Characterizing Pathological Changes of the Peripheral Airways in Mild & Moderate COPD.

Steven Booth[1, 2], Naoya Tanabe[1], Aileen Hsieh[1], Leila Mostaco-Guidolin[1], Hyun-Kyoung Koo[1,2], Dragoş M. Vasilescu[1,3], James C. Hogg[1,3], **Tillie-Louise Hackett**[1,2].

1. Centre for Heart Lung Innovation, University of British Columbia (UBC), Vancouver, B.C. Canada
2. Department of Anaesthesiology, Pharmacology and Therapeutics, UBC, Vancouver, B.C. Canada
3. Department of Pathology, UBC, Vancouver, B.C. Canada

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THE UNIVERSITY OF BRITISH COLUMBIA

Centre for Heart Lung Innovation
UBC and St. Paul's Hospital

Providence HEALTH CARE

INTRODUCTION

- Airflow obstruction, the hallmark characteristic of Chronic Obstructive Pulmonary Disease (COPD) has long been attributed to a combination of small airways disease and emphysematous destruction. McDonough et al. (NEJM, 2011), recently reported a significant reduction in terminal bronchiolar number in end-stage COPD compared to controls with normal lung function.
- Furthermore, it was shown that the pre-terminal bronchioles in these same severe COPD cases were significantly and heterogeneously narrowed (Tanabe et al, 2016).
- However, it remains unknown exactly what happens to these generations of terminal airways in the early disease stages.

SPECIFIC AIM

- The objective of this study was to characterize the morphological and molecular changes occurring in the terminal bronchioles in Mild/Moderate COPD.

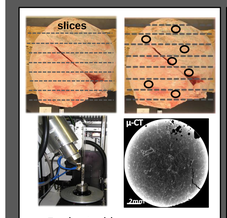
METHODS

Table 1. Patient characteristics

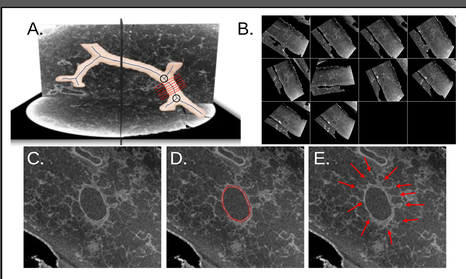
Characteristic	Control (Smokers with normal lung function) n = 10	Mild/Moderate COPD GOLD 1 n = 10	GOLD 2 n = 8	GOLD 4 n = 6
Gender (female:male)	6:4	4:6	3:5	1:5
Age (years)	62.0 ± 7.9	67.4 ± 7.3	62.9 ± 11.3	59.2 ± 2.1
Height (cm)	167.9 ± 8.7	168.6 ± 9.9	167.4 ± 6.7	170.3 ± 6.3
Weight (kg)	68.4 ± 14.7	77.1 ± 18.1	73.1 ± 15.6	71.5 ± 10.8
Smoking history (pack years)	34.5 ± 10.5	45.5 ± 25.3	33.6 ± 12.7	37.5 ± 15.1
FEV_1 (% predicted)	91.8 ± 6.4	88.3 ± 6.2	62.1 ± 9.5	22.3 ± 6.7
FEV_1/FVC (%)	74.9 ± 4.4	63.5 ± 4.7	60.1 ± 7.6	29.5 ± 7.8
DLCO/VA (ml/min/mmHg/L)	3.85 ± 0.9	2.83 ± 0.7	2.64 ± 0.9	1.71 ± 0.8
Total lung volume (L)	4.99 ± 1.5	5.11 ± 1.7 (n=8)	5.15 ± 1.0 (n=6)	6.46 ± 2.1

Table 1. Lung samples from donors with normal lung function and mild/moderate COPD (GOLD 1+2) were donated from patients undergoing surgical resection for lung cancer treatment. Lung samples from donors with severe COPD (GOLD 4) were donated from patients undergoing lung transplantation. All specimens were provided with consent to the James Hogg Research Lung Registry.

