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Airway morphometry in COPD with bronchiectasis: a view on all airway generations

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Summary:

Although terminal bronchioles were equally reduced in COPD lungs with and without bronchiectasis, significantly more large and small airways were found in COPD lungs with bronchiectasis.

ABSTRACT

The pathophysiological processes underlying bronchiectasis in COPD are not understood. In COPD, both small and large airways are progressively lost. It is currently not known to what extent the different airway generations of patients with COPD and bronchiectasis are involved.

COPD explant lungs with bronchiectasis were compared to COPD explant lungs without bronchiectasis and unused donor lungs as controls. In order to investigate all airway generations, a multimodal imaging approach using different resolutions was conducted. Per group, 5 lungs were frozen (n=15), underwent CT imaging for large airway evaluation with 4 tissue cores per lung imaged for terminal bronchioles measurements. Two additional lungs per group (n=6) were air-dried for lobar microCT images that allow airway segmentation and 3D quantification of the complete airway tree.

COPD lungs with bronchiectasis had significantly more airways compared to COPD lungs without bronchiectasis ($p<0.001$), with large airway numbers similar to control lungs. This difference was present in both upper and lower lobes. Lack of tapering was present ($p=0.010$) and larger diameters were demonstrated in lower lobes with bronchiectasis ($p=0.010$). MicroCT analysis of tissue cores showed similar reductions of tissue percentage, surface density and number of terminal bronchioles in both COPD groups compared to control lungs.

Although terminal bronchioles were equally reduced in COPD lungs with and without bronchiectasis, significantly more large and small airways were found in COPD lungs with bronchiectasis.

INTRODUCTION

Chronic obstructive pulmonary disease (COPD) is a prevalent disease, characterized by persistent respiratory symptoms, and caused by an abnormal inflammatory response to chronic inhalation of irritants. Chronic inflammation affects small airways and lung parenchyma, resulting in progressive airflow limitation (1) with both environmental and host factors contributing to the pathogenesis and heterogeneity of the disease (2, 3). Lately, the overlap between COPD and bronchiectasis has been receiving more attention as a potential COPD phenotype. Bronchiectasis is defined by permanently dilated and chronically inflamed middle-order bronchi, due to a vicious circle of transmural infection and inflammation. This translates into a clinical syndrome with productive cough, bacterial colonization, and recurrent respiratory infections. The diagnosis of bronchiectasis is primarily based on imaging with the gold standard being abnormal bronchial dilation on 1mm computed tomography (CT) images (4). Bronchodilation is assessed using Naidich's criteria: a broncho-arterial ratio >1, lack of tapering, and the presence of bronchi within 1cm of costal pleura or abutting the mediastinal pleura (5).

The reported prevalence of bronchiectasis in COPD cohorts varies between 4 to 70%, due to differences in study population and radiological criteria (6). On the other hand, COPD is present in 3-35% of patients in bronchiectasis cohorts (7–9). Importantly, patients with COPD and bronchiectasis have a more severe disease course with higher mortality and an

obvious need for adjusted therapeutic strategies (10–14). It is still unclear whether COPD facilitates bronchiectasis or if these two conditions co-exist in an individual patient.

Small airways, with a luminal diameter <2mm, are the major site of COPD pathogenesis and the main reason for airflow obstruction. It has been demonstrated that the numbers of small airways in COPD are reduced and that terminal bronchioles are narrowed in comparison with controls, even before the onset of emphysema (15, 16). Moreover, remodeling of small airways has been shown from early disease onwards (17). More recently, a reduced total airway count on CT has been demonstrated in patients with mild COPD compared to never-smokers and smokers without COPD, suggesting that large airways may also play a role in pathogenesis (18). In bronchiectasis, it is assumed that obliteration occurs in the small airways, causing airflow obstruction (19, 20). So far, no advanced imaging studies in bronchiectasis have focused on airway generations beyond the resolution of conventional clinical CT. MicroCT imaging, with its increased resolution, is therefore a promising tool enabling evaluation up to the level of the most distal conducting airways or terminal bronchioles (16).

We provide, for the first time, a detailed morphometric analysis of the entire bronchial tree in end-stage COPD lungs with and without bronchiectasis and controls. In 14 COPD explant lungs and 7 unused donor lungs, we used a threefold imaging approach with high resolution CT (~600µm resolution), whole lobe microCT (~140µm resolution) and microCT scanning of tissue cores (~10µm resolution) to investigate large and small airways up to the level of the terminal bronchioles.

MATERIALS AND METHODS

Study material

Explanted lungs of patients with end-stage COPD undergoing lung transplantation and of unused donors were prospectively collected in our centre after approval of the local Medical Ethics Board of University Hospitals Leuven, Belgium (ML6385, s52174). Patients were eligible for lung transplantation based on criteria proposed by the International Society of Heart and Lung Transplantation (21), and gave written informed consent to use their lungs for research purposes. According to Belgian law, declined organs from prospective donors can be used for research. The cause of donor death and reason for lung decline are summarized in the data supplement (Table E1). The donor lungs were carefully inspected during processing to make sure that there was no macroscopic disorder. The presence and morphology of bronchiectasis in COPD lungs was evaluated per lobe on clinical, pre-transplant inspiratory CT scans of the lungs using the modified Reiff score (lingula considered separately, tubular=1, varicose=2 or cystic=3 (22)) and confirmed by experienced chest radiologists (AD, JV). None of the patients had a history of bronchiectasis before the diagnosis of COPD and a thorough pre-transplant work-up could not reveal a specific cause for bronchiectasis. Based on availability and conservation, 5 COPD lungs with bronchiectasis, 5 COPD lungs without bronchiectasis and 5 unused donor lungs were retrospectively analysed (Figure 1A) and 2 lungs of each group were prospectively collected (Figure 1B). COPD lungs without bronchiectasis will be referred to as COPD lungs and unused donor lungs as controls hereafter.

Frozen whole-lung CT and tissue core microCT

15 explant lungs, obtained from 15 different individuals, were inflated and frozen as previously described (16, 23). A CT scan was obtained from the frozen lungs, resulting in images with a resolution of approximately 600µm (Figure 1A). Emphysema was quantified as the percentage of lung area below -950HU with customized in-house software. An open-source image viewer, Horos software version 3.0 (Horosproject, www.horosproject.org) was used for measurements and manual counting of airway bifurcations per generation by scrolling through the ex-vivo whole-lung CT images. The airway branches were counted starting from the main stem bronchus (generation 1), reporting each subsequent bifurcation as a new generation and recording the number of visible bifurcations per generation. Inner diameters were measured manually at the widest level in each segment.

After CT scanning, two apical and two basal cylindrical cores (2 cm height x 1.4 cm diameter) were obtained from each lung, as previously described (16, 23). MicroCT scans (Bruker Skyscan 1172, Bruker, Kontich, Belgium) of the frozen tissue cores were performed (10µm resolution) (24) (Figure 1A). Tissue percentage and surface density of the cores were calculated. Decreasing values of surface density reflect loss of normal tissue through disease (e.g. emphysema in COPD). Terminal bronchioles (TB), the last segments of conducting airways before alveolar buds become visible, were anatomically identified, counted and measured on the microCT images.

Air-dried whole-lobe microCT

2 lungs of each group, obtained from 2 different individuals, were processed in order to obtain microCT images of entire lobes (Figure 1B). Lung lobes rather than whole lungs were scanned due to limitations on the volume of tissue that can be imaged within a single scan. Lobes were separated, cannulated and air-dried for 7 days while continuously inflating near total capacity. In case of right-sided lungs, the middle lobe was processed as part of the upper lobe. Lobes were scanned with a custom microCT set-up developed at the Ghent University Centre for X-ray Tomography (UGCT) (25), resulting in images with a resolution of approximately 140µm (Figure 1B). Additional detail on this set-up is provided in an online data supplement. Volume of the air-dried lobes and percentage of area below -950HU was obtained by StratX® Lung Analysis Platform (Neuchâtel, Switzerland) on the in-vivo inspiratory CT scans. MicroCT images of the lung lobes were used for semi-automatic airway segmentation by region growing in itk-SNAP software (26) which resulted in 3D models of the bronchial tree. These models were used for automatic tracing and reconstruction in NeuronStudio software that applies deconvolution, skeletonization and the Rayburst algorithm to result in calculation of airway numbers per generation and average inner diameter per segment (27).

Statistics

GraphPad Prism 7.0 (GraphPad, San Diego, CA, USA) was used to calculate differences between groups with Mann-Whitney or Kruskal-Wallis and Dunn's post-hoc test. The numbers of airways per generation were compared using 2-way ANOVA with Tukey's post-hoc test. Analysis of diameters with 2-way ANOVA was limited to the generations that were present in all lungs, and due to the processing of the lungs, diameters of generations 1

and 2 (1-3 in case of lobes), were not considered reliable. MicroCT results of tissue cores were analysed with mixed models using SAS 9.4 (SAS Institute Inc., Cary, NC, USA) with subject as random effect. P-values <0.05 were considered significant.

RESULTS

Numbers and diameters of airways on CT images

Characteristics of subjects of which the lungs were analyzed are presented in Table 1. All patients suffered from end-stage COPD. The subjects were not matched for sex. Bronchiectasis was present in at least 2 lobes per lung, none of the subjects had cystic bronchiectasis. Lung mass was significantly higher in the group with bronchiectasis compared to COPD (p=0.030) (Table 2). In terms of emphysema, COPD lungs showed a higher % of lung with a density below -950HU compared to control lungs (p<0.05), whereas no significant difference was found compared to COPD lungs with bronchiectasis (Table 2).

Table 1: Characteristics of patients of which an explanted lung was used for CT

	CONTROLS	**COPD**	**COPD with BRONCHIECTASIS**	*p*-value
Number	5	5	5	
Sex, M/F	4/1	1/4	5/0	**0.020**
Age, y	60 (52 - 69)	60 (56 - 62)	60 (54 - 61)	0.95
Height, cm	175 (164 - 178)	161 (157 - 169)	173 (173 - 179)*	**0.031**
BMI, kg/m^2	24.8 (24.3 - 26.9)	22.0 (21.1 - 24.3)	21.0 (15.8 - 25.7)	0.16
Pack years	NA†	30 (30 - 38)	39 (28 - 65)	0.55
Sputum production	NA	1	4	0.058
Use of azithromycin	NA	1	4	0.058
Exacerbations per year	NA	2 (1 - 3)	3 (2 - 4)	0.41
FVC				
L	NA	1.83(1.66 - 2.07)	2.48 (2.02 - 3.47)	**0.032**
% pred	NA	65 (52 - 77)	61 (48 - 79)	1.00
FEV1				
L	NA	0.83 (0.60 - 0.86)	0.90 (0.62 - 1.18)	0.73
% pred	NA	30 (28 - 34)	22 (15 - 33)	0.31

FEV1/FVC	NA	0.40 (0.31-0.52)	0.32 (0.22-0.38)	0.15
DLCO, % pred	NA	34 (32 - 45)	28 (21 - 43)	0.42
Lobes with bronchiectasis	NA	0	2 (2 - 2.5)	
Modified Reiff score	NA	NA	5 (4-6)	

Results are presented as number or median (Q1-Q3). Chi square, Kruskal-Wallis with Dunn's post hoc test (* p<0.05 compared to COPD) and Mann-Whitney tests were used. COPD: Chronic Obstructive Pulmonary Disease, M: male, F: female, y: years, BMI: Body Mass Index, NA: not applicable, FVC: Forced Vital Capacity, FEV1: Forced Expiratory Volume in 1 second, DLCO: diffusion capacity for carbon monoxide. † these donors were registered as non-smokers at the time of allocation.

Table 2: Results of ex-vivo CT scans of the explanted lungs

	CONTROL	**COPD**	**COPD with BRONCHIECTASIS**	*p*-value
Left/Right lung	0/5	4/1	3/2	
Total lung volume, L	3.5 (2.7 - 3.6)	3.1 (2.6 - 3.4)	4.2 (3.5 - 5.0)	0.10
Lung mass, g	433 (316 - 486)	266 (227 - 300)	455 (370 - 561)**	**0.030**
Lung density, g/L	125 (117 - 134)	90 (78 - 99)	105 (82 - 151)	0.061
Area below -950HU, %	17.9 (11.2 - 22.1)	44.0 (35.4 - 49.4)*	31.2 (14.4 - 47.5)	**0.027**
Airway generations, n	16 (15 - 16.0)	12 (12 - 14)*	15 (15 - 16)**	**0.011**

Results are presented as median (Q1-Q3). *p*-values obtained by Kruskal-Wallis test. Significant results found by Dunn's post hoc test are presented with symbols: * p<0.05 compared to control, ** p<0.05 compared to COPD.

More generations were counted in lungs of COPD with bronchiectasis and lungs of controls compared to COPD (both p<0.05) (Table 2, Figure 2A). The number of bifurcations was higher in COPD lungs with bronchiectasis and control lungs compared to COPD lungs (p<0.001) (Figure 2A). No difference in bifurcation numbers was present between lungs with bronchiectasis and control lungs. Interestingly, the airway diameter was not different between the groups (p=0.23) (Figure 2B), unless the widest diameter per bifurcation was considered as a measure of lack of tapering (p=0.010) (Figure 2C).

