ERJ open research
THE BEST IN OPEN ACCESS BASIC, TRANSLATIONAL & CLINICAL RESPIRATORY RESEARCH

Early View

Original article

The importance of central airway dilatation in patients with bronchiolitis obliterans

Mariko Kogo, Hisako Matsumoto, Naoya Tanabe, Toyofumi F. Chen-Yoshikawa, Naoki Nakajima, Akihiko Yoshizawa, Tsuyoshi Oguma, Susumu Sato, Natsuko Nomura, Chie Morimoto, Hironobu Sunadome, Shimpei Gotoh, Akihiro Ohsumi, Hiroshi Date, Toyohiro Hirai

Please cite this article as: Kogo M, Matsumoto H, Tanabe N, *et al*. The importance of central airway dilatation in patients with bronchiolitis obliterans. *ERJ Open Res* 2021; in press (https://doi.org/10.1183/23120541.00123-2021).

The importance of central airway dilatation in patients with bronchiolitis obliterans

Mariko Kogo, MD[1)], Hisako Matsumoto*, MD, Ph.D[1)], Naoya Tanabe*, MD, Ph.D[1)], Toyofumi F Chen-Yoshikawa, MD, Ph.D[2)], Naoki Nakajima, MD, Ph.D[3)], Akihiko Yoshizawa, MD, Ph.D[3)], Tsuyoshi Oguma, MD, Ph.D[1)], Susumu Sato, MD, Ph.D[1)], Natsuko Nomura MD[1)], Chie Morimoto, MD[1)], Hironobu Sunadome MD, Ph.D[1)], Shimpei Gotoh MD, Ph.D[1)], Akihiro Ohsumi, MD, Ph.D[4)], Hiroshi Date, MD, Ph.D[4)], Toyohiro Hirai, MD, Ph.D[1)]
*Equally contributed to this manuscript

Affiliations
[1)]Department of Respiratory Medicine, Graduate School of Medicine Kyoto University, Kyoto, Japan
[2)] Department of Thoracic Surgery, Graduate School of Medicine, Nagoya University, Nagoya, Japan
[3)] Department of Diagnostic Pathology, Graduate School of Medicine Kyoto University, Kyoto, Japan
[4)] Department of Thoracic Surgery, Graduate School of Medicine Kyoto University, Kyoto, Japan

Correspondence: Hisako Matsumoto, MD, Ph.D
Department of Respiratory Medicine
Kyoto University, Kyoto, Japan
54 Shogoin Kawahara-cho, Sakyo-ku, Kyoto, Japan
E-mail: matsumoto.hisako.v92@kyoto-u.jp
Tel: +81-75-751-3803
Fax: +81-75-751-4643

Take-Home Message (195 characters)
In patients with BO, airway dilatation may reflect air-trapping and the pathological extent of obstructive bronchioles. More attention should be paid to airway dilatation in the management of BO.

Keywords

Obliterative Bronchiolitis, Computed Tomography, Bronchiectasis.

Abstract

Background

Bronchiolitis obliterans (BO) is a clinical syndrome characterized by progressive small airway obstruction, causing significant morbidity and mortality. Central airway dilatation is one of its radiological characteristics, but little is known about the clinical and pathological associations between airway dilatation and BO.

Methods

This retrospective study consecutively included patients who underwent lung transplantation due to BO at Kyoto University Hospital from 2009 to 2019. Demographic and histopathological findings of the resected lungs were compared between patients with and without airway dilatation measured by chest computed tomography (CT) at registration for lung transplantation.

Results

Of a total of 38 included patients (median age, 30 years), 34 (89%) had a history of hematopoietic stem-cell transplantation, and 22 (58%) had airway dilatation based on CT. Patients with airway dilatation had a higher frequency of *Pseudomonas aeruginosa* isolation with greater residual volume than those without airway dilatation. Quantitative CT analysis revealed an increase in lung volume to predictive total lung capacity and a percentage of low attenuation volume < −950 HU at inspiration in association with the extent of airway dilatation. Airway dilatation on CT was associated with an increased number of bronchioles with concentric narrowing of the lumen and thickening of the subepithelium of the walls on histology.

Conclusions

In patients with BO, airway dilatation may reflect increased residual volume or air-trapping and pathological extent of obstructive bronchioles, accompanied by a risk of *Pseudomonas aeruginosa* isolation. More attention should be paid to the development of airway dilatation in the management of BO.

Introduction

Bronchiolitis obliterans (BO) is a form of irreversible airflow obstruction following an injury to the respiratory and terminal bronchioles from various potential causes [1–3]. The BO's frequency is rare but is becoming more frequent as a complication of hematopoietic stem-cell transplantation (HSCT) and lung transplantation [2]. Although the impact of BO on post-transplantation prognosis is milder than that of restrictive forms, BO typically progresses and eventually causes death or requires lung transplantation [1–4].

Currently, the presence and progression of BO are primarily defined by spirometry. However, many studies have emphasized the usefulness of radiological findings [5–9]. Additionally to the low attenuation area at a single expiratory image, the recent development of computed tomography (CT) analysis enabled us to quantitatively evaluate air-tapping separately from emphysema by comparing the inspiratory and expiratory images on a voxel-by-voxel basis with a non-rigid registration technique or parametric response mapping [6, 7, 10].

Central airway dilatation, another radiological characteristic of BO, is less likely to receive attention than air-trapping. In the 1990s, several studies have focused on central airway dilatation and its association with airflow limitation in BO [11–14]. Although the sensitivity and specificity of central airway dilatation to detect the early phase of BO were lower than those of air-trapping, several studies have mentioned that central airway dilatation is one of the critical manifestations for detecting the development of BO [5, 15]. Furthermore, because the central airway dilatation is easily detectable on inspiratory CT, it might be informative during BO patients' follow-up, i.e., not only for detecting BO. Despite its potential roles in BO's management, studies on the clinical impacts of central airway dilatation and its association with pathological findings of BO are limited [16]. This study revealed the underlying clinical, radiological, and pathological aspects related to airway dilatation among BO patients.

Material and Methods

Study design and subjects

We conducted a historical cohort study enrolling patients who underwent lung transplantation due to BO that was pathologically confirmed with samples taken during lung transplantation at the Kyoto University Hospital between January 2009 and April 2019 (Figure 1). The pathological definition of BO is the existence of one or more bronchioles with concentric narrowing of the bronchiolar lumen by inflammation and fibrosis [1]. Demographic, clinical, respiratory functional, radiological at registration for lung transplantation were retrieved from clinical records. Bacterial cultures were evaluated with the spontaneous sputum at the time of registration when patients did not exhibit signs of acute infection. Standard laboratory protocols were used for the identification of pathogenic bacterial species that colonize the lower respiratory tract. Microbiological analysis was not performed on lung tissue obtained at the time of transplantation. When available, clinical and respiratory functional data and CT findings between registration and the transplantation were also collected. Patients were excluded from the quantitative CT analysis when they lacked high-resolution computed tomography (HRCT) in defined reconstruction (Supplementary Methods), were on ventilator support, or had a pneumothorax (Figure 1). Written informed consent for the examination was obtained from each patient at the time of lung transplantation for research using their clinical data and histological samples. The Ethics Committee approved the study of Kyoto University (approval No. G0469).

