MicroCT analysis of paraffin embedded lung tissue: Is small airway obstruction an early feature of COPD?

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ABSTRACT

Introduction: The airflow limitation in Chronic Obstructive Pulmonary Disease (COPD) is caused by small airways obstruction and emphysematous destruction. Using micro-CT (µCT) imaging, it was documented that terminal bronchiolar narrowing and loss precedes emphysema in very severe COPD leading to our hypothesis that *'Small airway narrowing and obliteration precedes emphysematous changes in COPD and begins in patients with mild to moderate disease'.* We developed a three-dimensional (3D) µCT imaging technique facilitating the assessment of terminal bronchioles and emphysema in paraffin embedded lung samples of patients with either normal lung function, or mild to moderate COPD.

Methods: Our donor lung samples, obtained from patients undergoing surgical resection for lung cancer treatment were formalin inflated, sliced and sampled into cores which were then paraffin embedded (FFPE). A Nikon Metrology µCT scanner was used to scan these FFPE cores in a non-destructive manner. The contiguous sections of each scan were examined to determine terminal bronchiolar number and presence of emphysema using mean linear intercept (Lm). A semi-automatic segmentation technique enabled the 3D reconstruction of the airways to characterize their structure and caliber. After scanning, the FFPE cores were sectioned and stained with Movat's pentachrome, allowing a more comprehensive analysis of airway remodeling and lung morphology.

Results: Our findings demonstrate that µCT FFPE scans provide adequate contrast to determine Lm values. These values were validated using Lm measured by histology on the same samples. Preliminary data indicate a decline in the number of terminal bronchioles/mL of lung tissue (TB/mL) with disease severity. Specifically, in ex-smokers with normal lung function, we found 5.37TB/mL compared to 4.02TB/ml in mild COPD (GOLD1) and 3.28TB/mL in moderate COPD (GOLD2). In addition, this terminal bronchiolar loss occurred in the presence of no emphysema as indicated by normal Lm (<489µm). A 3D reconstruction of the airway structures within the paraffin embedded core of the GOLD1 patient demonstrated an obliterated small airway with intact alveolar ducts and surrounding parenchyma compared to the ex-smoker. These images were confirmed by histology, using the µCT images to precisely locate these airway lesions, enabling efficient sectioning of the paraffin embedded samples and further characterization of the remodeling process.

Conclusions: It is evident that µCT examination of FFPE lung samples enables the assessment of small airway morphology and surrounding parenchyma. Preliminary findings are in keeping with the hypothesis that terminal bronchioles are narrowed and lost in the early stages of COPD and may precede emphysematous development.

INTRODUCTION

- Chronic obstructive pulmonary disease (COPD) is the 4th leading cause of death worldwide. COPD is defined as a chronic progressive disease, characterized by airflow limitation, caused by obstruction of the small airways and / or emphysematous destruction, that is not fully reversible.
- It has been documented that the narrowing and loss of terminal bronchioles precedes the appearance of emphysematous destruction in lungs of patients with very severe COPD (McDonough J. et al. NEJM 2011). This leads to the question: 'What comes first: Loss of terminal bronchioles or emphysema?' (Figure 1).

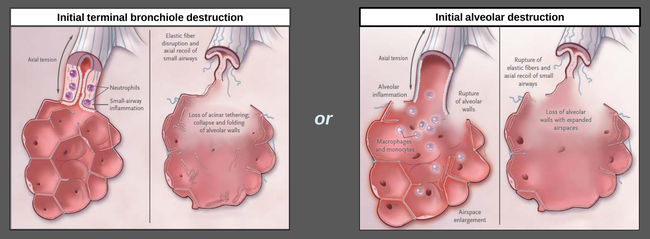


Figure 1. Potential Mechanisms of Airflow limitation in COPD. (Modified from Mitzner W. NEJM 2011).

- Clinical multi-detector computed tomography (MDCT) scanners do not have the resolution to visualize the small airways (<2.3 mm diameter) affected in disease. In contrast, microCT (µCT) provides the higher magnification required to image the lung microstructure.
- µCT has a significant advantage over traditional histology as it offers non-destructive 3D imaging. In addition, structures of interest can be localized within the volumetric µCT image, potentially decreasing the blinded, laborious and costly sectioning of entire samples.
- Traditionally, formalin fixation and paraffin embedding (FFPE) of tissue has been the standard for histology. Due to the low contrast between soft tissue and paraffin wax, FFPE samples have been precluded from µCT imaging.
- We have developed a µCT imaging protocol to enable non-destructive imaging of archival FFPE samples for the assessment of terminal bronchiolar number and emphysema in mild to moderate COPD.

HYPOTHESIS

Small airway narrowing and obliteration precedes emphysematous changes in COPD and begins in patients with mild to moderate disease

METHODS AND SAMPLE COHORT

Characteristic	Normal	GOLD 1 MILD	GOLD 2 MODERATE	GOLD 4 VERY SEVERE
No. of patients	10	10	10	10
FEV_1 (% of predicted)	98.1 ± 1.7	93.0 ± 1.3	67.5 ± 1.5	21.9 ± 0.6
FEV_1:FVC (% of FVC)	0.78 ± 0.009	0.65 ± 0.006	0.58 ± 0.016	0.30 ± 0.008
Age (years)	63 ± 1	67 ± 1	66 ± 2	66 ± 1
Smoking history (pack years)	40 ± 13	50 ± 5	60 ± 5	67 ± 5

Table 1. Lung samples from patients with normal lung function or COPD undergoing surgical resection for lung cancer treatment were donated with informed consent between the years 1980 and 2000 to the James Hogg Research Lung Tissue Registry.

Figure 2. Methods of Tissue collection, Image acquisition and re-construction.

Pre-operative MDCT scan prior to lung resection to determine whole lung volumes and lung densities	Lung sample fixed by formalin inflation and sliced evenly from apex to base	Random samples extracted uniformly over lung height, embedded in paraffin and stacked in a holder for µCT imaging	Sample holder placed inside the HMX 376 µCT scanner at the µ-VIS Centre, University of Southampton, UK	Imaging protocol parameters X-ray energy: 50-70kV. Current: 110-150µA (with no filtration). 3D reconstruction of resultant image with voxel dimension of 6.7µm

RESULTS

Specific Aim 1: Determine the feasibility of analyzing lung tissue morphometry in FFPE samples using a combination of µCT with stereology.

Figure 3.

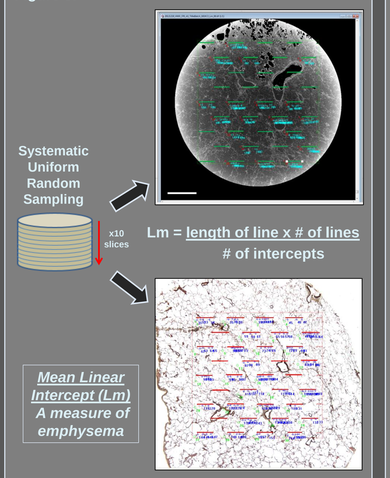


Figure 3. Images were captured at ten regularly spaced intervals within the µCT scans and the corresponding histological sections. Air-space enlargement was determined by the Mean Linear Intercept (Lm) using a formulated grid of test lines projected onto the images. Lm values obtained from the µCT images were compared with Lm values from histology for the validation of these measurements.

Figure 4.

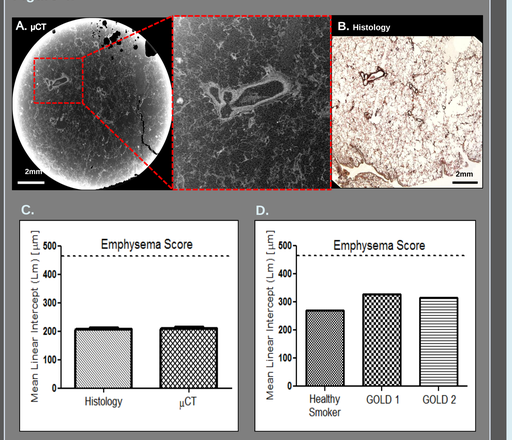


Figure 4A. µCT images allow visualization of small airways <2mm in diameter in FFPE samples.

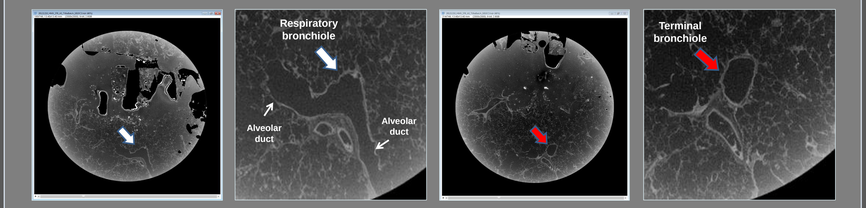
B: Following µCT scanning, FFPE lung samples can be sectioned and stained for histological examination using Movat's Pentachrome stain.