Numbers and diameters of airways on lobar microCT images

Characteristics of the patients of which lobar microCT was performed, are summarized in Table 3. Figure 3 shows airway segmentation on lower lobes of a control

lung, a COPD lung and a COPD lung with bronchiectasis. The higher resolution of these microCT scans, in comparison to the previously described CT evaluation, is reflected by the higher number of generations counted on these images (e.g. 22 and 19 versus 16 generations in lower lobes of control lung) and enables us to evaluate smaller airways in more detail. The 3D models of all lobar bronchial trees are shown in Supplement Figure E1. The % of area -950HU was similar in the upper lobes of COPD lungs with and without bronchiectasis, in contrast to the lower lobes in which the COPD lungs with bronchiectasis seem to demonstrate a lower percentage of emphysema (Table 3).

Table 3: Characteristics of subjects and results of airway segmentation on lobar microCT

	CONTROL		COPD		COPD with BRONCHIECTASIS	
Sex, M/F	M	M	M	M	M	M
Age, y	58	57	64	64	61	65
Height, cm	185	180	170	168	173	170
Weight, kg	95	80	70	62	78	60
BMI, kg/m^2	27.8	24.7	24.2	22.0	26.0	20.8
Pack years	NA*	NA*	40	40	50	40
FVC						
L	NA	NA	3.18	1.65	2.48	3.70
% pred	NA	NA	84	45	61	97
FEV1						
L	NA	NA	0.82	0.88	0.48	1.38
% pred	NA	NA	28	30	15	46
FEV1/FVC	NA	NA	0.26	0.53	0.19	0.37
DLCO, % pred	NA	NA	32	34	38	28
Lobes with bronchiectasis	NA	NA	0	0	2	3
Modified Reiff score	NA	NA	NA	NA	4	4
Right/left lung	Right	Left	Right	Left	Right	Left
UPPER LOBE†						
Volume, L	NA	NA	2.477	1.697	2.095	1.863
Area below -950HU, %	NA	NA	38	40	37	35
Generations, n	19	19	14	14	14	18
Airways, n	1478	958	324	224	602	628
LOWER LOBE						
Volume, L	NA	NA	1.625	1.317	1.329	0.847
Area below -950HU, %	NA	NA	33	38	24	23
Generations, n	22	19	19	17	20	17
Airways, n	1350	1129	364	384	683	926

Individual results are presented. COPD: Chronic Obstructive Pulmonary Disease, M: male, F: female, y: years, BMI: Body Mass Index, NA: not applicable, FVC: Forced Vital Capacity, FEV1: Forced Expiratory Volume in 1 second, DLCO: diffusion capacity for carbon monoxide. *These donors were registered as non-smokers at the time of allocation. †Sum of upper and middle lobe in case of right lung.

After segmentation, the COPD lungs with bronchiectasis showed considerably more airways in the upper and lower lobes compared to COPD lobes (Table 3, Figure 3, Figure 4A and 4B). In comparison with control lobes, both COPD lobes without as well as with bronchiectasis had lower airway numbers in upper and lower lobes. This difference with controls was clearly less pronounced in COPD lobes with bronchiectasis (Figure 4A and 4B). Throughout the first generations of the lower lobes, the diameters of COPD lungs with bronchiectasis were wider compared to COPD lungs and narrower in COPD lungs compared to control ($p=0.010$) (Figure 3, Figure 4D). This difference was not present in the upper lobes (Figure 4C) ($p=0.30$). If the airways in the lower lobes were subdivided by diameter of more or less than 2mm, the difference between COPD lungs with and without bronchiectasis was maintained, but particularly present in larger airways (>2mm), whereas the difference between COPD and control was more pronounced in the small airways (≤2mm) ($p<0.001$) (Figure 4E and Figure 4F).

Tissue characteristics and terminal bronchioles on microCT images of frozen tissue cores

Tissue percentage, surface density and number of TB per ml was significantly lower in both COPD groups compared to control (Supplement Figure E2A-C). Between tissue cores of COPD patients with or without bronchiectasis, no difference was found for these variables. Moreover, the number and diameter of TB per ml was similar between these groups (Supplement Figure E2A-D and Supplement Table E1). Taking spatial distribution into account, no difference was found in any of the groups between cores from upper versus lower lobes, neither between cores from regions with bronchiectasis and regions without bronchiectasis (Supplement Table E2).

DISCUSSION

To our knowledge, this is the first study investigating and comparing the entire bronchial tree in end-stage COPD lungs with and without bronchiectasis and the first report on microCT of large lung specimens. On the lower resolution clinical CT, we found that COPD with bronchiectasis showed little reduction in airway numbers, which was in contrast to COPD without bronchiectasis which showed extensive reduction in airway numbers, both compared to controls. When measured on the higher resolution microCT images of whole lobes, COPD with bronchiectasis showed only a moderate reduction in the large and small airways compared to controls. At the same time, the reduction in the terminal bronchioles was equal to what was found in COPD without bronchiectasis.

The finding of more airways in COPD lungs with bronchiectasis compared to COPD lungs without bronchiectasis suggests less severe emphysema at the moment of transplantation (16, 28). However, all patients had end-stage COPD, meeting the criteria for lung transplantation (21) without significant difference in forced expiratory volume in 1 second (FEV1), FEV1/FVC or diffusion capacity (DLCO). As well, the degree of emphysema measured using % of area below -950HU, a threshold commonly used to quantify emphysema on CT, or the surface density measured on microCT of tissue cores was not different between the groups. All together, these parameters indicate that our observation of higher airway counts

in lungs with bronchiectasis compared to COPD alone, could not be attributed to different disease severity.

One explanation for the higher number of airways in bronchiectasis could be a higher visibility because of increased airway diameter, as previously postulated in cystic fibrosis (29). However, our data do not fully support this hypothesis, as the number of airways in the lower lobes was significantly higher in bronchiectasis from generation 10 onwards, whereas diameters were mainly wider in the preceding generations. Furthermore, the increased airway count was not related to the presence of bronchiectasis, since upper lobes -which were less or not affected by bronchiectasis- also showed this difference.

An alternative explanation for the higher airway counts could be found in less pronounced airway loss in COPD lungs that develop bronchiectasis over time. However, it has been shown that airway loss occurs from the early stages of the disease (17, 18, 30–32), whereas bronchiectasis seems associated with more advanced stages of COPD (12, 13). Unfortunately, the use of end-stage disease lungs prevents us from drawing conclusions about early disease stages.

Even more intriguing would be to hypothesize that COPD patients with bronchiectasis have a more differentiated bronchial tree from early life onwards, which could predispose them to development of bronchiectasis rather than to loss of airways. Recently, the importance of early life events on lung structure and function is well accepted (33, 34). Variation in segmental airway branching (35), lung structure on CT (31) -both genetically determined- and reduced total airway count (18) have been suggested as risk factors for COPD. A lower total airway count is been associated with airflow limitation, independently of emphysema and smoking, which suggests that the composition of the airway tree may determine maximum attained lung function (18). Our data add to the idea that the composition of the

bronchial tree is also relevant in the development of specific phenotypes of respiratory disease. The sequence in which emphysema and bronchiectasis develop in patients with COPD is still unclear and there is obviously need for longitudinal imaging studies to answer these questions. Investigating lungs diagnosed with bronchiectasis before the development of COPD due to smoking, would also be valuable.

