Bronchial Thermoplasty in severe asthma

d’Hooghe, J.N.S.

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CHAPTER 8

Identification and quantification of airway wall layers with Optical Coherence Tomography: a histology based validation study.

d’Hooghe JNS
Goorsenberg AWM
de Bruin DM
Roelofs JJTH
Annema JT
Bonta PI

ABSTRACT

Background

High-resolution computed tomography has limitations in the assessment of airway wall layers and related remodeling in obstructive lung diseases. Near infrared-based Optical Coherence Tomography (OCT) is a novel imaging technique that combined with bronchoscopy generates highly detailed images of the airway wall. The aim of this study is to identify and quantify human airway wall layers both ex-vivo and in-vivo by OCT and correlate these to histology.

Methods

Patients with lung cancer, prior to lobectomy, underwent bronchoscopy including in-vivo OCT imaging. Ex-vivo OCT imaging was performed in the resected lung lobe after needle insertion for matching with histology. Airway wall layer perimeters and their corresponding areas were assessed by two independent observers. Airway wall layer areas (total wall area, mucosal layer area and submucosal muscular layer area) were calculated.

Results

13 airways of 5 patients were imaged by OCT. Histology was matched with 51 ex-vivo OCT images and 39 in-vivo OCT images. A significant correlation was found between ex-vivo OCT imaging and histology, in-vivo OCT imaging and histology and ex-vivo OCT imaging and in-vivo OCT imaging for all measurements (p < 0.0001 all comparisons). A minimal bias was seen in Bland-Altman analysis. High inter-observer reproducibility with intra-class correlation coefficients all above 0.90 were detected.

Conclusions

OCT is an accurate and reproducible imaging technique for identification and quantification of airway wall layers and can be considered as a promising minimal-invasive imaging technique to identify and quantify airway remodeling in obstructive lung diseases.
Identification and quantification of airway wall layers with Optical Coherence Tomography: a histology based validation study.

**ABBREVIATIONS LIST**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>AMC</td>
<td>academic Medical Center</td>
</tr>
<tr>
<td>ASM</td>
<td>airway smooth muscle</td>
</tr>
<tr>
<td>BT</td>
<td>bronchial thermoplasty</td>
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<tr>
<td>COPD</td>
<td>chronic pulmonary obstructive disease</td>
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<tr>
<td>H&amp;E</td>
<td>hematoxylin and eosin</td>
</tr>
<tr>
<td>HRCT</td>
<td>high resolution computed tomography</td>
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<tr>
<td>ICC</td>
<td>intra-class correlation</td>
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<tr>
<td>NSCLC</td>
<td>non-small cell lung cancer</td>
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<tr>
<td>OCT</td>
<td>optical coherence tomography</td>
</tr>
<tr>
<td>PET-CT</td>
<td>positron emission tomography-computed tomography</td>
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</table>
INTRODUCTION

Airway remodeling is defined by structural changes and thickening of the airway wall, which is seen in several pulmonary diseases, such as asthma and chronic obstructive pulmonary disease (COPD) [1-3]. The identification and severity of airway remodeling is important as it relates to disease severity[4]. Currently, airway remodeling can be assessed by high resolution computed tomography (HRCT)-scan of the chest. However this imaging technique requires patient exposition to ionizing radiation and has limited resolution that hampers visualization and quantification of the different airway wall layers. Bronchial mucosal biopsies taken during bronchoscopy, can visualize the different airway wall layers very precisely but are invasive. Furthermore these biopsies, provide only information of a small selected site of the airways and the processing of biopsies is time consuming and often causes artefacts [5].

