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# Thorax

## Detection of early sub-clinical lung disease in children with cystic fibrosis by lung ventilation imaging with hyperpolarized gas MRI

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Manuscripts

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3 **Detection of early sub-clinical lung disease in children with cystic fibrosis by lung**  
4 **ventilation imaging with hyperpolarized gas MRI**  
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10 Helen Marshall<sup>1</sup> / Alex Horsley<sup>1,2</sup>, Chris J Taylor<sup>3</sup>, Laurie Smith<sup>1,3</sup>, David Hughes<sup>3</sup>, Felix C  
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12 Stewart<sup>1</sup>, Guilhem J Collier<sup>1</sup>, Dawn Teare<sup>5</sup>, Steve Cunningham<sup>4</sup>, Ina Aldag<sup>3</sup> and Jim M Wild<sup>1</sup>.  
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**ABSTRACT**

Hyperpolarized  $^3\text{He}$  ventilation-MRI, anatomical lung MRI, lung clearance index (LCI), low-dose CT, and spirometry were performed on 19 children (6-16yrs) with clinically stable mild CF ( $\text{FEV}_1 > -1.96$ ), and 10 controls. All controls had normal spirometry, MRI and LCI. Ventilation-MRI was the most sensitive method of detecting abnormalities, present in 89% CF patients, compared to CT abnormalities in 68%, LCI 47% and conventional MRI 22%. Ventilation defects were present in the absence of CT abnormalities and in patients with normal physiology, including LCI. Ventilation MRI is thus feasible in young children, highly sensitive and provides additional information about lung structure-function relationships.

99 words (Max 100)

## BACKGROUND

Lung ventilation heterogeneity due to the obstruction of small airways is an early and potentially reversible step in the progression of cystic fibrosis (CF). Individually, multiple breath washout (MBW), hyperpolarised (HP) gas ventilation MRI, and conventional structural lung imaging by CT, have all been shown to be sensitive to early changes in the lungs before spirometry<sup>1-3</sup>. The information they provide is complementary and HP ventilation-MRI in particular has the potential to reveal airways obstruction and the functional consequences of regional structural changes detected with CT, as well as the nature of ventilation heterogeneity that prolongs gas washout in MBW<sup>4</sup>. The aim of this study was to investigate the relative sensitivity of imaging and physiology assessments for the detection of early-stage lung disease in children with CF. In addition, we explored what insight functional ventilation imaging provides about the nature of ventilation abnormalities in the lungs in early CF, and how these correlate with the more clinically scalable assessment of ventilation heterogeneity provided by MBW.

## METHODS

For full technical details, see the online supplement (OLS).

Nineteen CF children and ten controls were assessed. Subjects attended on a single occasion, when clinically stable, and were assessed with SF<sub>6</sub> MBW<sup>1</sup>, plethysmography, spirometry, HP <sup>3</sup>He MRI and <sup>1</sup>H MRI. CF patients also underwent inspiratory and expiratory chest CT<sup>5</sup>. All subjects had FEV<sub>1</sub> z-score > -1.96 and were aged between 6-16 years old. To test for significant differences between the healthy control and CF patient groups, 2-tailed paired tests were performed to reflect the age-matching of data. Sensitivity was defined as

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2  
3 the presence of an abnormal measurement (i.e. not within the normal range for that  
4 measurement) in the presence of the diagnosis of CF. This assumes that all patients should  
5 have some abnormality in at least one measurement, which may not be true in this  
6 population with very mild clinical expression of CF, and therefore provides a conservative  
7 estimation of sensitivity of the lung assessments. This study was approved by the National  
8 Research and Ethics Committee (REC 12/YH/0343) and parents/guardians provided written  
9 informed consent  
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## 19 20 **RESULTS**