An important strength of the study, is the use of microCT on whole lung lobes. We found reduced airway counts in the zone between generation 10 and the (pre-)terminal bronchioles, whereas previous studies only focused on the extremities of the bronchial tree. Furthermore, this study strengthens the idea that imaging may serve as a useful biomarker in COPD, as recently highlighted in this journal (36).

This study is suffering from a number of important limitations. Firstly, the relevance of bronchiectasis in COPD has been questioned because diagnostic criteria may overestimate bronchodilation (37, 38) and higher broncho-arterial ratios have been attributed to reduced vessel size rather than increased bronchial diameters in smokers (39). Unfortunately, our approach did not allow for quantification of vessel dimensions. Second, the use of rejected lungs as healthy controls may be questioned. Fortunately, most lungs were not declined because of poor quality but because of other, non-parenchymal, reasons. Even if we assume that the intensive care period -prior to organ donation- would affect lung quality, it will not likely affect the investigated variables such as airway counts. Third, lungs in the conventional CT analysis were not matched for gender, side (left/right) or FVC. We tried to confirm our results in the lobar microCT analysis in which we could match for gender and location, but not for lung function variables. This illustrates COPD's inherent heterogeneity, even in end-stage disease. Fourth, pre-lung transplant follow up was not standardized in

patients referred from different hospitals, which precludes us from a thorough comparison of clinical characteristics. Nevertheless, patients with bronchiectasis had a distinct clinical syndrome based on the use of azithromycin and the presence of sputum. Moreover, the lungs with bronchiectasis had a modified Reiff score of at least 4, meaning that 4 or more lobes had tubular bronchiectasis.

Finally, sample sizes are low, given the limited availability of unused donor lungs and of COPD lungs with bronchiectasis. The pronounced visual differences in a limited sample size are still striking and should therefore lead to future longitudinal studies focusing on associations between COPD, bronchiectasis, emphysema and initial airway counts.

In conclusion, our study shows a bronchial tree with more airway branches in end-stage COPD lungs with bronchiectasis compared to COPD lungs without bronchiectasis, despite similar degree of emphysema and loss of terminal bronchioles. Our findings indicate that the intrinsic composition of the airway tree may predispose to certain phenotypes in COPD.

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FIGURE LEGENDS

Figure 1: Overview of used materials and methods

21 explant lungs were collected, comprised of 7 unused donor lungs, 7 COPD lungs and 7 COPD lungs with bronchiectasis. (A) Of each group, 5 lungs of 5 different individuals were prepared for freezing, CT scanning and further processed to obtain 4 tissue cores per lung for microCT. (B) Of 2 lungs per group, obtained from 2 different individuals, lobes were separated and air-dried to obtain microCT scans of the entire lobes. COPD: Chronic obstructive Pulmonary Disease, CT: computed tomography, TB: terminal bronchioles.

Figure 2: Bifurcation numbers and diameters on CT images of frozen lungs

Results of measurements on CT images of control lungs (n=5), COPD lungs (n=5) and COPD lungs with bronchiectasis (n=5). (A) Number of bifurcations per generation. (B) Diameters of airway segments per generation. (C) Widest diameter per bifurcation, lack of tapering. Results are presented as mean ± sem. The reported p-value is the result of two-way ANOVA with symbols representing significant results of Tukey's post-hoc test. § $p<0.001$, §§ $p<0.05$ for COPD compared to control. * $p<0.001$, ** $p<0.05$ for COPD with bronchiectasis compared to control. $^{\#}$ $p<0.001$, $^{\#\#}$ $p<0.01$ and $^{\#\#\#}$ $p<0.05$ for COPD with bronchiectasis compared to COPD.

Figure 3: Airway segmentation of lower lobes on microCT images

Lower lobes of a (A) control lung, (B) COPD lung and (C) COPD lung with bronchiectasis. The upper panels show the bronchial tree after segmentation, projected on the contours of the lobe. The lower panels show the same bronchial tree after automated annotation with each color representing a different generation within the lobe.

Figure 4: Airway numbers and diameters as calculated on segmented airway models obtained by microCT of air-dried lobes

Results of measurements on microCT of control lobes (n=2 upper and n=2 lower lobes), COPD lobes (n=2 upper and n=2 lower lobes) and COPD lobes with bronchiectasis (n=2 upper and n=2 lower lobes). Graphs A and B show numbers of airways per generation and graphs C and D show average diameters of airway segments per generation in upper (A and C) and lower (B and D) lobes. In graphs E and F airways in the lower lobes were separated based on diameter ≤2mm and >2mm respectively. Results are presented as mean ± sem. The reported p-value is the result of two-way ANOVA with symbols representing significant results of Tukey's post-hoc test. § $p<0.001$, §§ $p<0.01$ COPD and §§§ $p<0.05$ for COPD compared to control. * $p<0.001$, ** $p<0.01$ and *** $p<0.05$ for COPD with bronchiectasis compared to control. $^{\#}$ $p<0.001$, $^{\#\#}$ $p<0.01$ for COPD with bronchiectasis compared to COPD.