Optical Coherence Tomography (OCT) is a promising real-time high-resolution imaging technique to assess airway remodeling[6, 7]. Using near-infrared light cross-sectional images are created by the backscattering of light by the tissue[8]. For example, in ophthalmology, OCT is used in clinical practice for retina assessment [9] and in cardiology for stent positioning during percutaneous coronary interventions [10]. Former studies have shown that OCT is able to visualize the different airway wall layers including mucosa (epithelium and lamina propria), submucosa (including airway smooth muscle, glands) and cartilage [11-15]. Only limited data are available on the quantification of total airway wall area and the correlation with histology [5, 16] and CT[5, 6]. The feasibility of OCT to quantify separate airway wall layers, including the mucosa and submucosa, and the correlation with histology in human airways is unknown. Furthermore correlating ex-vivo and in-vivo OCT images has never been done before. The aim of this study is to identify and quantify airway wall layers in ex-vivo and in-vivo OCT images and correlate these to histology, and assess the inter-observer reproducibility. We hypothesize that: 1) airway wall layer areas assessed on ex-vivo OCT images correlate well with matched histology sections, 2) airway wall layer areas assessed on in-vivo OCT images correlate well with both ex-vivo OCT images and histology sections, and, 3) there is a good inter-observer reproducibility for manually traced OCT airway wall layer perimeters and their corresponding areas.

METHODS
STUDY DESIGN

This is a prospective observational cohort study, performed in the Academic Medical Center (AMC) in Amsterdam, the Netherlands. Ethical approval was obtained from the Medical Ethics Committee of the AMC (NL51605.018.14). Fig 1 shows the flow of study conduct.
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Figure 1 Flow chart of study conduct.

STUDY SUBJECTS

Patients with a strong suspicion or tissue proven peripheral non-small cell lung cancer (NSCLC) staged cT1-3N0-1M0 based on a positron emission tomography-computed tomography (PET-CT) and in need of a standard diagnostic bronchoscopic work-up and lobectomy were eligible for the study. Signed informed consent was obtained prior to the procedure.

IN-VIVO OCT IMAGING

OCT images of in-vivo airways were acquired using a C7-XR St. Jude Medical Inc. system interfaced with a C7 Dragonfly catheter (Ø 0.9 mm diameter) (St. Jude Medical Inc., St. Paul, MN, USA). After standard diagnostic bronchoscopy, the OCT catheter was inserted through a guide sheath into the working channel of the bronchoscope into the airways of interest where an automated pullback of 5.4 cm was performed (S1A Fig). All airways in the lobe candidate for surgical resection were imaged from subsegmental to segmental airways. Each pullback was repeated at least two times.

EX-VIVO OCT IMAGING

Following surgical resection, the lobectomy specimen was subjected to OCT imaging within three hours after removal. In preparation for ex-vivo OCT imaging the airways were partially exposed and instilled with phosphate buffered saline. In order to correlate the ex-vivo OCT imaging with histology, two to four curved suture needles were inserted in the in-vivo OCT imaged airways (S1B Fig). These needles were clearly visible on ex-vivo OCT imaging (S1C Fig) and guided matching of ex-vivo OCT images with histology sections. Ex-vivo OCT imaging was performed similarly to in-vivo OCT imaging as described above.

HISTOLOGICAL PREPARATION

After performing ex-vivo OCT imaging of the airways, the lobectomy sample was fixed in phosphate buffered formalin overnight. Measured airways were dissected
Figure 2. Ex-vivo OCT cross-sectional image visualizing the different layers of the human airway wall and corresponding histology image. (A) Histology cross section, stained with H&E. (B) Higher magnification view of the square of histology image A, visualizing the different layers of the airway wall of the segmental LLL. (C) Histology cross section, stained with desmin. (D) Higher magnification view of the square of histology image C, visualizing the submucosal muscular layer of the airway wall. (E) Corresponding cross section of OCT of ex-vivo airway to histology airway image A and C. (F) Higher magnification view of the square of OCT image E, visualizing the corresponding layers of the airway wall.
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and sectioned according to the sutures needles. Subsequently the tissue samples were dehydrated with increasing concentrations of ethanol for ~4 hours, cleared in xylene and impregnated in paraffin, using a standard tissue processor (A82300001 Excelsior AS Tissue Processor, ThermoFisher Scientific, Waltham, MA, USA). Next, tissues were manually embedded in paraffin. Sections of 4 µm thickness were stained with hematoxylin and eosin (H&E) to visualize the airway wall structures (Figs 2A and 2B). Immunostaining with desmin was used to identify the airway smooth muscle layer (Figs 2C and 2D). We used Philips Digital Pathology Solution 2.3.1.1 to digitalize the histology slides (Philips Electronics, the Netherlands).

