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Patterns of regional lung physiology in cystic fibrosis using ventilation MRI and MBW

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Take home message: *Ventilation distribution on MRI improves at TLC and two distinct patterns of regional lung disease in CF are highlighted, where abnormal FEV_1 is associated with VDP >10%. Ventilation MRI & MBW are highly correlated*.

Word count: 3011

Glossary

CF – Cystic fibrosis

EIVt – End-inspiratory tidal volume

FEV_1 – Forced expiratory volume in 1 second

FRC -Functional residual capacity

^3He – Helium-3

LCI – Lung clearance index

MBW – Multiple breath washout

MRI – Magnetic resonance imaging

RV – Residual volume

S_{cond} - convection dependent ventilation heterogeneity

S_{acin} - convection-diffusion dependent ventilation heterogeneity

TCV – Thoracic cavity volume

TLC – Total lung capacity

VDP – Ventilation defect percentage

VH_I – Ventilation heterogeneity index

VV – Ventilated volume

Abstract (200 words)

Hyperpolarised helium-3 (^{3}He) ventilation MRI and multiple-breath washout (MBW) are sensitive methods for detecting lung disease in cystic fibrosis (CF). We aimed to explore their relationship across a broad range of CF disease severity and patient age, as well as assess the effect of inhaled lung volume on ventilation distribution.

32 children and adults with CF underwent MBW and ^{3}He-MRI at a lung volume of end-inspiratory tidal volume (EIVt). 28 patients also performed ^{3}He-MRI at total lung capacity (TLC). ^{3}He-MRI were quantitatively analysed for; ventilation defect percentage (VDP), ventilation heterogeneity index (VH_I), and the number and size of individual contiguous ventilation defects. From MBW, the lung clearance index (LCI), S_{cond} and S_{acin} were calculated.

VDP and VH_I at EIVt strongly correlated with LCI (r=0.89,r=0.88 respectively), S_{acin} (r=0.84,r=0.82) and FEV_1 (r=-0.79,r=-0.78). Two distinct ^{3}He-MRI patterns were highlighted; patients with abnormal FEV_1 had significantly ($p<0.001$) larger, but fewer contiguous defects than those with normal FEV_1 who tended to have numerous small volume defects. These two MRI patterns were delineated by a VDP of approximately 10%. At TLC, when compared to EIVt, VDP and VH_I reduced in all subjects ($p<0.001$), demonstrating improved ventilation distribution and also regions of volume-reversible and non-reversible ventilation abnormalities.

Introduction

Hyperpolarised gas ventilation MRI allows for detailed and sensitive quantitative assessment of regional lung ventilation abnormalities in patients with obstructive airways disease [1-4]. The MRI technique usually involves the inhalation of a fixed volume of hyperpolarised noble gas and the resulting distribution of ventilation is imaged during a short breath-hold. In patients with cystic fibrosis (CF) it has been shown to be highly sensitive to detect early lung disease in children [5, 6], to track disease progression [7] and to assess treatment response [8]. The use of a fixed inhalation volume however, will result in patients with smaller lung volume being closer to their total lung capacity (TLC) than patients with larger lung volume. The effect that this has on the distribution of ventilation has not yet been assessed in CF. In addition, understanding the nature and pattern of regional ventilation defects seen on MRI, across the spectrum of CF lung disease, will allow for a greater understanding of lung disease pathophysiology and progression.

The lung clearance index (LCI), derived from multiple-breath washout (MBW) is a physiological measure of global ventilation heterogeneity that is sensitive to early lung disease [9]. LCI correlates with structural abnormalities seen on CT [10-12] and MRI [13] and is also sensitive to treatment response [14]. The utility of LCI in CF has been established primarily in children and patients with mild disease. In more severe CF lung disease, when previously obstructed regions open up to tidal ventilation due to treatment, the LCI response is unpredictable and may worsen despite improvements in FEV_1 [15-19]. Comparing LCI to ventilation MRI may therefore help to better understand ventilation abnormalities expressed by the two methods.

In this work we therefore aimed to:

(1) Compare ventilation MRI and MBW, including LCI and the phase III slope outcomes S_{cond} (convection dependent ventilation heterogeneity) and S_{acin} (convection-diffusion dependent ventilation heterogeneity), in children and adults with CF with a broad range of lung disease severity.

(2) Assess ventilation MRI at two different lung volumes in order to investigate the nature of reversible airway obstruction in CF.

