Micro-Computed Tomography Measurements of Peripheral Lung Pathology in Chronic Obstructive Pulmonary Disease

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Background: The smaller airways, < 2 mm in diameter, offer little resistance in normal lungs, but become the major site of obstruction in chronic obstructive pulmonary disease (COPD).

Objective: To examine bronchiolar remodeling and alveolar destruction in COPD using micro-computed tomography (micro-CT).

Methods: Micro-CT was used to measure the number and cross-sectional lumen area of terminal bronchioles (TB) and alveolar mean linear intercept (Lm) in 4 lungs removed from patients with very severe (GOLD-4) COPD and 4 unused donor lungs that served as controls. These lungs were inflated with air to a transpulmonary pressure (P_L) of 30 cm H₂O and held at P_L 10 cm H₂O while they were frozen solid in liquid nitrogen vapor. A high resolution CT scan was performed on the frozen specimen prior to cutting it into 2-cm thick transverse slices. Representative core samples of lung tissue 2 cm long and 1 cm in diameter cut from each slice were fixed at -80°C in a 1% solution of glutaraldehyde in pure acetone, post-fixed in osmium, critically point dried, and examined by micro-CT.

Results: A 10-fold reduction in terminal bronchiolar number and a 100-fold reduction in their minimal cross-sectional lumen area were measured in both emphysematous and non-emphysematous regions of the COPD lungs.

Conclusions: The centrilobular emphysematous phenotype of COPD is associated with narrowing and obliteration of the terminal bronchioles that begins prior to the onset of emphysematous destruction.

Keywords: COPD, small airway obstruction; termination bronchioles, reduction of; emphysematous destruction

Chronic obstructive pulmonary disease (COPD) is defined by a reduction in the volume of air that can be forcibly expired from fully inflated lungs in one second (FEV₁) expressed as a ratio of the volume that can be forcibly expired without a time limit (FVC). When FEV₁/FVC falls below 0.70 and cannot be reversed by bronchodilator therapy, COPD is said to be present and can be classified into mild, moderate, severe, and very severe categories based on the level of deterioration in FEV₁ (1). The conducting airways leading to the gas-exchanging tissue vary in length and in the number of branch points between the trachea and terminal bronchioles (2); and the time required to empty regions of lung supplied by these different pathways is determined by the product of the airway resistance (cm H₂O/L/s) and the elastic recoil force (compliance) available to drive air out of the lung (L/cm H₂O). The product of resistance and compliance yields the units of time (3, 4) and the FEV₁ represents gas expelled from units that empty

early during a forced expiration, compared with the FVC containing the slower units that continue to empty right up until the subject is forced to take their next breath. A fall in the FEV₁/FVC indicates a shift toward units that empty more slowly, but neither the FEV₁ nor its ratio to FVC can determine whether slower lung emptying is caused by an increased resistance or to an increase in compliance due to emphysematous destruction of lung tissue. This means that other types of test are required to separate the emphysematous destruction from the airway obstructive phenotype of COPD.

Direct measurements have shown that the major site of increased airway resistance in COPD is in the small bronchi and bronchioles less than 2 mm in diameter in COPD (5–7). Although measurements of frequency dependence of compliance (8) and single breath nitrogen washout curves (9, 10) reflect the pathology found in the peripheral lung of everyone that smokes, the presence of these physiological abnormalities does not seem to identify the susceptible minority of heavy smokers who will undergo a rapid decline in FEV₁ to levels that reflect severe (GOLD-3) and very severe (GOLD-4) COPD.

The chronic inflammatory process that develops in all smokers increases in both extent and severity in association with progression of COPD through the GOLD categories (11). This inflammatory process is dominated by polymorphonuclear neutrophils, macrophages, and lymphocytes that show an increasing trend to form into follicles with germinal centers that define the presence of an adaptive immune response in the later stages of COPD. However, multivariate analyses showed that more of the variance in the relationship between decline in FEV₁ and these variables was explained by thickening of the airway walls and occlusion of the lumen by inflammatory exudates containing mucus than by the accumulation of any type of inflammatory cell. This close relationship between airway wall thickening and decline in FEV₁ suggests that structural changes produced by the tissue repair and remodeling of chronically damaged tissue cause the obstruction in the small airways.