Figure 1. Methods of tissue collection, image acquisition and analysis



- Explanted lungs were inflated, frozen and sliced evenly from apex to base.
- Eight random samples were extracted uniformly over the lung sample, formalin fixed and embedded in paraffin for micro-CT scanning.
- Micro-CT parameters:
  - Nikon micro-CT scanner
  - X-ray energy: 50-70kV
  - Current: 110-150μA
  - 3D reconstruction with voxel dimension of 6.7μm.



- 3D volumetric microCT scans were used to visualize the branching airway network and allowed terminal bronchioles to be identified and counted (**A**). (Terminal bronchioles were defined as the last purely conducting airway with no alveolar openings).
- Using custom software 10x systematically uniform random sample (SURS) cross sectional images were obtained along the length of each terminal bronchiole for analysis (**B**).
- Terminal bronchiole walls and lumens were segmented (**C+D**) and alveolar attachments were counted (**E**) on all cross sectional images for each airway.
- Histological sections of the same airways were analyzed using multiphoton microscopy to assess fibrillar collagen & elastin composition of the airway wall.

- To test for differences in airway morphometry or ECM composition between groups all data were analyzed in R using linear mixed-effect models with false discovery rate pvalue correction for multiple testing. FDR<0.05 was considered significant.

RESULTS

Figure 2. Terminal bronchioles are significantly destroyed in Mild/Moderate COPD and surviving bronchioles show signs of disease

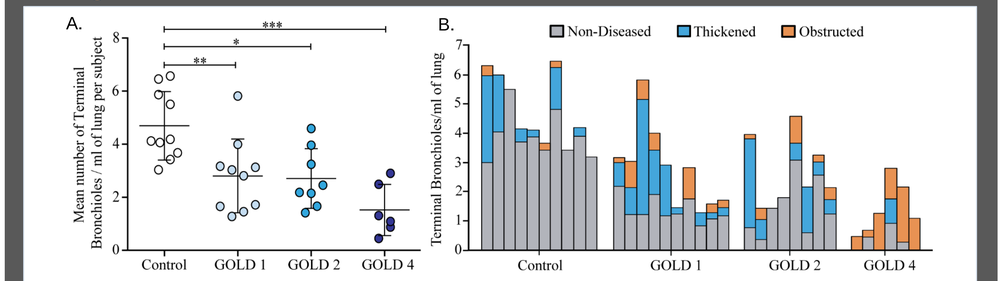


Figure 2A. . Mean number of terminal bronchioles per milliliter (TB/ml) of lung tissue per subject. Terminal bronchiolar number is significantly decreased in all stages of disease compared to the control group (GOLD 1: P<0.01, GOLD 2: P<0.05, GOLD 4: P<0.001).
Figure 2B. Phenotype of surviving terminal bronchioles. In mild and moderate COPD samples there is an increased proportion of surviving bronchioles which appear thickened. Whereas in very severe COPD the majority of surviving bronchioles are obstructed.

Figure 3. Mild/Moderate COPD terminal bronchioles have narrowed lumens and thickened walls

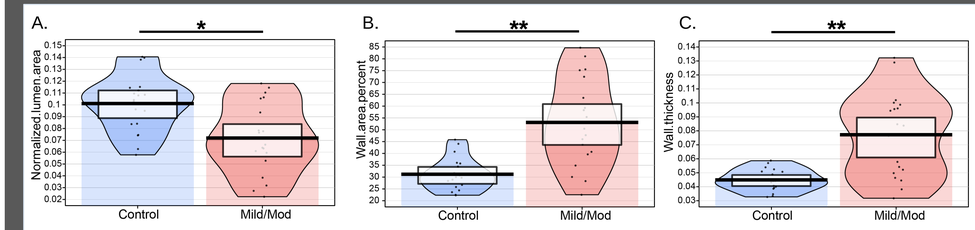


Figure 3A. Luminal area of terminal bronchioles. The luminal area is significantly decreased in mild/moderate COPD terminal bronchioles compared to controls (FDR<0.05).
Figure 3B. Wall area of terminal bronchioles. The percentage of airway wall area is significantly increased in mild/moderate COPD terminal bronchioles compared to controls (FDR<0.01).
Figure 3C. Wall thickness of terminal bronchioles. The percentage of airway wall area is significantly increased in mild/moderate COPD terminal bronchioles compared to controls (FDR=0.01).

