ORIGINAL ARTICLE

Small Airway Disease in Pre-Chronic Obstructive Pulmonary Disease with Emphysema

A Cross-Sectional Study

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Abstract

Rationale: Small airway disease is an important pathophysiological feature of chronic obstructive pulmonary disease (COPD). Recently, "pre-COPD" has been put forward as a potential precursor stage of COPD that is defined by abnormal spirometry findings or significant emphysema on computed tomography (CT) in the absence of airflow obstruction.

Objective: To determine the degree and nature of (small) airway disease in pre-COPD using microCT in a cohort of explant lobes/lungs.

Methods: We collected whole lungs/lung lobes from patients with emphysematous pre-COPD ($n = 10$); Global Initiative for Chronic Obstructive Lung Disease (GOLD) stage I ($n = 6$), II ($n = 6$), and III/IV ($n = 7$) COPD; and controls ($n = 10$), which were analyzed using CT and microCT. The degree of emphysema and the number and morphology of small airways were compared between groups, and further correlations were investigated with physiologic measures. Airway and parenchymal pathology was also validated with histopathology.

Measurements and Main Results: The numbers of transitional bronchioles and terminal bronchioles per milliliter of lung were significantly lower in pre-COPD and GOLD stages I, II, and III/IV COPD compared with controls. In addition, the number of alveolar attachments of the transitional bronchioles and terminal bronchioles was also lower in pre-COPD and all COPD groups compared with controls. We did not find any differences between the pre-COPD and COPD groups in CT or microCT measures. The percentage of emphysema on CT showed the strongest correlation with the number of small airways in the COPD groups. Histopathology showed an increase in the mean chord length and a decrease in alveolar surface density in pre-COPD and all GOLD COPD stages compared with controls.

Conclusions: Lungs of patients with emphysematous pre-COPD already show fewer small airways and airway remodeling even in the absence of physiologic airway obstruction.

Keywords: COPD; small airways; emphysema

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At a Glance Commentary

Scientific Knowledge on the Subject: Small airway loss and remodeling is important in the pathophysiology of chronic obstructive pulmonary disease.

What This Study Adds to the Field: Our study showed significant airway loss and remodeling in patients with pre–chronic obstructive pulmonary disease.

A recent *Lancet* Commission pointed out the importance of chronic obstructive pulmonary disease (COPD) and the need for fundamental changes in the way we think about this disease (1). COPD is a highly prevalent disease, with more than 384 million estimated cases globally. It is the third leading cause of death in 2019 according to the World Health Organization. COPD is characterized by persistent respiratory symptoms and airflow limitation due to airway and/or alveolar abnormalities that are usually caused by significant exposure to noxious particles or gases, most typically cigarette smoke (2).

COPD presents with increased alveolar airspace size (i.e., emphysema) and small airways and terminal bronchioles (TBs) and/or transitional bronchioles (TrBs) that typically obstruct and disappear (3, 4). Using micro–computed tomography (microCT), it is now possible to analyze and quantify these morphological small airways and parenchymal changes. Studies have demonstrated a 40–50% reduction in small airway count in mild (Global Initiative for Chronic Obstructive Lung Disease [GOLD] stage I) and moderate (GOLD stage II) COPD and a 70–90% reduction in severe COPD (GOLD stage IV) (3–5), as well as a reduction of alveolar attachments and narrowing of pre-TBs in severe COPD (6). It is the current belief that this reduction in small airways leads to parenchymal alterations.

The diagnosis of COPD currently requires poorly reversible airflow limitation, defined as a postbronchodilator FEV_1/FVC ratio lower than 0.7. However, significant lung damage may have already occurred before abnormalities in lung function are evident. Indeed, CT-detected small airway abnormalities, airway wall thickening, and emphysema may be helpful in identifying patients at increased risk for disease progression to COPD (7, 8). Furthermore, data from large cohorts suggest that approximately 35–50% of patients with preserved-ratio impaired spirometry (PRISm), defined as reduced FEV_1 with a normal FEV_1/FVC ratio, experience a progression to classically defined COPD (9, 10). More recently, the term "pre-COPD" has therefore been proposed to refer to these individuals in whom spirometry is unable to detect airflow obstruction but who are at increased risk of subsequently developing COPD based on their symptoms (dyspnea, cough, phlegm) and functional (i.e., low FEV_1 and/or low D_{LCO} without airflow obstruction) or structural abnormalities (i.e., radiologic evidence of emphysema) (8, 11, 12). The (airway) pathophysiology of these patients with so-called pre-COPD has not been investigated. Elucidating the morphologic changes could significantly assist in understanding why these cases progress to COPD and could provide new insights in the early pathophysiologic processes of COPD. We hypothesize that lungs of patients with pre-COPD will already show features of small airway disease. Using microCT, a cohort of explant lobes/lungs is thoroughly analyzed to determine the degree and nature of (small) airway disease in pre-COPD. Some of the results of these studies have been previously reported in the form of an abstract (European Congress of Radiology, not published).

Methods

Patient Selection and Characterization

Lungs or lung lobes from patients undergoing lung resection for primary lung cancer without evidence of metastasis (control, $n = 10$; pre-COPD, $n = 10$; GOLD stage I, $n = 6$; GOLD stage II, $n = 6$), patients with severe COPD undergoing lung transplantation (GOLD stage III, $n = 1$; GOLD stage IV, $n = 5$), or autopsy specimens (GOLD stage III, $n = 1$) were prospectively collected from May 2021 until December 2022 at Antwerp University Hospital. This study has been approved by the ethical committee of Antwerp University Hospital, and all patients provided written informed consent (approval ID, EDGE001693). All lungs were from patients with European ancestry except for two patients of Asian ancestry in the pre-COPD group. Pulmonary function testing was performed between 1 week and 3 months before surgery according to the American Thoracic Society/European Respiratory Society guidelines. Based on smoking status, pulmonary function, and chest CT, patients were subdivided into control (ever- and never-smokers, FEV_1 and FVC >80% predicted, FEV_1/FVC ratio >0.70, and <5% emphysema on CT), pre-COPD (smoking history >10 pack-years, FEV_1/FVC ratio >0.70, FEV_1 <80% predicted, and/or >5% emphysema on CT [11]), GOLD stage I (smoking history >10 pack-years, FEV_1/FVC ratio <0.70, FEV_1 >80% predicted), GOLD stage II (smoking history >10 pack-years, FEV_1/FVC ratio <0.70, FEV_1 <80% and >50% predicted), and GOLD stage III/IV (smoking history >10 pack-years, FEV_1/FVC ratio <0.70, FEV_1 <50% predicted) groups. Lungs that showed features compatible with another chronic respiratory disease (e.g., fibrosis on CT; $n = 2$) and never-smokers with emphysema >5% on CT ($n = 3$) were *a priori* excluded.

