CT evaluation of small pulmonary vessels area in COPD patients with severe pulmonary hypertension

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Supplemental methods

Study population

The design of the study was retrospective, and approved by our institutional review board. All patients were informed and agreed that their data can be used anonymously for further research studies. COPD patients were referred between January 2008 and December 2014 to our institution, a tertiary medical center for complete examination of PH, before initiation of any treatment. All patients underwent within 1 week: medical questioning, physical examination, 6-minutes walk tests, arterial blood gases, blood tests (including C-reactive protein [CRP], antinuclear antibodies and HIV serology), pulmonary function testing (PFT), trans-thoracic echocardiography, ventilation/perfusion scintigraphy (V/Q scan), right heart catheterization (RHC), and unenhanced computed tomography (CT) within a minimal period of 1 month of disease stability. For all patients final statement about disease aetiology was made by agreement between experienced cardiologists and pneumologists, after careful review of all information available. Diagnosis of COPD was based on PFT, according to a $FEV_1/FVC < 70\%$.

From a total of 198 selected patients, 105 COPD patients were included in the study, with no other condition susceptible to explain PH (See Figure 1 and Table 1, S1). Among the 105 COPD patients, 20 patients demonstrated severe PH, as defined by mPAP at RHC superior to 35 mmHg as the last world PH symposium proposed this cut-off [1]. These severe PH patients were FEV_{1-} (with a variability of 5%), and age-matched (more or less 5 years) to 20 other COPD patients without severe PH (mPAP < 35 mmHg) (Table 1). Sixty-five remaining patients were unable to be matched and were presented separately (Supplemental Table S1). Unmatched patients had the same hemodynamic and biological characteristics. However they had more severe airflow limitations at PFT ($p<0.05$, data not shown).

The fifty seven excluded patients with concomitant: sleep apnea syndrome (n = 10), evidence of thromboembolic vessel occlusion on V/Q scan (n = 3), liver cirrhosis with portal hypertension (n = 4), left heart disease as defined by history of coronaropathy and a left heart function on echocardiography inferior to 40% (n = 14), lung fibrosis (n= 13), familial history of primary pulmonary hypertension (n= 7), or use of appetite suppressant (n= 3). No patient appeared to have positive test on HIV serology and antinuclear antibody. No patient had intracardiac shunt on echocardiography. Three patients were further excluded because of rhedibitory motion artefacts on CT imaging (n = 1), presence of a lung tumor (n = 1), and diffuse lung micronodules (n = 1). Among the 40 COPD patients who composed our study population, 20 patients were found to have severe PH as defined by mPAP at RHC superior to 35 mmHg [1]. The 20 other COPD patients had mild-to-moderate PH (25≤ mPAP < 35 mmHg; n= 12) or no PH (mPAP < 25 mmHg; n=8) and were chosen as the control group (Table 1).

Pulmonary function tests

Lung mechanics were assessed by using body plethysmography (BodyBox, Medisoft, Belgium). The following functional parameters were measured in litres and expressed as percentages of predicted values: forced expiratory volume in 1 second (FEV_1), forced vital capacity (FVC), total lung capacity (TLC), residual volume (RV). HypAir Compact (Medisoft) was used to record transfert lung capacity of carbon monoxide (TLCO) and expressed as percentage of predicted value. Pulmonary functions variables were expressed in percent predicted. American Thoracic Society [2] and European Respiratory society guidelines [3] were chosen for reference values. Arterial blood (room air) was drawn for recording arterial blood gases PaO_2, $PaCO_2$ (mmHg). The 6 minute walk test (6MWT) expressed in meters was assessed in a standardized manner following ATS recommendation.

CT protocol

CT examinations of the chest were performed with a 64-section multidetector CT scanner (Somatom Definition; Siemens, Erlangen, Germany) without contrast medium administration by using the following parameters: 110-kV tube voltage, 50-mAs tube current and 0.75-mm collimation. Acquisitions were performed 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, pixel size 0.625mm^2, 320x320-mm field-of-view, and 512x512 matrix. CT scans were anonymized in a blinded fashion before further full automatic analysis.

Quantitative CT analysis of bronchial wall thickness and emphysema

Datasets of images reconstructed with high-spatial-frequency algorithm were transferred into a workstation and displayed with a parenchymal window width (1800 HU) and level (-600 HU). Quantitative analysis was performed in three dimensions by using dedicated and validated software [4, 5]. CT measurements of intrapulmonary airways were performed at the subsegmental level. Automatic quantification of bronchi wall area (WA), lumen area (LA), wall area percent (WA%) and wall thickness (WT) were obtained on orthogonal bronchial cross sections by using the Laplacian-of-Gaussian algorithm [5, 6].