CT analysis

Definition of central airway dilatation

Details of CT and histopathological analyses are described in the Supplementary Methods.

HRCT scanning was acquired at the time of the registration for lung transplantation. On full-inspiratory CT, airway dilatation was determined when at least one of the following criteria was fulfilled, independently of interstitial lung abnormalities [17]; (1) internal diameter of the bronchus greater than that of the adjacent pulmonary artery and (2) lack of tapering of the bronchial lumen toward the periphery for any generation. The extent of airway dilatation was semi-quantitatively assessed using a modified Reiff score (Supplementary Methods) [18].

Quantitative CT analysis

The total lung volume (CT-TLV) and the percentage of low attenuation volume < −950 Hounsfield units (HU) (LAV_{-950}%) were evaluated on full-inspiratory CT. The predicted total lung capacity (pred TLC) was calculated from the sex and height, and used to adjust the CT-TLV by the natural size of the lungs [19]. In a subgroup of patients whose full-expiratory CT scans were available, the percentage of low attenuation volume < −856 HU at expiration (LAV_{-856}%) was calculated to evaluate air-trapping. Additionally, non-emphysematous air-trapping, presumably a surrogate of small airway disease severity, termed functional small airway disease (fSAD) [20], was calculated by non-rigidly registering inspiratory and expiratory CT scans using custom software.

For the evaluation of longitudinal CT data before and after the clinical diagnosis of BO in representative patients, the lumen of a branch from the basal posterior bronchus ($B^{10}a$) was measured as reported previously [15].

Histopathological analysis

The lung tissue obtained at the time of lung transplantation was evaluated. Lungs were inflated with undetermined pressure and fixed by formalin, processed into paraffin blocks, and cut into a section of 4-μm thickness according to routine surgical pathology protocols [21]. According to a previous study [22], the six sections from affected lungs containing one or more bronchioles were stained with hematoxylin and eosin and either elastica van Gieson or elastica-Masson. On digital images of whole sections [23], relatively circular bronchioles (long axis/short axis < 3) smaller than 2 mm in long axis were evaluated [22, 24], and the perimeter of the basement membrane defined as an elastic layer of the bronchiole (Pbm) was measured. The airway wall thickness of three compartments, including the epithelium, subepithelium (from the bottom of the epithelium to the basement membrane), and lamina propria (from the basement membrane to the outer edge of the smooth muscle), was assessed by dividing each area by Pbm (Suppl. Figure 1) [22]. Bronchioles with concentric narrowing of the bronchiolar lumen by inflammation and fibrosis were identified. The percentage of the number of these bronchioles to the total number of bronchioles evaluated on sections was measured for each patient to evaluate the pathological extent of BO. The number of alveolar attachments to the outer wall of bronchioles was counted and adjusted by Pbm.

Statistical analysis

The patients with BO were divided into those with and without airway dilatation determined by CT and compared. Continuous variables were compared using the Wilcoxon rank-sum test, and categorical variables were compared using the Pearson chi-square test. The correlations between Reiff score and quantitative CT analysis variables or histological analysis were presented as Spearman correlation coefficients. The threshold of Reiff score in relation to the extent of

pathological BO (the cut-off value was set at the first tertile in our cohort) was estimated using a receiver operating characteristic (ROC) curve analysis. A two-tailed p-value < 0.05 was considered statistically significant. All statistical analyses were performed using JMP Pro 14 for Windows (SAS Institute, Inc., Cary, NC). Data are presented as median values with interquartile ranges (IQR) for continuous variables and as percentages for categorical variables.

Results

Patients' characteristics

During the study period, 38 patients underwent lung transplantation due to BO (Figure 1). The main cause of BO was HSCT (89%), followed by lung transplantation (11%). The median time from BO's clinical diagnosis to lung transplantation registration and from registration to lung transplantation was 2.1 years and 1.6 months, respectively. Twenty-nine patients (76%) received living-donor lung transplantation. The patients' clinical features are summarized in Table 1. Among these cases, 22 (58%) had developed airway dilatation (Reiff score ≥ 1) at the time of registration for lung transplantation with a median Reiff score of seven. Among the 22 cases, HRCT at BO's diagnosis was available for seven patients and two patients had already developed airway dilatation at the time of the diagnosis. In 21 patients whose pulmonary function was available, patients with airway dilatation (n = 12) had a higher residual volume (RV) and a tendency toward higher RV/TLC than those without airway dilatation (n = 9). However, FEV_1 did not differ between the two groups. The time from diagnosis of BO to registration was not significantly different between patients with and without airway dilatation among patients who had pulmonary function data (p = 0.48) as well as those of the total cohort. Sputum culture was obtained for 32 patients. Patients with airway dilatation (n =18) had a higher frequency of detection of *Pseudomonas aeruginosa* (Table 2).

Quantitative CT analysis

Quantitative CT analysis was performed in 24 patients (Figure 1). On inspiratory CT, patients with airway dilatation (n = 14) had greater CT-TLV/pred TLC and LAV_{-950}% than those without airway dilatation (n = 10) (Table 3A). Moreover, there were significant correlations between Reiff score and CT-TLV/pred TLC, LAV_{-950}%, (rho = 0.64, $p < 0.001$ and rho = 0.45, $p = 0.029$, respectively) (Figure 2A, 2B). A representative CT image of a BO patient with high LAV_{-950}% is shown in Suppl. Figure 2A. Analysis using expiratory CT (n = 17) revealed significantly higher LAV_{-856}% and a trend for increased fSAD in patients with airway dilatation (n = 10) than those without airway dilatation (n = 7) (Table 3B). Finally, the Reiff score was marginally correlated with fSAD (rho = 0.48, $p = 0.053$).

Longitudinal data from the onset of BO were available for three patients, all of whom developed BO after lung transplantation. Pulmonary function tests were discontinued due to recurrent pneumothorax. A patient with airway dilatation (Figure 3A–3D) showed rapid disease progression, expressed as an increase in RV/TLC and areas of fSAD in the following year after diagnosis of BO. In contrast, the patient's clinical course without airway dilatation (Figure 3E-3F) was relatively stable during the same period.

Pathological analysis

The number of bronchioles examined per patient and their mean Pbm were similar between patients with and without airway dilatation recognized on CT (Suppl. Figure 3A, 3B). As shown in Figure 4A, obstructive bronchioles on histology were more frequently found in patients with airway dilation than in those without (median [IQR] 65% [38–80] vs. 19% [5–39], $p < 0.001$). This difference was still significant even when adjusted for the time from BO’s diagnosis to lung

transplantation registration ($p = 0.002$). Obstructive bronchioles contained foamy macrophages (Suppl. Figure 1) as well as the deposition of extracellular matrix in the subepithelium (Figure 3C, 3D) when compared with normal bronchioles (Figure 3G, 3H). In patients with airway dilatation, the thicknesses of subepithelium, but not the lamina propria, were significantly greater, while that of the epithelium was smaller than those without airway dilatation (Figure 4B). Additionally, there were positive correlations between the Reiff score on CT and the pathological extent of BO or thickness of subepithelium ($rho = 0.55$, $p < 0.001$ and $rho = 0.57$, $p < 0.001$, respectively) (Figure 4C, 4D). The first tertile of the extent of BO in our cohort was 62%, and ROC analysis of Reiff score for this value showed an area under the curve (AUC) of 0.73 with the best cut-off value of Reiff score of 1 (sensitivity 0.92, specificity 0.60). The number of alveolar attachments per Pbm was comparable between the groups (Suppl. Figure 3C). On the pathological observation on dilated central airways, airway dilatation was not accompanied by traction ectasia due to fibrosis (Suppl. Figure 4). To minimize the effect of the period between data at registration and transplantation when the pathologies were collected, we limited the analysis to patients who received living-donor lung transplantation, which yielded similar results as those of the whole patients (Suppl. Figure 5).