Figure 1

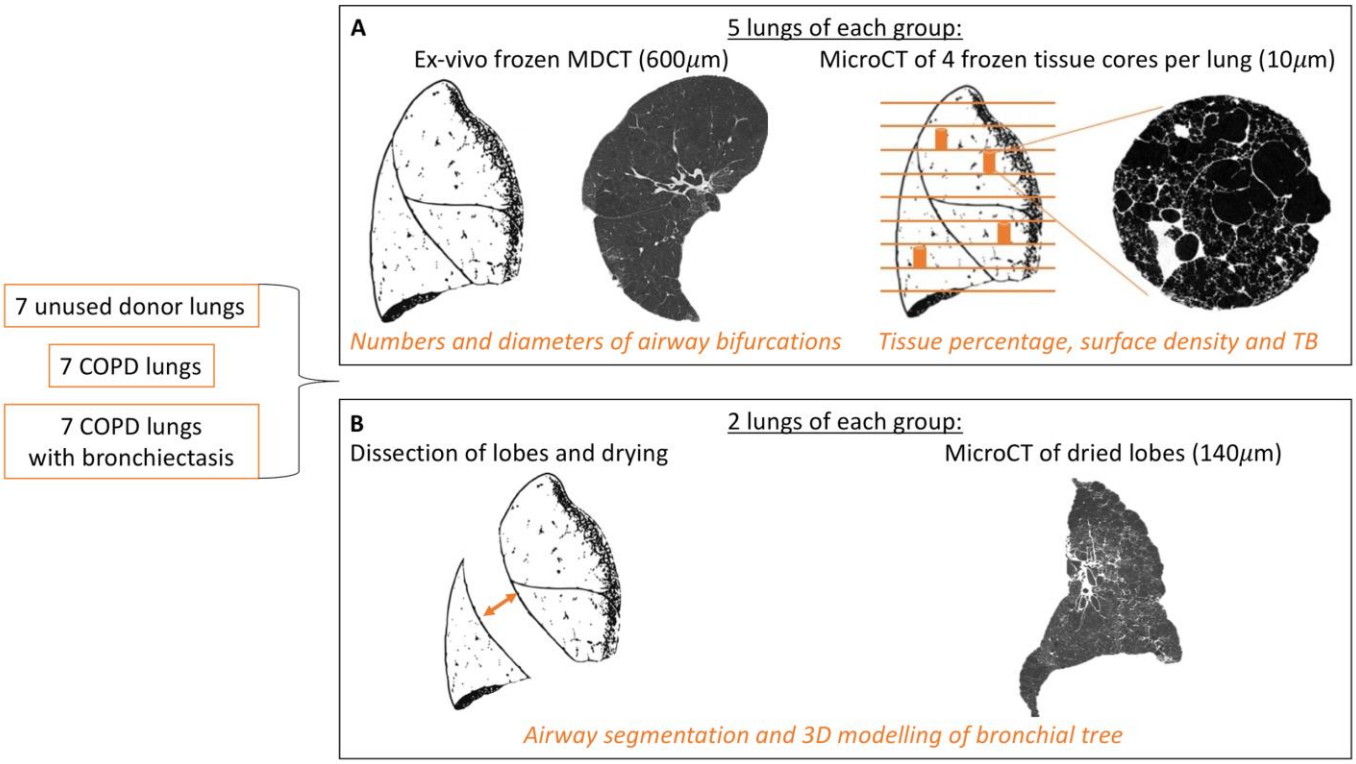
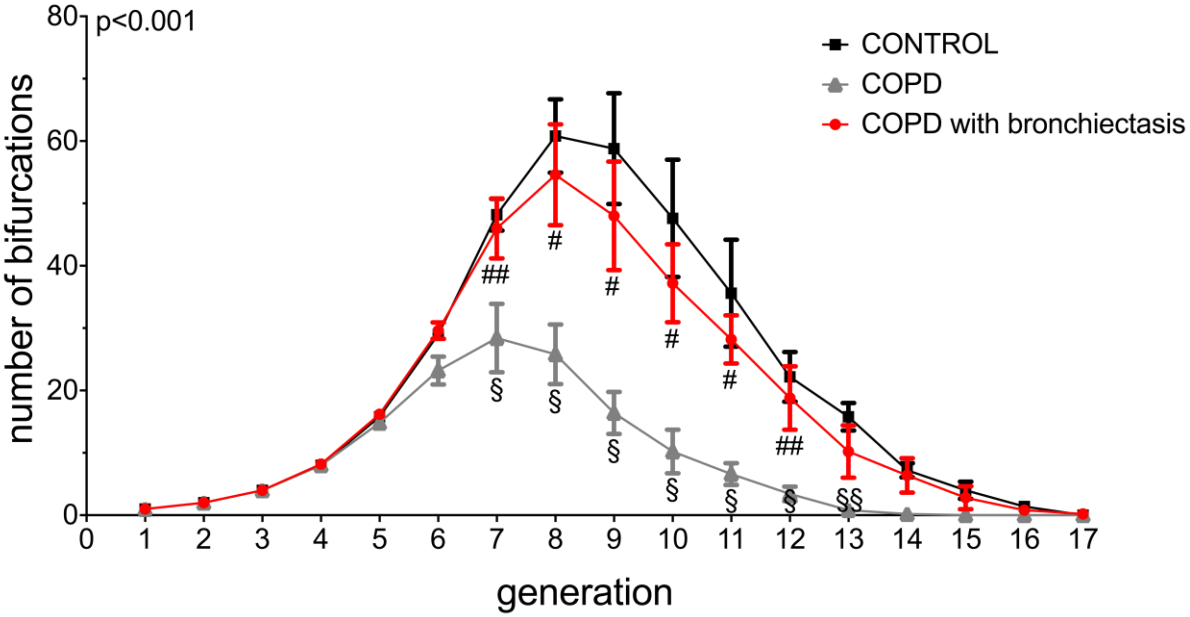
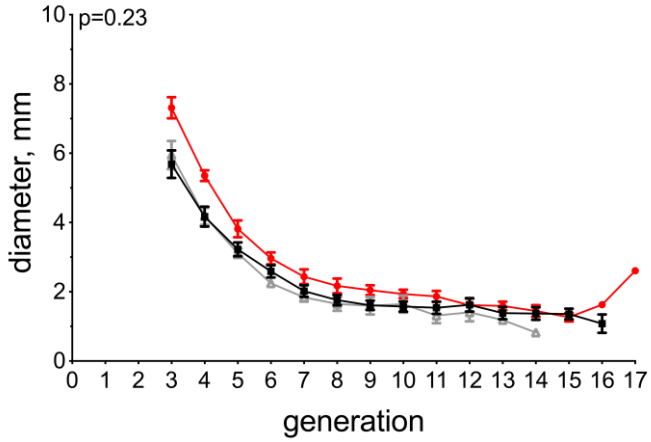

7 unused donor lungs
7 COPD lungs
7 COPD lungs with bronchiectasis
A
5 lungs of each group:
Ex-vivo frozen MDCT (600μm)
MicroCT of 4 frozen tissue cores per lung (10μm)
Numbers and diameters of airway bifurcations
Tissue percentage, surface density and TB
B
2 lungs of each group:
Dissection of lobes and drying
MicroCT of dried lobes (140μm)
Airway segmentation and 3D modelling of bronchial tree