21  
22 Full demographic and outcome data are presented in the OLS. CF patients had early-stage  
23 lung disease, with no significant differences between CF and controls for any lung function  
24 measures except LCI (Figure 1). In controls, no ventilation or structural abnormalities were  
25 detected on either  $^3\text{He}$  or  $^1\text{H}$  MRI (see OLS). Both  $^3\text{He}$  MRI quantitative measures were  
26 however significantly increased in CF patients when compared to controls (Figure 1).  
27 Ventilation heterogeneity and obstruction seen with ventilation-MRI generally  
28 corresponded to anatomical abnormalities detected by CT (Figure 4 OLS). In several  
29 instances however ventilation defects were present on ventilation-MRI with no  
30 corresponding features of structural pathology detectable on CT (Figure 2).  
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45 The presence of visible defects on ventilation-MRI had the greatest sensitivity for detecting  
46 evidence of CF airways disease: nine CF patients (47%) had elevated sitting LCI, when  
47 compared to 17 (89%) with ventilation-MRI abnormalities. In contrast, structural  
48 abnormalities were detectable in four patients (22%) with  $^1\text{H}$  MRI and 13 patients (68%)  
49 with CT. Ventilation-MRI detected abnormalities in four patients with normal CT; LCI was  
50 abnormal in only two of these. Two patients with mild variant disease (both R117H-7T  
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heterozygotes) had no abnormality detected by any technique; these were the only CF patients where  $^3\text{He}$  MRI was normal.

A table of correlations is presented in the OLS. LCI and  $^3\text{He}$  MRI metrics showed moderately significant correlation and  $^3\text{He}$  MRI %-unventilated lung correlated significantly with CT Brody score and CT gas trapping score. There was no significant correlation between LCI and CT scores.

## DISCUSSION

This is the first study to combine the powerful functional technique of ventilation imaging using HP gas MRI with assessments of structural lung disease from CT and the whole lungs assessment of ventilation heterogeneity provided by MBW. Although both  $^3\text{He}$  MRI and MBW measure aspects of ventilation heterogeneity in the lungs, the detailed spatial information provided by ventilation-MRI meant that abnormalities in ventilation distribution were visible even when these were insufficient to affect the LCI. Likewise, ventilation defects were visible in some cases in the absence of structural abnormalities on CT (e.g. Figure 2), which may be due to the inherent sensitivity of ventilation imaging to small airways obstruction that cannot be explicitly resolved on CT.

These are important findings for our understanding of how CF lung disease develops and how we interpret lung function data. In this cohort of CF patients, specifically selected to represent those with the mildest airways disease, LCI was abnormal in almost half of all subjects (47%), in keeping with prior observations<sup>1,6</sup>. Ventilation-MRI however was more sensitive than both LCI and CT and detected ventilation defects in all but two patients (89%), both of whom had genetic variants that may not be associated with CF lung disease until

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3 adulthood. The great advantage of ventilation-MRI is that it offers detailed regional  
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5 information about the nature and distribution of ventilation defects. Thus in patients with  
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7 early disease we predominantly detected patchy ventilation defects distributed throughout  
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9 the lung, with larger focal defects in areas where there was already evidence of structural  
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11 damage on CT or  $^1\text{H}$  MRI. In contrast, MBW represents a washout signal integrated over the  
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13 whole lungs, so that mild ventilation heterogeneity can inevitably be masked, and  
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15 differentiation of the signal into regional or anatomical lung compartments is at best  
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17 speculative<sup>7</sup>.  
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22 The  $^1\text{H}$  MRI protocol deployed here was used to determine lung boundaries and was not  
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24 optimised for structural imaging, where new methods like 3D ultra-short echo time imaging  
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26 should offer improvements. Alternative CT scoring systems and protocols also exist that may  
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28 offer additional sensitivity<sup>8</sup>. Finally, we did not perform gadolinium contrast enhanced  
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30 imaging, as we felt this would deter children from participating. Despite possible limitations  
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32 in the  $^1\text{H}$  MRI and CT protocol, it is clear that HP gas ventilation-MRI is a powerful tool for  
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34 detecting early lung changes. The superior sensitivity and detail of information provided by  
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36 ventilation-MRI also offers the prospect that this measurement will allow an earlier or more  
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38 detailed radiation-free appreciation of the response to novel therapies than even LCI<sup>9</sup>. The  
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40 advent of high quality HP gas ventilation-MR imaging using the cheaper and readily available  
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42  $^{129}\text{Xe}$  isotope means that the technology is now much more readily clinically deployed<sup>10</sup>. The  
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44 ventilation-MR images presented here are thus exemplars of what may become routine  
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46 assessments in detecting early disease and treatment effects.  
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54 In conclusion, in this population of CF children with very mild lung disease, we have shown  
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56 that ventilation-MRI is highly sensitive to detecting the consequences of airway disease.  
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3 Even patients with apparently pristine lungs by all current physiology and imaging standards  
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5 have evidence of ventilation-MRI abnormalities. HP gas MRI provides detailed regional  
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7 information about disease severity and physiological impairment.  
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26  
27 responsibility for its validity.  
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40 subjects.  
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**Figure captions**

