructions of datasets of images were transferred into a workstation and displayed with a parenchymal window width (1800 Hounsfield units (HU)) and level (-600 HU). Dedicated and validated software were used to quantitatively analyse CT scans<sup>5,6</sup>. CT measurements of intrapulmonary airways were performed at subsegmental level.

Automatic quantification of bronchi wall area (WA), lumen area (LA) and wall thickness (WT) were obtained on orthogonal bronchial cross sections perpendicular to bronchial main axis by using the Laplacian-of-Gaussian algorithm, as previously described <sup>6,7</sup>.

Emphysema was automatically quantified and was assessed on CT images reconstructed with standard algorithm by using whole-lung densitometry with Myrian<sup>®</sup> software (Intrasens, Montpellier, France). CT attenuation values of -500 and -1024 HU were used to isolate lungs from the rest of thoracic structures on a threshold-based technique. Low attenuation area (LAA%) was derived from the voxel frequency distribution histogram and represented the percentage of lung voxels less than -950 HU, as previously described <sup>1,8,9</sup>.

#### *Mosaic attenuation evaluation*

CT set of images reconstructed with sharp algorithm (B70f) were seen at window settings appropriate for parenchymal assessment (width: 1,500 HU, level: -700 HU). Mosaic attenuation score was built and derived from a semi-quantitative evaluation of each lobe using a published method <sup>10</sup>. Mosaic attenuation was determined for each lobe as a percentage score ranging from 0 to 100% of reduced attenuation with 10% increments. Reduced attenuation has been subjectively defined as a decrease in parenchymal attenuation conforming to the borders of secondary pulmonary lobules that contain vessels with caliber smaller than expected <sup>11</sup>.

A weighting factor was applied for each lung's lobe score to calculate a total mosaic attenuation score. Weighting factors were 3/20, 2/20 and 5/20 for each upper, middle and lower lobes, respectively <sup>10</sup>.

#### *Measurement of small pulmonary vessels area*

Automated measurement of small vessels was obtained from CT images, as previously described<sup>1,12-16</sup>. CT set of images reconstructed with sharp algorithm (B70f) were transferred into a workstation for post-processing and analysed by using the ImageJ software version 1.40g (a public domain Java image program available at <http://rsb.info.nih.gov/ij/>). First, in order to eliminate image noise, all CT images were smoothed by applying a Gaussian filter. A threshold technique to select pixels between -500 and -1024 Hounsfield units (HU) was used to segment the lung fields. The segmented lung fields were then converted into binary images with a window level of -720 HU. The ImageJ software “Analyze particles” function was applied. This function is useful to count and measure objects on binary images, here it detected the cross sectional area and number of vessels. The ImageJ software “Circularity” function was used to select objects within range of circularity between 0.9 and 1, and allowed selection of vessels running orthogonal to the axial plane.

The cross-section area (CSA) of small pulmonary vessels were automatically quantified separately for each CT slice at the sub-subsegmental levels using ImageJ software. The sub-subsegmental level is defined by a vessel area less than 5 mm<sup>2</sup>. At the same time, lung area was measured for each CT slices. Finally, quantifications were obtained after normalisation by the corresponding lung section area at each CT slice: the cross sectional area of small pulmonary vessels less than 5 mm<sup>2</sup> (%CSA<sub><5</sub>). Then, a mean of %CSA<sub><5</sub> of all CT slices for each patient was calculated.

#### *Measurement of main pulmonary artery ratio diameter*

Large vessels diameters were manually measured, at the level of pulmonary artery bifurcation, for both pulmonary arterial truncus (AP) and ascending aorta (AO) using the same image<sup>1,8</sup>.

The largest diameters were measured by one observer. The ratio AP/AO was then calculated<sup>1,8</sup>.