Figure 1 is from an older study (5) showing that the small airways less than 2 mm in diameter account for only approximately 10% total resistance of the airways below the larynx (Figure 1A), whereas these same airways (Figure 1B) contribute the majority of the approximate doubling of the total airways resistance in the lungs of a patient with COPD. As the small conducting airways are arranged in parallel the total resistance (R_T) of these airways is determined by the sum of the inverse of the resistance of the individual branches (i.e., $R_T = 1/R_1 + 1/R_2 + 1/R_3$, etc.). This means that removal of 50% of the existing airways is required to simply double small airway resistance. In contrast, generalized narrowing of all or most of the individual peripheral airways has a much greater effect on resistance based on the change in the radius of the individual airways raised to the 4th power. These arguments suggest that generalized narrowing and possibly removal of smaller airways was the most likely cause of the peripheral airways obstruction in COPD. Matsuba and Thurlbeck (12) conducted the only study we are aware of that

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Figure 1. Data from Reference 5, where a small catheter was placed in the periphery of postmortem lungs to compare the total resistance to flow below the larynx to the resistance beyond the smaller airways less than 2 mm in internal diameter. These data show (A) that the resistance in airways less than 2 mm in diameter accounts for approximately 10% of the total resistance to flow below the larynx in the normal human lung. In contrast, (B) shows results from a patient with chronic obstructive pulmonary disease where the total resistance to flow below the larynx is approximately double that observed in the control lung, and all of this increase in resistance is accounted for by the smaller airways less than 2 mm in diameter (see text for further explanation). Reproduced by permission from Reference 5.

attempted to test this hypothesis by quantifying small airway number and size in postmortem lungs. However, their data failed to support the hypothesis because they found that the number of conducting airways less than 2 mm in internal diameter was only slightly reduced and the changes in caliber relatively small in emphysematous lungs.

The introduction of computed tomography (CT), high-resolution computed tomography (HRCT), and more recently Micro-CT have provided much more effective tools for measuring both emphysematous destruction and airway wall thickness in patients with COPD. Volumetric CTs allow regional differences in lung density to be measured in Hounsfield units (HU), derived from a linear transformation of the attenuation coefficients observed between 0 HU defined as the radiodensity of distilled water at standard pressure and temperature (STP) and the radiodensity of air at STP defined as $-1,000$ HU. This convention allows the measured HU to be converted to density by adding 1,000 to the HU measured in any region and dividing the result by 1,000. Hayhurst and coworkers were the first to use CT to diagnose emphysematous destruction in living persons based on the frequency distribution of the HU (13). Müller and colleagues followed by using an individual cut-off to define the presence of emphysematous destruction (14). Others used the inverse of lung density to measure specific volume (ml air/gram tissue) that they used to estimate lung surface area (15) and still others defined the complexity of terminal airspace geometry by its fractal dimension (16).

The introduction of HRCT made it possible to measure airway wall thickness in living humans and use it as a surrogate for small airway obstruction and separate persons with COPD into emphysematous, airway obstructive, and mixed phenotypes (17–20). Nakano and coworkers showed that although HRCT lacks the resolution required to measure the dimensions of the smaller airways, measurements made on larger airways compared favorably to those measured on the same airways using histology and could be used to predict the histologic measurements of smaller airways of the same lung (21).

Micro-CT is similar in concept to CT and HRCT, but uses a micro-focused X-ray source and planar X-ray detectors. This procedure is commonly used to investigate animal lungs and has been introduced into the study of lung samples from patients with COPD (22). To achieve the resolution required to resolve the structure of the peripheral lung, the equipment is arranged slightly differently in that instead of patient or animal maintaining a constant position while the tubes and detectors rotate, the sample is rotated while the X-ray tubes and detectors remain stationary. The major advantage of micro-CT over CT and HRCT is that it can be used to acquire high-resolution images of both bronchiolar and alveolar microstructure that allow them to be measured accurately. The main disadvantage is that the radiation dose required to accomplish this goal with current technology is damaging to living tissue.

The purpose of this communication is to present preliminary results obtained from representative samples removed from intact human lungs from four patients with very severe (GOLD-4) COPD treated by lung transplantation and an equal number of unused donor lungs that served as controls. To obtain these samples, the entire lung was maintained in an inflated state near TLC while it was frozen solid in liquid nitrogen fumes. The frozen lung specimen was kept frozen on dry ice while it was cut into 2-cm-thick samples, and cores of tissue 1 cm in diameter and 2 cm in length were cut from these frozen slices. These tissue cores remained frozen at -80°C while they were fixed in a solution of 1% glutaraldehyde in pure acetone that has a freezing point of -94°C . These samples are then warmed to room temperature, post-fixed in osmium, washed several times, and critical-point

dried. The dried specimens were then examined by micro-CT and analyzed using software similar to that used to analyze CT and HRCT. A contiguous stack of approximately 1,000 cross-sectional images obtained from each of these samples were used to measure both the number and dimensions of the terminal bronchioles and the alveolar dimensions present in these lungs. The number of terminal bronchioles present in each lung sample was counted by identifying the number of times purely conducting airways branched into respiratory bronchioles identified by the presence of alveoli opening into the bronchiolar structure. The software was then used to manipulate the reconstructed image of each terminal bronchiole into the x-y plane, shift this image into the z-plane, where the entire length of the lumen was examined in contiguous 16- μ m-thick sections and measured at its narrowest point. The total number of terminal bronchioles in each lung was estimated by multiplying the average number/ml of lung in the samples by the total lung volume; and the total minimal cross-sectional area was estimated by multiplying the average minimal cross-sectional area of the terminal bronchioles by the total number of terminal bronchioles present in the lung. The average size of the alveoli was also estimated by measuring the mean linear intercept (Lm) of the alveoli at regular intervals in each tissue sample.