Figure 4. Luminal volume is decreased in COPD terminal bronchioles

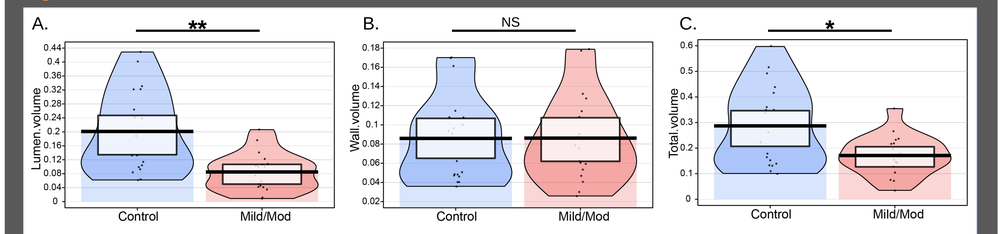


Figure 3A. Luminal volume of terminal bronchioles. The luminal volume along the airway branch length is significantly decreased in mild/moderate COPD terminal bronchioles compared to controls (FDR<0.01).
Figure 3B. Wall volume of terminal bronchioles. The airway wall volume along the airway branch length is not significantly different in mild/moderate COPD terminal bronchioles compared to controls.
Figure 3C. Total volume of terminal bronchioles. The total volume (wall+lumen) of mild/moderate COPD terminal bronchioles is significantly decreased compared to controls (FDR<0.05).

Figure 5. COPD terminal bronchioles are less circular and have decreased numbers of alveolar attachments

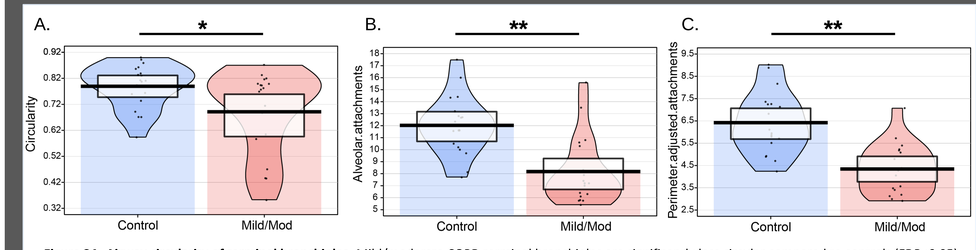


Figure 3A. Airway circularity of terminal bronchioles. Mild/moderate COPD terminal bronchioles are significantly less circular compared to controls (FDR<0.05).
Figure 3B. Raw alveolar attachment count. The number of alveolar attachments surrounding the airways is significantly decreased in mild/moderate COPD terminal bronchioles compared to controls (FDR<0.01).
Figure 3C. Alveolar attachment count adjusted for airway size. The number of alveolar attachments surrounding the airways when adjusting for airway perimeter is significantly decreased in mild/moderate COPD terminal bronchioles compared to controls (FDR<0.005).

Figure 6. Using micro-CT as a scouting tool to section terminal bronchioles in paraffin embedded tissue