**Figure 1** – Comparison of lung physiology and ventilation imaging metrics for healthy controls and cystic fibrosis (CF) patients.

Each point represents a single subject, error bars represent group means and 95% confidence interval of the means. (a) FEV<sub>1</sub> z-score and (b) FEV<sub>1</sub>/FVC z-score derived from spirometry. Horizontal dotted line indicates a z-score of zero. (c) Sitting lung clearance index (LCI) and (d) supine LCI from multiple breath washout. Horizontal dotted line represents upper limit of normal LCI. (e) Un-ventilated lung volume percentage (UVP) and (f) mean coefficient of variation (CV) of ventilation imaging signal, from <sup>3</sup>He MRI.

**Figure 2** - Hyperpolarised gas MRI and CT imaging: examples of discordance in patients with cystic fibrosis (CF).

Each row represents a single unique CF patient, with equivalent slices from hyperpolarised gas MRI, CT, and <sup>1</sup>H MRI in the columns from left to right. In the first subject (a-c), <sup>3</sup>He MRI showed heterogeneous ventilation with widely distributed patchy, semi-ventilated defects (a). CT for this slice was normal (expiratory shown in b) and <sup>1</sup>H MRI (c) was normal throughout. In the second CF patient, small, sub-segmental defects were observed throughout the lungs with <sup>3</sup>He MRI (d). CT for this slice was normal (expiratory shown in e) but elsewhere showed lingular atelectasis and minimal air-trapping. <sup>1</sup>H MRI was normal for this slice (f) but showed lingular atelectasis elsewhere. In the final patient, several small ventilation defects were seen on <sup>3</sup>He MRI (g), whilst CT (inspiratory shown in h), <sup>1</sup>H MRI (i), and lung clearance index were all normal.

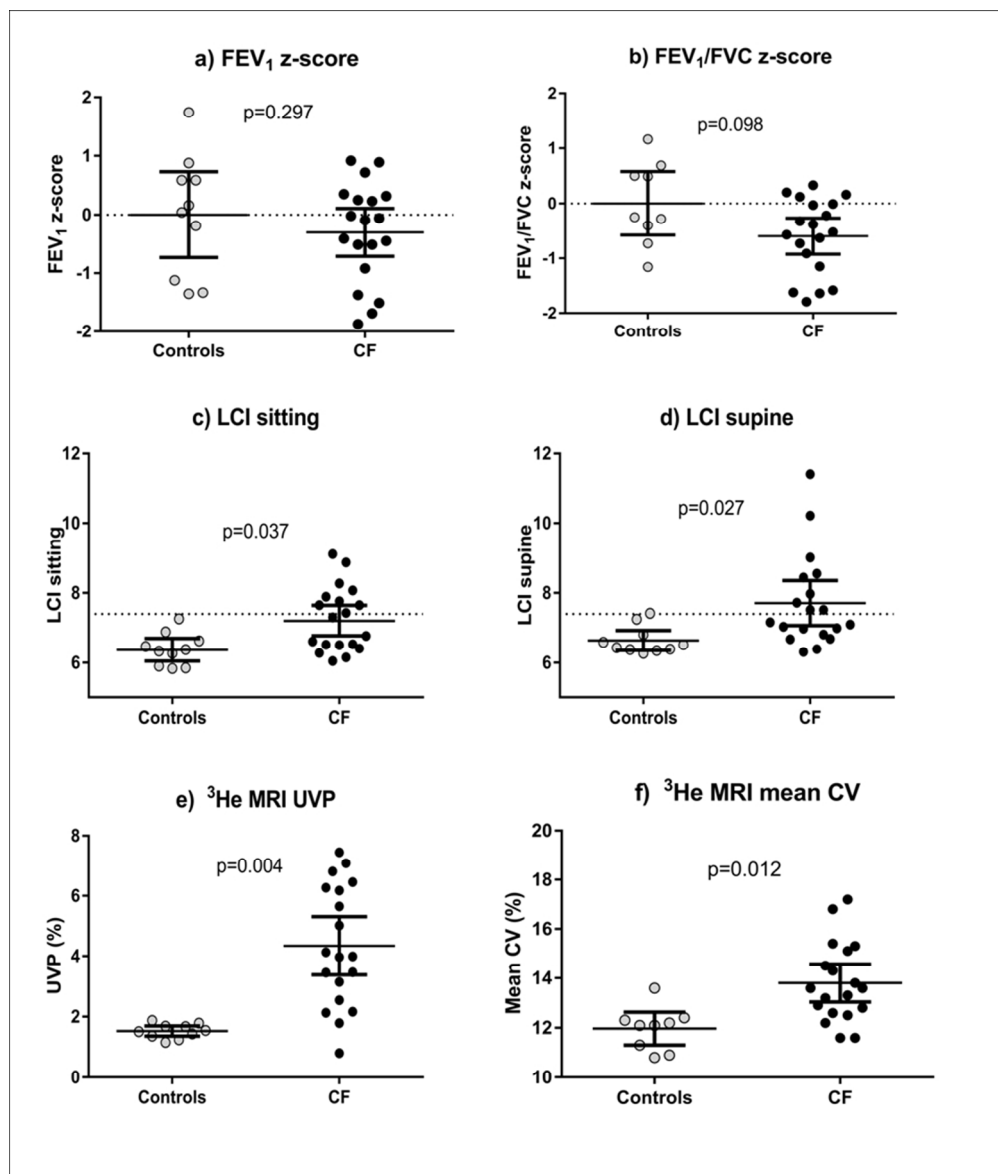


Figure 1 – Comparison of lung physiology and ventilation imaging metrics for healthy controls and cystic fibrosis (CF) patients.!! † Each point represents a single subject, error bars represent group means and 95% confidence interval of the means. (a) FEV<sub>1</sub> z-score and (b) FEV<sub>1</sub>/FVC z-score derived from spirometry. Horizontal dotted line indicates a z-score of zero. (c) Sitting lung clearance index (LCI) and (d) supine LCI from multiple breath washout. Horizontal dotted line represents upper limit of normal LCI. (f) Un-ventilated lung volume percentage (UVP) and (f) mean coefficient of variation (CV) of ventilation imaging signal, from <sup>3</sup>He MRI.

162x190mm (150 x 150 DPI)