Automatic quantification of emphysema was performed on CT images reconstructed with standard algorithm by using whole-lung densitometry with Myrian® software (Montpellier, France). A threshold-based technique was used to isolate lungs from the rest of thoracic structures using CT attenuation values of -500 and -1024 HU. Low attenuation area (LAA%) was derived from the voxel frequency distribution histogram and represented the percentage of lung voxels less than a threshold of -950 Hounsfield units, as previously described [7-9].

Measurement of small pulmonary vessels area and main pulmonary artery ratio diameter

Automated measurement of small vessels from CT images was obtained by using a method detailed elsewhere [10-12]. Briefly, 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/). CT images were first smoothed by applying a Gaussian blurring to eliminate image noise. The lung fields were segmented with a threshold technique to select pixels between -500 and -1024 Hounsfield units (HU). Next the segmented lung fields were converted into binary images with a window level of -720 HU. The ImageJ software "Analyze particles" function was used to count and measure objects on binary images and calculate the cross sectional area of vessels. Selection of vessels running orthogonal to the axial plane was obtained by using the ImageJ "Circularity" function to select objects within range of circularity between 0.9 and 1. The cross section area (CSA) of small pulmonary vessels were measured at subsegmental and at sub-subsegmental levels separately, the sub-subsegmental level corresponding to a vessel area less than 5 mm^2 (Figure 2 of the main manuscript), and the subsegmental level to an area between 5 to 10 mm^2. The following measurements were obtained: the cross sectional area of small pulmonary vessel less than 5 mm^2 ($\%CSA_{<5}$), and between 5 to 10 mm^2 ($\%CSA_{5-10}$), the mean number of cross sectioned vessels $CSN_{<5}$ and CSN_{5-10} normalized by the corresponding lung section area at each CT slice.

Manual measurements of large vessels were performed after multiplanar reconstruction strictly orthogonal to the main axis of the pulmonary arterial troncus (AP) and the ascending aorta (AO). Diameters were measured at three different locations by one observer (GD) with

8 years of experience in thoracic imaging, into their widest portion, and averaged to obtain a mean value. The AP and AP/AO values were calculated and recorded for analysis.

Right heart catheterization

A flow-directed balloon-tipped 7F Swan-Ganz catheter (131HF7; Baxter Healthcare Cop., Irvine, USA) inserted in the jugular vein was used in order to assess a right heart catheterization (RHC) [13]. Systolic, mean and diastolic pulmonary artery pressure (s-,m-,dPAP), pulmonary capillary wedge pressure (PCWP) were measured at end expiration.

The difference between dPAP and PCWP represent the gradient. Fick method allowed to determinate cardiac output (CO).

Echocardiography

Two-dimensional Doppler Echocardiography was performed at rest using standard techniques in order to assess right and left heart functions. The right ventricular systolic pressure (RVSP) was calculated after measurement of transcuspid pressure gradient and estimation of the right atrial pressure. The end-systolic and end-diastolic left ventricular volumes were determined to calculate the left ventricular ejection fraction (LVEF%).

Statistical analysis

NCSS software (NCSS 2001, Kaysville, UT, USA) was used to assess statistical analyses. *P* values less than 0.05 were considered significant. Results were expressed as mean with standard deviation. Comparisons were performed by using t-tests. Parameters that were not normally distributed were analysed by a Mann-Whitney tests. Univariate correlations were assessed by Pearson tests. Identification of the strength of the association between mPAP and variables, already found to correlate with mPAP at univariate analysis, was assessed using

forward/backward stepwise multiple regression analyses. The dependant variable was mPAP. Cross-correlated independent variables were not transferred in this model to prevent multicollinearity.

In order to predict severe PH, we built four scores (*i.e.*, Paw-scores), combining 2 to 3 variables, best correlated to mPAP, similarly to that described for the BODE index [14]. Each patients received points, ranging from 0 (minimal value) to 3 (maximal value) for each analyzed variable, according to equal-sized quartiles [15] (Table 2). Then, the scores ranged from 0 to 9 points (3 variables-scores) or from 0 to 6 points (2 variables-scores), the higher value indicating a higher risk of severe PH. Scores were compared by using areas under the curve (AUCs) of receiver operating characteristic (ROC) curves using 2 different prevalence of severe PH in COPD, previously published in the literature (*i.e.*, 5% [16] and 13% [17],).