Lung Processing

Immediately following lobar/lung resection, the lungs were collected. After macroscopic investigation and resection of the tumor by the pathologist, the lungs were transported to the laboratory. In the laboratory, the bronchi were cannulated, air leaks were plugged, and subsequently the lung was air-inflated using a compressed air source at a maximum pressure of 30 cm H_2O and fixed in liquid nitrogen fumes after gentle deflation to 10 cm H_2O, allowing storage at −80°C, as described previously (Figure 1A) (13, 14). The inflated specimen was then subjected to *ex vivo* CT scanning (Figure 1D) while surrounded with dry ice in a Styrofoam box (GE Healthcare Revolution). This CT scan was used for further scoring (as detailed later).

Subsequently, the lung was sliced into 2-cm-thick slices (Figure 1B), after which cores with a diameter between 14 and 20 mm were systematically extracted using a core

This article has a related editorial.

This article has an online supplement, which is accessible from this issue's table of contents at www.atsjournals.org.

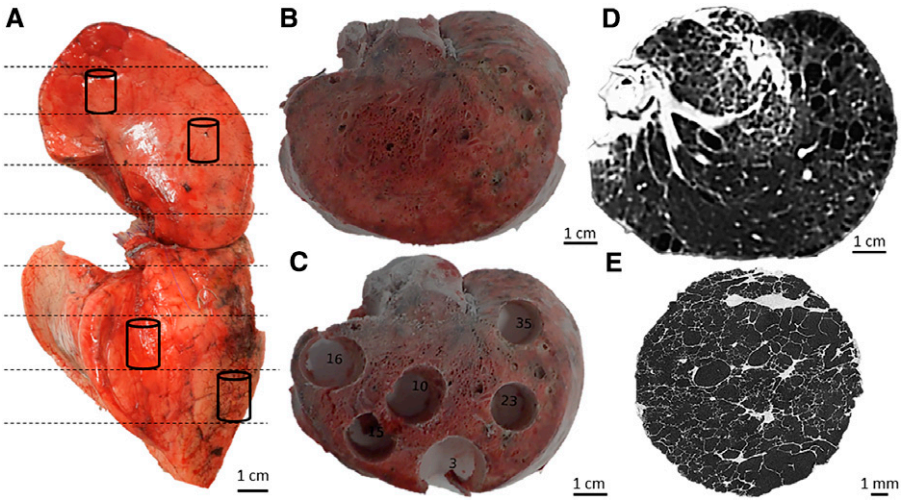

Figure 1. Study design illustrated with a lung from a patient with pre–chronic obstructive pulmonary disease (COPD). (*A*) Explant lungs/lung lobes were collected during surgery, cannulated, inflated with air, and frozen solid in liquid nitrogen vapors. (*B*) Following computed tomography (CT) and microCT scanning, the specimens were sectioned in 2-cm lung slices and (*C*) cores were randomly taken from these slices, with the location of every sample carefully tracked. (*D*) Representative *ex vivo* CT image of a pre–COPD lung (*D*) that was used to assess the extent of emphysema. (*E*) Four random cores per lung were selected (two from the upper lung areas and two from the lower lung areas) for microCT analysis to characterize transitional bronchiole numbers per milliliter of lung and airway morphology.

bore (Figure 1C). Per lung/lung lobe, four cores distant from the tumor site were randomly selected. To account for regional disease differences, two of these four cores were randomly derived from slices from the upper half of the lung/lobe and the other two cores were randomly selected from slices derived from the lower half of the lung. These cores were scanned using microCT (FlexCT; TESCAN UniTOM XL) (15) at a resolution of 15 μm while keeping the core frozen (Figure 1E).

CT Scoring

The following five features were assessed on explant CT: emphysema, bronchiectasis, bronchial wall thickness, mucus plugging, and tree-in-bud changes and/or centrilobular nodules. Scoring was performed by an experienced chest radiologist (C.M.) who was blinded to clinical status and further validated by a second experienced chest radiologist (J.J.). The CT features were scored visually on 1-mm-thick slices at 10-mm intervals; the specimens did not include patient information. Between 7 and 29 slices per lobe were evaluated depending on the size of the lung; in transplant lungs, all lobes were assessed. More information is provided in the online supplement.

MicroCT Analysis

Each lung core was used to visually quantify the number of TrBs per milliliter of lung and surface density as a measure of emphysema using CTAn software (Bruker); lower values represent more emphysema, which has been validated previously with the mean linear intercept (13). TrBs were identified by following the conducting airways until the airway wall was lost and alveolar buds were visually discernible. The parent airway was subsequently identified as a TB. Three to five airways were randomly selected (if available) per tissue core to further assess airway morphometry, resulting in 12–20 airways per lung that were analyzed in more detail. Measures were performed in the middle of the airway segment (TB, TrB) or at the end of the airway in case of TrBs. Our main analysis focused on the measures at the end of the TrBs. Cross-sectional microCT images of each of these airways perpendicular to the center line of their lumens were reconstructed at that point using DATAVIEWER (Bruker), and the wall thickness, minimal and maximal airway diameters, and number of alveolar attachments to their wall were manually measured with FIJI (16). The ratio of the minimal and maximal airway diameters was also calculated.

Histology

To further demonstrate the presence of emphysema or small airway disease, a portion of a core was fixed in 4% paraformaldehyde, dehydrated, and embedded in paraffin. Subsequently, a vibratome was used to create 8-μm slices, which were stained with hematoxylin and eosin. More details on the quantitative morphometry to measure chord length and alveolar surface density are provided in the online supplement (17, 18).