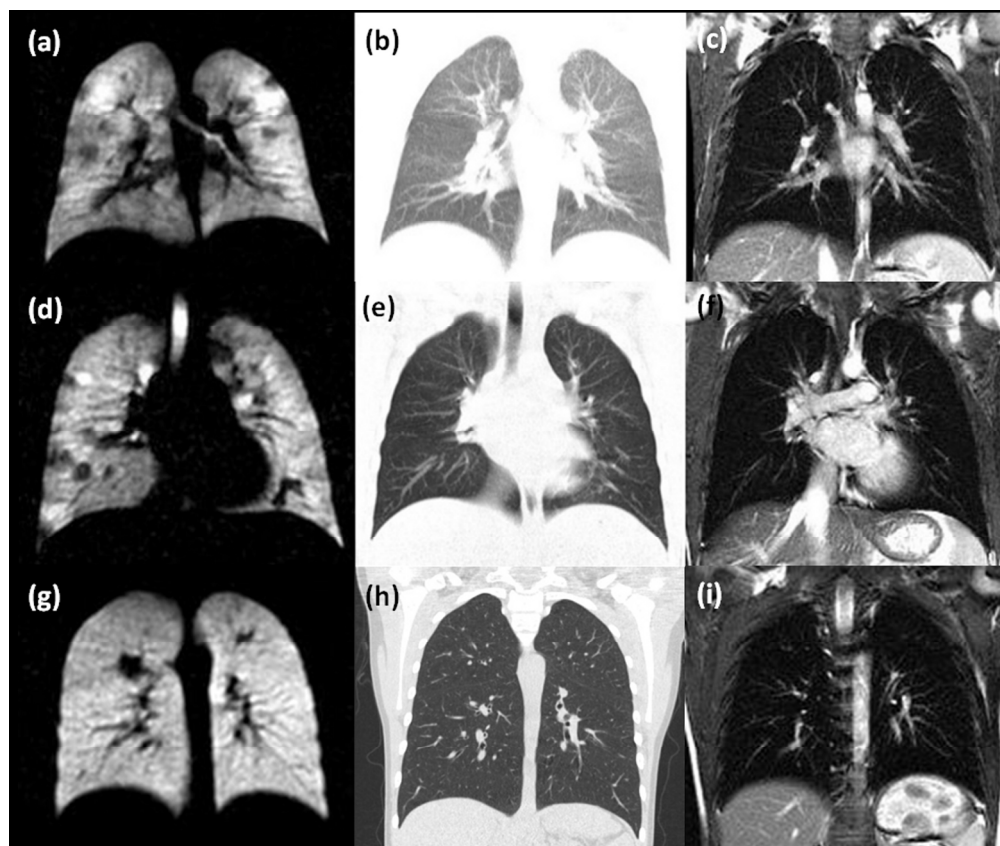


Figure 2 - Hyperpolarised gas MRI and CT imaging: examples of discordance in patients with cystic fibrosis (CF). Each row represents a single unique CF patient, with equivalent slices from hyperpolarised gas MRI, CT, and  $^1\text{H}$  MRI in the columns from left to right. In the first subject (a-c),  $^3\text{He}$  MRI showed heterogeneous ventilation with widely distributed patchy, semi-ventilated defects (a). CT for this slice was normal (expiratory shown in b) and  $^1\text{H}$  MRI (c) was normal throughout. In the second CF patient, small, sub-segmental defects were observed throughout the lungs with  $^3\text{He}$  MRI (d). CT for this slice was normal (expiratory shown in e) but elsewhere showed lingular atelectasis and minimal air-trapping.  $^1\text{H}$  MRI was normal for this slice (f) but showed lingular atelectasis elsewhere. In the final patient, several small ventilation defects were seen on  $^3\text{He}$  MRI (g), whilst CT (inspiratory shown in h),  $^1\text{H}$  MRI (i), and lung clearance index were all normal.

162x136mm (300 x 300 DPI)

Only



## Online Supplement - Full Study Report

# Detection of early sub-clinical lung disease in children with cystic fibrosis by lung ventilation imaging with hyperpolarized gas MRI

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## BACKGROUND

Infection and inflammation develop early in the lungs of patients with cystic fibrosis (CF) and typically precede clinical symptoms.<sup>1</sup> The process appears to start in the peripheral small airways (<2mm diameter), which are particularly susceptible to the pathological effects of mucus retention and airway wall inflammation. Over time, a dysregulated inflammatory

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3 response to chronic infection leads to progressive remodelling of the small airways and  
4 bronchiectasis. Early detection of lung disease is therefore vital in the effective  
5 management of CF so that interventions can be applied before this process becomes  
6 irreversible. A major challenge in clinical practice however is that conventional clinical  
7 measures of lung function and routine chest radiology are not sensitive enough to identify  
8 early-stage, predominantly reversible, lung disease.<sup>2</sup>

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11 Airway disease in CF does not progress evenly throughout the lungs, and an initial feature is  
12 uneven distribution of ventilation during tidal breathing. This is a consequence of variability  
13 in airway resistance and compliance, due to the heterogeneous distribution of obstruction  
14 and inflammation in the airways. Multiple-breath washout (MBW) of an inert tracer gas  
15 from the lungs, measured at the mouth during tidal breathing, is influenced by this  
16 heterogeneity of ventilation distribution.<sup>3</sup> Quantitative metrics derived from MBW, such as  
17 lung clearance index (LCI), appear to be particularly sensitive to early CF airways disease<sup>4</sup>  
18 and treatment response.<sup>5</sup> LCI provides a global lung index of overall ventilation  
19 heterogeneity, but is not regionally specific, i.e. it is not possible to determine whether  
20 abnormal elevation in LCI is a consequence of multiple small poorly ventilated regions of the  
21 lungs, or a few larger abnormally ventilated lung regions.<sup>6</sup> This may explain the variability in  
22 LCI response to some treatments, such as IV antibiotics given for a CF exacerbation.<sup>7</sup>

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25 Hyperpolarized (HP) gas ventilation MRI provides high-resolution images of inhaled tracer  
26 gas distribution within the lung airspaces, thereby directly imaging the distribution of  
27 ventilation heterogeneity. HP ventilation MRI using Helium-3 (<sup>3</sup>He) has been shown to be  
28 safe, repeatable and quick to perform in paediatric CF patients, requiring short breath-holds  
29 to acquire volumetric images of lung ventilation.<sup>8-10</sup> Obstructed lung regions appear black,  
30 and ventilated airspaces have a grey-scale signal intensity that is directly proportional to the  
31 local gas concentration in the lungs. The high spatial-resolution images obtained therefore  
32 provide regional quantitative information on lung ventilation and its heterogeneity. The  
33 technique has inherent sensitivity to small airways obstruction, is sensitive to early lung  
34 disease,<sup>11</sup> and has been used to demonstrate regional response to therapy in children with  
35 CF.<sup>8</sup>

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38 Individually, MBW and HP gas ventilation MRI, along with conventional structural lung  
39 imaging by CT, have all been shown to be sensitive to early changes in the lungs before  
40 spirometry<sup>4 11 12</sup>. The information they provide is however complementary in terms of  
41 functional and structural understanding of CF lung disease. HP gas MRI in particular has the  
42 potential to inform us about the functional consequences of regional structural changes  
43 detected with CT, and the nature of ventilation heterogeneity that prolongs gas washout in  
44 MBW. The aim of this study therefore was to investigate the relative sensitivity of HP gas  
45 MRI, structural <sup>1</sup>H MRI, CT and MBW for the detection of early-stage lung disease in children  
46 with CF. In addition, we explored what insight functional ventilation imaging provides about  
47 the nature of ventilation abnormalities in the lungs in early CF, and how these correlate with  
48 the more clinically scalable assessment of ventilation heterogeneity provided by MBW.  
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## METHODS

This was a cross-sectional observational study of patients with mild cystic fibrosis (CF) and healthy controls. All subjects attended on a single occasion, whilst clinically stable, and underwent a series of lung function and radiological assessments during the same visit. Visits took place between February 2013 – August 2014.

This study was approved by the Leeds East Research and Ethics Committee. Parents/guardians signed informed consent and paediatric subjects provided assent.