Figure 2

A


p<0.001
CONTROL
COPD
COPD with bronchiectasis
number of bifurcations
generation

B


p=0.23
diameter, mm
generation

C

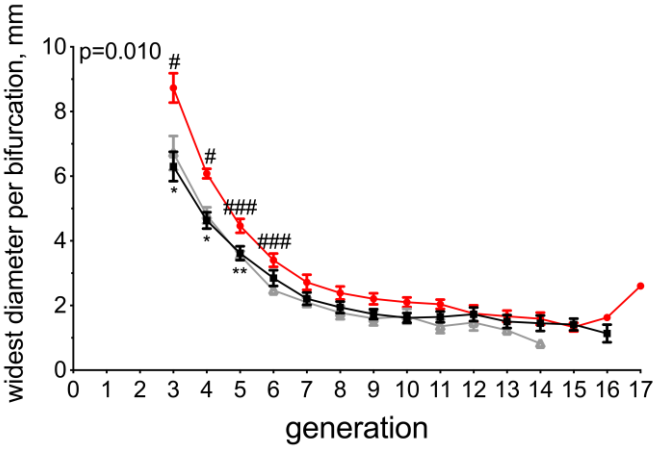

p=0.010
widest diameter per bifurcation, mm
generation

Figure 3

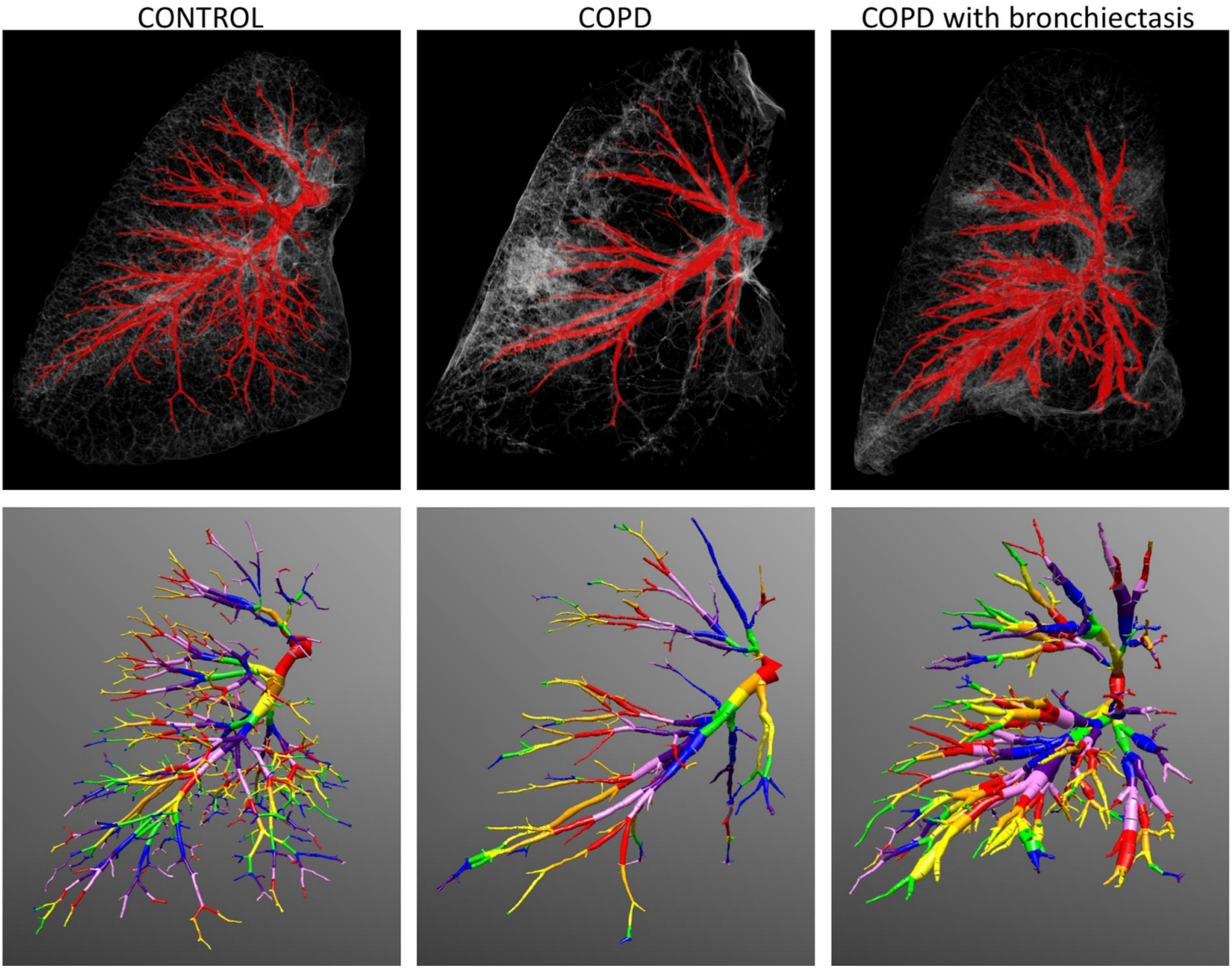
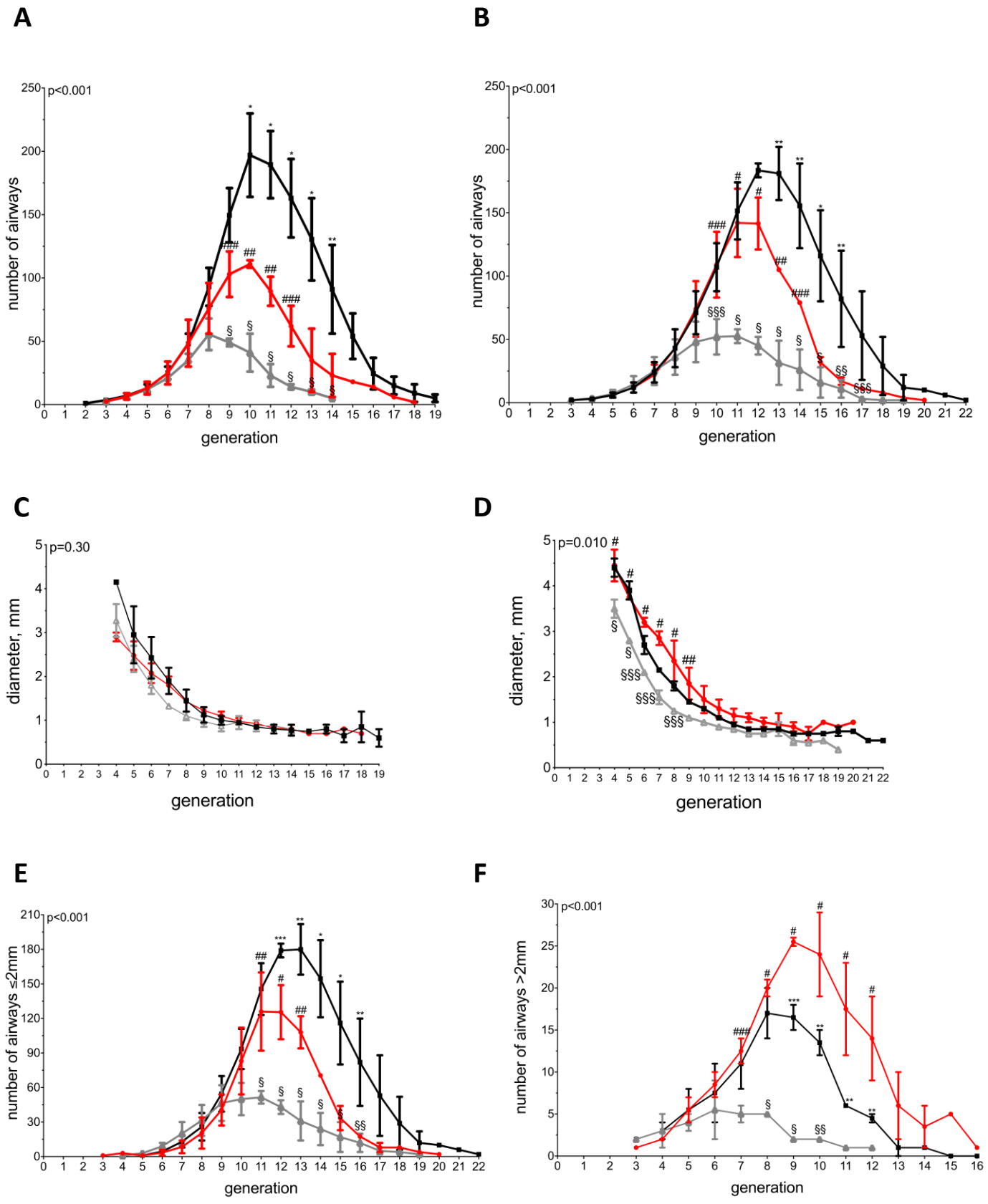