#### *Right heart catheterisation*

After jugular insertion, a flow-directed balloon-tipped 7F Swan-Ganz catheter (131HF7; Baxter Healthcare Cop., Irvine, USA) was moved in order to assess a right heart catheterisation (RHC)<sup>17</sup>. At end expiration, systolic, mean and diastolic pulmonary artery pressure (s-,m-,dPAP), pulmonary capillary wedge pressure (PCWP) were determined. Gradient was calculated by difference between dPAP and PCWP. Cardiac output (CO) was determined using the Fick method.

#### *Echocardiography*

Standard techniques of two-dimensional Doppler Echocardiography were used at rest to assess right and left heart functions. Measurement of maximal tricuspid regurgitation velocity (ITVmax) and determination of transtricuspid pressure gradient (ITGdmax) with the estimation of the right atrial pressure, allowed to calculate the systolic pulmonary arterial pressure (SPAP). Measurements of end-systolic and end-diastolic left ventricular volumes lead to calculation of the left ventricular ejection fraction (LVEF%).

#### *Statistical analysis*

Statistical analyses were performed using NCSS software (NCSS 2001, Kaysville, UT, USA). Results were expressed as mean with standard deviation and analysed using one-way ANOVA and Tukey post-hoc test. Parameters that were not normally distributed were expressed as median with inter quartile ranges and tested by Kruskal-Wallis and multiple comparisons z-value post-hoc test. Categorical variables were tested with Fisher's exact tests. Univariate

correlations were assessed using r coefficient of Pearson, when data were not normally distributed a log-transformation was applied.

Paw score was calculated to predict severe PH, combining 3 variables (*ie*, PaO<sub>2</sub>, WT and %CSA<sub><5</sub>)<sup>1</sup>. Briefly, each patient received points, ranging from 0 (minimal value) to 3 (maximal value) for every variable, as previously published<sup>1</sup> (See supplemental Table 1). Thresholds were determined previously using interquartile ranges of each variables<sup>1</sup>. Then, the scores ranged from 0 to 9 points, the higher value indicating a higher risk of severe PH. Finally, scores and sensitivity were compared. *P* values less than 0.05 were considered significant.

## References

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**Supplemental Table 1:** Paw score variables and range

Paw score points	Variables		
	PaO <sub>2</sub> (mmHg)	%CSA <sub>&lt;5</sub>	WT
0	≥ 64.5	< 0.313	< 1.095
1	[51.5-64.4]	[0.313-0.432]	[1.095-1.179]
2	[46.5-51.4]	[0.433-0.547]	[1.180-1.324]
3	< 46.5	≥ 0.548	≥ 1.325

Definition of abbreviations: PaO<sub>2</sub>: Arterial partial pressure in oxygen; %CSA<sub><5</sub>, percentage of total lung area taken up by the cross-sectional area of pulmonary vessels less than 5mm<sup>2</sup>; WT, mean Wall Thickness.

**Supplemental Table 2:** Univariate correlation between mPAP and different clinical, biological and functional parameters

	COPD with severe PH			Severe iPAH			Severe CTEPH		
	n	Coef	p-value	n	Coef	p-value	n	Coef	p-value
Age (years)	24	0.27	<b>0.04</b>	16	-0.24	0.37	16	-0.17	0.53
BMI (kg.m <sup>-2</sup> )	24	0.08	0.67	16	-0.24	0.38	16	0.41	0.12
PFT									
FEV <sub>1</sub> (% pred)	24	0.27	0.21	15	0.06	0.84	16	-0.31	0.25
FEV <sub>1</sub> /FVC (%)	24	0.25	0.24	15	0.13	0.65	16	0.23	0.38
FVC (%)	24	0.14	0.50	15	0.24	0.39	16	-0.36	0.16
TLC (%)	23	-0.29	0.19	15	0.01	0.97	16	0.12	0.65
RV (%)	23	-0.39	0.07	15	-0.03	0.92	16	0.24	0.37
TLCO (%)	22	0.08	0.72	15	0.28	0.31	13	0.59	<b>0.03</b>
Arterial blood gases									
PaO <sub>2</sub>	24	-0.15	0.49	16	0.35	0.19	16	-0.04	0.88
PaCO <sub>2</sub>	24	-0.33	0.11	16	0.19	0.49	15	-0.14	0.62
Biology									
CRP	24	0.40	<b>0.05</b>	12	-0.01	0.98	9	0.43	0.25
BNP	24	0.42	<b>0.04</b>	16	0.03	0.91	16	0.36	0.17
Echoardiography									
sPAP (mmHg)	23	0.49	<b>0.02</b>	16	0.27	0.32	13	0.21	0.51
ITVmax (m/s)	23	0.38	0.07	16	0.65	<b>0.01</b>	13	0.28	0.35
ITGdmax (mmHg)	23	0.37	0.07	15	0.65	<b>0.01</b>	13	0.26	0.38
LVEF (%)	20	-0.17	0.46	12	0.32	0.31	10	0.07	0.85
Six-minutes walk test									
Distance (m)	19	0.18	0.45	15	-0.09	0.74	13	-0.59	<b>0.03</b>