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Supplemental tables, figures and legends

Table S1: Characteristics of unmatched COPD subjects

		COPD subjects without severe PH
n		65
Age	Years	57 ± 9
Sex ratio	Men/Women	40 / 25
BMI	$kg.m^{-2}$	22 ± 4
Tobacco	smoking status (Y/N)	65 / 0
	curent smoker (Y/N)	14 / 51
	pack year (no)	33 ± 23
PFT	FEV_1 (% pred)	29 ± 15
	FEV1/FVC (%)	38 ± 11
	TLC (%)	125 ± 30
	RV (%)	216 ± 74
	TLCO (%)	30 ± 10
6-MWT	Distance (meters)	326 ± 126
Arterial blood gases (ambient air)	PaO_2 (mmHg)	66 ± 14
	$PaCO_2$ (mmHg)	45 ± 9
RHC	mPAP (mmHg)	25 ± 6
	sPAP (mmHg)	37 ± 9
	dPAP (mmHg)	16 ± 3
	PCWP (mmHg)	7 ± 3
	Gradient (mmHg)	9 ± 4
	PVR (Wood unit)	4 ± 2
	PVRi (Wood $unit.m^{-2}$)	6 ± 3
	Cardiac output ($L.min^{-1}$)	5 ± 1.3
	Cardiac index (L.min-	3 ± 0.7
Biology	CRP (pg/ml)	5 ± 4
	BNP (mg/ml)	84 ± 63

COPD without severe PH is defined mPAP < 35 mmHg. Data are means ± standard deviation for categorical variables. Definition of abbreviations: PH= Pulmonary hypertension; BMI= Body Mass Index; PFT= Pulmonary function test; FEV_1= Forced Expiratory Volume in 1 second; pred= predicted; FVC= Forced Vital Capacity; TLC= Total Lung Capacity; RV= Residual Volume; TLCO= Transfert Lung capacity of Carbon Monoxide; 6-MWT= 6 Minute Walk Test; RHC= Right Heart Catheterism; m,s,dPAP= mean, systolic, diastolic Pulmonary Arterial Pressure; PCWP= Pulmonary Capillary Wedge Pressure; Gradient= dPAP-PCWP;

PVR= Pulmonary Vascular Resistance; PVRi= indexed PVR; BNP= Brain natriuretic peptide; CRP= C-reactive protein.

Table S2: Univariate analysis of mPAP in COPD subjects with and without severe PH

		COPD subjects with severe PH			COPD subjects without severe PH		
		n	Coefficient	p-value	n	Coefficient	p-value
PFT	**TLCO%**	18	0.17	0.49	19	-0.47	0.04
	FEV1%	20	0.37	0.11	20	-0.02	0.93
	FEV1/FVC	20	0.36	0.12	20	- 0.03	0.9
Arterial blood gases	**PaO2**	20	-0.21	0.37	20	-0.51	0.02
Biology	**BNP**	20	0.22	0.34	20	0.53	0.01
	CRP	20	0.26	0.27	20	-0.31	0.19
Tomodensitometry	**LAA%**	20	-0.37	0.11	20	0.42	0.06
	WA	20	0.32	0.16	20	0.43	0.058
	LA	20	0.06	0.81	20	0.12	0.6
	WA%	20	0.05	0.85	20	0.27	0.24
	WT	20	0.52	0.02	20	0.6	0.005
	AP/AO	20	-0.04	0.87	20	0.64	0.002
	%CSA$_{<5}$	20	0.53	0.015	20	-0.57	0.009
	%CSA$_{5-10}$	20	0.42	0.06	20	-0.47	0.03
6-MWT	**Distance (m)**	16	0.02	0.92	16	-0.58	0.02

Severe PH in COPD is defined mPAP ≥ 35 mmHg, without < 35 mmHg.

Data are rho correlation coefficient of Pearson.

Definition of abbreviations: mPAP= mean Pulmonary Arterial Pressure; PH= Pulmonary hypertension; PFT= Pulmonary function test; FEV_1= Forced Expiratory Volume in 1 second; pred= predicted; FVC= Forced Vital Capacity; TLC= Total Lung Capacity; TLCO= Transfert Lung capacity of Carbon Monoxide; BNP= Brain natriuretic peptide; CRP= C-reactive protein; LAA%= Low lung Attenuation Area; WA= mean Wall Area; LA= mean lumen area; WA%= mean Wall Area Pourcentage; WT= mean Wall Thickness; AP= Pulmonary Artery Troncus; AO= Aorta; $\%CSA_{<5}$= percentage of total lung area taken up by the cross-sectional area of pulmonary vessels less than 5 mm^2; $\%CSA_{5-10}$= percentage of total lung area taken up by the cross-sectional area of pulmonary vessels between 5 and 10 mm^2.