OCT MEASUREMENTS PROTOCOL AND TRAINING

Before analyzing the ex-vivo and in-vivo OCT images, a protocol was written that defined how to identify and quantify the airway wall layers in OCT images. For training purposes, according to this protocol a test series of OCT images were analyzed by two independent OCT image experts (JH and AG). The test OCT imaging set contained 51 randomly selected OCT images which were obtained from another study. Luminal perimeter (PL), outer perimeter of the mucosa (P muc) and outer perimeter of the submucosal muscular layer (P submusc) were traced.

OCT - HISTOLOGY MATCHING

Ex-vivo OCT images and histology sections were matched by one observer (JH) based on the needles which were clearly visible in OCT. In-vivo OCT images were linked with ex-vivo OCT images and histology by matching luminal perimeters and corresponding areas in mm² combined with the distance from reference points and segmentations in corresponding airways.

OCT AND HISTOLOGY MEASUREMENTS

We used ImageJ software for Windows (National Institutes of Health, Bethesda, MD, USA) to manually trace the luminal perimeter (PL), mucosal perimeter (P muc) and submucosal muscular perimeter (P submusc) in the desmin stained histology images of our study population (Figs 3A and 3B). The same perimeters were traced in in-vivo and ex-vivo OCT images using St. Jude Medical Inc. software (Figs 3C and 3D). The criteria for tracing the airway wall layer perimeters in OCT images were based on the differences in light intensity as shown in S2 Fig. From the inside of the airway lumen to the outer wall, the first thin low intensity layer identified is the epithelial layer. The second, high intensity, layer matches the lamina propria.
Figure 3. *Ex-vivo* OCT cross-sectional image and corresponding histology image of human airway. (A) Clean histology cross section of human airway of the segmental LLL, stained with desmin. (B) Cross section images of histology, stained with desmin, with manually traced perimeters; $P_L$: lumen perimeter, $P_{muc}$: mucosal perimeter, $P_{submuc}$: submucosal muscular perimeter. (C) Corresponding cross section of OCT of ex-vivo airway to histology airway image A. (D) Cross section images of OCT and with manually traced perimeters; $P_L$: lumen perimeter, $P_{muc}$: mucosal perimeter, $P_{submuc}$: submucosal muscular perimeter and OCT probe in situ.

The mucosal layer (the mucosal layer includes the epithelial layer and lamina propria layer). The third, lower intensity, layer is the submucosa layer which includes the airway smooth muscle. The next, very low intensity, layer is the cartilage layer identified by a surrounded thin high intensity layer, the perichondrium. Subsequently, the luminal areas $A_L$ (in mm$^2$), mucosal areas $A_{muc}$ (in mm$^2$) and submucosal muscular areas $A_{submuc}$ (in mm$^2$) corresponding to the traced perimeters $P_L$, $P_{muc}$, $P_{submuc}$ were automatically calculated. These areas were used to calculate the surface areas of
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the different layers; total airway wall area (WA_t = A_{submusc} - A_L), mucosal wall layer area (WA_{muc} = A_{muc} - A_L) and submucosal muscular wall layer area (WA_{submusc} = A_{submusc} - A_{muc}) in mm². Both JH and AG analysed the blinded histology, OCT ex-vivo and OCT in-vivo images independently in order to assess the inter-observer reproducibility.

PRIMARY ENDPOINT

The primary endpoint was the correlation between ex-vivo OCT and histology for the above described parameters (A_L, A_{muc}, A_{submusc}, WA_t, WA_{muc} and WA_{submusc} in mm²).