Data are Pearson correlation coefficients.

Definition of abbreviations: COPD, chronic obstructive pulmonary disease; iPAH, idiopathic pulmonary arterial hypertension; CTEPH, chronic thromboembolic pulmonary hypertension; Coef, Coefficient; BMI, body mass index; BNP, brain natriuretic peptide; CRP, C reactive protein; FEV<sub>1</sub>, forced expiratory volume in 1 second; FVC, forced volume capacity; ITVmax, maximal tricuspid regurgitation velocity; ITGdmax, maximal transtricuspid pressure gradient; LVEF, left ventricular ejection fraction; sPAP, systolic pulmonary arterial pressure; PFT, pulmonary function test; pred, predicted; PVR, pulmonary vascular resistance; PVR<sub>i</sub>, indexed PVR; RV, residual volume; TLC, total lung capacity; TLCO, transfer lung capacity of carbon monoxide.

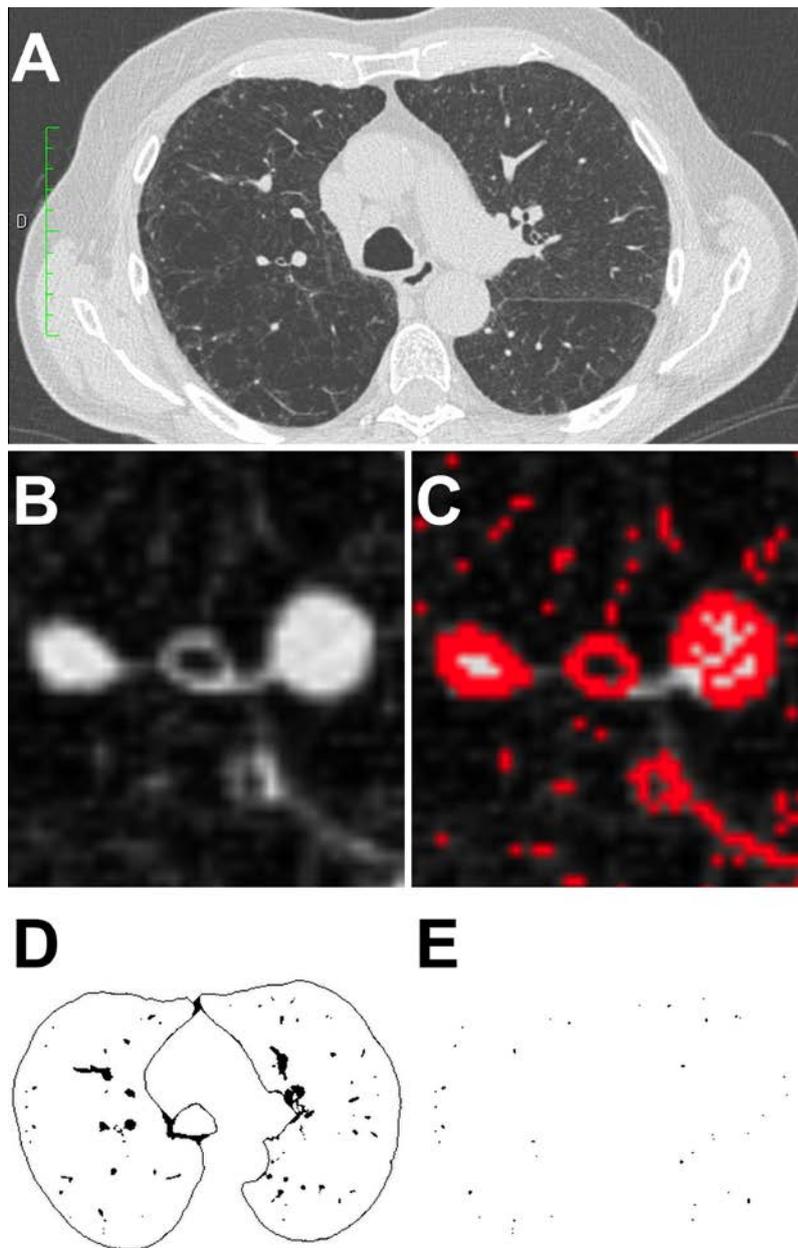
**Supplemental Table 3:** Sensitivity of ultrasound echocardiography and Paw score to detect severe PH