Figure 4


A
B
C
D
E
F
p<0.001
p=0.30
p=0.010
number of airways
number of airways ≤2mm
number of airways >2mm
diameter, mm
generation

DATA SUPPLEMENT

Materials and methods: Air-dried whole-lobe microCT

These dried lobes were scanned with HECTOR, a custom designed microCT set-up developed at the Ghent University Centre for X-ray Tomography (UGCT) (25). The setup consisted of an open-type X-ray tube with directional target (X-RAY WorX, Garbsen, Germany) and a PerkinElmer amorphous-Si flat panel detector (2048x2048 pixels of 200^2 μm^2 each) with a CsI scintillator. The X-ray tube and flat panel detector were static, while the lobe was rotated over 360° and two thousand projection images were recorded. The tube was operated at 80keV and 200μA target current, and the reconstruction was performed with Octopus Reconstruction (XRE, Ghent, Belgium) (1) resulting in datasets of around 2000×2000×1500 voxels with a resolution of approximately 140µm (Figure 1B).

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Figure E1: Segmented airway models of all lobes

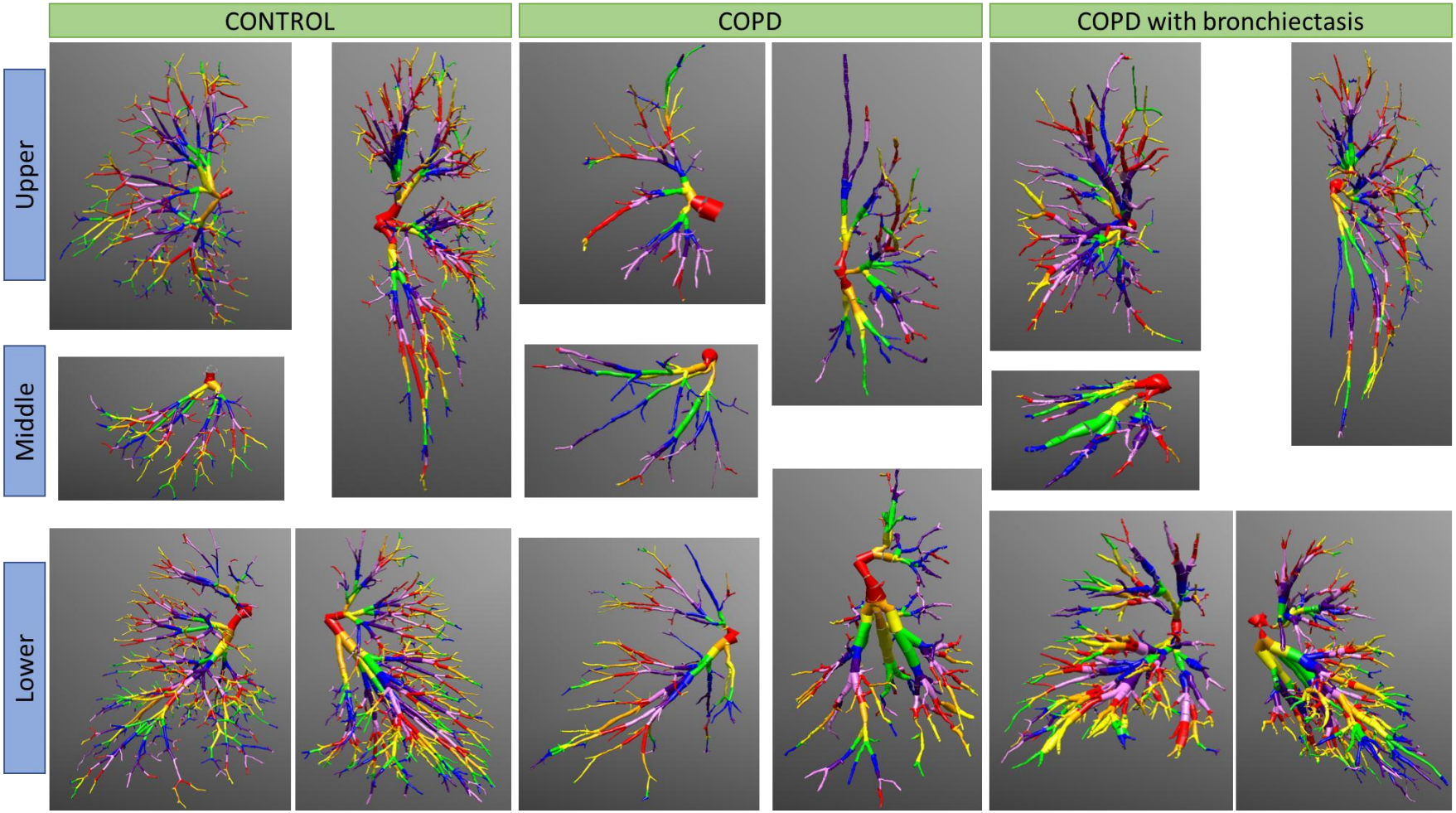
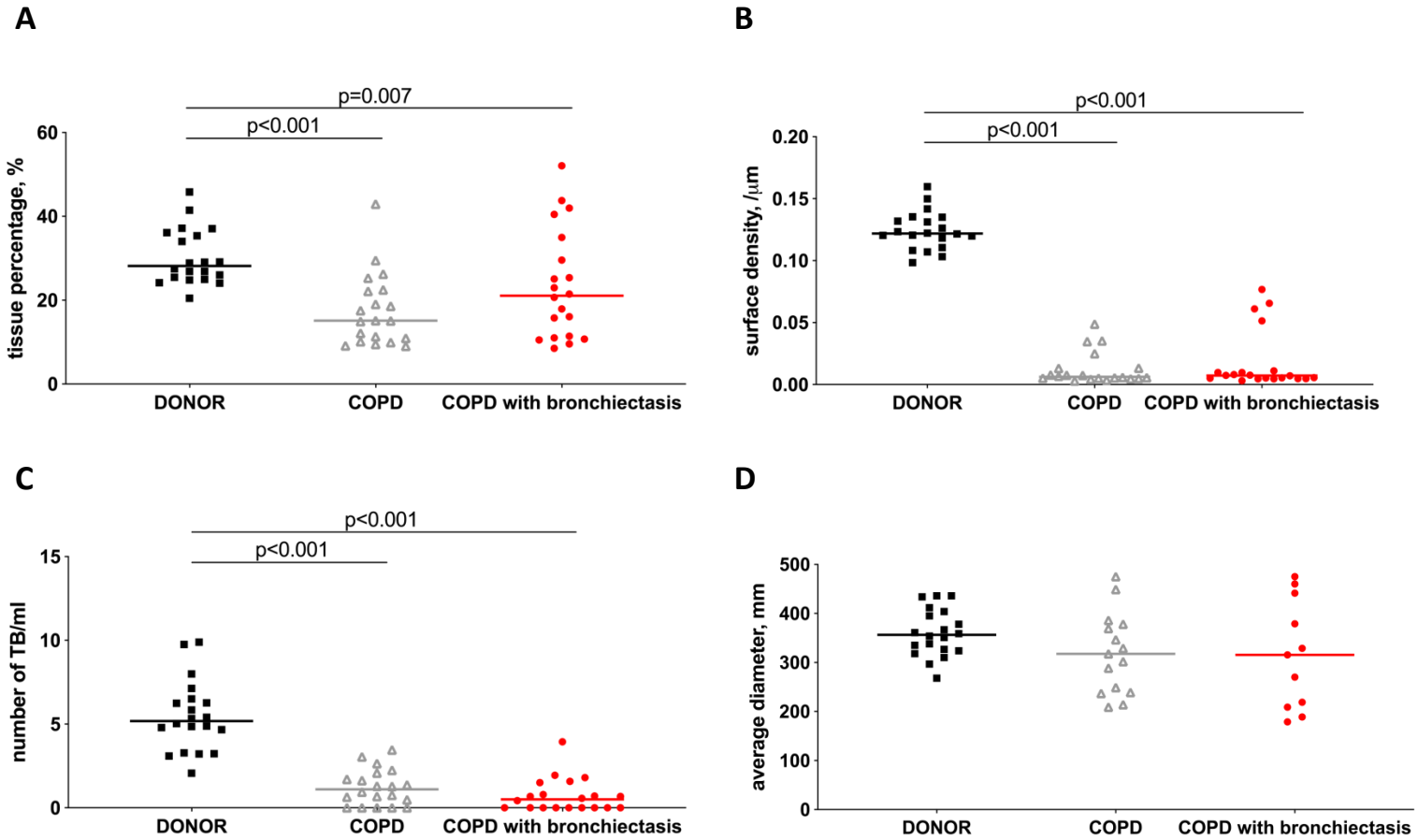