Statistics

All results are displayed as mean ± standard deviation or median (IQR) where appropriate. Airway measures are averaged over the entire lung, resulting in one value for each parameter per lung, unless otherwise indicated. Normality of the variables was assessed with the Shapiro-Wilk test. Comparison of the CT and microCT measures between groups was done using one-way ANOVA in combination with Tukey *post hoc* testing to assess significance compared with controls. Correlation analysis was performed with the Spearman test. A P value <0.05 was considered significant. Analysis was performed with Graph Pad Prism 9.0. To assess the robustness of our main findings, a linear mixed effect model with random intercept was constructed, correcting for covariates including patient age, sex, and smoking history using the lme4 package in R (R Foundation for Statistical Computing), with the findings averaged over the entire lung or on a per-sample basis with subject as a random effect variable.

Results

Patient Population

A total of 39 lungs/lung lobes were included in the study. Detailed patient characteristics are shown in Table 1. Patients with pre-COPD were classified as such based on spirometry compatible with PRISm ($n=3$) or CT evidence of significant (>5%) emphysema ($n=7$). Because all patients with PRISm also showed >5% emphysema, the PRISm and pre-COPD group were analyzed together. Seven patients with pre-COPD also showed low diffusion (<80% predicted).

As expected, patients with pre-COPD showed a FEV_1/FVC ratio comparable to that in controls, whereas the FEV_1/FVC ratio was decreased in patients with GOLD stage I COPD ($P<0.0001$) compared with control

Table 1. Patient Characteristics

Characteristic	Control	Pre-COPD	GOLD I	GOLD II	GOLD III/IV
Number of patients	10	10	6	6	7
Age, yr	65 ± 10	66 ± 7	64 ± 7	67 ± 6	63 ± 4
BMI, kg/m^2	26.1 ± 4.1	25.0 ± 3.0	22.4 ± 3.3	23.5 ± 2.5	22.1 ± 3.0
Sex (M//F)	4//6	5//5	3//3	5//1	0//7
Ever-smoker	5	10	6	6	7
>5% CT emphysema	0	10	5	6	7
Smoking, pack-years	37 ± 29	38 ± 17	33 ± 12	40 ± 8	35 ± 10
Tissue					
LUL	2	5	2	1	0
RUL	2	2	2	4	0
LLL	1	0	1	1	0
RLL	5	3	1	0	0
Left lung	0	0	0	0	3
Right lung	0	0	0	0	4
FEV_1, L	2.72 ± 0.84	2.60 ± 0.94	2.60 ± 0.60	2.08 ± 0.26	0.56 ± 0.17
FEV_1, % predicted	98 ± 13	93 ± 20	92 ± 11	70 ± 9	24 ± 8
FVC, L	3.41 ± 1.24	3.12 ± 1.11	4.10 ± 1.14	3.70 ± 0.64	1.89 ± 0.52
FVC, % predicted	98 ± 14	90 ± 19	113 ± 16	96 ± 17	65 ± 19
FEV_1/FVC ratio	0.80 ± 0.06	0.79 ± 0.06	0.64 ± 0.03	0.57 ± 0.08	0.29 ± 0.02
D_{LCO}, % predicted	100 ± 14	81 ± 16	74 ± 21	75 ± 15	36 ± 8
TLC, % predicted	102 ± 10	104 ± 20	119 ± 13	110 ± 10	144 ± 14
RV, % predicted	112 ± 22	128 ± 37	134 ± 27	137 ± 38	237 ± 22

Definition of abbreviations: COPD = chronic obstructive pulmonary disease; GOLD = Global Initiative for Chronic Obstructive Lung Disease; LLL = left lower lung; LUL = left upper lobe; RLL = right lower lung; RUL = right upper lobe; RV = residual volume; TLC = total lung capacity. Data are presented as mean ± SD where applicable.

subjects and patients with pre-COPD. In addition, diffusion capacity was decreased in patients with pre-COPD and GOLD stage I COPD compared with controls ($P = 0.033$ and $P = 0.011$), without a difference between pre-COPD and GOLD stage I ($P = 0.49$). An overview of the CT and microCT features in the different study groups is shown in Figure 2 (control, Figure 2A; pre-COPD, Figure 2B; GOLD stage I, Figure 2C; GOLD stage II, Figure 2D; GOLD stage III/IV, Figure 2E)

Radiologic Assessment of *ex Vivo* Specimen CT Scans

The percentage of emphysema was higher in the pre-COPD ($P = 0.011$), GOLD stage I ($P = 0.017$), GOLD stage II ($P = 0.008$), and GOLD stage III/IV ($P < 0.0001$) groups compared with the control group. There was no difference between the pre-COPD and GOLD stage I groups.

The other parameters (bronchiectasis, airway wall thickening, mucus plugging, and tree-in-bud changes) on *ex vivo* CT did not differ between the groups (Table 2). There was a correlation between the two radiologic observers regarding the degree of emphysema ($R = 0.87$) and bronchiectasis ($R = 0.44$), whereas airway wall thickening ($R = 0.22$), mucus plugging ($R = 0.17$), and tree-in-bud changes ($R = 0.15$) were not associated.

Airway and Parenchymal Morphology

An overview of the microCT measures is shown in Table 3, and the respective effect sizes are shown in Table E1 in the online supplement.

The pre-COPD group showed a 35% reduction in the number of TBs compared to controls. The GOLD stage I, stage II, and stage III/IV groups also showed significantly lower numbers of TBs per milliliter, with 32%, 53%, and 95% fewer TBs, respectively, compared with control (Figure 3A).

Compared with the control group, there was a 45% reduction in the number of TrBs in the pre-COPD group ($P < 0.0001$), a 39% decrease in the GOLD stage I group ($P = 0.008$), a 56% reduction in the GOLD stage II group ($P < 0.0001$), and a 94% reduction in the GOLD stage III/IV group ($P < 0.001$) (Figure 3B).