(3) Assess the relationship between size and number of individual contiguous ventilation defects from ventilation MRI in CF patients across a broad range of disease severity to aid understanding of regional imaging data and disease status.

Methods

Children and adults with CF were recruited from three UK specialist centres (Sheffield Children's Hospital and Northern General Hospital, Sheffield, UK and Manchester Adult CF Centre, Manchester, UK). Patients were required to be aged over five years, be clinically stable for four weeks prior to their visit and achieve an FEV_1 >30% predicted within the previous six months. This study was approved by the Yorkshire and Humber - Leeds West Research Ethics Committee (REC reference: 16/YH/0339). Parents/guardians of children and all adult patients provided written informed consent.

MRI acquisition

Ventilation MRI was performed on a 1.5T GE HDx scanner (GE, Milwaukee, USA) using hyperpolarised helium-3 (^{3}He) using a transmit-receive vest coil (CMRS, Milwaukee, USA) and a 3D ventilation imaging sequence as described previously [20]. Images were acquired at two different lung inhalation volumes during separate breath-holds. Firstly, images were acquired at a lung volume corresponding to end-inspiratory tidal volume (EIVt), by inhaling a pre-determined fixed volume of test gas from their resting functional residual capacity (FRC). This corresponds to the approximate inspired volume most typically reported in studies to date [1, 5, 6, 8, 21-28]. The volume of gas was titrated based on the subject's height and consisted of scaled doses of ^{3}He and balanced with nitrogen (N_2) (E-Table 1). Secondly, images were acquired at TLC by repeating the EIVt-breathing manoeuvre, immediately followed by a full inhalation of room air. Further methodological details are described in the OLS.

MRI Post-Processing

For both the EIVt and TLC ^{3}He and ^{1}H image pairs, image metrics were calculated from a semi-automated segmentation [29]. The ^{3}He images were segmented in order to calculate the ventilated lung volume (VV) and the ^{1}H images were used to calculate the thoracic cavity volume (TCV). From these two segmentations the ventilation defect percentage (VDP) and the ventilation heterogeneity index (VH_I) were calculated (See Table 1 and OLS).

Contiguous individual ventilation defects in 3D were assessed. Defects that contributed to <1% of total VDP were discarded. The number of remaining defects

($N_{defects}$), as well as the volume of individual defects were calculated. The image analysis workflow is summarised in E-Figure 2.

To describe the degree of ventilation change from EIVt to TLC, the reversible-volume index was calculated from the EIVt and TLC images by:

$$\text{Reversible-volume index} = \frac{(\Delta VV)}{(\Delta TCV)}$$

Where $\Delta VV = VV_{(TLC)} - VV_{(EIVt)}$ and $\Delta TCV = TCV_{(TLC)} - TCV_{(EIVt)}$. Finally, the difference in VH_I (ΔVH_I) was quantified by: $\Delta VH_I = VH_{I\,(TLC)} - VH_{I\,(EIVT)}$.

Pulmonary Function

MBW was performed as previously described using a modified open-circuit Innocor and 0.2% SF_6 [30]. MBW was performed in triplicate both seated and supine [31]. From MBW, the metrics; lung clearance index (LCI), S_{cond} and S_{acin} were calculated and the average taken from at least two technically acceptable trials. Spirometry and body plethysmography were performed to international standards [32, 33] using a 'PFT Pro' (Vyaire, Basingstoke, UK) and recommended reference equations [34]. All tests were performed on the same day. Either MBW or MRI was performed first, followed by the other. Spirometry was always performed last.

Statistical analysis

Metrics were assessed for normality using the Shapiro-Wilks test and expressed as either the mean (SD) or the median (range). Patients were grouped into three groups; group 1 consisted of patients with normal FEV_1 (>-1.64 z-score) and normal LCI (<7.4 [30]); group 2 had normal FEV_1 but abnormal LCI ($\geq$7.4); and group 3 had both abnormal FEV_1 and LCI. Group comparisons were assessed using the Kruskal-Wallis with Dunn's multiple comparisons test. As a result of this analysis, two refined groups are referred to throughout the results; those with normal FEV_1 (z-score >-1.64) and those with abnormal FEV_1 (z-score $\leq$-1.64). Spearman's correlation analysis was performed to assess the relationship between metrics. In total 13 metrics were considered, therefore after Bonferroni adjustment [35], a p-value <0.004 was considered significant for correlation analysis. The Wilcoxon-signed rank test was used to assess the difference in MRI metrics between EIVt and TLC. All analyses were performed in GraphPad Prism (V7.0, San Diego, US).

Table 1: Description of metrics calculated from ^{3}He and ^{1}H MRI

Thoracic Cavity Volume (TCV)	Calculated from the segmentation of the ^{1}H anatomical MRI. The TCV is the lung volume at which ^{3}He-MRI is performed and is measured in litres.
Ventilated Volume (VV)	Calculated from ^{3}He ventilation image segmentation. VV represents the volume of ventilated lung and is measured in litres.
Ventilation Defect Percentage (VDP)	VDP is the percentage of the TCV that is not ventilated in the 3He MR images. Areas of the ^{3}He image that contribute to VDP appear black. It is calculated as: VDP = 100-((VV/TCV)*100). Larger VDP values are associated with increased lung disease.
Ventilation Heterogeneity Index (VH_I)	VH_I is a marker of the heterogeneity of the ^{3}He signal within ventilated regions of the ^{3}He MR images. For each ventilated pixel, a local coefficient of variation of signal intensity in the surrounding pixels is computed. VH_I is the inter-quartile range of the distribution of those values. Increased VH_I is associated with increased ventilation heterogeneity and therefore increased lung disease.
Number of ventilation defects ($N_{defects}$)	The number of individual 3D contiguous ventilation defects within the subject's lung. This only includes un-ventilated lung areas contributing to VDP. Defects were counted if the defect volume was >1% of total VDP.
Largest ventilation defect	This is the volume of the largest contiguous ventilation defect within the lungs. It is measured in litres and also as a percentage of the TCV.
Reversible-volume index	This represents the relative change that occurs in VV in response to the increase in TCV when comparing EIVt to TLC images. The reversible-volume index is equal to at least 1.0, the larger the value above 1.0 the greater the degree of EIVt ventilation defects that have resolved at TLC. In a healthy subject's lungs or in the lungs of a patient with non-reversible ventilation defects (resulting from complete obstruction), an increase in TCV due to deep inhalation, will result in equal increase in VV and the reversible-volume index will be 1. In contrast, any ventilation defect present at EIVt that at least partially resolves at TLC, will produce a reversible-volume index >1 (E-Figure 3).

Results

32 patients with CF were recruited and assessed (17 (53%) female). Patient demographics, lung function and MRI metrics are presented in Table 2. Of the 32 patients studied, all but one child had visible ventilation abnormalities on ventilation MRI at EIVt. E-Figure 4 shows representative ^{3}He images for all patients. 30 patients (94%) had a VDP >2% at EIVt, the upper value from healthy controls previously reported [6]. In contrast, 26 (81%) patients had raised LCI and 14 (44%) patients had abnormal FEV_1. This resulted in six patients with normal FEV_1 and LCI (Group 1), 12 patients with normal FEV_1 but abnormal LCI (Group 2) and 14 patients with abnormal FEV_1 and LCI (Group 3). At EIVt, group 3 had significantly higher VDP, VH_I, largest individual defect and a significantly lower total number of individual defects ($p<0.001$), than groups 1 and 2. There was however no significant difference between groups 1 and 2 for these metrics, though a trend towards higher VH_I was seen in group 2 (Figure 1). The only metric to significantly distinguish groups 1 and 2 was S_{cond} ($p=0.03$).

Table 2: *Patient demographics, lung function and ventilation MRI metrics at End-inspiratory tidal volume (EIVt). Results are displayed for the whole population and for the three groups of patients. Group 1 = patients with normal spirometry and LCI. Group 2 = patients with normal spirometry but abnormal LCI and group 3 = abnormal spirometry and LCI. Metrics are presented as mean ± standard deviation (SD), or median (range) depending on the distribution of individual metrics. FEV_1 = forced expiratory volume in one second. RV/TLC = residual volume/total lung capacity. LCI = lung clearance index. S_{cond} = convection dependent ventilation*

*heterogeneity. S_{acin} = convection-diffusion dependent ventilation heterogeneity. VDP = ventilation defect percentage. VH_I = ventilation heterogeneity index. TCV = thoracic cavity volume. $N_{defects}$ = number of defects. * denotes p<0.05 versus Group 1. ^ denotes p<0.05 versus Group 2.*

	Mean ± SD or Median (Range)			
	All patients	Group 1	Group 2	Group 3
N. (N. (%female))	32 (53%)	6	12	14
Age (years)	16.7 (6.4, 43.1)	10.1 (6.4, 16.5)	12.7 (8.3, 17.4)	29.9 (14.9, 43.1)*^
Height (cm)	156.2 ± 17.7	137.0 ± 16.1	153.7 ± 16.3	166.4 ± 11.7*
Weight (kg)	49.7 ± 18.4	32.8 ± 12.5	45.1 ± 15.1	60.9 ± 16.2*
FEV_1 (z-score)	-1.8 ± 2.03	0.5 ± 1.2	-0.6 ± 0.7	-3.9 ± 0.9*^
RV/TLC (%)	33.9 (17.8, 52.6)	23.5 (19.9, 26.5)	24.9 (17.8, 35.3)	47.7 (31.6, 52.6)*^
LCI	10.0 (6.0, 17.8)	6.7 (6.0, 7.0)	7.9 (7.4, 10.3)	13.3 (8.3, 17.8)*^
S_{cond}	0.07 ± 0.03	0.04 ± 0.02	0.08 ± 0.02*	0.09 ± 0.03*
S_{acin}	0.14 (0.04, 0.55)	0.08 (0.04, 0.10)	0.10 (0.05, 0.19)	0.30 (0.14, 0.55)*^
LCI_{supine}	9.6 (6.2, 20.2)	6.9 (6.2, 8.3)	9.1 (7.0, 10.6)	14.1 (7.7, 20.2)*^
VDP (%)	14.9 (0.2, 45.0)	2.7 (0.2, 3.3)	4.2 (1.5, 9.2)	29.0 (9.5, 45.0)*^
VH_I (%)	15.1 (6.7, 22.2)	8.9 (6.7, 11.3)	12.1 (9.3, 17.8)	20.1 (15.0, 22.2)*^
Largest defect (%TCV)	3.1 (0.03, 26.8)	0.7 (0.03, 1.3)	1.2 (0.1, 3.5)	15.2 (3.8, 26.8)*^
$N_{defects}$	7 (2, 24)	16 (9, 18)	13 (4, 24)	3 (2, 7)*^

Figure 2 demonstrates 3D ventilation MR images at EIVt with contiguous ventilation defects highlighted, examples shown are for a patient in group 2 and a patient in group 3.

Correlations between lung function and MRI at EIVt

VDP demonstrated significant correlation ($p<0.001$, Figure 3) with: LCI (r=0.89) and S_{acin} (r=0.84), but not S_{cond} (r=0.32); VDP also correlated with RV/TLC (r=0.80) and FEV_1 (r=-0.79). VH_I demonstrated significant correlations with LCI (r=0.88) and S_{acin} (r=0.82), but not S_{cond} (r=0.46 E-Figure 5); and with RV/TLC (r=0.78) and FEV_1 (r=-0.78). Supine MBW results also demonstrated significant equivalent correlations and are documented in E-Table 2.

The volume of the largest defect correlated significantly ($p<0.001$) with VDP (r=0.97, Figure 4), LCI (r=0.85), FEV_1 (r=-0.80) and S_{acin} (r=0.79) and the number of defects demonstrated a significant correlation with VDP (r=-0.86), LCI (-0.75), FEV_1 (r=0.75) and S_{acin} (r=-0.62).

Ventilation MRI comparison between EIVt and TLC

Ventilation images at both EIVt and TLC were successfully acquired from 28 patients (Table 3). Two patients were excluded due to acquisition errors and two patients could not successfully coordinate the TLC breathing manoeuvre. The median (range) TCV measured from ^1H MRI at EIVt was 78.2 (61.2,95.0)% of the TCV at TLC, which was significantly correlated to the FRC_{pleth}/TLC ratio (a marker

of lung hyperinflation) measured during body plethysmography (r=0.68). The TCV at TLC was 97.7 (85.0,107.7)% of the TLC measured during body plethysmography.

Table 3*: Ventilation MRI metrics for the 28 patients with images acquired at both end-inspiratory tidal volume (EIVt) and total lung capacity (TLC). Data is presented as median (range). VDP = ventilation defect percentage (%). VH_I = ventilation heterogeneity index. TCV = thoracic cavity volume. * denotes p<0.05 between lung volumes.*

	EIVt	**TLC**
VDP (%)	8.5 (1.5, 45.0)	4.2 (0.2, 35.3)*
VH_I (%)	15.2 (7.4, 22.2)	9.7 (5.7, 18.0)*
Largest defect size (%TCV)	3.3 (0.1, 26.8)	2.0 (0.0, 20.8)*
$N_{defects}$	9 (2, 24)	8.5 (2, 19)
Reversible-volume index		1.1 (1.0, 2.4)
ΔVH_I		-4.1 (-1.5, - 8.3)

At TLC there was a marked reduction in ventilation abnormalities. Signal intensity in ventilated regions of the lungs appeared more homogeneous, and in most patients some areas of un-ventilated lung became ventilated at TLC. This resulted in fewer ventilation defects at TLC for some patients whilst in others ventilation abnormalities remained (Figure 5). At TLC when compared to EIVt, there was a significant decrease (p<0.001, Figure 6) in MRI markers (expressed as median difference [95% confidence interval]) including: VDP -4.7 [-11.0,-2.2]%; VH_I -4.1

[-5.6,-3.1]%; volume of the largest defect -47.3 [-160.1, -17.1]mL, and largest defect expressed as a percentage of TCV -1.7 [-4.3,-0.8]%. 10/28 patients had a reduction in $N_{defects}$ at TLC (p=0.2), all of whom had normal FEV_1. The reversible-volume index, but not ΔVH_I, significantly correlated with VDP at EIVt (r=0.85) and with LCI (r=0.82, E-Figure 6), S_{acin} (r=0.75) and FEV_1 (r=-0.74). The reversible-volume index was significantly higher in group 3 than in groups 1 and 2 (p<0.001), but not between groups 1 and 2. ΔVH_I was not significantly different between groups.

VDP at TLC significantly correlated with LCI (r=0.85), S_{acin} (r=0.77), FEV_1 (r=-0.79) and RV/TLC (r=0.86). VH_I at TLC significantly correlated with LCI (r=0.82), S_{acin} (r=0.74), FEV_1 (r=-0.84) and RV/TLC (r=0.86).

Discussion

In this study we present a detailed analysis of the relationship between MBW and hyperpolarised gas ventilation MRI in patients with CF, across a broad range of age and disease severity. This analysis confirms the strong relationships between global MRI and MBW metrics that have previously been reported in smaller cohort studies, performed across narrower ranges of age and disease severity [5-7]. Previous work has documented that patients with CF have ventilation defects evident on hyperpolarised gas MRI [1, 8, 21, 24, 36, 37], that the technique is reproducible and repeatable [21, 24] and that it can be used to assess regional response to treatment [8, 28]. Here we demonstrate what appear to be two distinct ventilation MRI patterns of CF lung disease; (i) patients who have

numerous smaller defects (and normal FEV_1); and (ii), patients who have fewer but much larger contiguous defects, where FEV_1 is invariably reduced. We have also demonstrated that many of these ventilation abnormalities are lung volume dependent. When larger volumes are inhaled, some apparently obstructed airways open and allow gas to ventilate previously un-ventilated areas.

This last observation has important implications from an imaging methodology perspective. Studies utilising hyperpolarised gas are often performed by inhaling fixed gas volumes, with one litre of gas inhaled from FRC being common [21-25]. This however results in smaller subjects being closer to their TLC than taller subjects, potentially reducing VDP and making cross-sectional comparisons challenging. Recent paediatric studies have titrated the inhaled volume based on measured or predicted lung volume [6, 7, 26, 27] and we recommend this practice for all patients with CF. It is important to note however that we have not directly compared the inhaled volume given in this study to a fixed 1 litre inhalation.

The finding that only some ventilation defects decrease in size at TLC implies that regions of volume-reversible airways obstruction and regions of complete airways obstruction (that are fixed on the time-scale of the imaging session) co-exist in CF lungs. The reversibility of defects with deep inhalation highlights the probable value of physiotherapy and exercise in opening these lung regions, and also the potential for ventilation MRI to aid targeted therapies to be applied to specific lung regions. The change in ventilation from EIVt to TLC was not uniform across the population, with some patient's ventilation remaining distinctly abnormal at TLC. Assessing the transient nature of these lung-volume dependent

ventilation defects longitudinally may provide insight into the progression of disease pathophysiology.