SECONDARY ENDPOINTS

Secondary endpoints were the correlation between in-vivo OCT and ex-vivo OCT and between in-vivo OCT and histology for the above described parameters (A_L, A_{muc}, A_{submusc}, WA_t, WA_{muc} and WA_{submusc} and in mm²). Furthermore the inter-observer reproducibility of the manually traced perimeters and corresponding areas between two observers was analyzed (A_L, A_{muc}, A_{submusc} in mm²).

STATISTICAL ANALYSIS

Data were tested for normality using a D’Agostino and Pearson omnibus normality test and histograms. The relationship between histology and OCT images (ex-vivo and in-vivo) and the inter-observer reproducibility was determined by using a Pearson correlation coefficient (r) for normally distributed data and the Spearman’s rank correlation coefficient (r) for non-normally distributed data with its least squares linear regression models. The agreement between measurements is shown in Bland-Altman plots. Both analysis were performed in GraphPad Prism version 5.01 (GraphPad Software Inc, San Diego, CA, USA). To analyze if both observers indeed measured the same values we calculated the intra-class correlation coefficient (ICC) using SPSS statistics for Windows version 23.0. The used P values in our analysis were all two sided and sat at a level of statistical significance of P < 0.05.

RESULTS

From April 2015 until November 2015 5 patients with NSCLC who underwent an lobectomy were included in this study. Patient characteristics are shown in Table 1.
Table 1. Patient characteristics undergoing OCT and lobectomy

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age (years)</th>
<th>Sex</th>
<th>FEV1% predicted</th>
<th>COPD GOLD status</th>
<th>Resected lung lobe</th>
</tr>
</thead>
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<tr>
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<td>97</td>
<td>n.a.</td>
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<tr>
<td>Patient 2</td>
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<td>Male</td>
<td>94</td>
<td>n.a.</td>
<td>LLL</td>
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<tr>
<td>Patient 3</td>
<td>71</td>
<td>Female</td>
<td>90</td>
<td>n.a.</td>
<td>LLL</td>
</tr>
<tr>
<td>Patient 4</td>
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<td>Male</td>
<td>36</td>
<td>GOLD III</td>
<td>LLL</td>
</tr>
<tr>
<td>Patient 5</td>
<td>64</td>
<td>Male</td>
<td>48</td>
<td>GOLD III</td>
<td>RUL</td>
</tr>
</tbody>
</table>

FEV1: forced expiratory volume in one second. COPD: chronic obstructive pulmonary disease. n.a.: not applicable. RUL: right upper lobe. LLL: left lower lobe

**PRIMARY ENDPOINT**

In total 13 ex-vivo airways in 5 patients were imaged with OCT, resulting in 51 matching cross sectional OCT images and histology sections. Airway wall layers could be identified as shown in Figs 2 and 3. Linear regression analysis showed a significant correlation between ex-vivo OCT imaging and histology for all parameters ($A_l r=0.96, p<0.0001$, $A_{muc} r=0.92, p<0.0001$, $A_{sub} r=0.87, p<0.0001$, $WA, r=0.79, p<0.0001$, $WA_{muc} r=0.78, p<0.0001$ and $WA_{submuc} r=0.62, p=0.0001$) (Table 2, Fig 4 A-F left graphs). Bland-Altman analysis showed a minimal bias for (all measurements of) these parameters (Fig 4 A-F right graphs). A proportional error was found for mucosal wall area ($WA_{muc}$ in mm$^2$) and submucosal muscular wall area ($WA_{submuc}$ in mm$^2$).