Regarding the surface density, a measure of emphysema, lower values were found in pre-COPD ($P = 0.0002$), GOLD stage I ($P = 0.013$), GOLD stage II ($P = 0.0006$), and GOLD stage III/IV ($P < 0.0001$) groups compared with controls, indicative of an increase in the distance between the alveolar airspaces (i.e., emphysema) (Figure 3E).

The number of alveolar attachments at the levels of the TBs and TrBs was also lower in pre-COPD, GOLD stage I, GOLD stage II, and GOLD stage III/IV groups compared with controls (Figure 3C). The ratio of minimal to maximal airway luminal diameter was also different across groups, with mainly lower ratios at the end of the TrBs in the pre-COPD and COPD groups compared with controls (Figure 3D). Airway wall thickness was also different among groups at the levels of the TrBs and TBs, specifically with increased airway wall thickness in the pre-COPD group compared with controls in the middle of the TBs and at the end of the TrBs.

A regression model adjusting for age, sex, and smoking history confirmed the robustness of the observed differences in the different COPD stages (control, pre-COPD, GOLD stage I, GOLD stage II, and GOLD stage III/IV) based on averages over the entire lung or the core level (Table E2).

The reproducibility of the microCT measures was formally assessed on 10 random tissue cores (control, pre-COPD, and GOLD stages I, II, and IV; $n = 2$ each) and showed excellent correlation (alveolar attachments, $R = 0.87$, $P = 0.0011$; wall thickness, $R = 0.94$, $P < 0.0001$) and minimal to maximal airway ratio ($R = 0.94$, $P < 0.0001$) at the end of the TrBs (Figure E1).

Comparison between Control Never-Smokers and Ever-Smokers

In our control group ($n = 10$), there were five never-smokers and five ever-smokers. There

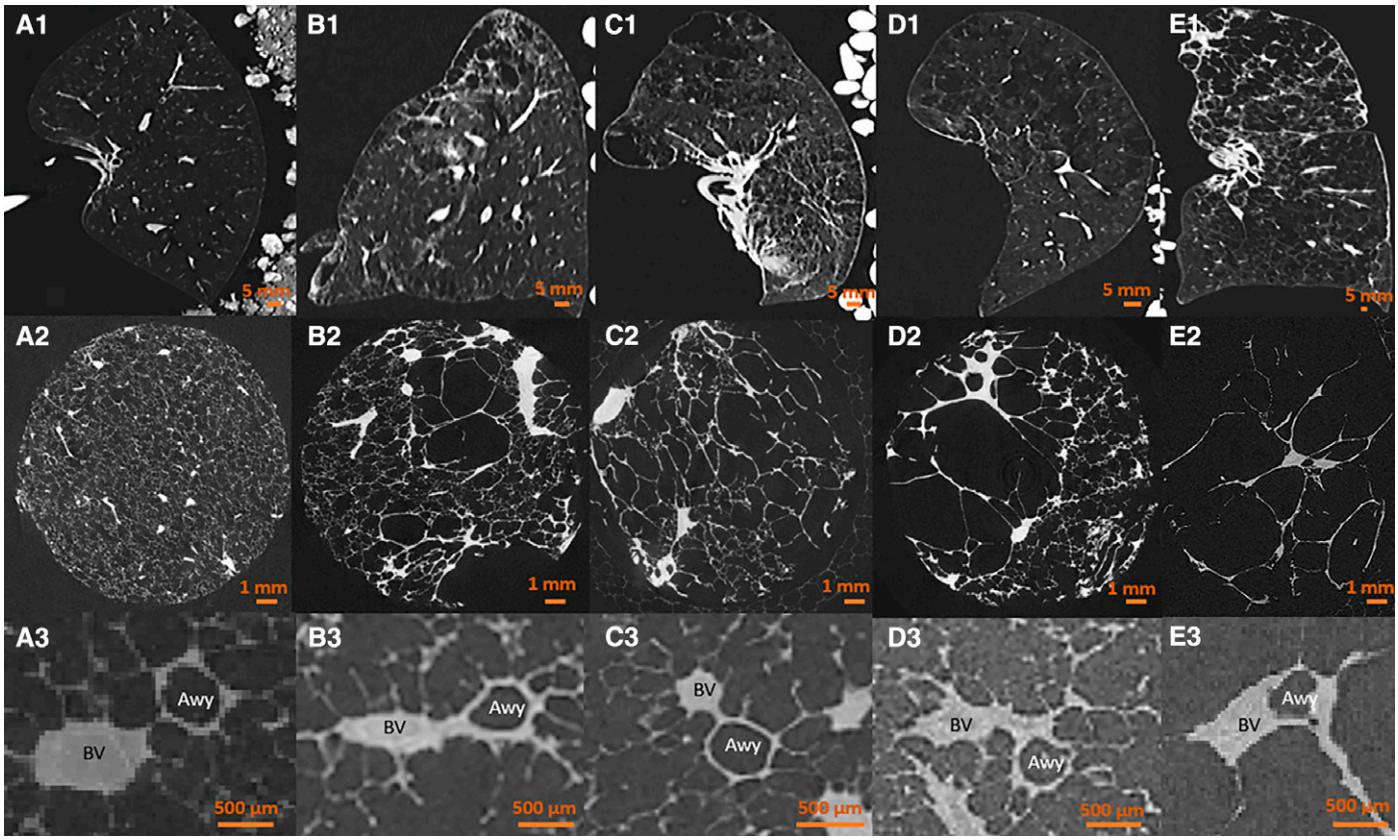

Figure 2. Overview of the computed tomography and micro–computed tomography presentation of the different study groups (control, *A*; pre–chronic obstructive pulmonary disease [COPD], *B*; Global Initiative for Chronic Obstructive Lung Disease [GOLD] stage I, *C*; GOLD stage II, *D*; GOLD stage III/IV, *E*) demonstrating visible emphysema beginning with the pre-COPD group and advancing with more advanced GOLD stage. The airways are shown with their accompanying blood vessels showing a more irregular lumen and fewer alveolar attachments with more severe COPD. Awy = airway; BV = blood vessel.

were no differences in pulmonary function parameters in ever- versus never-smokers. The number of TrBs per milliliter ($P = 0.67$), number of alveolar attachments ($P = 0.54$), airway wall thickness ($P = 0.78$), and ratio of minimal to maximal airway diameter ($P = 0.40$) at the ends of the TrBs were not different between ever- and never-smokers.