C. Comparison of mean linear intercept (Lm) measured by µCT and histology on matched µCT images and histological sections. (P = 0.987)

D. Lm score measured on µCT images of FFPE lung tissue from healthy smokers, mild (GOLD 1) and moderate (GOLD 2) disease.

Specific Aim 2: Determine if the significant reduction in terminal bronchioles reported in end stage COPD begins in mild (GOLD1) disease.

Figure 5. ***Terminal bronchiole:*** *The last bronchiole without alveolar openings from their walls*



	Control [non-smoker] (n=4, McDonough *et al.*)	Healthy Smoker (n=5)	COPD GOLD 1 (n=3)	COPD GOLD 2 (n=2)	COPD GOLD 4 (n=4, McDonough *et al.*)
Terminal Bronchioles (No. / mL)	**6.9 (SE 1.2)**	**5.37**	**4.02**	**3.28**	**0.7 (SE 0.1)**

Figure 5A. Measure of Terminal Bronchiolar number. Contiguous sections of each µCT scan are examined to identify terminal bronchioles.

B. Table 2 demonstrates the number of terminal bronchioles per mL of lung tissue from healthy, mild, moderate and severe disease.

Specific Aim 3: Define the structural pathological changes of terminal bronchioles and surrounding peripheral lung structures in mild COPD using a combination of µCT and histology.

Figure 7.

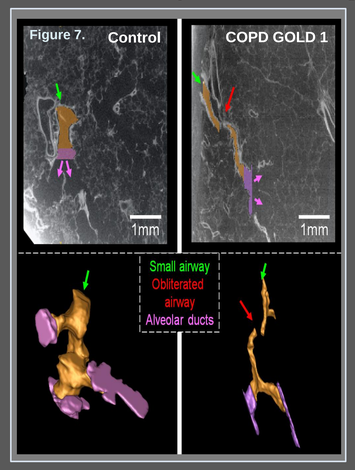


Figure 8.

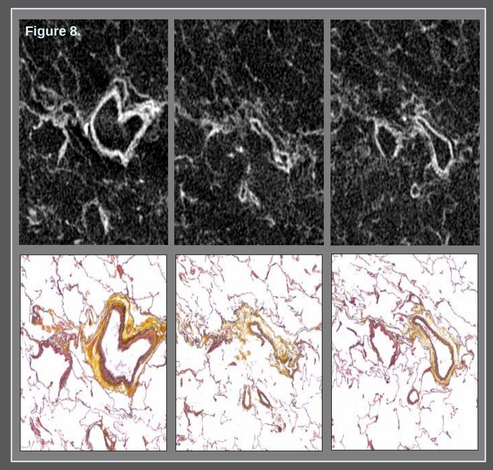


Figure 7. Three dimensional reconstruction of airway lesions in FFPE samples. The 3D rendering of a small airway (orange) from a healthy smoker demonstrates a normal branching structure, leading into the alveolar ducts (purple). In contrast, the airway from a GOLD 1 patient demonstrates a narrowed and obliterated terminal bronchiole (red arrow), with intact respiratory bronchioles and alveolar ducts.

Figure 8. Key features identified by µCT can be verified and further characterized by histology. µCT images provide a comprehensive analysis of airway lesions, enabling efficient serial sectioning. Histological sections are stained with Movat's Pentachrome to identify structural cells and extracellular matrix components.

CONCLUSIONS and CLINICAL SIGNIFICANCE

- µCT scans of FFPE lung tissue can be used to identify the number of terminal bronchioles and measurements of airspace enlargement (Lm) to determine emphysema.
- Our preliminary data indicates that there is a decreased number of terminal bronchioles in mild and moderate COPD lung tissue with no presence of emphysematous destruction.
- On completion of this study, we hope that a further understanding of the interaction between small airways and emphysema in the early stages of COPD will be achieved.
- COPD patients are currently not treated until GOLD stage 2. Our data indicate that therapeutic interventions may need to be implemented early to change clinical outcomes.

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