Table 2. Correlation between ex-vivo OCT and histology for airway wall area measurements

<table>
<thead>
<tr>
<th>Parameter</th>
<th>r</th>
<th>P-value</th>
</tr>
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<tr>
<td>$A_l$</td>
<td>0.96</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>$A_{muc}$</td>
<td>0.92</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>$A_{submuc}$</td>
<td>0.87</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>$WA_t$</td>
<td>0.79</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>$WA_{muc}$</td>
<td>0.78</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>$WA_{submuc}$</td>
<td>0.62</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

$A_l$: luminal area. $A_{muc}$: mucosal area. $A_{submuc}$: submucosal muscular area, $WA_t$: total airway wall area. $WA_{muc}$: mucosal wall layer area. $WA_{submuc}$: submucosal muscular wall layer area.
Figure 4. Linear regression analysis and Bland-Altman plots for histology and OCT ex-vivo airway wall area measurements (n=51). (A) $A_l$ lumen area in mm$^2$. (B) $A_{muc}$ mucosal area in mm$^2$. (C) $A_{submuc}$ submucosal muscular area in mm$^2$. (D) $W_A$ total airway wall area in mm$^2$. (E) $W_{A_{muc}}$ mucosal wall area in mm$^2$. (F) $W_{A_{submuc}}$ submucosal muscular wall area in mm$^2$. 
SECONDARY ENDPOINTS

Ex-vivo OCT and in-vivo OCT images
A total of 39 in-vivo OCT cross sectional images could be compared with their corresponding ex-vivo OCT images. Images were matched according to luminal perimeters for the same airways. High correspondence between in-vivo and ex-vivo OCT imaging for the luminal area was shown (A_l r=0.99, p<0.0001). Linear regression analysis showed a significant correlation for all other parameters (A_{muc} r=0.97, p<0.0001, A_{submuc} r=0.88, p<0.0001, WA_r=0.54, p<0.0001, WA_{muc} r=0.68, p<0.0001 and WA_{submuc} r=0.40, p=0.0001). Bland-Altman analysis showed a negligible bias between OCT ex-vivo and OCT in-vivo images (S3 Fig). Similar results were found when in-vivo OCT was compared with histology (S4 Fig).

INTER-OBSERVER REPRODUCIBILITY

All 51 ex-vivo OCT images and 39 in-vivo OCT images were analyzed independently by the two observers. The intra-class correlation coefficients to assess the accuracy of ex-vivo and in-vivo OCT measurements by the two observers were high for all parameters (S1 Table). Linear regression analysis of the ex-vivo OCT measurements between the two observers showed a significant correlation (r ≥0.98, p< 0.0001 for all measurements) (S5 Fig). Bland-Altman plots showed a minimal bias for all three parameters (A_l -0.02 (95% CI = -0.22-0.19), A_{muc} -0.06 (95% CI = -0.52-0.41) and A_{submuc} 0.02 (95% CI = -0.99-1.04)(S5 Fig). Comparable results were found for the in-vivo OCT measurements between the two observers (S6 Fig).

DISCUSSION

To the best of our knowledge, this is the first study to show the feasibility of OCT to identify and subsequently quantify separate human airway wall layers showing a strong and significant correlation with histology for both ex-vivo and in-vivo OCT images. Besides, this is the first report comparing ex-vivo and in-vivo OCT imaging and showing its significant correlation. Importantly, a high inter-observer reproducibility was detected between two independent observers. The correlations for separate airway wall areas measured by OCT and histology were less strong when compared to the previous animal study performed in porcine lungs, which are known to have more widespread airway cartilage than human airways [16]. This makes it easier to distinguish the different perimeters in OCT images of porcine airways [17]. The proportional error in Bland-Altman analysis of WA_{submuc} (mm²) and WA_r (mm²) for ex-vivo OCT imaging versus histology, suggests that a small systematic difference between histology and OCT, is present. Probably, this is the result of formalin fixation, alcohol dehydration and paraffin embedding, which are known to cause tissue shrinkage, however without disturbing the intrinsic proportions of the various tissue layers. The high correlation
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we found for the lumen and total airway wall quantification is comparable to the in-vivo OCT study performed by Chen et al[5].

Since we could not use suture needles as landmarks for matching the OCT in-vivo images with histology, we used a corresponding inner luminal area from the same airway instead. A decrease of the airway lumen after resection and especially after histological processing can be expected. This can contribute to the observed differences between the in-vivo OCT and ex-vivo OCT measurements and in-vivo OCT and histological measurements. However, the correlation for $A_{\text{muc}}$ and $A_{\text{submuc}}$ remained strong and significant for in-vivo OCT imaging compared to histology and suggests that an exact match is not necessary when you measure the same airway with a corresponding lumen. A previous study assessing insertion-reinsertion reproducibility of the total airway wall area in OCT found similar results and stated that heterogeneity in airway wall structure seemed to be relatively small[18].

This study has multiple strengths. First, all measurements were independently assessed by two observers, making it possible to analyze the inter-observer reproducibility. By using an anonymized test series of different patients, we avoided creating bias by already seeing the OCT images from our own cohort. Based on histology and the OCT test series both observers with knowledge of the histology of airway walls were able to analyze OCT images of the airway wall. With high intra-class correlation coefficients and a negligible bias for all measurements between two independent observers this study shows that OCT imaging has a strong inter-observer reproducibility. The strong inter-observer reproducibility in both ex-vivo and in-vivo OCT imaging, confirms data from previous studies[16, 18]. Second, a large sample size of 51 matched cross sectional OCT and histology images were analyzed. Third, the unique study method, where human airways were measured with OCT both in-vivo and ex-vivo and subsequently compared with each other and with histology, ensured a reliable comparison. Finally, we believe that the method of airway wall layer quantification as executed in this study is of interest. Since subtracting airway wall area measurements from one another results in areas of the airway wall layer that are independent of the shape of the airway.

There are several limitations to this study. First, not all ex-vivo airways were also measured with OCT in-vivo, therefore 39 of the 52 ex-vivo OCT images had a corresponding in-vivo OCT image. Since it was not possible to use sutures during OCT in-vivo imaging, the luminal area and distance to reference points and segmentations was used to match with histology. In addition, matching was done by a single person. Second, our cohort contained a heterogeneous population with both subjects with a non-obstructive lung function and subjects with COPD. However, in these 5 patients several airways were imaged, creating 51 histology-
OCT ex-vivo matches. Since the aim of this study was to correlate histology to OCT images independent of the health status of the airway wall, the heterogeneity of the study population does not interfere with the study aim. Another possible limitation is generated by artefacts after processing histology tissue. A well-known artefact is shrinkage of histology tissue caused by fixation[19]. This could potentially have caused the small proportional error seen in the Bland-Altman analysis. In addition, OCT imaging artifacts can contribute to the found differences between OCT and histological measurements. As shown in Figs 2 and 3 the sensitivity decreases with the distance between the probe and the airway wall. Furthermore minimal artefacts are expected from the angle of the airway wall surface relative to the light beam, the refractive index radial calibration and refractive effects.

One major advantage of OCT over histology is that OCT is able to measure airway segments real-time in their natural state in-vivo. With OCT being significantly correlated to histology and easy to learn with high reproducibility, it could be an ideal instrument to assess airway wall remodeling, understand airway disease pathogenesis and eventually monitor and evaluate treatment results over time in patients with airway diseases such as asthma[20]. For instance, there is evidence that Bronchial Thermoplasty (BT) induces a reduction in airway smooth muscle (ASM) mass in severe asthma[21]. As OCT is able to visualize and quantify the different airway wall layers in a specific airway segment, it could potentially detect changes in the submucosal muscular layer, containing the ASM after BT. As such OCT may serve as an ideal BT treatment evaluation instrument and might be used for identification of patients that have a large ASM mass. In the future, combining OCT with technical advancements such as polarisation will make it possible to visualize and quantify the ASM itself, which could be of added value in these patients [22]. For this purpose, although the high inter-observer reproducibility of OCT measurements are reassured, automated software for airway wall measurements is highly needed.

In conclusion, OCT is an accurate and reproducible imaging technique for identification and quantification of the airway wall areas in total and in sublayers. OCT can be considered a promising non-invasive imaging technique to identify and quantify airway remodeling in patients with obstructive lung diseases.
ACKNOWLEDGMENTS

The authors would like to acknowledge and thank O. de Boer and T. Dirksen for their committed and professional work.