Associations between Small Airway Counts and Physiological and Radiologic Measures

We also sought to determine the associations between the number of small airways and clinical variables. The percent predicted values were used because these already correct for patient age, sex, and height. The numbers of TrBs per milliliter were associated with FEV_1 ($R = 0.66$, $P < 0.0001$), FVC ($R = 0.36$, $P = 0.026$), FEV_1/FVC ratio ($R = 0.73$, $P < 0.0001$), total lung capacity (TLC) ($R = -0.62$, $P < 0.0001$), D_{LCO} ($R = 0.73$, $P < 0.0001$), and residual volume (RV) ($R = -0.66$, $P = 0.0014$) (Figures E2A and E2B). The strongest correlations with the number of TrBs per milliliter were found with the percentage of emphysema on CT

Table 2. Summary of *ex vivo* Computed Tomography Scoring

	Control	Pre-COPD	GOLD I	GOLD II	GOLD III/IV	Correlation (*R*) with Second Observer
Emphysema, %	1.3 ± 1.9	22.0 ± 16.9	22.9 ± 16.0	31.3 ± 12.7	85.2 ± 11.2	0.87 (0.75 to 0.93)
Bronchiectasis, %	4.3 ± 4.1	3.5 ± 3.0	4.2 ± 3.6	2.3 ± 1.5	1.7 ± 1.4	0.44 (0.13 to 0.67)
Airway wall thickening, %	4.7 ± 2.8	7.8 ± 6.1	9.7 ± 6.4	5.7 ± 1.9	10.5 ± 3.3	0.22 (−0.12 to 0.51)
Mucus plugging, %	0.0 ± 0.0	1.7 ± 4.4	0.0 ± 0.0	0.5 ± 1.3	1.4 ± 1.3	0.17 (−0.17 to 0.47)
Tree-in-bud changes, %	2.5 ± 7.9	6.1 ± 9.4	4.0 ± 7.6	0.7 ± 1.6	1.4 ± 2.5	0.15 (−0.19 to 0.46)

Definition of abbreviations: COPD = chronic obstructive pulmonary disease; GOLD = Global Initiative for Chronic Obstructive Lung Disease. The scoring system is described in more detail in METHODS and the online supplement. Percentages indicates the percentage of the total lung/lobe. Correlation analysis was performed with a Spearman rank test, and the result is shown as an *R* value (95% CI).

Table 3. Summary of MicroCT and Histologic Measures

	Control	Pre-COPD	GOLD I	GOLD II	GOLD III/IV	ANOVA *P* Value
MicroCT measures						
Number of TrBs/mL, *n*	11.45 ± 1.90	6.32 ± 3.03*	6.97 ± 1.75†	5.00 ± 1.77*	0.70 ± 0.54*	<0.0001
Morphometry TrB middle						
Alveolar attachments, *n*	9.2 ± 0.6	7.5 ± 1.0*	7.4 ± 1.0†	7.1 ± 1.0*	4.8 ± 0.8*	<0.0001
Airway wall thickness, μm	52.4 ± 6.2	81.0 ± 15.4	65.5 ± 13.2	85.4 ± 14.1	108 ± 68†	0.0101
Min/max airway diameter	0.86 ± 0.03	0.79 ± 0.03	0.83 ± 0.04	0.77 ± 0.04	0.80 ± 0.17	0.0009
Morphometry TrB end						
Alveolar attachments, *n*	8.9 ± 0.5	7.5 ± 1.1†	7.0 ± 1.0*	6.9 ± 1.0*	5.1 ± 0.4*	<0.0001
Airway wall thickness, μm	53.9 ± 10.6	97.1 ± 39.1‡	68.1 ± 9.7	97.3 ± 19.4‡	82.5 ± 48.6	0.017
Min/max airway diameter	0.84 ± 0.02	0.79 ± 0.03‡	0.77 ± 0.05‡	0.74 ± 0.04*	0.77 ± 0.07‡	0.0007
Number of TBs/mL, *n*	5.53 ± 0.94	3.63 ± 1.47*	3.79 ± 0.70†	2.61 ± 1.05*	0.27 ± 0.17*	<0.0001
Morphometry TB middle						
Alveolar attachments, *n*	10.0 ± 0.7	8.2 ± 1.3‡	7.8 ± 1.2†	7.9 ± 1.1‡	4.8 ± 2.2*	<0.0001
Airway wall thickness, μm	61.5 ± 7.8	97.5 ± 40.0‡	75.3 ± 14.9	98.3 ± 21.1‡	68.2 ± 31.1	0.022
Min/max airway diameter	0.86 ± 0.03	0.81 ± 0.04	0.84 ± 0.03	0.76 ± 0.03‡	0.56 ± 0.20*	<0.0001
Surface density, 1/μm	0.0157 ± 0.0016	0.0117 ± 0.0035*	0.0122 ± 0.0036‡	0.0109 ± 0.0024*	0.0046 ± 0.0008*	<0.0001
Histologic measures						
Chord length, μm	96.3 ± 7.9	132.6 ± 12.1†	164.2 ± 11.9*	170.2 ± 25.0*	314.3 ± 44.9*	<0.0001
Alveolar surface density, 1/μm	0.0112 ± 0.0097	0.080 ± 0.0080‡	0.0747 ± 0.0089‡	0.0715 ± 0.0097†	0.0049.0 ± 0.0117*	<0.0001

Definition of abbreviations: COPD = chronic obstructive pulmonary disease; GOLD = Global Initiative for Chronic Obstructive Lung Disease; TB = terminal bronchiole; TrB = transitional bronchiole.
Data presented as mean ± SD.
*$P < 0.001$.
†$P < 0.01$.
‡$P < 0.05$.

