Lung morphometry: quantitative assessment of tissue changes in mice exposed to chronic whole body cigarette smoke

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Aims
Chronic tobacco smoke exposure induces structural changes (oedema, atelectasia, emphysema, fibrosis, etc.) in the human lung related to chronic obstructive pulmonary disease (COPD). We investigate the pathophysiological mechanisms and neuro-immune interactions in a predictive mouse model of COPD using an integrative approach. Besides several functional, biochemical, immunological and histopathological techniques, we did microCT imaging to follow the morphological changes in vivo non-invasively. Here we show a new quantitative method of evaluating chronic cigarette smoke-induced changes in the mouse lung adopting and modifying the parameters originally described for bone morphometry.

Method
Twelve-week-old male C57Bl/6 mice were used for the study, which were exposed to whole body smoke exposure (Kentucky Research Cigarette type 3R4F, University of Kentucky, USA) twice a day for 5 days per week for 2 months. MicroCT scans of the lungs were performed before the exposure and after two month of smoking with Skyscan 1176 in vivo high resolution microCT (Kontich, Belgium) under sodium pentobarbital (70 mg/kg i.p.) anaesthesia. The following parameters were used for scanning: 50 kV tube voltage, 500 µA current, 0.5 mm Al filter, 35 µm pixel size, 52 ms exposure time, 0.7 degree rotation step, 180 degree scan, 10 images per rotation step (retrospective synchronization, breath gating, list mode). Reconstruction settings after sorting the images into five bins: post-alignment compensation: -0.5; beam hardening: 35; ring artifact correction: 20; smoothing: 6; contrast limit was -1000 – 50 HU. Images in bins Nr. 2 were used as the sharpest ones for the analysis. A water-containing Eppendorf tube and an empty tube were scanned and reconstructed with the same settings and used for calibrating the datasets. Custom image processing was used for segmenting out the lung and complete 3D morphometrical analysis was performed with CTan software.

Results
The following parameters proved to be useful indicators of chronic smoke-induced morphological changes in the mouse lung: 1) The aerated lung volume increased ominously, which refers to the increased residual air volume at the end of the expiration. 2) Lung surface did not change significantly, but surface to volume ratio remarkably decreased. 3) Structure Model Index showed significant shrinkage. 3) Structure thickness elevated, which correlates with the mean alveolar diameter. 4) Connectivity decreased after two month of smoking. 5) The structure thickness distribution showed a right shift toward thicker values caused by the increased mean airway/alveolar diameter. These findings well correlate with the histopathological analysis.
Figure 1. Lung morphometry
Conclusion
The determined parameters are valuable markers of cigarette smoke-induced lung damage of the mouse to follow and quantify the structural changes in our predictive COPD model in vivo even after a longer follow-up period.