Discussion

To the best of our knowledge, this is the first study that demonstrated associations between central airway dilatation on CT and pathological severity of BO as well as air-trapping in patients with BO. Furthermore, central airway dilatation, which was observed in 58% of BO patients in this study, was associated with isolations of *P. aeruginosa* from the airway.

Airway dilatation on CT in patients with BO has been studied since the 1990s [8, 11–16]. Gazourian L et al. recently re-focused on the importance of central airway dilatation by showing its association with air-trapping represented as RV/TLC [15]. With our results of increased %RV and CT-TLV/pred TLC in patients with airway dilatation, the relationship between the presence of airway dilatation and air-trapping in BO was confirmed. We further demonstrated that airway dilatation was significantly associated with LAV_{-950}% and marginally with fSAD, an index of air-trapping by CT analysis, using a non-rigid registration technique or parametric response mapping. We newly revealed that the pathological progression of BO could be one of the underlying mechanisms of central airway dilatation recognized on CT; the presence of airway dilatation indicated that 62% of bronchioles were affected with a BO median of 1.6 months later with a sensitivity of 92% and specificity of 60%. Thus, the present data extended the previous findings by clearly showing an association between the development of airway dilatation and the histopathological extent of BO. Patients with airway dilatation had a higher proportion of emphysema-like lesions detected by CT than those without airway dilatation. Of interest, as shown in the Suppl. Figure 2A, in BO patients the emphysema-like lesion was homogeneously distributed, and the secondary pulmonary lobule was ill-defined. This spatial pattern is different from the centrilobular emphysema commonly found in smokers with chronic obstructive respiratory disease (COPD), which is radiologically characterized by well-defined low attenuation regions surrounded by normal lung regions. (Suppl. Figure 2B). These findings suggest that the reduction in lung tissue density revealed by CT in BO patients is not associated with parenchymal destruction. We speculate that the relatively increased collateral ventilation due to reduced direct ventilation from conducting airway on parenchyma [25] and the regional hypoxic vasoconstriction due to local hypoxia might decrease local tissue density [26], which

could be recognized by CT as low attenuation region. Furthermore, impaired lung development may also induce insufficient alveolization and reduce lung tissue density and CT values in these regions. Washko G et al. examined patients from 18 to 30 years old until their middle age, demonstrating that a failure to reach the predicted level of peak FEV_1 or accelerated decline in FEV_1 after that, was associated with the development of airspace dilatation, which was considered a result of early life inflammatory and immunological events [27]. Although further study is needed to clarify the relationship between emphysema-like lesion and airway dilatation in BO, this study is one of the first focusing on emphysema in association with airway dilatation in BO.

In previous studies of BO, the prevalence of airway dilatation widely ranges between 11%–71% [8, 12, 15], which may be due to the differences in the timing of its detection from the diagnosis of BO as well as disease severity of BO; one study that reported the lowest prevalence of airway dilatation included patients around only one year after the diagnosis of BO, which was about half the time of 2.1 years in our cohort [12]. Indeed, Gazourian L et al. reported deterioration of airway dilatation over time after the diagnosis of BO in patients with a progressive decline in FEV_1, although they did not assess pathologies [16]. Additionally, our study's ROC analysis showed the relationship between airway dilatation on CT and pathologically extensive BO. We concluded that the development of airway dilatation might be a phenomenon occurring in the relatively progressive or late phase of BO. It might be more meaningful during BO's follow-up, such as better allocation for lung (re-) transplantation, than for the early diagnosis.

The mechanisms underlying the associations between central airway dilatation and the progression of BO remain unclear. When considering our result of the decreased epithelial thickness of bronchioles in patients with prominent airway dilatation, one possible explanation is

that severe and irreversible injury due to chronic graft-versus-host disease denuded epithelium and caused chronic low-grade inflammation of the airways, which resulted in an architectural weakening of proximal airway walls [28, 29]. The microbiome change during the BO's progression, inducing colonization of *P. aeruginosa*, could be the second hit for developing airway dilatation [1, 29, 30].

The assessment of airway dilatation is thus essential. Meanwhile, there is no consensus in the valid scoring system for the extent of airway dilatation in BO patients. Previous studies of BO calculated the mean diameter of airway lumen using a specific software [15, 16], while we adopted the modified Reiff score, which is often used in the evaluation of bronchiectasis. A previous report of bronchiectasis showed that the extent of bronchiectasis graded similarly using the Reiff score was significantly associated with airflow limitation, RV, and reduction in gas transfer, independently of airway wall thickening and air-trapping on CT. At the same time, these associations were not observed for the diameter of the dilated airways [26]. Therefore, we consider the Reiff score to be a useful and clinically applicable index for grading the extent of airway dilatation in BO.

Several limitations should be addressed. First, this is a historical cohort study with missing data, particularly, the data of HRCT for quantitative analysis and pulmonary function. Indeed, the results of pulmonary function tests at registration were available only for 53% of our cohort due to their severe respiratory conditions or recurrent pneumothorax. The results of gas transfer, which are important to assess emphysema physiologically, were also lacking in most patients. Second, the small sample size from a single center made it difficult to evaluate confounding factors that may have affected the results. However, this study is the first to statistically evaluate the pathologies of BO, particularly in association with airway dilatation. Moreover, we could confirm our results in a subgroup of patients who received living-donor lung

transplantation, who had a little time gap between CT evaluation and pathology. Further studies will be required to clarify the mechanisms underlying the association between central airway dilatation and the progression of BO. Although these limitations warrant careful interpretation of our results, the clinical, radiological, and pathological findings of this study re-emphasize the importance of airway dilatation recognition in BO's management. This would be particularly important because many patients with severe BO repeat pneumothorax in their clinical courses, which hamper pulmonary function tests.

Conclusions

Central airway dilatation on CT may reflect the histopathological extent of BO and isolations of *Pseudomonas aeruginosa* from the airway, emphasizing the importance of its recognition in the management of BO. The mechanisms underlying the associations between central airway dilatation and the development of BO may need further study.

Acknowledgment

The authors want to thank Maki Isomi as a coordinator for lung transplantations.

References

1. Aguilar PR, Michelson AP, Isakow W. Obliterative Bronchiolitis. Transplantation 2016; 100: 272-283.
2. Barker AF, Bergeron A, Rom WN, Hertz, MI. Obliterative bronchiolitis. N Engl J Med 2014; 370: 1820-1828.
3. Chen-Yoshikawa TF, Sugimoto S, Shiraishi T, Minami M, Matsuda Y, Chida M, Maeda S, Aoyama A, Okada Y, Okumura M, Iwasaki A, Miyoshi S, Oto T, Date H. Prognostic factors in lung transplantation after hematopoietic stem cell transplantation. Transplantation 2018; 102:154-161.
4. Verleden SE, Sacreas A, Vos R, Vanaudenaerde BM, Verleden GM. Advances in understanding bronchiolitis obliterans after lung transplantation. Chest 2016; 150: 219-225.
5. de Jong PA, Dodd JD, Coxson HO, Storness-Bliss C, Pare PD, Mayo JR, Levy RD. Bronchiolitis obliterans following lung transplantation: early detection using computed tomographic scanning. Thorax 2006; 61: 799-804.
6. Belloli EA, Degtiar I, Wang X, Yanik GA, Stuckey LJ, Verleden SE, Kazerooni EA, Ross BD, Murray S, Galban CJ, Lama VN. Parametric response mapping as an imaging biomarker in lung transplant recipients. Am J Respir Crit Care Med 2017; 195: 942-952.
7. Cheng GS, Selwa KE, Hatt C, Ram S, Fortuna AB, Guerriero M, Himelhoch B, McAree D, Hoffman TC, Brisson J, Nazareno R, Bloye K, Johnson TD, Remberger M, Mattsson J, Vummidi D, Kazerooni EE, Lama VN, Galban S, Boeckh M, Yanik GA, Galban CJ. Multicenter evaluation of parametric response mapping as an indicator of bronchiolitis obliterans syndrome after hematopoietic stem cell transplantation. Am J Transplant 2020; 20: 2198-2205.

8. Gunn ML, Godwin JD, Kanne JP, Flowers ME, Chien JW. High-resolution CT findings of bronchiolitis obliterans syndrome after hematopoietic stem cell transplantation. J Thorac Imaging 2008; 23: 244-250.

9. Song I, Yi CA, Han J, Kim DH, Lee KS, Kim TS, Chung MJ. CT findings of late-onset noninfectious pulmonary complications in patients with pathologically proven graft-versus-host disease after allogeneic stem cell transplant. AJR Am J Roentgenol 2012; 199: 581-587.

10. Galban CJ, Boes JL, Bule M, Kitko CL, Couriel DR, Johnson TD, Lama V, Telenga ED, van den Berge M, Rehemtulla A, Kazerooni EA, Ponkowski MJ, Ross BD, Yanik GA. Parametric response mapping as an indicator of bronchiolitis obliterans syndrome after hematopoietic stem cell transplantation. Biol Blood Marrow Transplant 2014; 20: 1592-1598.

11. Leung AN, Fisher K, Valentine V, Girgis RE, Berry GJ, Robbins RC, Theodore J. Bronchiolitis obliterans after lung transplantation: detection using expiratory HRCT. Chest 1998; 113: 365-370.

12. Ooi GC, Peh WC, Ip M. High-resolution computed tomography of bronchiolitis obliterans syndrome after bone marrow transplantation. Respiration 1998; 65: 187-191.

13. Lentz D, Bergin CJ, Berry GJ, Stoehr C, Theodore J. Diagnosis of bronchiolitis obliterans in heart-lung transplantation patients: importance of bronchial dilatation on CT. AJR Am J Roentgenol 1992; 159: 463-467.

14. Sargent MA, Cairns RA, Murdoch MJ, Nadel HR, Wensley D, Schultz KR. Obstructive lung disease in children after allogeneic bone marrow transplantation: evaluation with high-resolution CT. AJR Am J Roentgenol 1995; 164: 693-696.

15. Gazourian L, Coronata AM, Rogers AJ, Weinhouse GL, Soiffer RJ, Antin JH, Ritz J, Ho VT, Baron RM, Washko GR. Airway dilation in bronchiolitis obliterans after allogeneic hematopoietic stem cell transplantation. Respir Med 2013; 107: 276-283.
16. Gazourian L, Ash S, Meserve EEK, Diaz A, Estepar RSJ, El-Chemaly SY, Rosas IO, Divo M, Fuhlbrigge AL, Camp PC, Ho VT, Bhatt AS, Goldberg HJ, Sholl LM, Washko GR. Quantitative computed tomography assessment of bronchiolitis obliterans syndrome after lung transplantation. Clin Transplant 2017; 31: 12943.
17. Bedi P, Chalmers JD, Goeminne PC, Mai C, Saravanamuthu P, Velu PP, Cartlidge MK, Loebinger MR, Jacob J, Kamal F, Schembri N, Aliberti S, Hill U, Harrison M, Johnson C, Screaton N, Haworth C, Polverino E, Rosales E, Torres A, Benegas MN, Rossi AG, Patel D, Hill AT. The BRICS (Bronchiectasis Radiologically Indexed CT Score): A Multicenter Study Score for Use in Idiopathic and Postinfective Bronchiectasis. Chest 2018; 153: 1177-1186.
18. Chalmers JD, Goeminne P, Aliberti S, McDonnell MJ, Lonni S, Davidson J, Poppelwell L, Salih W, Pesci A, Dupont LJ, Fardon TC, De Soyza A, Hill AT. The bronchiectasis severity index. An international derivation and validation study. Am J Respir Crit Care Med 2014; 189: 576-585.
19. Quanjer PH, Tammeling GJ, Cotes JE, Pedersen OF, Peslin R, Yernault JC. Lung volumes and forced ventilatory flows. Eur Respir J 1993; 6 Suppl 16: 5-40.
20. Bhatt SP, Soler X, Wang X, Murray S, Anzueto AR, Beaty TH, Boriek AM, Casaburi R, Criner GJ, Diaz AA, Dransfield MT, Curran-Everett D, Galban CJ, Hoffman EA, Hogg JC, Kazerooni EA, Kim V, Kinney GL, Lagstein A, Lynch DA, Make BJ, Martinez FJ, Ramsdell JW, Reddy R, Ross BD, Rossiter HB, Steiner RM, Strand MJ, van Beek EJ, Wan ES, Washko GR, Wells JM, Wendt CH, Wise RA, Silverman EK, Crapo JD,

Bowler RP, Han MK, Investigators CO. Association between Functional Small Airway Disease and FEV1 Decline in Chronic Obstructive Pulmonary Disease. Am J Respir Crit Care Med 2016; 194: 178-184.

21. Takeuchi Y, Miyagawa-Hayashino A, Chen F, Kubo T, Handa T, Date H, Haga H. Pleuroparenchymal fibroelastosis, and non-specific interstitial pneumonia: frequent pulmonary sequelae of haematopoietic stem cell transplantation. Histopathology 2015; 66: 536-544.
22. Hogg JC, Chu F, Utokaparch S, Woods R, Elliott WM, Buzatu L, Cherniack RM, Rogers RM, Sciurba FC, Coxson HO, Pare PD. The nature of small-airway obstruction in chronic obstructive pulmonary disease. N Engl J Med 2004; 350: 2645-2653.
23. Zheng PP, van der Weiden M, Kros JM. Fast -tracking of co-localization of multiple markers by using the nanozoomer slide scanner and NDPViewer. J Cell Physiol 2014; 229: 967-973.
24. Tanabe N, Vasilescu DM, Kirby M, Coxson HO, Verleden SE, Vanaudenaerde BM, Kinose D, Nakano Y, Pare PD, Hogg JC. Analysis of airway pathology in COPD using a combination of computed tomography, micro-computed tomography, and histology. Eur Respir J 2018; 51: 1701245.
25. Culiner, MM. Obliterative bonchitis and bronchiolitis with bronchiectasis. Dis Chest 1963; 44: 351-360.
26. Roberts HR, Wells AU, Milne DG, Rubens MB, Kolbe J, Cole PJ, Hansell DM. Airflow obstruction in bronchiectasis: correlation between computed tomography features and pulmonary function tests. Thorax 2000; 55: 198-204.

27. Washko GR, Colangelo LA, Estepar RSJ, Ash SY, Bhatt SP, Okajima Y, Liu K, Jacobs DR, Jr., Iribarren C, Thyagarajan B, Lewis CE, Kumar R, Han MK, Dransfield MT, Carnethon MR, Kalhan R. Adult Life-Course Trajectories of lung function and the development of emphysema: The CARDIA lung study. Am J Med 2020; 133: 222-230 e211.

28. King PT. The pathophysiology of bronchiectasis. Int J Chron Obstruct Pulmon Dis 2009; 4: 411-419.

29. Whitwell F. A study of the pathology and pathogenesis of bronchiectasis. Thorax 1952; 7: 213-239.

30. Richardson H, Dicker AJ, Barclay H, Chalmers JD. The microbiome in bronchiectasis. Eur Respir Rev 2019; 28: 190048.

Table 1. Patient characteristics at registration for lung plantation.

	Airway dilatation- n = 16	Airway dilatation+ n = 22	p-value
Male	9 (56)	9 (41)	0.35
Age, year	33 (14 – 47)	25 (15 – 42)	0.43
Height, cm	158 (141 – 169)	159 (143 – 164)	0.89
Weight, kg	39.3 (25.7 – 50.6)	40.0 (28.9 – 49.6)	0.70
Ex-smokers	1 (6)	3 (14)	0.46
Causes of BO			
HSCT	14 (88)	20 (91)	0.74
Lung transplantation	2 (13)	2 (9)	
Bilateral / Right / Left	0 (0) / 1 (6)/ 1 (6)	2 (9) / 0 (0) / 0 (0)	
BO with prominent fibrosis	3 (19)	1 (5)	0.16
Prednisolone use	11 (69)	19 (86)	0.19
dose, mg/day	15 (7.5 – 15)	8 (4 – 12.5)	0.13
Immunosuppressive agent use	6 (38)	12 (55)	0.30
Inhaled corticosteroid use	10 (63)	12 (55)	0.62
Time from the causes of BO to diagnosis of BO, year	2.3 (0.8 – 5.2)	1.3 (0.7 – 2.9)	0.17
Time from BO's diagnosis to registration, year	1.5 (0.6 – 4.1)	2.8 (1.1 – 6.0)	0.15
Age at diagnosis of BO, year	31 (13 – 44)	21 (12 – 37)	0.31
Time from registration to lung transplantation*, month	2.4 (0.6 – 18.8)	1.5 (1.2 – 17.3)	0.73
Pulmonary function test†			
FVC, % of predicted	48.3 (16.3 – 68.4)	46.5 (38.9 – 50.8)	1.00
FEV_1, % of predicted	20.1 (16.7 – 44.0)	22.0 (18.3 – 23.0)	0.76
FEV_1/FVC, %	68.5 (35.0 – 96.1)	42.7 (34.5 – 50.1)	0.15
RV, % of predicted	87.0 (54.6 – 166.2)	176.8 (131.7 – 236.1)	0.03
RV/TLC, %	46.4 (41.9 – 50.8)	53.2 (46.9 – 58.9)	0.12
Pneumonia within a past year	6 (38)	8 (36)	0.94
Reiff score	0	7 (4 – 10)	-

BO; bronchiolitis obliterans, HSCT; hematopoietic stem cell transplantation, FVC; forced vital capacity, FEV_1; forced expiratory volume 1.0, RV; residual volume, TLC; total lung capacity. All values are expressed as median (interquartile range) except categorical variables, which are expressed as n (%). *29 patients received living-donor lung transplantation. †available for 21 patients (airway dilatation+ n = 12, airway dilatation- n = 9).

Table 2. Bacterial culture of the sputum at registration.

	Airway dilatation- n = 14	Airway dilatation+ n = 18	p-value
Streptococcus ssp.	0 (0)	1 (6)	0.37
Staphylococcus ssp.	0 (0)	2 (11)	0.20
Pseudomonas aeruginosa	0 (0)	7 (39)	0.01
Other gram-negative bacilli	2 (14)	3(17)	0.85
Haemophilus influenzae	1 (7)	0	
Klebsiella oxytoca	1 (7)	0	
Escherichia coli	0	2 (11)	
Stenotrophomonas maltophilia	0	1 (6)	
Normal flora only	12 (86)	8 (44)	0.02
No growth	0 (0)	1 (6)	0.37

All values are expressed as n (%).

Table3. Quantitative analysis for inspiratory CT (A) and paired inspiratory and expiratory CT (B) among patients with and without airway dilatation.

A

	Airway dilatation- n = 10	Airway dilatation+ n = 14	p-value
CT-TLV/pred TLC, %	42.0 (21.3 – 58.9)	69.7 (56.7 – 97.0)	0.009
LAV_{-950}%, %	15.3 (5.7 – 25.1)	29.3 (15.1 – 41.7)	0.050

B

	Airway dilatation- n = 7	Airway dilatation+ n = 10	p-value
LAV_{-856}%, %	46.3 (0.6 – 57.2)	66.5 (54.1 – 72.6)	0.036
fSAD, %	10.0 (0.2 – 33.9)	34.7 (28.3 – 36.4)	0.057

CT-TLV; total lung capacity calculated on inspiratory CT, pred TLC; predicted total lung capacity, LAV_{-950}%; low-attenuation volume < -950 Hounsfield units (HU) at end inspiration, LAV_{-856}%; low-attenuation volume < -856 HU at end-expiration, fSAD; functional small airway disease. All values are expressed as median (interquartile range).

Figure legends

Figure 1

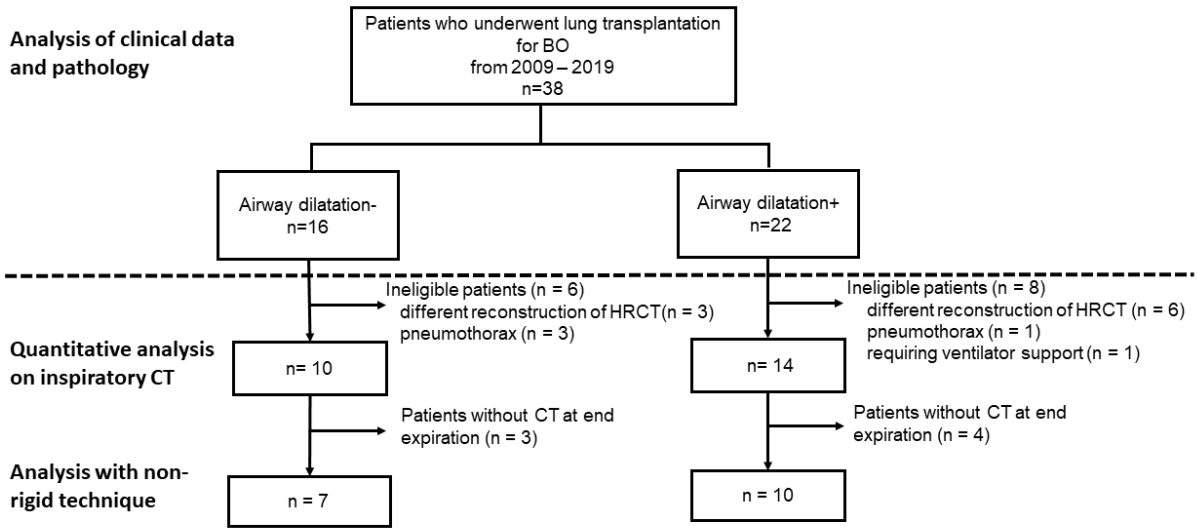


Figure 1. Patient flow chart. Thirty-eight patients were included and 22 of them had airway dilatation on CT. Twenty-four and seventeen patients were quantitatively analyzed for inspiratory CT and paired inspiratory-expiratory CT, respectively. BO; bronchiolitis obliterans, HRCT; high-resolution computed tomography.

Figure 2

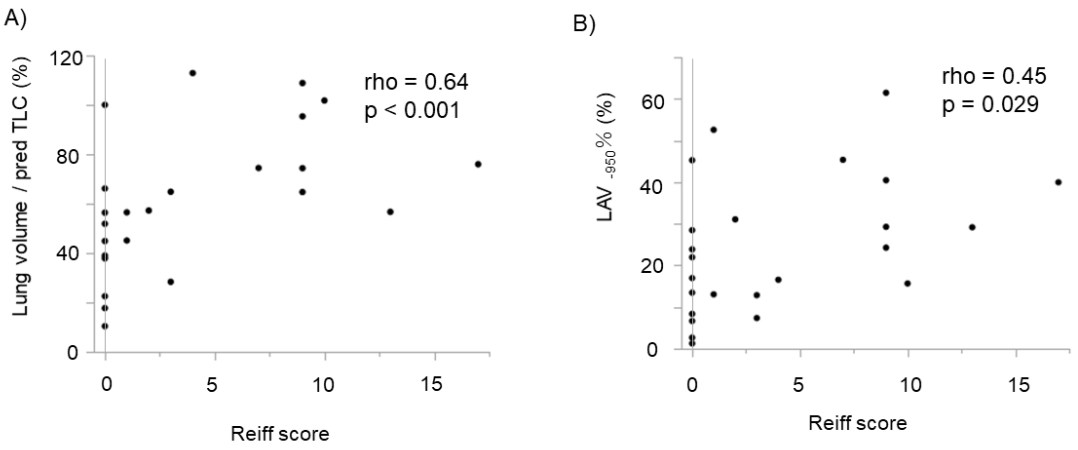


Figure 2. Associations between the Reiff score and variables on inspiratory CT. (A) CT-TLV/pred TLC and (B) LAV_{-950}%. The extent of airway dilation was expressed by the Reiff score, which assessed the number of lobes involved (with the lingula considered to be a separate lobe) and the degree of dilatation (tubular = 1, varicose = 2, and cystic = 3). The maximum score is 18, and the minimum score is 1 for a patient with airway dilatation. Patients without airway dilatation were scored as 0. CT; computed tomography, TLC; total lung capacity, LAV_{-950}%; percentage of low attenuation volume < -950 Hounsfield units.

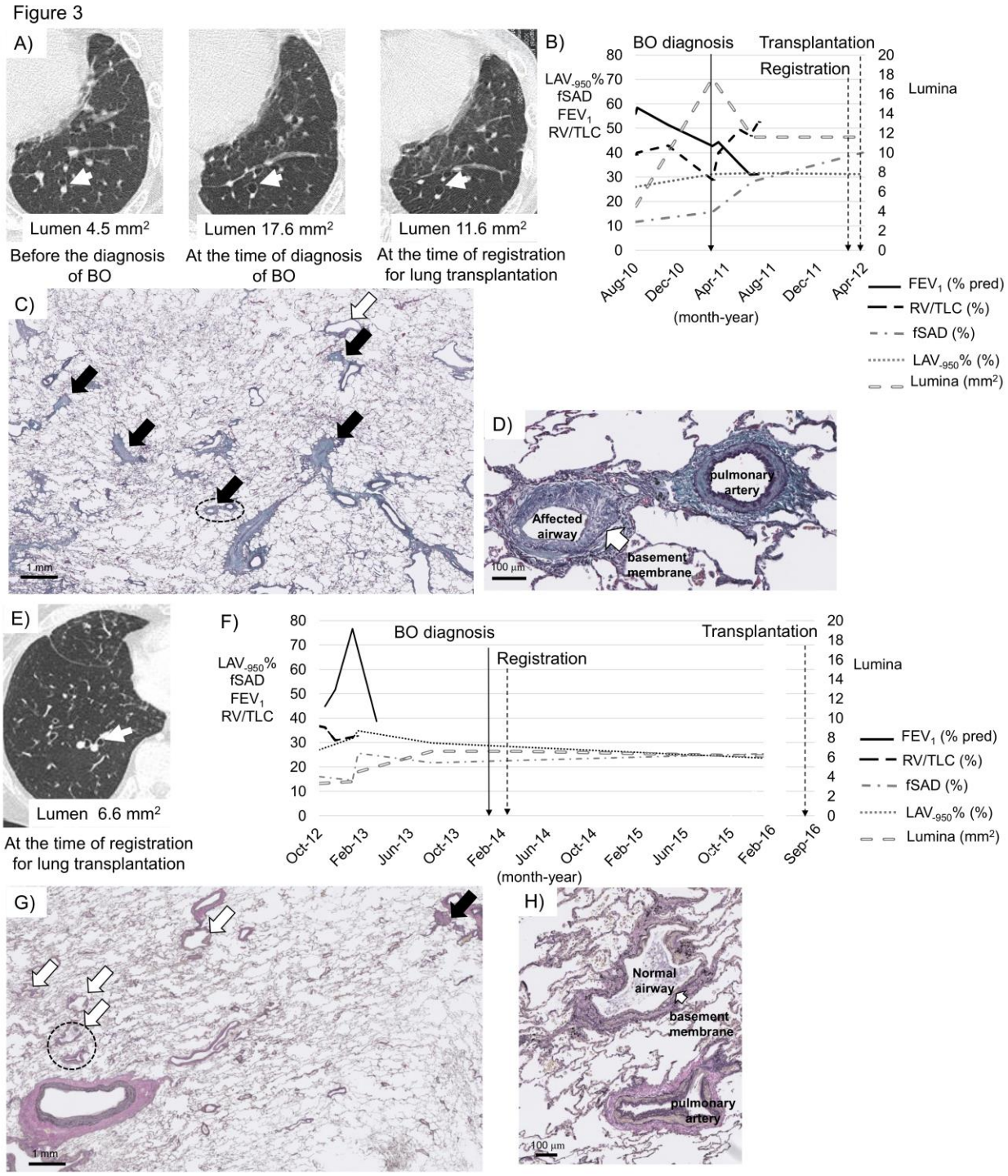


Figure 3. Longitudinal data of patients with (A–D) and without (E–H) airway dilatation. (A) and (E) show HRCT of each patient. The airways with a white arrow were measured for lumen. The longitudinal changes in pulmonary function test (unavailable from the middle due to repeating

pneumothorax in both patients) and indices on HRCT are presented in (B) and (F). (C) and (G) show representative lung histology of the patients. The small airways < 2 mm in diameter are shown with arrows (white arrow: normal airway, black arrow: narrowing or obstructive airway). [(C) elastica Masson stain, (G) elastica van Gieson stain]. (D) and (H) show one of the narrowing and normal airways, respectively, at high magnification of circled airways in (C) and (G) [(D) elastica Masson stain, (H) elastica van Gieson stain]. HRCT; high-resolution computed tomography, LAV_{-950}%; percentage of low attenuation volume < -950 Hounsfield units, fSAD; functional small airway disease, RV; residual volume, TLC; total lung capacity.

Figure 4

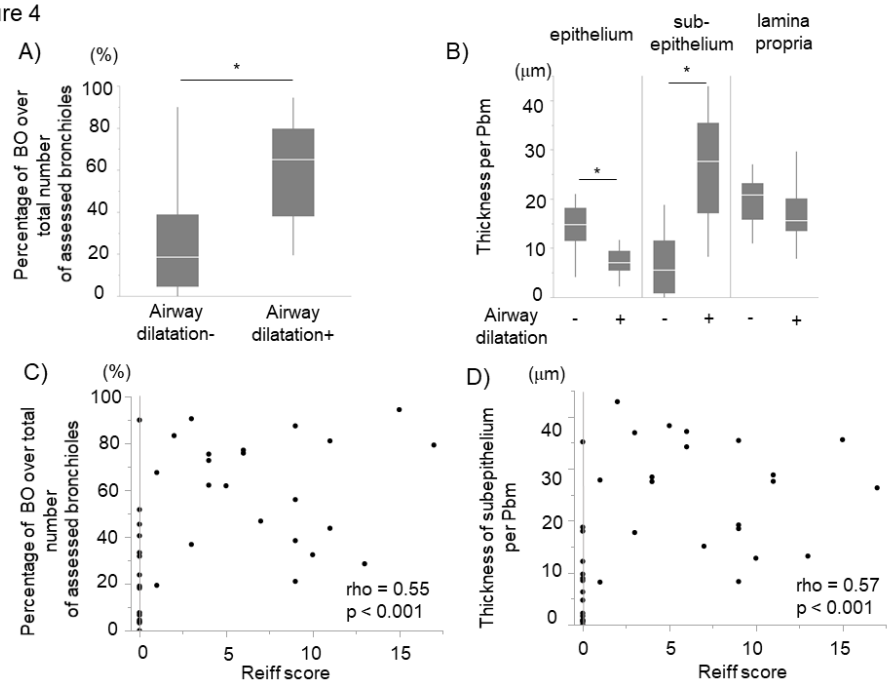


Figure 4. Pathological measurements related to BO of relatively circular bronchioles. (A) The percentage of narrowing or obstructive bronchioles due to BO among the total number of assessed bronchioles per patient and (B) thickness of airway wall compartments adjusted by Pbm. (C) and (D) show the associations between Reiff score and the extent of BO and thickness of subepithelium. The Reiff score assesses the number of lobes involved (with the lingula considered to be a separate lobe) and the degree of dilatation (tubular = 1, varicose = 2, and cystic = 3). The maximum score is 18, and the minimum score is 1 for a patient with airway dilatation. Patients without airway dilatation were scored as 0. The boxes represent the interquartile range with a median (the horizontal line). BO; bronchiolitis obliterans, Pbm; perimeter of the basement membrane. *; $p < 0.05$

Supplemental files

Methods

CT analysis

High-resolution computed tomography (HRCT) was acquired with an Aquilion 64 (Toshiba; Tokyo, Japan); the scanning conditions were as follows: 0.5-mm collimation, 500-millisecond scan time, 120 peak kilovoltage, and auto-exposure control. Reconstruction was performed with a high spatial frequency algorithm (FC51 or FC85). HRCT findings were independently reviewed by two pulmonologists, who were unaware of any prior clinicopathological report. The rate of concordance for the presence of airway dilatation between the two observers was 89%. When there was disagreement between the two observers, a third blind observer was consulted. The extent of airway dilation was expressed by a modified Reiff score, which assessed the number of lobes involved (with the lingula considered to be a separate lobe) and the degree of dilatation (tubular = 1, varicose = 2, and cystic = 3). The maximum score is 18, and the minimum score is 1 for a patient with airway dilatation. Patients without airway dilatation were scored as 0. Inter- observer reproducibility of Reiff was assessed using the intraclass correlation coefficient ($r = 0.95$).

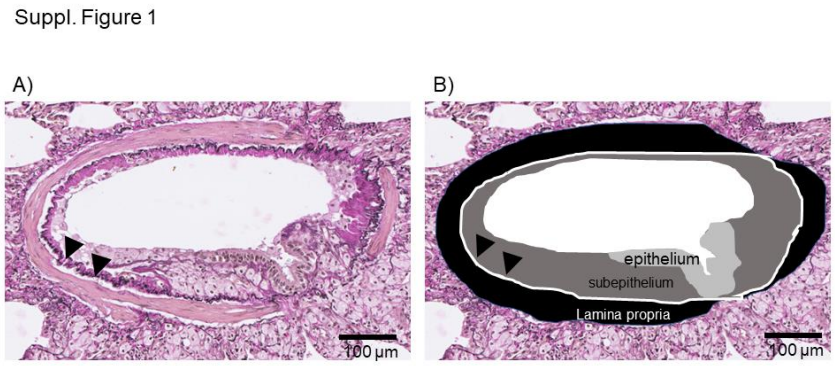
Quantitative CT analysis

HRCT reconstructed by FC 51 with, a slice thickness of 0.5 mm or 1 mm were quantitatively analyzed by the SYNAPSE VINCENT volume analyzer Ver 5.3 (FUJIFILM Medical, Tokyo, Japan). When both full expiratory and inspiratory CT were available, analysis with non-rigid registration technique was performed. Briefly, voxels with values $\geq$ -950 HU at inspiration and $<$ -856 HU at expiration were classified as fSAD. Relative volumes of each classification were used as global measures, which were calculated as a sum of voxels within a class normalized to the sum of all voxels within the expiratory lungs multiplied by 100.

Histopathological analysis

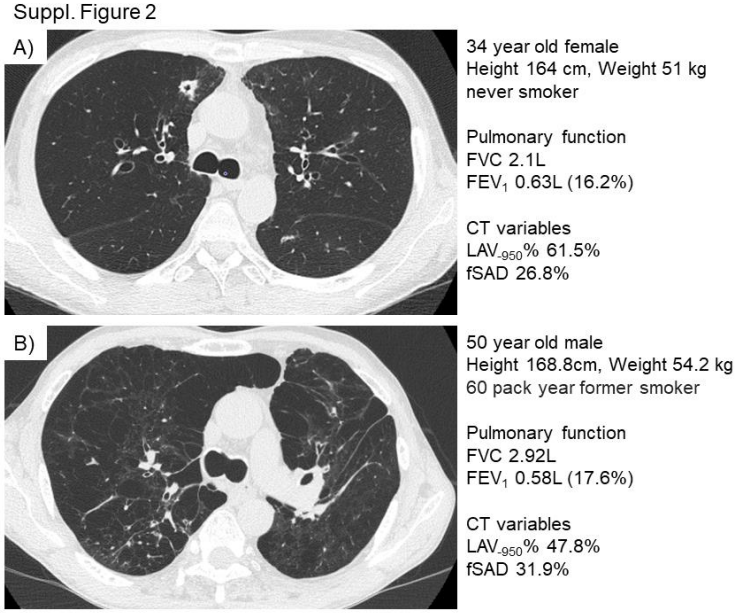
A pulmonologist trained by pathologists evaluated the lung tissue, and a pathologist additionally confirmed the diagnosis of BO. The slides' whole slide imaging was scanned using the Hamamatsu NDP slide scanner (Hamamatsu Nanozoomer S360) and assessed on the virtual slides by NDP View 2 ™.

Figure Legends



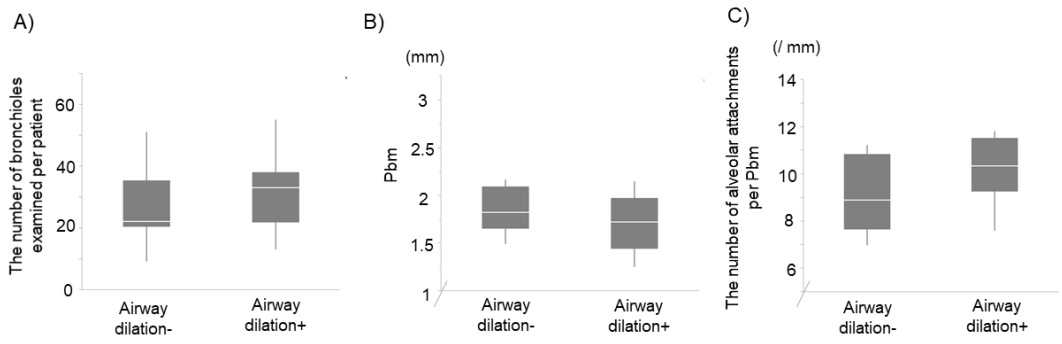
Suppl. Figure 1. An obstructive airway from a patient with bronchiolitis obliterans (A) showing each fractional area (B) (elastica van Gieson stain); arrowheads show basement membrane.

Suppl. Figure 2



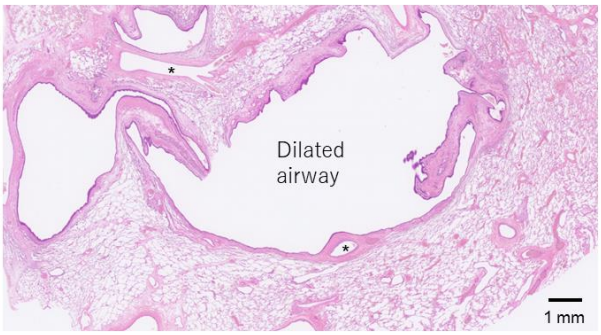
Suppl. Figure 2. (A) Representative CT image of a bronchiolitis obliterans patient with airway dilatation with high LAV_{-950}%. (B) Reference CT image of chronic obstructive pulmonary disease with comparable pulmonary function for the better understanding of radiological characteristics of bronchiolitis obliterans patients. CT; computed tomography, LAV_{-950}%; the percentage of low attenuation volume < -950 Hounsfield units, fSAD; functional small airway disease.

Suppl. Figure 3

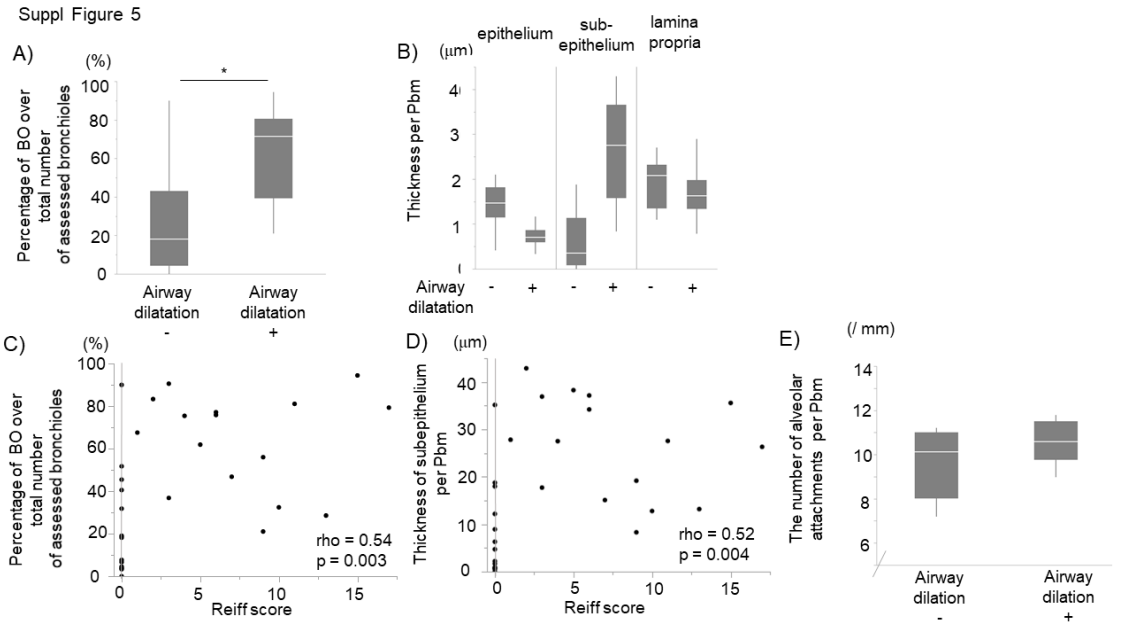


Suppl. Figure 3. Pathological measurements of relatively circular bronchioles. (A) The number of bronchioles examined per patient, (B) mean perimeter of the basement membrane (Pbm), and (C) the number of alveolar attachments per Pbm. The boxes represent the interquartile range with a median (the horizontal line). There were no significant differences between patients with and without airway dilatation.

Suppl Figure4



Suppl. Figure 4. The dilated central airway of a patient with bronchiolitis obliterans (hematoxylin and eosin stain). *; pulmonary artery.



Suppl. Figure 5. Pathological measurements related to BO in patients who received living-donor lung transplantation (Airway dilatation +; n = 16, Airway dilatation-; n = 13). (A) The percentage of the number of narrowing or obstructive bronchiole due to BO among the total number of assessed bronchioles per patient and (B) thickness of airway wall compartments adjusted by Pbm. (C) and (D) show the associations between Reiff score and the extent of BO and thickness of subepithelium. (E) shows the number of alveolar attachments per Pbm. BO; bronchiolitis obliterans. *; $p < 0.05$.