Table S3: Cross correlation in severe PH in parameters that correlates to mPAP

	$\%CSA_{<5}$	$\%CSA_{5-10}$
$\%CSA_{5-10}$	0.47*	
WT	- 0.09	0.01

Data are rho correlation coefficient of Pearson. * indicates a significant p-value. Definition of abbreviations: mPAP= mean Pulmonary Arterial Pressure; PH= Pulmonary hypertension; $\%CSA_{<5}$= percentage of total lung area taken up by the cross-sectional area of pulmonary vessels less than 5 mm^2; $\%CSA_{5-10}$= percentage of total lung area taken up by the cross-sectional area of pulmonary vessels between 5 and 10 mm^2; WT= mean Wall Thickness.

Table S4: Cross correlation in non severe PH in parameters that correlates to mPAP

	PaO_2	TLCO	BNP	6-MWT	AP/AO	$\%CSA_{<5}$	$\%CSA_{5-10}$
TLCO	0.27						
BNP	- 0.12	- 0.26					
6-MWT	- 0.07	0.25	- 0.41				
AP/AO	- 0.70*	- 0.54*	0.44	- 0.12			
$\%CSA_{<5}$	0.33	0.59*	- 0.08	0.21	- 0.50*		
$\%CSA_{5-10}$	0.40	0.53*	- 0.01	0.25	- 0.50*	0.69*	
WT	- 0.03	- 0.02	0.46*	- 0.01	0.23	- 0.20	0.01

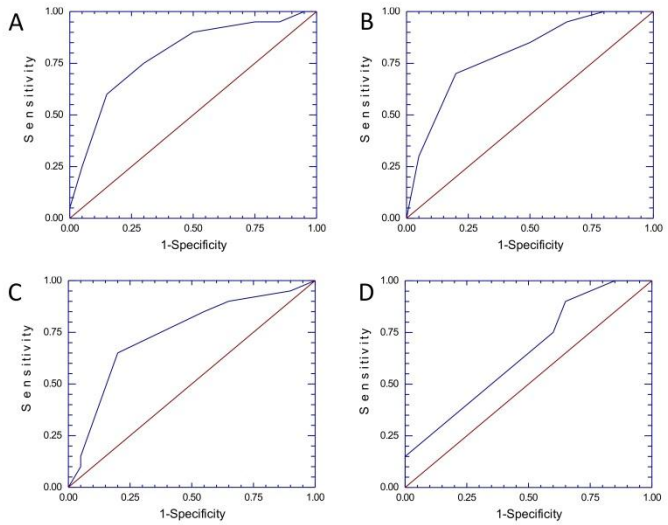
Data are rho correlation coefficient of Pearson. * indicates a significant p-value. Definition of abbreviations: mPAP= mean Pulmonary Arterial Pressure; PH= Pulmonary hypertension; TLCO= Transfert Lung capacity of Carbon Monoxide; BNP= Brain natriuretic peptide; 6-MWT= 6 Minute Walk Test; AP= Pulmonary Artery Troncus; AO= Aorta; $\%CSA_{<5}$= percentage of total lung area taken up by the cross-sectional area of pulmonary vessels less than 5 mm^2; $\%CSA_{5-10}$= percentage of total lung area taken up by the cross-sectional area of pulmonary vessels between 5 and 10 mm^2; WT= mean Wall Thickness.

Figure legend

Figure S1: Receiver operating characteristic curves for the 40 matched COPD patients were built in order to predict the presence of mPAP < 35 mmHg. A, represents the paw-score including 3 parameters: PaO_2, $\%CSA_{<5}$ (= percentage of total lung area taken up by the cross-sectional area of pulmonary vessels less than 5 mm^2) and WT (= mean bronchial Wall Thickness). B, represents 2 parameters of the paw-score: PaO_2, $\%CSA_{<5}$. C, represents 2 parameters of the paw-score: $\%CSA_{<5}$, WT. D, represents 2 parameters of the paw-score: PaO_2, WT.

Figure S2: Receiver operating characteristic curves for all the 105 COPD patients were built in order to predict the presence of mPAP < 35 mmHg. A, represents the paw-score including 3 parameters: PaO_2, $\%CSA_{<5}$ (= percentage of total lung area taken up by the cross-sectional area of pulmonary vessels less than 5 mm^2) and WT (= mean bronchial Wall Thickness). B, represents 2 parameters of the paw-score: PaO_2, $\%CSA_{<5}$. C, represents 2 parameters of the paw-score: $\%CSA_{<5}$, WT. D, represents 2 parameters of the paw-score: PaO_2, WT.

Supplementary Figure 1



Supplementary Figure 2