Figure E2: MicroCT analysis of tissue cores



Results of measurements on images of tissue core microCT (n=4 per lung, 5 lungs per group) of control lungs, COPD lungs and COPD lungs with bronchiectasis.
(A) Tissue percentage, (B) surface density, (C) number of terminal bronchioles per ml and (D) average diameter of visible terminal bronchioles. *p*-values were obtained by mixed models applied on individual core data with lung as random effect, comparing with the control group. The same model was performed for comparison of each group to COPD with bronchiectasis, no significant difference was found between COPD without versus with bronchiectasis.

Table E1: Cause of donor death and reason for lung decline

Donor	Cause of death	Reason for rejection
1	Cardiac arrest	Kidney tumor
2	CVA	Recipient died
3	CVA	Suspicion of beginning fibrosis in contralateral lung
4	CVA	Emboli
5	CVA	Emboli
6	CVA	Suspicion of infection in contralateral lung
7	CVA	Emboli

CVA: cerebrovascular accident

Table E2: Results of tissue core microCT measurements

	COPD LUNGS			**COPD LUNGS with BRONCHIECTASIS**		
	estimate	lower-upper limit	*p*-value	estimate	lower-upper limit	*p*-value
Tissue percentage, %	-11.32	-17.33 - -5.30	<0.001	-8.70	-14.94 - -2.47	0.007
Surface density, /µm	-0.11	-0.12 - -0.098	<0.001	-0.11	-0.12 - -0.10	<0.001
Visible TB, n/ml	-4.14	-5.050 - -3.22	<0.001	-4.99	-5.93 - -4.046	<0.001
Diameter of TB, µm	-34.75	-87.70 - 18.20	0.19	-58.63	-118.75 - 1.49	0.056

Results are presented as estimates with upper and lower limits. *p*-values were obtained by mixed models applied on individual core data with lung as random effect, comparing with the control group. The same model was performed for comparison to COPD with bronchiectasis, no significant difference was found between COPD without versus with bronchiectasis.

Table E3: MicroCT results of upper versus lower lobes and bronchiectatic versus non-bronchiectatis regions

	Upper lobe	**Lower lobe**	*p*-value
CONTROL cores, n	15	5	
Tissue percentage, %	26.88 (24.99 - 35.35)	29.13 (25.78 - 41.46)	0.43
Surface density, /µm	0.12 (0.12 - 0.13)	0.12 (0.11 - 0.15)	0.93
TB/ml, n	5.028 (3.28 - 6.27)	5.84 (3.94 - 8.88)	0.60
COPD cores, n	10	10	
Tissue percentage, %	11.72 (9.80 - 18.66)	20.59 (13.73 - 26.33)	0.11
Surface density, /µm	0.0053 (0.0041 - 0.012)	0.0077 (0.0056 - 0.019)	0.11
TB/ml, n	0.38 (0 - 1.84)	1.33 (0.67 - 2.22)	0.18
COPD with bronchiectasis cores, n	8	12	
Tissue percentage, %	24.01 (20.87 - 49.54)	16.87 (10.82 - 32.58)	0.25
Surface density, /µm	0.0079 (0.0060 - 0.048)	0.0052 (0.0046 - 0.041)	0.20
TB/ml, n	0.28 (0 - 0.77)	0.56 (0 - 1.57)	0.78
	Non-bronchiectatic region	**Bronchiectatic region**	
COPD with bronchiectasis cores, n	10	10	
Tissue percentage, %	24.01 (11.27 - 45.85)	20.83 (10.66 - 27.77)	0.49
Surface density, /µm	0.0077 (0.0052 - 0.024)	0.0066 (0.0047 - 0.052)	0.68
TB/ml, n	0 (0 - 0.82)	0.097 (0.033 - 1.58)	0.088

Results are presented as n or median (Q1-Q3). P-values were obtained by Mann-Whitney test