Quantitative 3D-Micro-CT Imaging of the Human Lung Fibrosis

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Aims: Interstitial lung diseases (ILD) are characterized by a chronic inflammation of interstitial structures leading to a growth of connective tissue with lung fibrosis as its final result. Prevalence of ILD is estimated with 80.9 per 100,000 male/year and 67.2 per 100,000 females (1). Amongst the over 200 different entities of ILDs, idiopathic interstitial pneumonia (IIP) with its seven different subtypes (2) is a heterogeneous group of diseases with unknown cause. The major manifestation of IIP is Idiopathic pulmonary fibrosis (IPF) with its radiological and pathological pattern of usual interstitial pneumonia (UIP). Current diagnosis in cases with ILDs depends on anamnesis, clinical observations, radiological and histopathological patterns. Therefore, the aim of the present study was to evaluate the feasibility of micro-CT imaging for analysis of the human lung fine structure.

METHODS
Preparation of native lungs: Six subpleural lung samples from six individuals (fibrotic lungs (n = 3), healthy lungs (n = 3)) were fixed in buffered 3.7 % formalin. Samples were cut with a sharp mechanical stamp into cylindrical shape of 10 mm length and 8 mm diameter. Tissue specimens were dried with a critical point dryer as described (10) to remove excessive fluid without tissue deformation.
Preparation of intravascular contrasted lungs: The pulmonary artery in the lower lobe (segment 8) was cannulated and perfused with heparinized saline until venous effluent was free from blood. A lead containing compound polymer (Microfil® MV-122, Flow Tech, Carver, MA, USA) was infused with a nominal pressure of ~30 mmHg. After polymerization 28 lung samples from 6 individuals (fibrotic lungs (n = 3), healthy lungs (n = 3)) were fixed in formalin, dried and cut as mentioned above.
Preparation of osmium contrasted lungs: Nine lung samples from 3 patients with severe fibrosis were immersed in tubes filled with a solution of 1% osmium tetroxide as described (2). Tubes were rotated for three hours; afterwards specimens were washed threefold in purified water to remove excessive Osmium.
Preparation of HgCl2 contrasted lungs: Lung samples (n = 9) from three patients with severe lung fibrosis were threefold immersed in tubes filled with Bouin’s solution. Before cutting into cylindrical shape, probes were dried in compartment air, shrinking of the sample was avoided by fixation with needles on a cork board.
Image acquisistion: All specimens were scanned in a micro-computed tomograph (micro-CT) manufactured by SkyScan (SkyScan 1072_80kV; Kontich, Belgium). Micro-CT was performed as previously
described (3) with a tungsten microfocus tube using a tube voltage of 80 kV. On a computer controlled rotation stage samples were scanned over 180 degrees in steps of 0.45 degrees with an acquisition time of 2.6 seconds per step. The detector of our system (SkyScan 1072_80kV) is equipped with a 12-bit digital CCD-camera with a resolution of 1024 x 1024 pixels. Geometric magnification of the system is determined by object-source distance and leads up to an 80-fold magnification with a minimum pixel size of 8 µm. A modified Feldkamp cone-beam reconstruction modus was used for raw data editing. Each picture consists of a Matrix of 1024 x 1024 pixels with a cubic voxel size of 12 µm.

Histopathology:
After CT-scanning, samples were embedded in Paraffin, serial sectioned at 8 µm slice thickness and stained with hematoxylin-eosin and Goldner. Histological sections were examined by light microscopy and digitized afterwards.

Comparisons of Histopathology and Micro-CT Imaging:
To evaluate soft tissue fraction, vascular volume fraction and air space fraction image reformation and analysis i.e. multiplanar reformation (MPR), maximum intensity projection, volume rendering, region of interest measurements and object extraction was carried out with Analyze software package (Biomedical Imaging Resource, Mayo Foundation, USA, Version 7.0). Digitized histological sections were imported into Analyze and matched with the corresponding micro-CT data. Differences on structural changes of the lung were analyzed by variance statistics (ANOVA).

RESULTS: Assured by pathological affirmation, samples of fibrotic human lungs with UIP-pattern and disease free lungs were admitted to the study. As demonstrated in figure 1, significant differences in total soft tissue volume, vascular and air space volume fraction are detectable with micro-CT imaging. Fibrotic lung samples demonstrated a significant augmentation of the soft tissue volume (fibrosis 87 ± 10 % vs. controls 17 ± 9 %; p < 0.001) and a significant reduction in total air space (fibrosis 13 ± 8% vs. controls 83 ± 23%; p < 0.001) determined by micro-CT. Simultaneously, samples with lung fibrosis showed a significant reduction in vascular volume fraction compared to controls (fibrosis 4 ± 2% vs. control 14 ± 8%; p < 0.03). No significant difference between native vs. osmium stained samples were observed for air space fraction or soft tissue fraction in fibrotic lungs. Deviant to this finding we found a discrete but statistically significant difference between Osmium and HgCl2 staining (p < 0.04). As illustrated in figure 2, micro-CT obtained images that closely approximated histological microscopy and that, with spatial resolutions of 8 microns voxel size, allowed precise visualization of the lung fine structure. Every case of histopathological proven fibrosis was confirmed by micro-CT.

Using tomographic reconstruction algorithms, three-dimensional images of the human lung can be generated which allow total stereoscopic visualization of the lung 3D microarchitecture as demonstrated in figure 3. Irrespective of the differences in vascular volume fraction, contrast filled vessels showed another, morphologic discrepancy: vessels in non-fibrotic lungs exhibit a rectilinear distribution whereas vessels in fibrotic lungs featured a corkscrew like pattern (figure 4).

CONCLUSION: We demonstrated feasibility of using micro-CT to assess quantitative information in human lung samples with or without fibrosis with different staining methods. Micro-CT should be considered as an additional tool for ex-vivo studies of human lung fibrosis. Thus, micro-CT should be evaluated for further analysis of vascular and parenchymal changes in different types and degrees of human lung fibrosis.
Figure 1
Quantitative micro-CT analysis demonstrates significant differences between fibrotic and non-fibrotic lungs in soft tissue and air space fraction (p < 0.001), vascular volume fraction (p < 0.03). HgCl2 and Osmium staining shows significant differences in fibrotic lung samples concerning soft tissue fraction and air space fraction (p < 0.04). The total vascular volume fraction is significantly reduced in fibrotic lungs compared to controls (p < 0.02).

Figure 2
Grey scale inversed single slice micro-CT (A) and corresponding histology (B) demonstrating increased soft tissue contingent in fibrotic lung tissue (B, HE staining, magnification x 10).

Figure 3.

Micro-CT imaging of controls (A, B, C) and fibrotic lung samples (D, E, F). Summed voxel projection (A, D), maximum intensity projection (B, E) and axial single slice (C, F) demonstrates inflated alveoli in controls compared to the increase in soft tissue fraction in fibrotic lungs.
Figure 4.

Maximum intensity projection (A, B) and single slice micro-CT images (C, D) in controls (A, C) and fibrotic lungs (B, D). After intravascular contrast perfusion (Microfil), fibrotic lungs demonstrated a significant decrease in the total vascular volume fraction compared to controls. Moreover, fibrotic lung samples show irregular branching patterns as well as irregular vessel diameter and classical corkscrew like pattern.

References: