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Alveolar airspace size in healthy and diseased infant lungs measured via hyperpolarized ^3He gas diffusion MRI

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Abstract

Background: Alveolar development and lung parenchymal simplification are not well characterized *in vivo* in neonatal patients with respiratory morbidities, such as bronchopulmonary dysplasia (BPD). Hyperpolarized (HP) gas diffusion MRI is a sensitive, safe, non-ionizing, and non-invasive biomarker for measuring airspace size *in vivo* but has not yet been implemented in young infants.

Objective: This work quantified alveolar airspace size via HP gas diffusion MRI in healthy and diseased explanted infant lung specimens, with comparison to histological morphometry.

Methods: Lung specimens from 8 infants were obtained: 7 healthy left upper lobes (0–16 months, post-autopsy) and 1 left lung with filamin-A mutation, closely representing BPD lung disease (11-months, post-transplantation). Specimens were imaged using HP ^3He diffusion MRI to generate

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Author Contributions

NSH/RPT/ADH/SBF/JCW planned experiments. SBF/GSP/JCW supervised work. NSH/RPT performed experiments. NSH/RPT/HLH prepared samples. NSH/RPT/JDQ/JCW interpreted results. NSH wrote manuscript. All authors provided feedback and shaped analysis and manuscript.

Statement of Ethics

This study was performed with the approval of the Cincinnati Children's Hospital (IRB2014-6279) and University of Rochester Medical Center Institutional Review Boards (RSRB00047606) and with written, informed consent from families in compliance with the Helsinki Declaration.

Disclosures

The authors have no conflicts of interest that compromise this study.

apparent diffusion coefficients (ADC) as biomarkers of alveolar airspace size, with comparison to mean linear intercept (L_m) via quantitative histology.

Results: Mean ADC and L_m were significantly increased throughout the diseased specimen ($ADC=0.26\pm0.06\text{cm}^2/\text{s}$, $L_m=587\pm212\mu\text{m}$) compared with healthy specimens ($ADC=0.14\pm0.03\text{cm}^2/\text{s}$, $L_m=133\pm37\mu\text{m}$; $P<1\times10^{-7}$); increased values reflect enlarged airspaces. Mean ADC in healthy specimens were significantly correlated to L_m ($r=0.69$, $P=0.041$).

Conclusions: HP gas diffusion MRI is sensitive to healthy and diseased regional alveolar airspace size in infant lungs, with good comparison to quantitative histology in *ex vivo* specimens. This work demonstrates the translational potential of gas MRI techniques for *in vivo* assessment of normal and abnormal alveolar development in neonates with pulmonary disease.

Keywords

Hyperpolarized gas; helium-3; gas diffusion MRI; infant lungs; alveolar airspace; bronchopulmonary dysplasia; lung development; magnetic resonance imaging; prematurity

Introduction

The majority of infants with prolonged stay in the neonatal intensive care unit have pulmonary morbidities related to preterm birth (bronchopulmonary dysplasia [BPD]) or congenital abnormalities (e.g., congenital diaphragmatic hernia [CDH]) [1,2]. These multifaceted neonatal conditions typically involve abnormal development and simplification of the alveolar airspaces, which are important determinants of poor clinical outcomes. Survivors of BPD and CDH in early life often face increased morbidity and mortality, with impaired lung function that persists into childhood and adulthood [1].

The development of normal and abnormal alveolarization in infancy has not been extensively studied *in vivo*, largely due to a lack of feasible *in vivo* methods for evaluating lung microstructure. As a result, most studies rely on small samples from biopsy or autopsy, which typically represent more severely diseased cases and are subject to sampling error. Current and emerging techniques for pulmonary imaging, such x-ray computed tomography (CT) and ^1H ultrashort-echo time (UTE) magnetic resonance imaging (MRI), have been shown to visualize macroscopic abnormalities of parenchymal structure and can predict respiratory outcomes [3-5]. However, these modalities do not probe the alveolar microstructure. A safe, non-invasive technique for sensitively assessing normal and abnormal microstructural airspaces in infants may provide valuable links between respiratory status in early neonatal life and later clinical outcomes, particularly if implemented serially.