The analysis of contiguous ventilation defects allows quantification and tracking of individual defects over time. Two distinct ventilation MRI patterns at EIVt are highlighted, which may represent different ends of the disease spectrum in CF. Figure 4 demonstrates that in those patients with normal FEV_1, VDP is <10% and predominantly consists of numerous small-volume defects (possibly due to predominantly peripheral airways disease), which also appear more likely to be reversible. By the time FEV_1 becomes abnormal however, VDP is invariably >10%. In these patients, VDP is dominated by fewer larger contiguous defects (suggesting widespread airways disease and lobar destruction), possibly in part due to smaller defects merging with disease progression. In addition, these larger defects are less likely to disappear with full inspiration, suggesting a significant proportion of peripheral lung is not being routinely ventilated during tidal breathing. Such regions are likely to harbour reservoirs of trapped mucus and inflammatory exudate, encouraging further lung inflammation and damage. These findings in part are in contrast to previous work [37-39], which reported that the number of ventilation defects increased with worsening lung disease. This is likely due to the two dimensional (2D) assessment of ventilation images in previous studies, resulting in individual defects that span multiple slices being classed as multiple defects. Whereas in this 3D assessment of ventilation defects, defects are shown to be contiguous and not independent between slices, resulting in fewer individual defects.

There is an intuitive relationship between ventilation MRI and MBW metrics, as they both assess the distribution of inhaled gas within the lungs. These data suggest this relationship is stronger when MRI was performed at EIVt when compared to TLC, due to the EIVt manoeuvre most closely representing the end-inspiratory cycle of quiet breathing performed during MBW. Ventilation MRI has the advantage that the exact regional nature of this ventilation distribution can be assessed, including lung regions that are entirely blocked (and hence silent to MBW testing). In this cohort we found that LCI and S_{acin} had strong relationships with VDP and VH_I and also with reversible-volume index. S_{cond} however showed poor correlations due to the 'plateau effect' evident in E-figure 5, which occurs with increasing disease. This plateau has been reported previously [40], and suggests S_{cond} is useful primarily as a marker of very early CF lung disease, highlighted by the finding that S_{cond} was the only metric to significantly differentiate between groups 1 and 2. Convection-dependent ventilation heterogeneity therefore seems to be an early event in disease progression. Figure 3 suggests a similar relationship may be evident when comparing LCI and VH_I, albeit the plateau effect for VH_I occurs at much higher levels of LCI. Up to this point, VH_I is strongly associated with increasing LCI. It is possible that with increasing lung disease in an individual, areas of increased VH_I become non-ventilated and contribute directly to VDP instead.

Higher values for the reversible-volume index indicate that a greater proportion of EIVt ventilation defects receive ventilation at TLC, implying volume-reversible airway obstruction. Reversible-volume index showed significant correlations with both LCI and S_{acin}. We hypothesise that this positive correlation may indicate that

ventilation defects present at EIVt, which become ventilated at TLC, may be lung regions responsible for delayed gas washout during MBW. On the other hand, patients with large VDP but relatively low LCI may be explained by the presence of defects that are unable to achieve ventilation at TLC (i.e. low reversible-volume index), therefore these defects may not significantly contribute to the dynamic LCI signal (E-figures 6 and 7). The reversible-volume index may therefore help explain why an unpredictable LCI response is seen with treatments in more severe and acute CF lung disease, despite clinical and spirometric improvements. We postulate that a lower reversible-volume index in a subject with significant ventilation abnormalities will result in a relatively low LCI for their level of lung disease (E-figure 7). In response to treatment, we hypothesise that both the reversible-volume index and the LCI would increase in this case, caused by the opening of previously blocked lung regions to the MBW signal.

There are limitations to this study that require consideration. Whilst we have reported large numbers of patients for a ventilation MRI study [1, 5, 6, 8, 21, 22, 26, 27, 36, 37], there are still relatively small numbers of subjects in each sub-group, which inevitably limits the generalizability of this comparison. In comparing ventilation MRI with MBW we also acknowledge that the inert gases used have different diffusivity within the lung. ^{3}He has higher diffusivity in air when compared to SF_6, suggesting SF_6 may reveal larger ventilation defects if used in MR imaging. This has been reported when comparing ^{3}He with ^{129}Xe ventilation imaging in COPD [41]. A limitation of the individual defect analysis is that it assesses contiguous areas of signal void within the ventilation images, which are not necessarily anatomically contiguous. The larger defects evident in more

severe disease can in some cases be seen to merge across different lung lobes which are fed by distinct conducting airways and therefore do not represent a physiologically discrete defect caused by blockage of a single airway. Finally, in order to validate these cross-sectional findings, longitudinal data are required to determine whether the different patterns of ventilation observed behave as we predict on an individual basis over time.