Notation of prior abstract presentation

Results were reported in part during the European Respiratory Society (ERS) congress in London, 06/09/2016 abstract number: 1436, and during the American Thoracic Society (ATS) congress in San Francisco, 16-05-2016 abstract number: A3389.
REFERENCES


Identification and quantification of airway wall layers with Optical Coherence Tomography: a histology based validation study.


Identification and quantification of airway wall layers with Optical Coherence Tomography: a histology based validation study.

S1 Table. Inter-observer reproducibility of OCT measurements between two independent observers.

Inter-observer reproducibility of OCT measurements between two independent observers.

<table>
<thead>
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<th>ICC</th>
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<th>P-value</th>
<th>ICC</th>
<th>95% CI</th>
<th>P-value</th>
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<td>0.999-1.000</td>
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<tr>
<td>A_muc (mm²)</td>
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<td>0.995-0.998</td>
<td>&lt;0.0001</td>
<td>0.997</td>
<td>0.993-0.999</td>
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<tr>
<td>A_submuc (mm²)</td>
<td>0.991</td>
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<td>0.983</td>
<td>0.959-0.992</td>
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S1 Figure Bronchoscopic OCT imaging technique. (A) Bronchoscopic view: In-vivo OCT imaging with OCT catheter (left arrow) outside the sheet (right arrow) in the posterior segment (RB9) of the right lower lobe. The medial-basal segment (RB7) is used as reference point for the end of the pullback track of 5.4 cm marked by a metal part (left arrow). (B) Resected lung lobe with 3 suture needle marks (long arrows) through the lumen of the airway. OCT catheter placed in airway with needle marks (short arrow). (C) OCT cross-section of an ex-vivo imaged airway with a needle mark visible (long arrow). OCT probe visible in the center of the airway (short arrow).
S2 Figure. OCT criteria for identification of airway wall structures and layers. (A) OCT image of the airway wall of a segmental airway of the left lower lobe. (B) Manual tracing of perimeters based on differences in light intensities of the airway wall layers. From right to left the dotted lines represent; luminal perimeter, epithelial perimeter, mucosal perimeter, submucosal perimeter. (C) Corresponding annotated airway wall layers based on differences in light intensities. From right to left: first, low intensity, layer is the epithelial layer. The second, high intensity, layer matches the lamina propria layer. The third, low intensity, layer the submucosa including the airway smooth muscle. The next, very low intensity, layer is the cartilage layer which is identified by a surrounded thin high intensity layer, the perichondrium.
S3 Figure. Linear regression analysis and Bland-Altman plots for OCT ex-vivo and OCT in-vivo airway wall area measurements (n=39). (A) $A_L$ lumen area in mm$^2$. (B) $A_{muc}$ mucosal area in mm$^2$. (C) $A_{submuc}$ submucosal muscular area in mm$^2$. 
S4 Figure. Linear regression analysis and Bland-Altman plots for histology and OCT in-vivo airway wall area measurements (n = 39). (A) $A_L$ lumen area in mm$^2$. (B) $A_{muc}$ mucosal area in mm$^2$. (C) $A_{submuc}$ submucosal muscular area in mm$^2$. 
S5 Figure. Linear regression analysis and Bland-Altman plots for ex-vivo OCT airway wall measurements between two observers (n = 51). (A) $A_{l}$ lumen area in mm$^2$. (B) $A_{muc}$ mucosal area in mm$^2$. (C) $A_{submuc}$ submucosal muscular area in mm$^2$. 

Identification and quantification of airway wall layers with Optical Coherence Tomography: a histology based validation study.
S6 Figure Linear regression analysis and Bland-Altman plots for in-vivo OCT airway wall measurements between two observers (n = 39). (A) A_lumen area in mm². (B) A_muc mucosal area in mm². (C) A_submuc submucosal muscular area in mm².