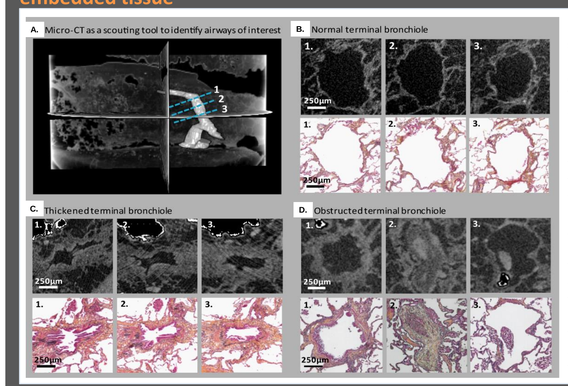
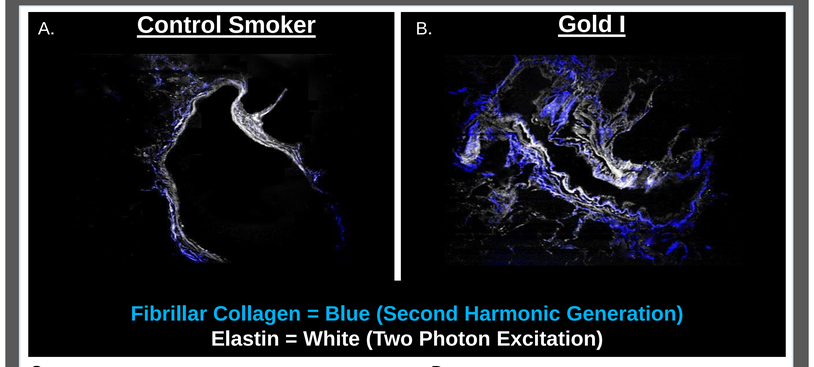


Figure 4A. Micro-CT 3D imaging and segmentation provides precise locations of airways, enabling efficient serial histological sectioning. The numbered lines indicate where corresponding sections were taken. **Figures 4B-D.** Matched micro-CT and histological sections of a control (B), thickened (C) and obstructed (D) terminal bronchiole, each stained with Movat's Pentachrome stain. Note the thickened/remodeled airway wall and folded/restricted lumen in the thickened airway. Note the remodeled appearance of the obstructed bronchiole, which at the top of the branch has an open lumen, but then becomes 100% obstructed further along the airway length in section 2. and then opens again further along in section 3.

Figure 7. Multiphoton microscopy analysis of terminal bronchiole extracellular matrix structure



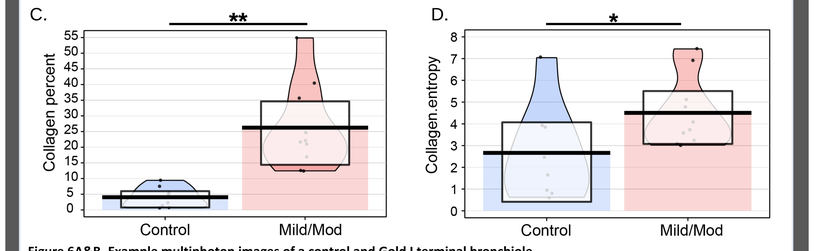


Figure 6A&B. Example multiphoton images of a control and Gold I terminal bronchiole.
Figure 6C. Fibrillar collagen in terminal bronchiole walls. The amount of fibrillar collagen in mild/moderate COPD terminal bronchiole walls is significantly increased compared to controls (p<0.01).
Figure 6D. Texture analysis of bronchiole fibrillar collagen. The fibrillar collagen in COPD terminal bronchioles is significantly disorganized compared to controls (p<0.05).

CONCLUSIONS and CLINICAL SIGNIFICANCE

- Micro-CT scans of FFPE lung tissue enables the visualization of small airways and parenchymal structures, not amenable by clinical CT.
- We demonstrate that there is a significant decrease in the number of terminal bronchioles in mild to moderate COPD (GOLD 1+2) lungs.
- Using custom microCT processing software we were able to analyze the morphology of terminal bronchioles along their branch length. COPD terminal bronchioles had narrowed lumens, thickened walls, decreased circularity and reduced alveolar attachments.
- MicroCT was used to guide efficient sectioning of terminal bronchioles in FFPE tissue. In these sections we observed a significant accumulation of fibrillar collagen in the airway wall, and this collagen had a disorganized structure.
- Our findings suggest that pathological and irreversible structural alterations occur in the terminal bronchioles in Mild and Moderate COPD, emphasizing the importance of early diagnosis and development therapeutics targeted to small airways to modify the progression of this debilitating respiratory disorder.