Small Airway Determinants of Airflow Limitation in Chronic Obstructive Pulmonary Disease

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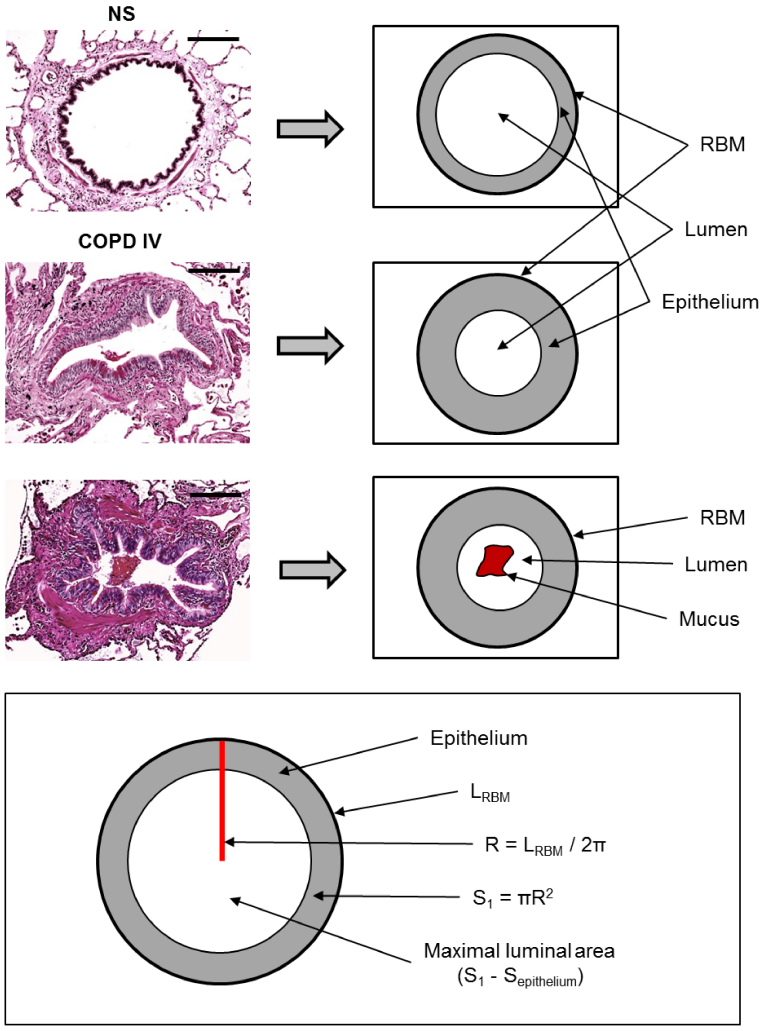
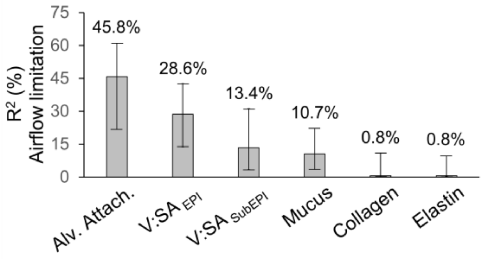
Online Supplement

Immunohistochemistry

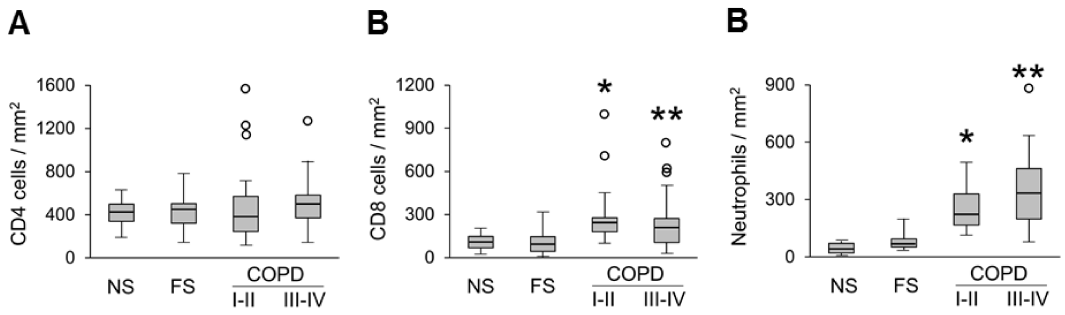
Additional serial sections were used for immunostaining with primary antibodies against neutrophil elastase for detection of neutrophils [mouse monoclonal, #GTX72042 (NP57), GeneTex, Irvine, CA], CD4 for detection of T helper cells [rabbit polyclonal, #ab133616 (EPR6855), Abcam, Cambridge, MA], CD8 for detection of T cytotoxic cells [mouse monoclonal, #GTX75393 (4B11), GeneTex] or CD19 for detection of B cells [mouse monoclonal, #GTX42325 (LE-CD19), GeneTex]. A standard immunoperoxidase/avidin-biotin complex protocol (Vectastain ABC kit, Vector Laboratories, Burlingame, CA) was used for detection of primary antibodies. To detect elastin, we used an immunofluorescence technique with mouse monoclonal antibody [ab237989 (ELN/1981), Abcam], a secondary biotinylated antibody (Vectastain ABC kit) and fluorochrome labelled with Cy3 (#016-160-084, Jackson ImmunoResearch Lab, West Growe, PA). The specificity of immunohistochemistry was verified using an isotype control antibody by replacing the primary antiserum with an identical concentration of non-immunized mouse or rabbit serum (Invitrogen Corporation, Camarillo, CA).