Gas diffusion MRI using inhaled hyperpolarized (HP) noble gases (e.g., ^3He or ^{129}Xe) is an established research technique with an extensive safety record [6-8]. This method can sensitively measure microstructural properties of alveolar airspaces on a regional level via a straightforward metric known as the apparent diffusion coefficient (ADC) [9]. Gas diffusion MRI has been implemented in various adult and older pediatric conditions, including interstitial lung disease, chronic obstructive pulmonary disease subclinical emphysema in smokers, cystic fibrosis, asthma, and age-dependent changes in healthy adults, with

comparisons between diffusion MRI and quantitative histology in older lung specimens [6,10-13].

Recent studies have implemented gas diffusion MRI in older children and adults with pulmonary sequelae stemming from lung conditions in infancy, including BPD and CDH, with findings that suggest impaired ventilatory function and enlarged alveoli compared to healthy subjects [14,15]. In a comparison of preterm and term school-aged children, diffusion MRI demonstrated heterogeneous alveolar dimensions in preterm subjects that was not detected by conventional methods (FEV₁ and multiple-breath washout) [16]. The feasibility of inhaled gas MRI in challenging infant and young pediatric populations was recently demonstrated using ventilation MRI – a technique that is distinct from gas diffusion MRI and can visualize inhaled gas distribution [17]; however, rigorous validation of gas diffusion MRI in infant lungs, with a smaller airspace regime than older pediatrics or adults, has not yet been investigated.

In this study, we investigated the relationship between alveolar airspace size and ADC measurements from ³He gas diffusion MRI in seven healthy and one diseased *ex vivo* infant human lung specimens, with comparison to quantitative histology. The diseased lung specimen was obtained from a patient with filamin-A (FLNA) gene mutation. FLNA mutation is characterized by poor alveolar development on histological presentation, providing a close representation of diffuse lung disease seen in BPD [18].

While *ex vivo* specimens were used here for quantitative histological comparison, this work demonstrates that HP gas diffusion MRI may have high translational value in regionally assessing alveolar airspaces in live infant patient populations with heterogeneous lung disease, which may offer the potential for *in vivo* monitoring of therapeutic efficacy and inform individualized clinical care decisions.

Methods

Lung Specimens

Seven *ex vivo* left upper lung lobes from healthy infants (3 female, 4 males) were obtained ~24 hours after death. Mean age was 6.3±6.4 months (range 0-16 months). One *ex vivo* left lung from an 11-month-old female patient with FLNA gene mutation and mild prematurity was obtained ~3 hours after transplantation. Specimen procurement and further condition details are provided in the online supplementary materials. Informed consent was obtained from all families. The work was approved by the Cincinnati Children's Hospital and University of Rochester Medical Center Institutional Review Boards (IRB2014-6279 and RSRB00047606, respectively).

³He MRI Preparation, Acquisition, and Analysis

Details on the preparation of lung specimens and HP gas prior to imaging are provided in the online supplementary materials. After preparation, the lung specimen was manually inflated with a ³He/N₂ gas mixture to approximately total lung capacity (TLC). Diffusion-weighted ³He MRI was acquired immediately after inflation. Details on hardware, acquisition, and lung inflation for MRI are shown in Table 1 [13,19-21]. Diffusion timing and b-values were

chosen to provide a 1D free diffusion length smaller than that required for an adult human but larger than an adult mouse [22,23], with compromise between short diffusion times and a wide range of b-values. Diffusion-weighted MRI signals were fit to a monoexponential decay model using all b-values to generate ADC values in all lung voxels.

Histological Morphometry

After imaging, lung specimens were inflation-fixed in formalin at 25 cmH₂O. One H&E-stained section was generated for each healthy specimen, and three sections were generated for the diseased specimen, due to disease heterogeneity. Twenty measurements of mean linear intercept (L_m), the accepted standard for alveolar airspace size in lung morphometry, [24] were collected per section. Further details on histological analysis are provided in the online supplementary materials.

Statistical Analysis

Mean ADC and L_m values were compared for individual healthy specimens using a one-tailed Pearson's correlation with permutation test. One-tailed, Wilcoxon rank-sum tests were used to compare ADC and L_m measurements between each individual healthy specimen and the diseased specimen. Mean ADC and L_m values were compared to specimen age in healthy specimens with two-tailed Spearman rank correlation. Null and alternative hypotheses for each test are provided in the online supplementary materials. The threshold for statistical significance was set at $P < 0.05$ for all analyses.

Results

All lung specimens underwent successful imaging and histology, with individual specimen details and findings shown in Table 2. Figure 1 demonstrates representative imaging and histological findings for a healthy 16-month-old specimen and a diseased 11-month-old specimen. Average ³He signal-to-noise ratio on the $b=0$ s/cm² MR image was 23 ± 10 . Figure 2 demonstrates significant correlation between mean ADC and L_m values in healthy specimens (Pearson $r=0.69$, $P=0.041$).

In the healthy specimen group, mean ADC was 0.14 ± 0.03 cm²/s and mean L_m was 133 ± 37 μ m (standard deviation of all specimens in the healthy group). ADC and L_m values were elevated and more heterogeneous in the diseased specimen (ADC = 0.26 ± 0.06 cm²/s and $L_m = 587 \pm 212$ μ m; standard deviation of all measurements from the single specimen in the diseased group). Figure 3 demonstrates a significant difference between the ADC and L_m values from each individual healthy specimen compared to the diseased specimen (Wilcoxon rank-sum $P < 1 \times 10^{-7}$ for each comparison). The increased mean ADC and L_m values in the diseased specimen are indicative of reduced restriction to gas diffusion (i.e., increased airspace sizes).

Spearman correlation was weak for both L_m ($\rho = -0.16$, $P = 0.72$) and ADC ($\rho = 0.36$, $P = 0.42$) when compared with healthy specimen age (Figure 4).

Discussion

This study demonstrates that ADC values obtained from non-invasive gas diffusion MRI are sensitive to relative differences in alveolar airspace size in normal and abnormal infant lung specimens at ages 0-16 months old, with comparison to histology. Further, this work represents the first application of gas diffusion MRI in the airspace size regime of infant lungs. This *ex vivo* study is an important proof-of-concept for gas diffusion MRI in neonatal alveolar airspaces, demonstrating sensitivity to airspace size abnormalities associated with FLNA gene mutation that can mimic those seen in infants with prematurity-related BPD [18,25], such as alveolar simplification, parenchymal cysts, and diffuse lung disease observed on histology, chest radiograph, and chest CT [25]. These results support the *in vivo* application of gas diffusion MRI to measure abnormalities in alveolar airspace microstructure in vulnerable infant and neonatal populations, a measurement that currently cannot be performed non-invasively by any other technique.

The analysis in this study is straightforward but includes some limitations. The number of *ex vivo* lung specimens was small (7 healthy and 1 FLNA), due in large part to the paucity of donated and/or transplanted organs in this age group. While the FLNA specimen in this study came from an infant that was mildly preterm, most of the lung abnormalities are likely attributable to the genetic mutation rather than BPD. The airspace sizes seen in this study's FLNA specimen may exceed typical BPD values, but previous measurements demonstrate a marked difference in L_m between control and BPD infants [26]; further, with established sensitivity in detecting and quantifying mild and early lung disease [27,28], gas diffusion MRI will likely characterize BPD-related lung disease effectively.

The gas mixture in this *ex vivo* work had a higher concentration of ^3He (50%) than previous studies in adults and children that used dilute-limit ^3He concentrations [9,10,13-15,29]. This higher concentration was used primarily for improved SNR, but also resulted in increased ^3He diffusivity (1.2 cm^2/s compared with 0.88 cm^2/s) and increased free diffusion length (467 μm compared with 400 μm). As such, inter-study comparisons of absolute ADC values are somewhat limited. Additionally, the relative proportion of ^3He in the gas mixture may have slightly varied between specimens, with small concomitant changes in the ^3He gas diffusivity. However, this effect was likely small across the cohort, with the greatest reduction in ^3He proportion being ~10% in the small 1-day-old specimens (~10% SNR decrease, ~8% diffusivity decrease). The potential for tissue structural distortion from histological processing is present but was addressed through shrinkage corrections via tissue dimension comparisons before and after processing. The inflation pressure used in this study (~25 cmH_2O) is consistent with typical peak inspiratory pressures in ventilated infants; given the elastic limit of the lungs at TLC, the risk of hyperinflation during imaging was likely low.