Inclusion criteria for CF patients:

- 6 years < age < 16 years
- Diagnosis of CF confirmed by a characteristic phenotype in conjunction with sweat test and/or genotyping.
- FEV<sub>1</sub> z-score > -1.96
- Clinically stable and free of symptoms of respiratory exacerbation

Exclusion criteria for CF patients:

- Any contraindication to MRI

CF patients were reviewed on the day of the study procedures by a clinician to confirm that they were clinically stable.

Inclusion criteria for healthy volunteers:

- 6 years < age < 16 years

Exclusion criteria for healthy volunteers:

- History of respiratory illness, prematurity, or previous assisted lung ventilation
- Any contraindication to MRI

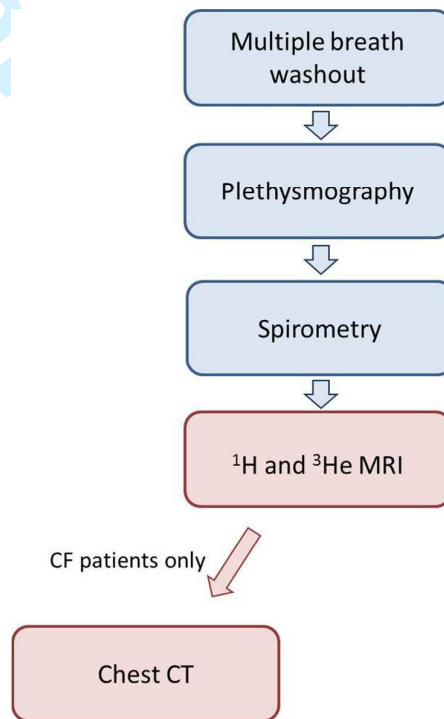
### Sample size

The original target sample size was 20 CF patients, and 10 healthy controls. This size was selected based on the known sensitivities of both the <sup>3</sup>He HP gas MRI technique and MBW. In previous studies in paediatric CF patients of the repeatability of <sup>3</sup>He-MRI lung ventilation volume, we observed a standard deviation of 5% between scans<sup>8</sup>. Thus 15 subjects were sufficient to detect a difference of one SD in lung volume when compared to healthy controls with 90% power at the 5% significance level. We therefore aimed to recruit 20 patients to allow some reserve in case of technical difficulties. This number was also felt to be realistically achievable, given the small size of the target population (CF children with well-preserved spirometry) and the intensive nature of the study visit. Healthy controls were recruited 1:2 compared to patients with age within +/-2 years.



### Study protocol

Subjects completed a series of non-invasive physiological and radiological assessments, see Figure 1. All subjects underwent physiology assessments together in a single session in the following order: multiple breath washout (MBW) sitting followed by supine; plethysmography; spirometry. The order of lung function testing was designed to ensure that dynamic spirometry manoeuvres did not impact on the MBW, as performing dynamic spirometry manoeuvres immediately or shortly prior to MBW may alter LCI.<sup>13</sup> Hyperpolarised (HP) <sup>3</sup>He and <sup>1</sup>H MRI were performed on the same day, within 3 hours of the physiology tests, though the order of these was not fixed. Children with CF, but not healthy controls, also underwent a chest CT scan. Data on microbiology, genotyping and sweat chloride were retrieved from the clinical record.



**Figure 1:** Summary of study flow and assessments.

MRI: Magnetic Resonance Imaging. CT: Computed tomography

### Multiple Breath Washout

Multiple breath washout was performed as previously described, using a modified Innocor™ gas analyzer and 0.2 % sulfur hexafluoride (SF<sub>6</sub>) as the tracer gas.<sup>14</sup> Unlike the earlier study, a faster-responding system was used, with a gas analyser response time of ≤120ms. For this reason no speeding correction was applied to the flow gas delay. Signals were aligned using a mechanical solenoid-activated gas pulse, rather than performing the