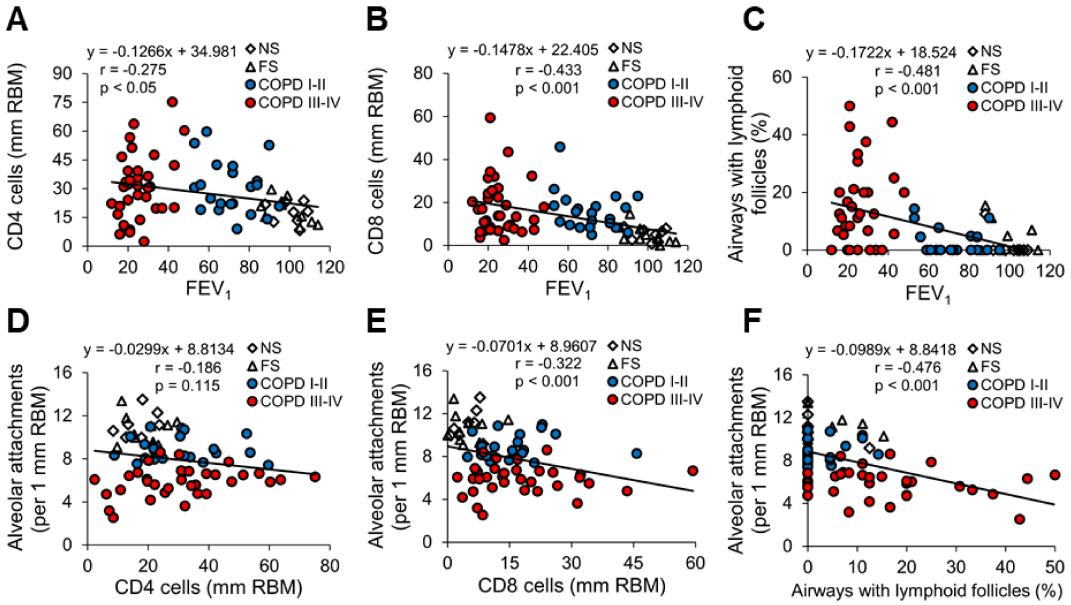
Supplemental Figure 1. Schematic illustration for determining the effects of increased epithelial height and luminal mucus accumulation on airway luminal area. The maximal luminal area of airways was calculated as the area of a circle formed by the full length of the RBM (L_{RBM}) minus the area occupied by the epithelium. Reduction of luminal area due to increased epithelial height (epithelial expansion) was calculated as a ratio of the maximal luminal area to the area of the circle formed by the full length of the RBM and presented as a percentage. Luminal area occupied by mucus plugging (luminal mucus occlusion) was calculated as a ratio of mucus content area to the maximal luminal area and presented as a percentage.



Supplemental Figure 2. Relative contribution of small airway pathological findings to airflow limitation in different stages of COPD based on multivariable linear regression model including cases with mild/moderate COPD (GOLD stages I-II). $R^2 = 65.4\%$. The relative contribution of pathological parameters on airflow limitation (measured as FEV_1) plotted as the proportion of FEV_1 variability and its corresponding 95% confidence interval explained by each parameter.



Supplemental Figure 3. Quantification of immune/inflammatory cells in small airways of COPD patients (normalization to 1 mm^2 of airway wall). A-C – box and whisker plots show immune/inflammatory cell counts in airway walls from all patient groups. Boxes represent the interquartile range, whiskers extend to the most extreme data point which is no more than 1·5 times the interquartile range from the box, and circles beyond the whiskers are extreme values, the line within the box represents the median. Groups were compared pairwise using Mann-Whitney U test. Estimated p-values were Bonferroni-adjusted. The threshold for significance was 0·05. *significantly different compared to NS controls, **significantly different compared to all other groups.



Supplemental Figure 4. A-C – Scatter plots showing correlation and line of best fit between FEV_1 and (A) CD4 T cells in airway walls, (B) CD8 T cells in airway walls, and (C) percent of airways with B cell-containing bronchus-associated lymphoid follicles. D-F – Scatter plots showing correlation and line of best fit between alveolar attachments and (D) CD4 T cells in airway walls, (E) CD8 T cells in airway walls, and (F) percent of airways with B cell-containing bronchus-associated lymphoid follicles.