Sampling bias in the histological analysis was likely smaller in healthy lung specimens, due to higher homogeneity in normal infant pulmonary development compared with the diseased specimen. Since imaging slices are inherently much thicker than histological slices (~1 cm compared to ~5 μm), the relative spread in L_m distributions is likely to be smaller than that of ADC distributions for an individual specimen. Due to the small histological sampling

region, ADC measurements may yield a more accurate representation of overall disease throughout the lung organ, particularly in cases of heterogeneous disease distribution. We note, however, that the difference between healthy and enlarged alveoli in these samples is smaller than shown previously in adult disease, with implications for advantages of ^{129}Xe in future studies (discussed below). Despite these limitations, the correlation between healthy L_m and ADC in this study ($r = 0.69$) is consistent with previous gas diffusion MRI studies (e.g., $r=0.60$ and $r=0.59$) [9,11], and likely would improve with inclusion of a larger sample of different stages of lung development within the healthy group. The lack of significant correlation for L_m and ADC with age in healthy specimens may be due in part to inter-individual variation in the healthy cohort, and also likely reflects imperfect sensitivity of both histological L_m and radiological ADC measurements in detecting subtle airspace size changes during normal development from 0-16 months of age. However, utility of the non-invasive ADC measurements from gas diffusion MRI may lie in identifying abnormally developed airspaces, as demonstrated with the diseased lung specimen here.

The present study provides an important proof-of-concept for quantifying alveolar airspace size in infants using ^3He gas diffusion MRI using a monoexponential fit to measure ADC. Previous investigators have developed and implemented non-monoexponential models in humans and small animals that reflect diffusion anisotropy within small airways, including Yablonskiy et al., among others [22,23,30]. These models are valuable and can provide regional estimates of morphometric airway dimensions. However, application to the young infant lung in normal or abnormal development may be inappropriate, since the existing models' phenomenologically-derived mathematical relationships are specific to the size regime of adult humans and adult mice. Further, alveolar airspaces and septal walls in young and developing infants are likely less accurately represented by cylindrical ducts with alveolar sleeves, as is assumed by the cylinder model.

In the future, ^{129}Xe may be preferred over ^3He as the nucleus of choice for HP gas lung imaging due to relatively higher availability, decreased cost, and shorter durations needed to polarize the gas. While ^3He has a larger gyromagnetic ratio and thus higher SNR than ^{129}Xe , the smaller diffusivity of ^{129}Xe allows the use of imaging protocols with longer diffusion times that are achievable on a broader range of MRI hardware; thus ^{129}Xe may provide greater sensitivity to the smaller airspace sizes of infant lungs in more numerous experimental set-ups [22].

The neonatal-sized MRI scanner used in this study is not necessary to conduct HP gas diffusion MRI, as has been demonstrated by numerous studies performed on adult-sized scanners with multi-nuclear capabilities and appropriate MR coils [6-8,10,12,13,15,17,27,29]. Further, noble-gas hyperpolarization systems are now commercially available, making such studies feasible at a variety of institutions (<https://cpir.cchmc.org/xemrictc>). Future comparisons of regional HP gas MRI findings and parenchymal tissue abnormalities via UTE MRI [4,5] could be performed with minimal patient risk.

We have shown that HP gas diffusion MRI can quantify alveolar airspace size in *ex vivo* infant lungs, with direct comparison to quantitative histology. This work demonstrates that

gas diffusion MRI may have high translational value in the future as a non-invasive biomarker of regional alveolar airspace in neonatal patients with pulmonary pathologies, such as those with BPD or CDH. With an established safety record in adults and pediatrics, HP gas diffusion MRI has strong translational potential in the neonatal population for cross-sectional study and serial monitoring of normal and abnormal alveolar development.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1: Results from two representative lung specimens: a left upper lobe from a 16-month-old healthy infant (left) and a left lung from an 11-month-old diseased infant with filamin-A (FLNA) gene mutation (right). Shown are ^3He gas images from MRI (top row) and apparent diffusion coefficient (ADC) maps for the corresponding image slices (middle row). ADC values are increased and have more regional heterogeneity in the diseased specimen compared to the healthy specimen. This is indicative of abnormally enlarged alveolar airspaces, as confirmed via regionally matched quantitative histology (bottom row).

Figure 2:

Comparison of mean values of apparent diffusion coefficient (ADC) from ³He gas diffusion MRI and mean linear intercept (L_m) from quantitative histology, with standard deviations represented by error bars. Left: Data from seven healthy infant lung specimens, with significant correlation (Pearson $r = 0.69$, $P = 0.041$). Right: Data from one diseased specimen with filamin-A (FLNA) gene mutation (red) is added to the healthy data (blue), demonstrating the increased value and relative heterogeneity of both ADC and L_m in the diseased case compared to healthy cases.

Figure 3:

Measurements of mean linear intercepts from quantitative histology (L_m , left) and apparent diffusion coefficients from 3He gas diffusion MRI (ADC, right) for healthy and diseased infant lung specimens (“H” and “D”, respectively, with diseased condition of filamin-A gene mutation [FLNA]). Distributions for individual specimens are shown in color (healthy in blue, diseased in red) and are labeled with specimen numbers (e.g., “H1” or “D1”).

Individual healthy specimens have significantly lower L_m and ADC values compared to the diseased specimen (Wilcoxon rank-sum $P < 1 \times 10^{-7}$ for each comparison), with elevated L_m and ADC values in the FLNA specimen indicating enlarged airspaces.

Figure 4:

Mean values of mean linear intercept (L_m , left) and apparent diffusion coefficients (ADC, right) as functions of lung specimen age (months) in healthy (blue) and diseased (red, filamin-A [FLNA] gene mutation) infant lung specimens, with standard deviations represented by error bars. Spearman correlation was weak for L_m ($\rho = -0.16$, $P = 0.72$) and ADC ($\rho = 0.36$, $P = 0.42$) compared with healthy specimen age (blue). The elevated ADC and L_m values for the specimen with FLNA gene mutation are clear, compared to healthy lungs across the age range of this cohort.

Table 1:

Hardware, acquisition, and lung inflation parameter details for MRI.

Scanner	
MRI System	1.5T, GE Healthcare HDx software (Waukesha, WI, USA) [19]
Details	Neonatal-sized, small-footprint, multinuclear
Coil	
Design	Home-built switched-frequency $^1\text{H}/^3\text{He}$ high-pass birdcage body coil with 18-cm inner diameter, with mechanical actuator to switch between modes without removal [20]
Tuning	Tuned to resonate at ^1H and ^3He frequencies on network analyzer (~ 63.9 MHz and ~ 48.7 MHz at 1.5T, respectively)
^3He Acquisition ^a	
Sequence	Axial diffusion-weighted 2D fast gradient-recalled echo
Diffusion weighting	Bipolar gradient pulses, 9 b-values spaced evenly from 0-7.6 s/cm ²
Read-out ordering	Centric ordering with line-by-line interleaved b-values [13]
Flip angle	6° (constant), calibrated via decay of hyperpolarized $^3\text{He}/\text{N}_2$ phantom signal over a series of constant-power excitation pulses [21]
Resonance frequency calibration	Determined from frequency spectrum acquired during initial in-vivo inflation
Repetition/echo time	$\sim 5.7/3.8$ ms
Field of view	10 – 14 cm
Acquisition matrix	64
In-plane resolution	(1.6 x 1.6)-(2.1 x 2.1) mm
Slice thickness	10 mm
Number of slices acquired	4-9
Diffusion time $\Delta t/\tau$	0.91/0.91/0.17 ms
Lung inflation	
Duration	~ 15 s (sustained inflation)
Pressure	25 cmH ₂ O (approximately total lung capacity, TLC)
Volume ^a	15-150 mL

^aSmall changes due to variations in bulk specimen size.

Individual lung specimen details, with findings from ^3He gas diffusion MRI (apparent diffusion coefficient, ADC) and quantitative histology (mean linear intercept, L_m).

Table 2:

Subject ID	Chronological age (months)	Sex	ADC (cm ² /s) ^a						L _m (μm) ^b					
			Mean	Median	SD ^c	IQR ^d	Q1 ^e	Q3 ^f	Mean	Median	SD ^c	IQR ^d	Q1 ^e	Q3 ^f
Healthy 1	4	M	0.176	0.176	0.014	0.016	0.168	0.184	185	174	30	40	164	204
Healthy 2	0 (1 day)	F	0.111	0.112	0.028	0.030	0.092	0.122	77	73	11	14	70	84
Healthy 3	6	M	0.140	0.141	0.012	0.016	0.132	0.148	119	111	22	19	105	124
Healthy 4	16	M	0.161	0.159	0.017	0.020	0.150	0.170	115	110	15	16	106	122
Healthy 5	0 (1 day)	F	0.137	0.134	0.048	0.041	0.117	0.158	148	144	21	25	132	157
Healthy 6	4	M	0.144	0.136	0.049	0.040	0.119	0.159	154	151	15	15	147	162
Healthy 7	14	F	0.116	0.117	0.028	0.036	0.099	0.135	130	129	11	10	123	133
Diseased 1	11	F	0.257	0.252	0.063	0.092	0.211	0.303	587	517	212	179	432	611

^a Apparent diffusion coefficient
^b Mean linear intercept
^c Standard deviation
^d Interquartile range
^e 1st quartile
^f 3rd quartile