	COPD with severe PH	Severe iPAH	Severe CTEPH
SPAP> 50 mmHg	69.6%	87.5%	100%
SPAP> 60 mmHg	60.9%	75.0%	85.7%
SPAP> 65 mmHg	52.2%	68.8%	78.6%
Paw score≥ 5 points	87.5%	43.8%	62.5%

Values are sensitivity to detect severe PH based on RHC using either sPAP assessed by echocardiography or Paw score.

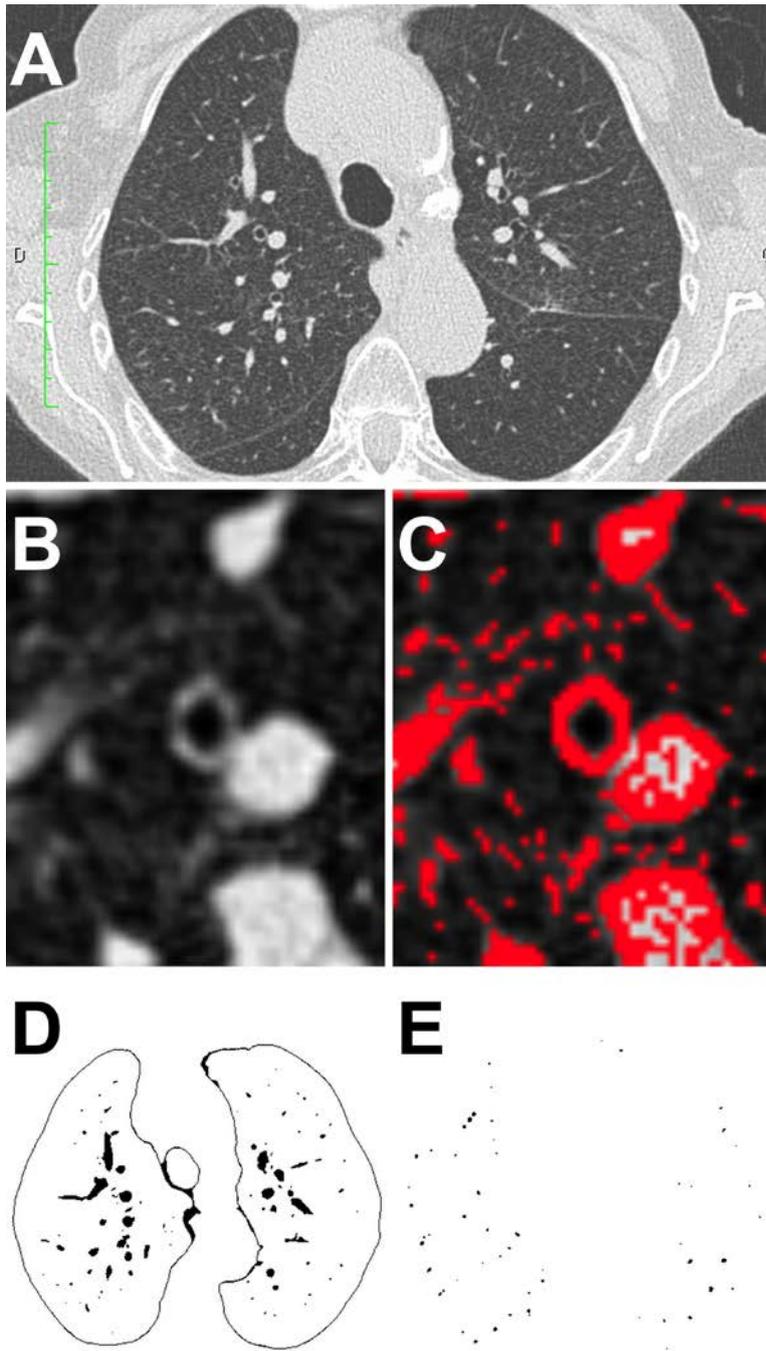
Definition of abbreviations: COPD, chronic obstructive pulmonary disease; iPAH, idiopathic pulmonary arterial hypertension; CTEPH, chronic thromboembolic pulmonary hypertension; SPAP, systolic pulmonary arterial pressure on echocardiography.

## Figures legends



**Supplemental Figure 1:** CT scans representative of a COPD patient with severe PH.

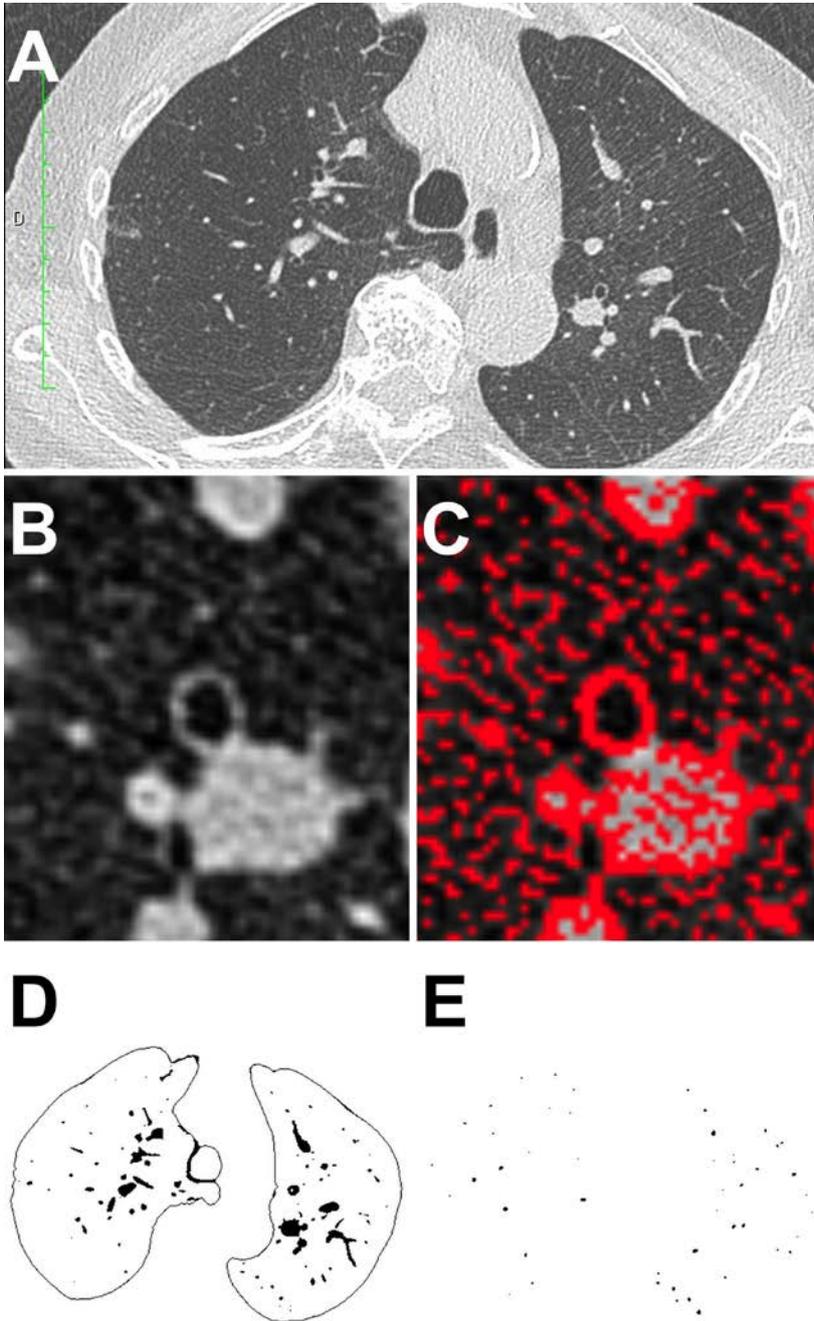
(A) Native transverse thin-section CT-image. (B) Reformatted section perpendicular to the main bronchial axis before and (C) after segmentation for bronchial wall measurement. (D) Converted binary image after bi-thresholding (E) Mask image analysis for small vessels ranging from 0 to 5 mm<sup>2</sup>.



**Supplemental Figure 2:** CT scans representative of a severe iPAH patient.

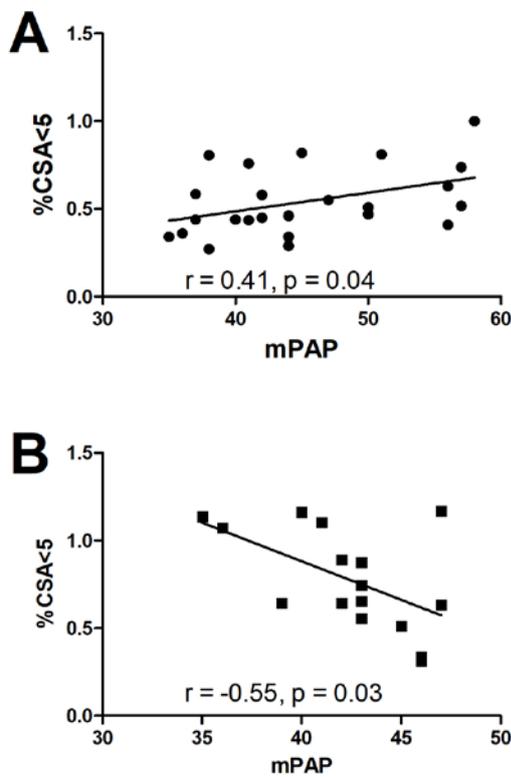
(A) Native transverse thin-section CT-image. (B) Reformatted section perpendicular to the main bronchial axis before and (C) after segmentation for bronchial wall measurement.

(D) Converted binary image after bi-thresholding (E) Mask image analysis for small vessels ranging from 0 to 5 mm<sup>2</sup>.



**Supplemental Figure 3:** CT scans representative of a severe CTEPH patient.

(A) Native transverse thin-section CT-image. (B) Reformatted section perpendicular to the main bronchial axis before and (C) after segmentation for bronchial wall measurement. (D) Converted binary image after bi-thresholding (E) Mask image analysis for small vessels ranging from 0 to 5 mm<sup>2</sup>.



**Supplemental Figure 4:** Correlations between mean pulmonary arterial pressure (mPAP) and percentage of total lung area taken up by the cross-sectional area of pulmonary vessels less than 5 mm<sup>2</sup> (%CSA<sub><5</sub>)

(A) Correlations for COPD subjects and (B) Correlations for iPAH subjects with severe PH.