In conclusion, this work adds to a growing body of work highlighting the use of ventilation MRI in CF, and specifically the role of VDP as a potential clinical tool and endpoint in clinical trials. In particular two key novel aspects are highlighted that help define the clinical meaning and utility of the methodology. Firstly, we highlight a VDP value of 10%, which separates normal and abnormal FEV_1 values, (the current clinical gold standard) and also appears to delineate a boundary between the two distinct ventilation imaging patterns described, i.e. numerous small defects versus fewer large defects. Secondly, we demonstrate the coexistence of reversible and non-reversible regions of airway obstruction in CF using ventilation imaging at different lung volumes. This has direct impact on the longitudinal monitoring of an individual's lung health and delivery of regionally specific treatment.

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Figure Captions

Figure 1: Kruskal-Wallis group comparison for VDP, VH_I, number of defects and largest individual contiguous defect, calculated from ventilation MRI at EIVt. Group 1 have both normal FEV_1 and LCI. Group 2 have normal FEV_1 but abnormal LCI. Group 3 have abnormal FEV_1 and LCI. In all graphs, group 3 is significantly different when compared to groups 1 and 2 ($p<0.001$), but there was no statistically significant difference between groups 1 and 2.

Figure 2: Demonstrates representative ventilation images at EIVt from a patient from group 2 (top) and group 3 (bottom). For both patients, the first row is a 3D ventilation image and the second row is a 3D ventilation image with the segmented individual contiguous ventilation defects added in colour. The colours associated with defects are arbitrary, but each different colour represents a single different radiologically contiguous defect. For each patient the ventilation image is observed from various anatomical angles. The top patient has VDP = 5.4%, largest defect (pink) = 1.5% of the TCV, $N_{defects}$ = 18. LCI = 7.7, FEV_1 z-score = -0.5. The bottom patient has VDP = 45.0%, largest defect (red) = 26.8% of the TCV, $N_{defects}$ = 3, LCI = 14.9, FEV_1 z-score = -5.4.

Figure 3: Scatter plots of VDP (top row) and VH_I (bottom row), both performed at EIVt, against pulmonary function metrics, with Spearman correlation values ($p<0.001$ in all).

Figure 4: Scatter plots of the number of individual contiguous ventilation defects ($N_{defects}$, top row) and the size of the largest individual contiguous defect (bottom row) at EIVt; against VDP at EIVt, LCI and FEV_1. In each graph patients are divided into those with an FEV_1 z-score <-1.64 (closed circles), or >-1.64 (open circles). For FEV_1 the dashed line represents the lower limit of normal (-1.64) and for LCI the dashed line represents the upper limit of normal (7.4). All Spearman correlations have a p-value <0.001.

Figure 5: Representative ^{3}He ventilation MR images acquired at EIVt and TLC from three subjects, with representative ^{1}H MRI. Subject A: FEV_1 z-score = -0.5, LCI = 7.7, RV/TLC = 22.4%. This subject has ventilation defects present at EIVt (VDP=5.4%), which largely disappear at TLC (VDP=0.4%), reversible-volume index = 1.2. Subject B: FEV_1 z-score = -3.7, LCI = 17.8, RV/TLC = 50.3%. This subject has ventilation defects present at EIVt (VDP=39.5%), some of which remain at TLC (VDP=18.2%), reversible-volume index = 2.0. Subject C: FEV_1 z-score = -1.1, LCI = 7.4, RV/TLC = 24.8%. This subject has ventilation defects present at EIVt (VDP=6.5%), which largely remain at TLC (VDP=5.3%) and therefore has a low reversible-volume index (1.0). The cause of part of the non-reversible ventilation can be seen in the left lung on the ^{1}H image where an area of significant mucus is present, corresponding to the ventilation defect seen on both EIVt and TLC ventilation images (see arrow).

Figure 6: A comparison of ventilation MRI metrics between EIVt and TLC. At TLC there was a significant reduction in: VDP, VH_I, and the largest individual

contiguous defect ($p<0.001$). The number of defects declined only for some subjects with normal FEV_1 (10/28 patients).