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Title: Structural differences in airways during chronic rejection after lung transplantation: A (micro)-CT analysis

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Body: Chronic rejection is a major problem after lung transplantation (Tx) and is accepted to be a small airway disease. Recently a distinction between an obstructive (fBOS) and restrictive phenotype (RAS) has been made. We aimed to investigate structural differences. Human explant lungs from 5 fBOS and 3 RAS patients were collected during reTx, were air-inflated to TLC, frozen solid in liquid nitrogen vapor and frozen specimens were examined by HRCT. Unused donor lung (n=1) served as control. The lungs were kept frozen while cut in 2cm slices and cylinders of 1.4cm diameter were removed. These were fixed at -20°C in a solution of acetone/1% gluteraldehyde and warmed to 25°C, dried and scanned with microCT (skyscan1172, Belgium). HRCT images showed that RAS lungs displayed a lower number of airways starting at generation 5. In patients with fBOS >50% of the airways get obstructed at generation 6 (figure 1), mainly in airways <2mm (82%). MicroCT analysis shows a lower number of terminal bronchioles in RAS patients (2/ml of tissue vs 4.5/ml in fBOS and 5/ml in control, p<0.0001) and a lower average cross-sectional area of remaining terminal bronchioles (110mm² vs 178 in fBOS vs 175 in control). In conclusion: in RAS lungs, both airways>1mm and terminal bronchioles shrink and/or disappear while in fBOS lungs, the airways become obstructed. There was no difference in terminal bronchioles between fBOS and normal lung.