($R = -0.84$, $P < 0.0001$) and surface density on microCT ($R = 0.84$, $P < 0.0001$) (*see* Figures E2C and E2D). Similar associations were found for the number of TBs per milliliter with FEV_1 ($R = 0.58$, $P < 0.001$), FEV_1/FVC ratio ($R = 0.72$, $P < 0.0001$), TLC ($R = -0.69$, $P < 0.0001$), D_{LCO} ($R = 0.65$, $P < 0.001$), RV ($R = -0.62$, $P = 0.001$), and emphysema on CT ($R = -0.83$, $P < 0.0001$).

Considering only the patients without airflow limitation (controls and pre-COPD; $n = 20$), the number of TrBs per milliliter did not correlate with FEV_1 ($P = 0.99$), FVC ($P = 0.72$), TLC ($P = 0.22$), or RV ($P = 0.10$). However, there was an association between the number of TrBs per milliliter and D_{LCO} ($R = 0.46$, $P = 0.041$) and emphysema on CT ($R = -0.77$, $P < 0.001$). The number of TBs per milliliter correlated with TLC ($R = -0.51$, $P = 0.020$), D_{LCO} ($R = 0.45$, $P = 0.045$), and degree of CT emphysema ($R = -0.72$, $P = 0.0005$). Correlation analysis including only patients with pre-COPD or GOLD stage I or II COPD ($n = 22$ in total) demonstrated no association between the number of alveolar attachments and FEV_1% predicted ($P = 0.94$) or FEV_1/FVC ratio ($P = 0.25$).

Histopathologic validation of small airway pathology

To further demonstrate the histopathological presence of emphysema, Figure 4 shows representative hematoxylin and eosin–stained images of the different study groups illustrating control lung tissue (Figure 4A) and mild microscopic emphysema in pre-COPD and GOLD stage I COPD (Figures 4B and 4C), which is more severe in GOLD stage II and GOLD stage III/IV COPD (Figures 4D and 4E). Assessment of the chord lengths showed that the airspace was increased in pre-COPD ($P = 0.0043$) and GOLD stage I ($P < 0.0001$), stage II ($P < 0.0001$), and stage III/IV COPD ($P < 0.0001$) compared with the controls. Alveolar surface density was also decreased in pre-COPD ($P = 0.032$), GOLD stage I ($P = 0.013$), GOLD stage II ($P = 0.0037$), and GOLD stage III/IV COPD ($P < 0.0001$) compared with controls (Figure E3). To further validate our microCT findings of small airway disease in pre-COPD, serial sectioning was performed on an area showing airway disease typical for COPD on microCT, where the presence of airway wall thickening, airway narrowing, and complete mucosal obliteration was observed on histopathological examination (Figure 5).

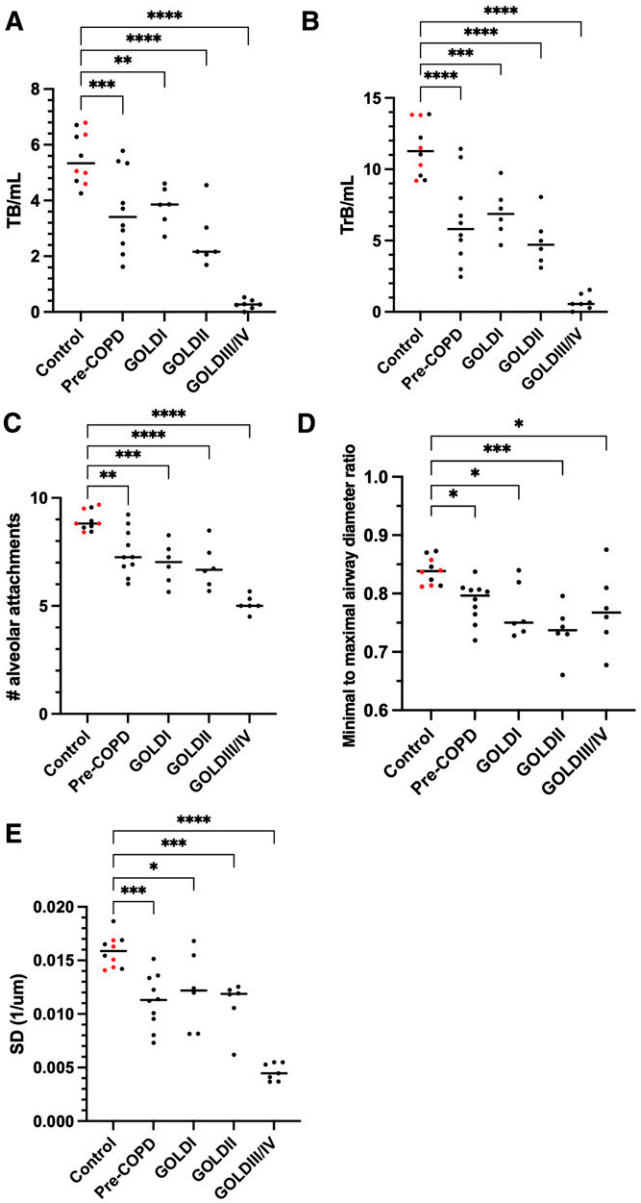

Figure 3. Overview of main results. (*A*) The number of terminal bronchioles per milliliter was decreased in pre–chronic obstructive pulmonary disease (COPD), Global Initiative for Chronic Obstructive Lung Disease (GOLD) stage I, GOLD stage II, and GOLD stage III/IV COPD compared with controls. (*B*) Numbers of transitional bronchioles per milliliter were decreased in pre-COPD, GOLD stage I, GOLD stage II, and GOLD stage III/IV obstruction compared with controls. (*C*) The number of alveolar attachments was lower in pre-COPD and GOLD stages I and III/IV COPD compared with controls. (*D*) Minimal to maximal airway diameter ratio was decreased in GOLD stages I and II compared with controls. (*E*) Surface density was decreased in pre-COPD and GOLD stage II and stage III/IV COPD compared with controls. Never-smokers are shown in red. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, and ****$P < 0.0001$. SD = surface density; TB = terminal bronchiole; TrB = transitional bronchiole.

Discussion

This is the first study showing that patients with pre-COPD with emphysema show significant small airway loss and remodeling. The number of TBs or TrBs per milliliter of lung in pre-COPD is, in fact, similar to what is found in patients with established GOLD stage I COPD. In addition, we found strong correlations between the number of TBs and TrBs per milliliter and physiologic measures such as pulmonary function and radiologic scorings. Significant emphysema on CT (>5%) constitutes an important parameter because lungs with emphysema in the absence of physiologic obstruction already show a lower number of small airways.

Currently, it is not clear why airflow obstruction is not yet observed in patients with pre-COPD because the extent of emphysema on CT, microCT, and histopathology, as well as the number of small airways, is comparable between pre-COPD and GOLD stage I COPD. In addition to the numbers of TBs and TrBs per milliliter, the number of alveolar attachments also showed significant decreases in the pre-COPD group compared with the control group. In fact, a recent pathology-based study indicated that a loss of alveolar attachments of the small airways is mostly related to airflow obstruction in COPD (19), which was recently confirmed by Booth and colleagues (20). Interestingly, the number of alveolar attachments also showed a strong correlation with the FEV_1/FVC ratio in our study. However, it is of interest that the number of alveolar attachments was already decreased in pre-COPD, in which the FEV_1/FVC ratio was not (yet?) decreased.

It is common knowledge that there is a significant proportion of (heavy) smokers without airway obstruction even though emphysema is already present on CT. These patients show greater rates of lung function decline in the future and are therefore more likely to develop airway obstruction in the (near) future (21). Similarly, population-based studies have demonstrated that patients with PRISm have a higher mortality risk and higher risk of the development of airway obstruction (22–24). Indeed, between 10% and 25% of patients with PRISm will continue to develop established COPD, making this a very relevant and interesting population to study as a precursor stage of COPD (25). These observations are also paired with physiologic observations in symptomatic smokers with respiratory symptoms without COPD, who show physiological changes (maximum mid-expiratory flow between 25% and 75% of vital capacity) comparable with pathological changes in the lung before the diagnosis of COPD (26). Impulse oscillometry has similarly demonstrated that small airway disease was present in a significant proportion of heavy smokers without airflow obstruction (27). Specifically in patients with

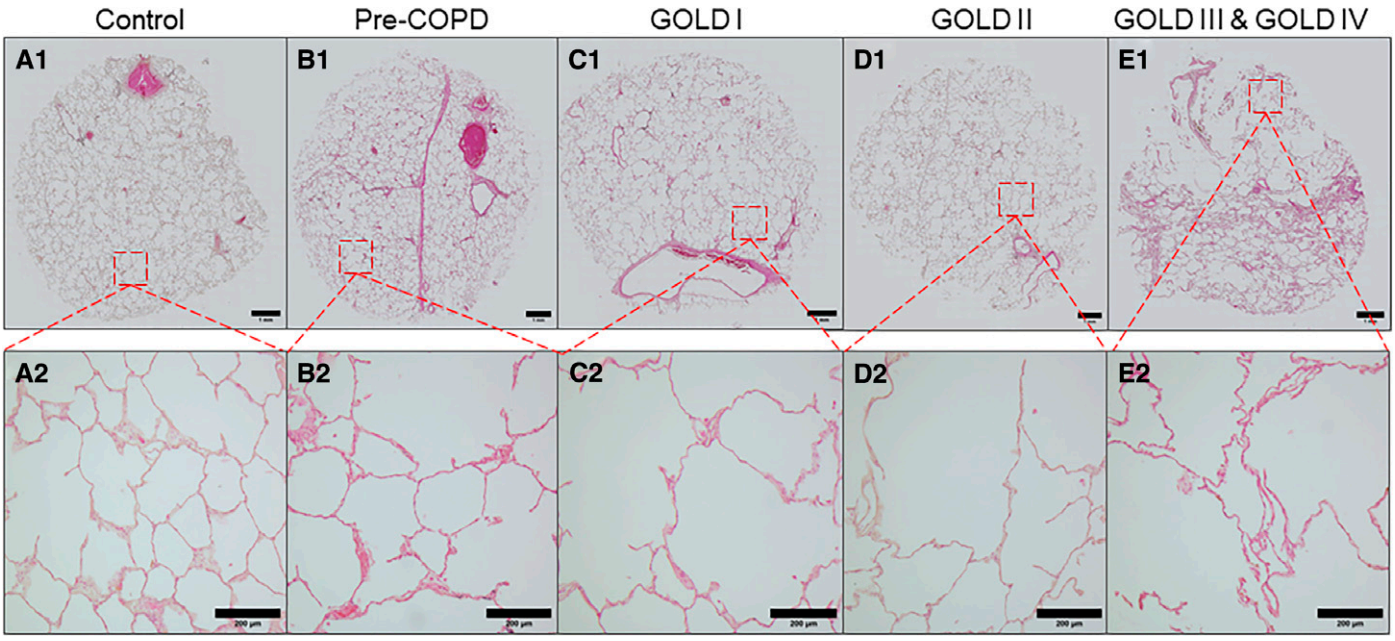

Figure 4. Histopathological correlates of the different study groups. Overview of representative hematoxylin and eosin staining of the cores that were scanned with micro–computed tomography showing inconspicuous parenchyma in controls (*A*) and mild emphysema in pre–chronic obstructive pulmonary disease (COPD) (*B*) and Global Initiative for Chronic Obstructive Lung Disease (GOLD) stage I COPD (*C*), which gets more severe in GOLD stage II COPD (*D*) and GOLD stage III/IV COPD (*E*).

PRISm, Zhao and colleagues recently showed spirometric small airway disease assessed with maximal mid-expiratory flow, forced expiratory flow of 50%, and forced expiratory flow, in addition to increased air trapping on CT (28). Given all this indirect evidence of ongoing pathophysiological processes in the lungs of patients with pre-COPD, this is the first direct evidence of small airway involvement in a group of patients who are at risk of experiencing COPD. Our observations also bring into

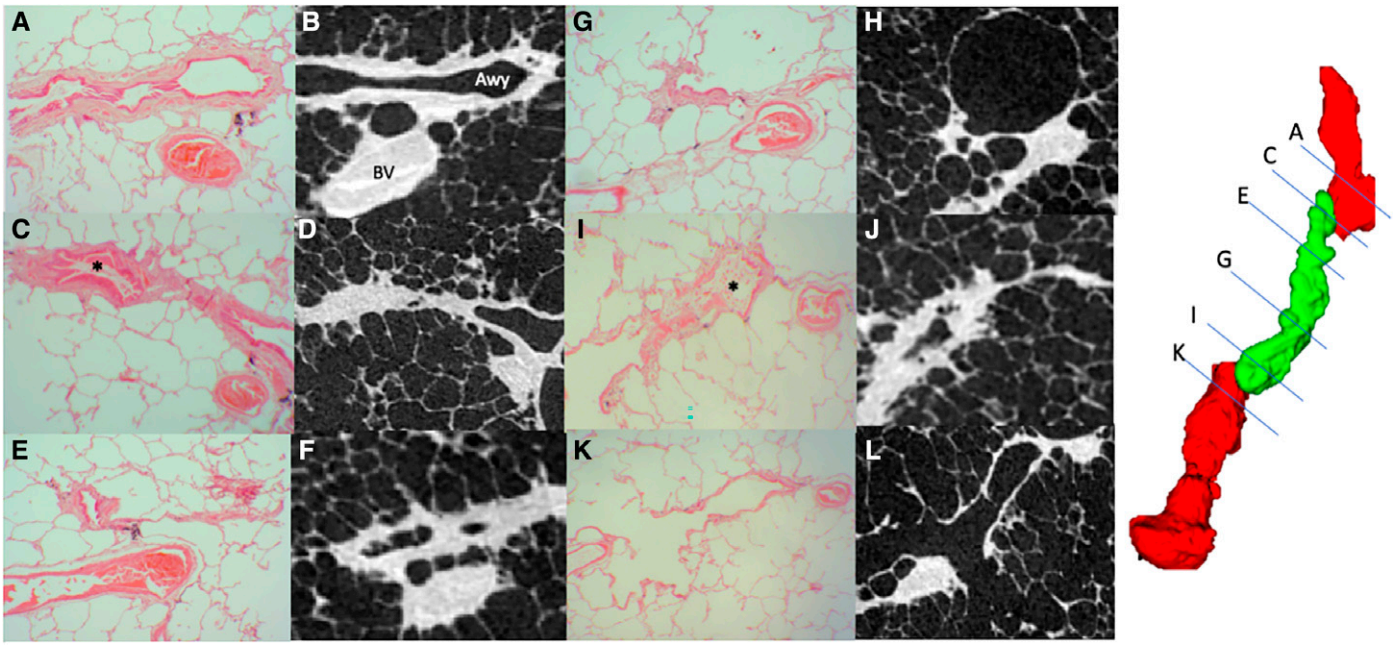

Figure 5. Small airway pathology in pre–chronic obstructive pulmonary disease. The airway showed variable airway pathology across a short segment, with the blood vessel shown as a reference. First, diffuse thickening of the airway wall was observed (*A* and *B*) before narrowing (*C–H*) and eventual complete obliteration of the small airway lumen with mucus (asterisk) (*I* and *J*). Eventually, the airway reopened and gave rise more distally to a normal-appearing transitional bronchiole and respiratory bronchioles (*K* and *L*). Awy = airway; BV = blood vessel.

question earlier studies that included healthy smokers as controls because these smokers were likely only considered healthy based on the absence of airflow obstruction. We are convinced that our observations of ongoing airway remodeling in pre-COPD will also lead to better investigations of the immunological and molecular changes in lungs with pre-COPD, especially the presence of inflammatory changes in those lungs. However, this was beyond the scope of the present work. It is noteworthy that 2 of 10 patients with pre-COPD showed a normal number of small airways, which we speculate are patients with a low risk of future progression to COPD. It also noteworthy that, within our cohort, the majority of patients with significant smoking history without COPD showed emphysema on CT and were hence classified as having pre-COPD instead of as healthy smokers.

We acknowledge certain limitations to the present study. Most importantly, we were not able to sample the resection specimens according to the standard of stereology because of logistical constraints related to the further processing of the lung to obtain fast pathological staging of the concurrent tumor. Second, we used only four samples per lung, which could be considered a low number. However, we leveraged this strategy previously and demonstrated limited differences when including more samples per lung (13). In addition, we formally tested whether there were differences in the microCT measures of four samples from a GOLD stage I lung compared with nine samples of the same lung but could not find any differences in the number of TrBs per milliliter, surface density, wall thickness, minimal to maximal airway diameter, or number of alveolar attachments at the end of the TrBs (Figure E4). In addition, we were not able to quantify the degree of small airway disease and emphysema on the *in vivo* CT using standardized software packages because the preoperative imaging was not standardized across the different study groups (transplantation, tumor resection), resulting in different slice thicknesses and different imaging modalities (CT vs. positron emission tomography/CT) among patients. However, we used rigorous scoring of a standardized *ex vivo* CT scan by an experienced chest radiologist as an alternative. In addition, we do not know the pulmonary function trajectory or the respiratory symptoms of the patients, so we cannot be certain what proportion of patients will formally develop COPD in the future. Also, it is possible that patients with pre-COPD start with a lower number of small airways and do not lose small airways. It remains to be established if these findings can be generalized to all patients with pre-COPD. We also combined patients with GOLD stages III and IV COPD into a single group because the number of subjects with GOLD stage III COPD was low ($n = 2$). These patients often do not qualify for lobectomy because of their poor respiratory function, but their COPD is mostly not severe enough to make the patient eligible for lung transplantation. By design, our analysis was limited to the analysis of a single lung lobe even though COPD is known to be a heterogeneous disease. The scanning resolution is also on the lower end to reliably identify the appearance of alveolar buds. Finally, the number of included lungs is also relatively low, especially given the fact that they were classified in several groups. Nevertheless, our analysis resulted in statistically meaningful comparisons.

In conclusion, we have shown that patients with pre-COPD, who, based on population-based studies, are at risk for the development of COPD, already have significant small airway disease and emphysema. Identifying pre-COPD and focusing on mechanistic investigation in pre-COPD could revolutionize our understanding and treatment of COPD (29). ■

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