Published in final edited form as:

Pediatr Transplant. 2017 May ; 21(3): . doi:10.1111/petr.12886.

WHAT MAKES A GOOD PEDIATRIC TRANSPLANT LUNG: INSIGHTS FROM *IN VIVO* LUNG MORPHOMETRY WITH HYPERPOLARIZED ³HE MRI (WHAT MAKES A GOOD PEDIATRIC TRANSPLANT LUNG)

Emily F. Fishman, M.D.¹, James D. Quirk, Ph.D.², Stuart C. Sweet, M.D. Ph.D.¹, Jason C. Woods, Ph.D.^{3,4}, David S. Gierada, M.D.², Mark S. Conradi, Ph.D.^{4,2}, Marilyn J. Siegel, M.D.², and Dmitriy A. Yablonskiy, Ph.D.^{2,*}

¹Department of Pediatrics, Washington University, St. Louis, MO, USA

²Mallinckrodt Institute of Radiology, Washington University, St. Louis, MO, USA

³Cincinnati Children's Hospital Medical Center, Cincinnati, OH, USA

⁴Department of Physics, Washington University, St. Louis, MO, USA

Abstract

Background—Obtaining information on transplanted lung microstructure is an important part of the current care for monitoring transplant recipients. However, until now this information was only available from invasive lung biopsy. The objective of this study was to evaluate the use of an innovative non-invasive technique *in vivo* lung morphometry with hyperpolarized ³He MRI - to characterize lung microstructure in the pediatric lung transplant population. This technique yields quantitative measurements of acinar airways' (alveolar ducts and sacs) parameters, such as acinar airways radii and alveolar depth.

Methods—Six pediatric lung transplant recipients with cystic fibrosis underwent *in vivo* lung morphometry MRI, pulmonary function testing, and quantitative CT.

Results—We found a strong correlation between lung lifespan and alveolar depth - patients with more shallow alveoli were likely to have a negative outcome sooner than those with larger alveolar depth. Combining morphometric results with CT we also determined mean alveolar wall thickness and found substantial increases in this parameter in some patients that negatively correlated with DLCO.

Conclusion—*In vivo* lung morphometry uniquely provides previously unavailable information on lung microstructure that may be predictive of a negative outcome and has a potential to aid in lung selection for transplantation.

*Corresponding Author: Dmitriy A. Yablonskiy, Ph.D., Mallinckrodt Institute of Radiology, Washington University, 4525 Scott Avenue, Campus Box 8227, St. Louis MO, 63110, yablonskiy@mir.wustl.edu, Tel.: +1(314) 362-1815, Fax: +1(314) 362-0526.

All authors report no conflicts of interest with this study.

Authorship Statement: Study design: MJS, DAY and SCS; Data acquisition: DAY, JDQ, JCW, DSG and MSC; Data analysis and interpretation: EFF, JDQ and DAY; Drafting manuscript: all authors; Critical revision of manuscript: all authors.

Keywords

Lung transplantation; pediatrics; *in vivo* lung morphometry; hyperpolarized gas magnetic resonance imaging; diffusion magnetic resonance imaging; acinar airways; alveoli

Introduction

Cystic fibrosis is a leading cause of lung transplantation in the pediatric population with up to 70% of lung transplants being attributed to this disease and the majority cause of lung transplants in children 6–17 years of age¹. Pediatric lung transplant survival has steadily increased and is now similar to median survival in adult recipients¹.

Bronchiolitis obliterans or chronic allograft dysfunction is the most frequent cause of morbidity and mortality in the lung transplant population. Bronchiolitis obliterans in pediatric patients is present in at least 35% of survivors within 5 years of the initial lung transplant and accounts for at least 48% of deaths in this population¹. In all lung transplant recipients, prevalence is as high as 70%^{2,3}. It is initially a clinical diagnosis with a decrease in FEV₁ (forced expiratory volume in 1 second) to less than 80% of the patient's best post-transplant baseline, and is often confirmed by histology^{4,5}.

Because early diagnosis and treatment of bronchiolitis obliterans have produced the best outcomes and that 1-year and 5-year survival is worse after re-transplantation, there is an interest in developing sensitive and non-invasive techniques that can be used to monitor lung transplant recipients¹. ³He MRI of lungs provides important information on lung function without ionizing radiation by detecting ventilation defects in emphysema, cystic fibrosis, and bronchiolitis obliterans^{6–11}. McAdams et al, in a study of 6 adult patients, found a correlation between the extent of ventilation defects shown by ³He MRI and the severity of bronchiolitis obliterans seen with pulmonary function testing¹¹. Gast et al qualitatively compared ³He ventilation MRI and CT in 14 adult patients with bronchiolitis obliterans and reported increased finding of ventilation defects with ³He MRI⁹. A more recent study completed by Gast et al concluded that ³He MRI could predict bronchiolitis obliterans prior to detection with clinical testing by estimating the ventilation defect area and using a quantitative threshold segmentation technique¹⁰.

While ventilation scans provide important information on regional lung function, they are not sensitive to lung microstructural changes in the distal airways – alveolar ducts and sacs forming lung acini that occupy 90% of lung volume¹². To study the structure and function of acinar airways and alveoli we have previously developed the *in vivo* lung morphometry technique¹³. Based on MRI diffusion measurements of hyperpolarized ³He gas inhaled in lung airspaces, this technique yields quantitative measurements of acinar airway parameters, such as acinar airway radius, R , and alveolar sleeve depth, h , that are used to calculate standard morphometric characteristics such as mean linear intercept, surface-to-volume ratio and alveolar density.¹³

Bronchiolitis obliterans is characterized by bronchiolar changes but patients after lung transplantation also develop other parenchymal changes including fibrosis^{14–17}. By

combining computed tomography (CT) data with ^3He diffusion MRI we also estimate an apparent alveolar wall thickness, which may be an important parameter in diagnosis and prognosis of lung diseases¹⁸.

In this study we applied the ^3He MRI-based *in vivo* lung morphometry technique to detect lung microstructure at the alveolar level in the pediatric post-lung transplant population. Our data suggest that ^3He MRI based *in vivo* lung morphometry provides unique new information on the alterations in lung microstructure that can serve as a potential target for improving care of this patient population.

Methods

Patient Selection

This prospective cohort study was approved by the Washington University in St. Louis School of Medicine institutional review board and all patients provided assent and parents provided informed consent. At St. Louis Children's Hospital, lung transplant recipients underwent pulmonary function testing and CT scanning as standard of care. Lung transplant recipients were recruited from the pulmonary clinic. The inclusion criteria for this preliminary study were the diagnosis of cystic fibrosis, age between 6 and 21 years old and history of a bilateral lung transplant. The exclusion criteria for this study were those who underwent unilateral lung transplantation, heart-lung transplantation, had a known pulmonary infection at the time of recruitment or were unable to comply with breathing maneuvers. Patients for whom exposure to a strong magnetic field would be a health risk (cardiac pacemakers or metallic implants for example) and those with significant claustrophobia were excluded.

Pulmonary function testing

Pulmonary function tests including FEV₁ (forced expiratory volume in 1 second), and diffusing capacity of the lung for carbon monoxide (DLCO) were obtained on the same day as MRI scans.

MR Imaging Procedures

Each of the selected patients underwent one MRI session. Timing of MRI was not standardized between patients with a range of 18 to 72 months post lung transplant. Vital signs, pulse oxygen saturation and three lead ECG were recorded before and after the MRI session and monitored during scanning.

All studies with hyperpolarized helium-3 (^3He) gas at Washington University School of Medicine in St. Louis are carried out according to FDA guidelines, under Investigational New Drug (IND) number 59,269-helium-3. Hyperpolarized ^3He gas is prepared using a custom spin-exchange optical pumping polarizer or a commercial IGI.9600.He polarizer (General Electric, Fairfield, CT).

Helium diffusion studies were conducted on a 1.5 T Siemens Sonata MRI scanner using a custom-built ^3He volume transmitter / 8-channel receiver RF coil (Stark Contrast MRI Coils Research, Erlangen, Germany). Initially, conventional ^1H MRI scans were obtained for

anatomic reference and to select the slices and orientations for the ^3He images at the same lung volumes that ^3He studies completed. To standardize the lung volume during imaging, patients were asked to exhale to functional residual capacity and inhale 1L of a 50/50% $^3\text{He}/\text{N}_2$ gas mixture from a flexible bag through a flexible tube and mouthpiece. Nose clips were applied to control inhalation volume and minimize mixing with room air. Imaging began at the start of a nine-second breath hold. ^3He diffusion images with 6 b-values were acquired with the following parameters: resolution = $4.7 \times 4.7 \times 30 \text{ mm}^3$; TR/TE = 13/8.45 ms; diffusion time = 1.8 ms; rise/fall time of the diffusion sensitizing gradient of 0.3 ms and no gap, b-values = 0, 2, 4, 6, 8, and 10 s/cm^2 .

MR image analysis

The ^3He diffusion MRI images from each channel of the receiver coil were individually phased and the real data were jointly analyzed on a pixel-by-pixel basis utilizing Bayesian probability theory¹⁹ and previously developed theory of gas diffusion in lung acinar airways¹³. In this approach acinar airways (alveolar ducts and sacs) are described in terms of the Weibel model^{20,21} as cylindrical air passages covered with alveolar sleeve (see Figure 1). *In vivo* lung morphometry provides measurements of alveolar sleeve depth h , and acinar airway radii R , that are further used to calculate the standard morphometric parameters such as surface to volume ratio S/V , mean linear intercept L_M , alveolar surface area S_a , lung volume per alveolus V_a and some other morphometric parameters¹³. In this paper we only use basic parameters (R and h) and S/V that is calculated as follows with alveolar diameter L ¹³:

$$S/V = S_a/V_a; S_a = \frac{\pi}{4} R \cdot L + \frac{\pi}{4} h \cdot (2R - h) + 2h \cdot L; V_a = \frac{\pi}{8} R^2 L; L = 2R \sin \frac{\pi}{8} \quad [1]$$

Since lung morphometric parameters depend on the lung inflation level²², the results of measurements were adjusted to each patient's FRC volume using previously established relationships²². Specifically, for each patient, measured values of average acinar airway radii R , were reduced by 6 μm and measured values of alveolar sleeve depth h , were increased by 11 μm (these adjustments correspond to a 1 L difference between FRC and the lung volume at which MRI experiment was conducted). The MRI data analysis for this study was identical to previous adult studies completed by our research group^{22,32}.

Evaluation of Apparent Alveolar Wall Thickness by Combining ^3He *in vivo* Lung Morphometry and CT

Clinical inspiratory CT scans were performed at total lung capacity (TLC) on a Siemens Sensation 16 scanner with in-plane resolution of approximately $0.6 \times 0.6 \text{ mm}$ and were reconstructed to 1 mm slices using a B70s filter. Quantitative CT provides signal attenuation in Hounsfield Units (HU),^{23–27} that can be converted to the density of lung tissue per unit lung volume (tissue plus gas) as follows:

$$\rho = \frac{HU + 1000}{1000} \cdot \rho_0; \rho_0 = 1 \text{ g/ml} \quad [2]$$

The density of lung tissue per unit lung volume can also be estimated using S/V values from our MRI measurements. Attributing all tissue (parenchymal and non-parenchymal¹²) to alveolar walls, we obtain:

$$\rho = \frac{S}{V} \cdot \frac{d}{2} \cdot \rho_{t0}; \quad [3]$$

In this equation $\rho_{t0}=1.065$ g/ml is the actual density of the lung tissue and d is the apparent thickness of alveolar walls. Thus, using Eqs. [2] and [3] we arrive at:

$$d = \frac{2}{S/V} \cdot \frac{\rho_0}{\rho_{T0}} \cdot \frac{(HU+1000)}{1000} \quad [4]$$

The surface-to-volume ratio (S/V) can be obtained from our *in vivo* lung morphometry technique using Eq. [1].

Statistical analysis was completed using IBM SPSS Statistics version 22. Pearson's correlation coefficient was computed when appropriate and linear regression was completed. A p-value of < 0.05 was considered statistically significant.

Results

All six patients enrolled in the study have successfully completed all tests. There were no adverse events during ^3He diffusion MRI lung imaging. These patients underwent ^3He diffusion MRI lung imaging at median time of 34 months after initial lung transplantation with a range of 18 to 72 months. For five of the patient's studied, CT imaging was completed 1 day prior to the MRI study. One patient underwent CT imaging 1 month prior to the MRI for this study. All patients were clinically followed for several years before and after MRI exam. Three patients had the diagnosis of bronchiolitis obliterans at the time of the study.

The median transplanted lung lifespan or length of time after initial transplantation until death or re-transplantation (terminal event) was 49 months with a range of 29 to 135 months. Demographic information including gender, age, height and weight for all patients is presented in Table 1. All patients were Caucasian. The last column in Table 1 represents lung lifespan.

Pulmonary function testing was also obtained during the same visit as ^3He MRI. Table 2 provides FEV_1 (forced expiratory volume in 1 second), percent predicted FEV_1 , diffusing capacity of the lung for carbon monoxide (DLCO), and percent predicted DLCO for each patient²⁸⁻³¹. Percent predicted FEV_1 was relatively stable between these measurements and the prior six months. Two patient's FEV_1 improved, one by 4% and one by 8%, three patients showed less than 10% decrease in FEV_1 (range 3–6% decrease) and one patient had a decline of 10% in the six months prior to the MRI. Overall, the percent predicted FEV_1 for

all patients was decreased from their best pulmonary function testing obtained post-lung transplant with median decrease of 10% (range 3–49% decrease).

The results obtained with in-vivo lung morphometry are summarized in Table 3 and illustrated in Figure 2. We observed no ventilation defects in these patients that would prohibit quantitation of lung morphometry values. Images of acinar airway radii R , alveolar sleeve depth h , and alveolar tissue surface-to-volume ratio (S/V) demonstrate values different for different patients. Table 3 shows the actual measured values of lung morphometric parameters and the values adjusted to each patient's FRC. Corresponding CT images are also shown in Figure 2.

Combining results of our ^3He -based *in vivo* lung morphometry and quantitative CT, we calculated the apparent alveolar wall thickness – Eq.[4]; results are presented in the last column of Table 3. Since CT and MR data were acquired at different lung inflation levels, CT defined density, Eq.[2], was scaled by the ratio of these volumes.

As a measure of gas diffusion across the interface between lung airspaces and alveolar capillary bed, percent predicted DLCO was compared with the apparent alveolar wall thickness determined in our study. The correlation between these parameters is presented in Figure 3.

The morphometric lung measurements, alveolar depth and acinar airway radius obtained from ^3He lung morphometry were correlated with the clinical outcome - transplanted lung lifespan from initial transplantation to the terminal event, as shown in Figure 4. A linear regression model with alveolar depth as the sole predictor of lung lifespan yielded an F-statistic = 14.992 and p value = 0.018 and associated $R = 0.888$.

Discussion

This study demonstrates that the ^3He -based *in vivo* lung morphometry technique¹³ yields previously unavailable clinical information in pediatric patients that have undergone lung transplantation.

One of our most intriguing findings is a strong correlation between lung lifespan and the alveolar depth (Figure 4). Patients with more shallow alveoli were likely to have a negative outcome sooner than those with larger alveolar depth. Shallowing of the alveolar sleeve of acinar airways was reported previously in emphysema patients^{13,32} and emphysema-like lung pathology can also be caused by inflammation³³. Since inflammation and lung injury are the pathophysiologies behind this finding in emphysema patients, we can hypothesize that this may play a role in bronchiolitis obliterans and its manifestations at the alveolar level. However, since our morphometric measurements were obtained only at a single time point during lung lifespan, it is not clear if these lung characteristic were inherited from the donor lung or developed during lung adjustment in a new environment.

Another interesting finding of our work is the increased thickness of alveolar walls characterized in our measurements by the parameter d – apparent alveolar wall thickness. While the thickness of alveolar walls in normal lungs is approximately $d_w = \mu\text{m}$ ³⁴, our data

(Table 3) show increased values for most patients. One would expect that increased alveolar wall thickness would impair diffusion across the blood-alveolar membrane causing decreased DLCO, which is indeed the case in our patient population. Results in Fig. 3 show very strong negative correlation between DLCO and the apparent alveolar wall thickness which in our measurements includes both parenchymal and non-parenchymal lung tissue.

Our finding of increased apparent alveolar wall thickness in transplanted lungs is also in agreement with the existing literature. Recent mouse models have been studied to replicate bronchiolitis obliterans and to evaluate lung histology^{35–37}. In an orthotopic, allogeneic lung transplantation mouse model, Atanasova et al. demonstrated fibrosis surrounding arteries and bronchioles and matrix deposition in the alveolar walls³⁷. In addition, with Heidenhain's azan and orcein stains, the allografts at 90 days post transplantation had fibrotic changes in the alveolar walls³⁷. Similarly, Junggraithmayr et al, utilizing a syngeneic rat model with skin sensitization prior to lung transplantation displayed moderate alveolar wall thickening on histology 60 days after transplant³⁸. These rodent models suggest that there may be a component of fibrosis, increased non-alveolar tissue or increased alveolar wall thickness that may explain the results seen in our study.

Increased interstitial fibrosis has been seen in human lung pathology specimens after lung transplantation. Several histology studies recognized that a portion of adult lung transplant recipients develop some degree of interstitial fibrosis within two years. In a retrospective study analyzing trans-bronchial biopsies by Burton et al, 66% of patients developed interstitial fibrosis within 2 years and this fibrosis was correlated with bronchiolitis obliterans which has been seen in earlier post-lung transplant studies^{14,15}. Additional studies report the presence of varying degrees of fibrosis along with areas of distinct bronchiolitis obliterans^{16,17}. This association of interstitial fibrosis and bronchiolitis obliterans may also explain the findings of increased average alveolar thickness in our study.

Pathology on the explanted lungs of four of our patients who underwent re-transplantation revealed varying degrees of bronchiolitis obliterans and interstitial fibrosis. However a formal morphometric analysis was not completed secondary to specimens not obtained at universal inflation.

All images were free of significant artifacts and there was minimal loss of signal in the ventilation maps (predominantly at the anterior of the middle slice in subject 5). To evaluate the quality of the MRI images, we analyzed the image SNR (from the sum of the phased images across all receiver channels). The median SNR for our patients was 95 with a range of 45 to 106. This quality analysis has been previously presented to describe the effect of SNR on parameter estimates^{39,40}.

A limitation to this study was the small sample size. Pediatric lung transplantation at a single center is a rare event and recruiting more patients is time and cost limited. In addition, during this study, ³He diffusion lung MRI imaging was not completed at a standard time in a patient's clinical course. This was the first non-invasive study utilizing *in vivo* lung morphometry with hyperpolarized ³He MRI to evaluate alveolar microstructure in the pediatric post-lung transplant population and suggests that this technology may provide new

clinical information. There are ongoing studies evaluating chronic rejection in lung transplantation utilizing other technologies including confocal microscopic imaging⁴¹. Future multi-site studies should be conducted to identify key time points to complete imaging both before and after lung transplantation and to establish ideal time interval to identify alveolar microstructure changes. Overall, alveolar depth has a potential to serve as a predictive measure for transplanted lung lifespan and warrants further investigation.

Conclusion

While further studies with larger number of lung transplant recipients are in order, our preliminary results suggest that *in vivo* lung morphometry provides unique and previously unavailable information on lung microstructure parameters that can improve care of lung transplant recipients. Indeed, a strong correlation between lung lifespan and the alveolar depth that we found has a potential to serve as a guide for lung selection and post-operative patient treatment; increased alveolar wall thickness that we report in some patients impairs alveolar wall permeability and can also be a target for therapy.

Acknowledgments

Primary Source of Funding: This study was supported by Phillips Medical Systems and the RSNA Research Seed Grant (awarded to M.S.). Research reported in this publication was supported by the Washington University Institute of Clinical and Translational Sciences grant UL1TR000448 from the National Center for Advancing Translational Sciences (NCATS) of the National Institutes of Health (NIH). The content is solely the responsibility of the authors and does not necessarily represent the official view of the NIH.

References

1. Benden C, Goldfarb SB, Edwards LB, et al. The registry of the international society for heart and lung transplantation: seventeenth official pediatric lung and heart-lung transplantation report-2014; focus theme: retransplantation. *J Heart Lung Transplant*. 2014 Oct; 33(10):1025–1033. [PubMed: 25242126]
2. Meyers BF, Lynch J, Trulock EP, Guthrie TJ, Cooper JD, Patterson GA. Lung transplantation: a decade of experience. *Ann Surg*. 1999 Sep; 230(3):362–370. discussion 370–361. [PubMed: 10493483]
3. Arcasoy SM, Kotloff RM. Lung transplantation. *N Engl J. Med*. 1999 Apr 8; 340(14):1081–1091. [PubMed: 10194239]
4. Cooper JD, Billingham M, Egan T, et al. A working formulation for the standardization of nomenclature and for clinical staging of chronic dysfunction in lung allografts. International Society for Heart and Lung Transplantation. *J Heart Lung Transplant*. 1993 Sep-Oct;12(5):713–716. [PubMed: 8241207]
5. Estenne M, Maurer JR, Boehler A, et al. Bronchiolitis obliterans syndrome 2001: an update of the diagnostic criteria. *J Heart Lung Transplant*. 2002 Mar; 21(3):297–310. [PubMed: 11897517]
6. Gierada DS, Saam B, Yablonskiy D, Cooper JD, Lefrak SS, Conradi MS. Dynamic echo planar MR imaging of lung ventilation with hyperpolarized (3)He in normal subjects and patients with severe emphysema. *NMR Biomed*. 2000 Jun; 13(4):176–181. [PubMed: 10867693]
7. Kauczor HU, Ebert M, Kreitner KF, et al. Imaging of the lungs using 3He MRI: preliminary clinical experience in 18 patients with and without lung disease. *J Magn Reson Imaging*. 1997 May-Jun; 7(3):538–543. [PubMed: 9170039]
8. Donnelly LF, MacFall JR, McAdams HP, et al. Cystic fibrosis: combined hyperpolarized 3He-enhanced and conventional proton MR imaging in the lung--preliminary observations. *Radiology*. 1999 Sep; 212(3):885–889. [PubMed: 10478261]
9. Gast KK, Viallon M, Eberle B, et al. MRI in lung transplant recipients using hyperpolarized 3He: comparison with CT. *J Magn Reson Imaging*. 2002 Mar; 15(3):268–274. [PubMed: 11891971]

10. Gast KK, Zaporozhan J, Ley S, et al. (3)He-MRI in follow-up of lung transplant recipients. *Eur Radiol.* 2004 Jan; 14(1):78–85. [PubMed: 14564471]
11. McAdams HP, Palmer SM, Donnelly LF, Charles HC, Tapson VF, MacFall JR. Hyperpolarized 3He-enhanced MR imaging of lung transplant recipients: preliminary results. *AJR Am J Roentgenol.* 1999 Oct; 173(4):955–959. [PubMed: 10511156]
12. Weibel, ER. *Morphometry of the Human Lung.* Springer-Verlag; 1963.
13. Yablonskiy DA, Sukstanskii AL, Woods JC, et al. Quantification of lung microstructure with hyperpolarized 3He diffusion MRI. *J Appl Physiol* (1985). 2009 Oct; 107(4):1258–1265. [PubMed: 19661452]
14. Burton CM, Iversen M, Carlsen J, Andersen CB. Interstitial inflammatory lesions of the pulmonary allograft: a retrospective analysis of 2697 transbronchial biopsies. *Transplantation.* 2008 Sep 27; 86(6):811–819. [PubMed: 18813106]
15. Yousem SA, Burke CM, Billingham ME. Pathologic pulmonary alterations in long-term human heart-lung transplantation. *Hum Pathol.* 1985 Sep; 16(9):911–923. [PubMed: 3928482]
16. Martinu T, Howell DN, Davis RD, Steele MP, Palmer SM. Pathologic correlates of bronchiolitis obliterans syndrome in pulmonary retransplant recipients. *Chest.* 2006 Apr; 129(4):1016–1023. [PubMed: 16608952]
17. Pakhale SS, Hadjiliadis D, Howell DN, et al. Upper lobe fibrosis: a novel manifestation of chronic allograft dysfunction in lung transplantation. *J Heart Lung Transplant.* 2005 Sep; 24(9):1260–1268. [PubMed: 16143243]
18. Stone KC, Mercer RR, Freeman BA, Chang LY, Crapo JD. Distribution of lung cell numbers and volumes between alveolar and nonalveolar tissue. *The American review of respiratory disease.* 1992 Aug; 146(2):454–456. [PubMed: 1489139]
19. Quirk JD, Sukstanskii AL, Bretthorst GL, Yablonskiy DA. Optimal decay rate constant estimates from phased array data utilizing joint Bayesian analysis. *J Magn Reson.* 2009 May; 198(1):49–56. [PubMed: 19181549]
20. Haefeli-Bleuer B, Weibel ER. Morphometry of the human pulmonary acinus. *Anat. Rec.* 1988 Apr; 220(4):401–414. [PubMed: 3382030]
21. Rodriguez M, Bur S, Favre A, Weibel ER. Pulmonary acinus: geometry and morphometry of the peripheral airway system in rat and rabbit. *Am J Anat.* 1987 Oct; 180(2):143–155. [PubMed: 3673919]
22. Hajari AJ, Yablonskiy DA, Sukstanskii AL, Quirk JD, Conradi MS, Woods JC. Morphometric changes in the human pulmonary acinus during inflation. *J Appl Physiol.* 2012 Mar; 112(6):937–943. [PubMed: 22096115]
23. Gierada DS, Bierhals AJ, Choong CK, et al. Effects of CT section thickness and reconstruction kernel on emphysema quantification relationship to the magnitude of the CT emphysema index. *Academic radiology.* 2010 Feb; 17(2):146–156. [PubMed: 19931472]
24. Madani A, Zanen J, de Maertelaer V, Gevenois PA. Pulmonary emphysema: objective quantification at multi-detector row CT--comparison with macroscopic and microscopic morphometry. *Proc Natl Acad Sci U S A.* 2006 Mar; 238(3):1036–1043.
25. Wang Z, Gu S, Leader JK, et al. Optimal threshold in CT quantification of emphysema. *European radiology.* 2013 Apr; 23(4):975–984. [PubMed: 23111815]
26. Hogg JC, Nepszky S. Regional lung volume and pleural pressure gradient estimated from lung density in dogs. *J Appl Physiol.* 1969 Aug; 27(2):198–203. [PubMed: 5796307]
27. Coxson HO, Rogers RM, Whittall KP, et al. A quantification of the lung surface area in emphysema using computed tomography. *Am J Respir Crit Care. Med.* 1999; 159(3):851–856. [PubMed: 10051262]
28. Wang X, Dockery DW, Wypij D, Fay ME, Ferris BG Jr. Pulmonary function between 6 and 18 years of age. *Pediatric pulmonology.* 1993 Feb; 15(2):75–88. [PubMed: 8474788]
29. Dockery DW, Berkey CS, Ware JH, Speizer FE, Ferris BG Jr. Distribution of forced vital capacity and forced expiratory volume in one second in children 6 to 11 years of age. *The American review of respiratory disease.* 1983 Sep; 128(3):405–412. [PubMed: 6614634]
30. Morris JF, Koski A, Johnson LC. Spirometric standards for healthy nonsmoking adults. *The American review of respiratory disease.* 1971 Jan; 103(1):57–67. [PubMed: 5540840]

31. Zapletal A, Paul T, Samanek M. Significance of contemporary methods of lung function testing for the detection of airway obstruction in children and adolescents (author's transl). *Zeitschrift für Erkrankungen der Atmungsorgane*. 1977 Aug; 149(3):343–371. [PubMed: 613549]
32. Quirk JD, Lutey BA, Gierada DS, et al. In vivo detection of acinar microstructural changes in early emphysema with 3He lung morphometry. *Radiology*. 2011 Sep; 260(3):866–874. [PubMed: 21734160]
33. Mouded M, Egea EE, Brown MJ, et al. Epithelial cell apoptosis causes acute lung injury masquerading as emphysema. *Am J Respir Cell Mol Biol*. 2009 Oct; 41(4):407–414. [PubMed: 19188661]
34. Chang YV, Quirk JD, Ruset IC, Atkinson JJ, Hersman FW, Woods JC. Quantification of human lung structure and physiology using hyperpolarized Xe. *Magnetic resonance in medicine : official journal of the Society of Magnetic Resonance in Medicine / Society of Magnetic Resonance in Medicine*. 2014 Oct 23;71:339–344.
35. De Vleeschauwer S, Jungraithmayr W, Wauters S, et al. Chronic rejection pathology after orthotopic lung transplantation in mice: the development of a murine BOS model and its drawbacks. *PLoS. One*. 2012; 7(1):e29802. [PubMed: 22238655]
36. Fan L, Benson HL, Vittal R, et al. Neutralizing IL-17 prevents obliterative bronchiolitis in murine orthotopic lung transplantation. *Am J Transplant*. 2011 May; 11(5):911–922. [PubMed: 21521466]
37. Atanasova S, Hirschburger M, Jonigk D, et al. A relevant experimental model for human bronchiolitis obliterans syndrome. *J Heart Lung Transplant*. 2013 Nov; 32(11):1131–1139. [PubMed: 24050896]
38. Jungraithmayr W, Vogt P, Inci I, et al. A model of chronic lung allograft rejection in the rat. *Eur Respir J*. 2010 Jun; 35(6):1354–1363. [PubMed: 19840961]
39. Chang YV, Quirk JD, Yablonskiy DA. In vivo lung morphometry with accelerated hyperpolarized (3) He diffusion MRI: a preliminary study. *Magnetic resonance in medicine*. 2015 Apr; 73(4):1609–1614. [PubMed: 24799044]
40. Sukstanskii AL, Bretthorst GL, Chang YV, Conradi MS, Yablonskiy DA. How accurately can the parameters from a model of anisotropic 3He gas diffusion in lung acinar airways be estimated? Bayesian view. *Journal of magnetic resonance*. 2007 Jan; 184(1):62–71. [PubMed: 17030132]
41. Yserbyt J, Doods C, Decramer M, et al. Acute lung allograft rejection: diagnostic role of probe-based confocal laser endomicroscopy of the respiratory tract. *J Heart Lung Transplant*. 2014 May; 33(5):492–498. [PubMed: 24656287]

Figure 1.

Schematic structure (longitudinal at left and transverse at right) of the Weibel acinar airways geometry model^{20,21} used for parameter estimate in the *in vivo* lung morphometry approach¹³. The model's major parameters: acinar airways external radii (R), acinar airways lumen radii (r), the depth of the alveolar sleeve ($h = R - r$), alveolar diameter (L) and alveolar volume (V_A).

Figure 2.

Ventilation maps, parametric maps of alveolar depth (h), acinar airway radius (R), surface to volume ratio (S/V), and CT images for all patients (only the central slices are shown).

Figure 3.

Correlation between the apparent alveolar wall thickness and % predicted DLCO adjusted for patient's hemoglobin. R^2 is displayed for this association. Each point represents average value for a single patient.

Figure 4.

Mean acinar airway radius (R) and mean alveolar depth (h) are plotted against transplanted lung lifespan. R^2 is displayed for both associations. Each point represents average value for a single patient.

Table 1

Demographic information for all patients

Demographic information for all patients. Lung lifespan is defined as a time from initial transplantation until a terminal event (death or re-transplantation).

Patient	Gender	Age at transplant (years)	Age at MRI (years)	Height at MRI (cm)	Weight at MRI (kg)	Months from transplant to study	Lung lifespan (months)
1	Female	16	19	151	43.3	35	57
2	Female	15	17	162	54.0	18	33
3*	Female	10	16	156	49.5	72	135
4	Male	16	19	175	52.5	32	41
5*	Female	11	14	140	33.4	25	29
6*	Female	16	19	160	58.5	40	88

* Patients with clinical evidence of Bronchiolitis Obliterans at the time of the study.

Table 2**Pulmonary function testing for all patients**

Pulmonary function tests completed during the same visit as ^3He MRI. FEV₁ (forced expiratory volume in 1 second), DLCO (diffusing lung capacity for the carbon monoxide), TLC (total lung capacity) and RV (residual volume). Predicted measurements at St. Louis Children's Hospital are referenced from Dockery, Zapletal and Morris²⁸⁻³¹.

Patient	FEV ₁ in L (% predicted FEV ₁)	% FEV ₁ /FVC	TLC in L (% predicted TLC)	RV in L (% predicted RV)	DLCO adjusted for patient's hemoglobin in mL/mmHg/min (% adjusted predicted DLCO)
1	2.1 (74)	82	3.7 (87)	1.0 (97)	15.2 (61)
2	3.1 (98)	81	4.9 (110)	1.1 (100)	16.2 (67)
3	1.8 (61)	89	3 (73)	0.7 (73)	14.3 (64)
4	3.4 (75)	73	6.7 (95)	1.7 (106)	24.7 (70)
5	1.6 (74)	89	2.3 (74)	0.4 (44)	10.8 (61)
6	2.5 (79)	79	4.2 (83)	0.7 (51)	18.2 (67)

Table 3
Measured values of lung morphometric parameters and the values adjusted to each patient's FRC (functional residual capacity)

Morphometric characteristics for all patients: alveolar depth, h , in μm , acinar airway radius, R , in μm , surface to volume ratio in cm^{-1} , apparent alveolar wall thickness in μm . Mean CT values displayed for all patients in Hounsfield units. Data for R , h and S/V and CT values are measured mean, (measured standard deviation).

Patient	Alveolar depth h in μm	Acinar airway radius R in μm	Surface to volume ratio S/V in cm^{-1}	Mean CT values in Hounsfield Units	Apparent alveolar wall thickness d in μm
1	146 (37) * ₁₅₇	302 (42) * ₂₉₆	213 (32) * ₂₂₈	-777 (111)	19.7
2	137 (30) * ₁₄₈	323 (33) * ₃₁₇	184 (21) * ₁₉₇	-863 (111)	14
3	156 (38) * ₁₆₇	288 (35) * ₂₈₂	237 (30) * ₂₅₅	-754 (111)	16.8
4	129 (32) * ₁₄₀	305 (24) * ₂₉₉	193 (23) * ₂₀₉	-869 (84)	12.7
5	132 (36) * ₁₄₃	292 (35) * ₂₈₆	210 (32) * ₂₂₇	-602 (104)	21.8
6	153 (36) * ₁₆₄	306 (29) * ₃₀₀	211 (19) * ₂₂₉	-869 (117)	11.6

(*) denotes mean values adjusted to each patient's functional residual capacity (FRC).