The preliminary results obtained show (23–25) that mean alveolar dimensions (Lm) are normally distributed in the control (Donor) lungs with a range of 150 to 600 μ m, with no difference between top and bottom of these fully inflated lungs. In contrast, the distribution of Lm in lungs from patients with the centrilobular form of emphysema was right shifted with a range of 150 to over 1,200 μ m, with progressively higher values from the base to the apex of the lung. These preliminary data also showed that the number of terminal bronchioles in four control lungs ($22,255 \pm 3,893$) was similar to the 27,992 reported by Horsfield and Cumming from a single lung cast (2). In sharp contrast, this number of terminal bronchioles was reduced by approximately 10-fold ($2,436 \pm 626$) in four explanted lungs from patients with the centrilobular emphysematous phenotype of severe COPD, and the total cross-sectional area of the terminal bronchioles was reduced approximately 100-fold compared with the control lungs. Most importantly, the changes observed in the terminal bronchioles was apparent in regions of the COPD lungs where the Lm remained within the normal range, indicating that no emphysema was present.

Clearly, a 10-fold reduction in terminal bronchiolar number and 100-fold reduction in the minimal cross-sectional area of lumen of the remaining terminal bronchioles provides a much better explanation for the older physiological data, showing that these airways account for very little of the resistance in the normal lung (5, 7) but increase their resistance many fold to become the major site of airway obstruction in COPD (5–7). In attempting to reconcile these findings with the earlier work of Matsuba and Thurlbeck, several points stand out. First, their measurements were based on the number of airways counted/mm² of lung tissue before the realization that counting numbers of histologic structures requires knowledge of the volume of tissue examined. This requires the use of a three-dimensional probe and can be accomplished by cutting a pair of histologic sections a known distance apart and counting the object only if it appears in one section and not in the other. Unfortunately, this approach to counting objects dispersed in three dimensions was not introduced into histologic studies until 1984 (26), a full 12 years after the article by Matsuba and Thurlbeck was published. Micro-CT provides the opportunity to take what stereologists refer to as a brute force approach to counting objects in three dimensions (27). Because we serially examined a known volume of tissue (i.e., between 8 and 20 1-cm-diameter, 2-cm-long cores of lung tissue from each case) to count the

terminal bronchioles. However, the technical advances provided by micro-CT allowed this volume of tissue to be examined much more quickly, accurately, efficiently, and nondestructively than can be achieved using serial section histology. A second advantage of micro-CT is that the software allows the reconstructed image of the terminal bronchiole to be adjusted into the x-y plane and then shifted into the z plane to allow the examination of the airway lumen along its entire length. This approach showed that instead of having the shape of a tube the terminal bronchiolar lumen is shaped like a catenoid with a discrete minimum value near the middle that widens at both ends. This type of observation would be difficult if not impossible to make using random histologic sections because of the bias introduced by the increased probability of sampling the open ends of the catenoid rather than the narrow middle. An additional advantage is that we were able to count terminal bronchioles specifically and exclusively by identifying them anatomically, whereas Matsuba and Thurlbeck examined bronchioles from many different generations with much greater variation in size. Therefore, in our opinion the technological advances provided by the micro-CT provide much more accurate information concerning both number and minimal caliber of a specific branch of airways than could have been obtained using the technology employed by Matsuba and Thurlbeck in 1972.

In summary; micro-CT examination of the peripheral lung micro structure indicates that COPD is associated with a marked reduction in number of terminal bronchioles in the lungs of patients with very severe (GOLD-4) COPD that is accompanied by an even greater reduction in the minimal lumen caliber of those that remain. Moreover, the fact that these changes in the terminal bronchioles were present in regions of the COPD lungs not yet affected by emphysema strongly suggests that they begin before the onset of emphysematous destruction and possibly at very early stages in the natural history of COPD. Whether or not terminal bronchiolar narrowing and obliteration begin early enough to be responsible for the rapid decline in forced expiratory flow that is associated with the development of severe (GOLD-3) and very severe (GOLD-4) COPD remains to be determined.

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