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3 on board flow-gas delay measurement. This has previously been shown to have superior  
4 accuracy and repeatability.<sup>15</sup>  
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7 Multiple breath washout tests were performed both seated and supine, with the subjects  
8 suitably distracted by watching television. A nose clip was applied and tidal breathing  
9 established whilst the subject breathed through a mouthpiece attached to a low dead-space  
10 filter (air safety medical, UK) and flow meter (Hans Rudolph paediatric pneumotach 0 – 160  
11 L/min flow range, Kansas, USA).  
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15 During the wash-in, the subject inspired 0.2% SF<sub>6</sub> in air from a flow-past circuit attached to  
16 the end of the mouthpiece and flow meter apparatus. Wash-in gas was supplied from a  
17 compressed gas cylinder (BOC, Guildford, UK), with the gas flow rate adjusted to ensure that  
18 rebreathing did not occur. The wash-in phase was continued until inspiratory and expiratory  
19 SF<sub>6</sub> concentrations differed by less than 0.004% (absolute difference in SF<sub>6</sub> concentration).  
20 Once wash-in was complete, the flow-past circuit was manually detached during expiration,  
21 and the washout commenced.  
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26 During the washout the subject breathed room air until the end tidal SF<sub>6</sub> concentration had  
27 fallen to less than 0.005% (1/40th of the SF<sub>6</sub> concentration at the end of wash-in). Each  
28 subject completed a minimum of six washouts. Sitting washouts were performed first,  
29 followed by supine washouts conducted whilst the patient lay horizontal on an examination  
30 couch, with the MBW mouthpiece supported in the correct position above them. Further  
31 wash-outs were performed if any suspected measurement errors occurred during testing, to  
32 ensure a total of three good quality trials were available for analysis in each postural  
33 position.  
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38 Functional residual capacity (FRC) was calculated from the total volume of expired tracer  
39 gas, and end tidal tracer gas concentrations at start and end of the washout,<sup>3</sup> and adjusted  
40 for BTPS.  
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44 Lung Clearance Index (LCI) is defined as the cumulative expired volume required to reduce  
45 the end tidal tracer gas to 1/40<sup>th</sup> of the starting concentration, divided by the FRC. LCI is  
46 quoted as the mean of at least two reproducible repeats from washouts of satisfactory  
47 quality. As an additional quality control measure, washouts whose FRC differed by more  
48 than 10% from both of the other two repeats were excluded from analysis.  
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### 52 53 **Plethysmography**

54 Body plethysmography and spirometry were performed on a CareFusion Jaeger 'PFT Pro',  
55 using SentrySuite software version 2.10 (Basingstoke, UK). All testing was performed to  
56 ATS/ERS recommended standards.<sup>13 16 17</sup> Flow was measured using a Lilly-type heated  
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3 screen pneumotachograph and volume measurements obtained using a software-based  
4 integrator. Calibration of the system was performed prior to each subject in accordance  
5 with manufacturer's instructions and in line with ATS/ERS criteria.<sup>13 16</sup>  
6  
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8  
9 For spirometry and the plethysmography pneumotach, a three litre air syringe was  
10 repeatedly discharged at a steady flow (approximately 6lps) and then verified for linearity  
11 over a range of flows. Body plethysmography was performed with an 830 litre constant-  
12 volume body plethysmograph (CareFusion, Basingstoke, UK) and was calibrated using an  
13 automated system for the air leak time decay and volume displacement.  
14  
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16  
17 The subject was seated in the plethysmograph in an upright position with a nose clip in  
18 place and lips tightly sealed around the mouthpiece. This was connected to a single use  
19 bacterial filter attached to a pneumotachograph located within the plethysmograph.  
20 Following a short period of tidal breathing the airway was then occluded for three seconds  
21 at FRC by a shutter attached to the distal side of the pneumotachograph and the patient  
22 instructed to make continued respiratory efforts against the occlusion. Thoracic gas volume  
23 (TGV) was calculated from the change in the plethysmograph pressure that occurred during  
24 the respiratory efforts against the occlusion. On releasing the occlusion the subject was then  
25 instructed to perform slow, maximal inhalation followed by a maximal exhalation to RV. TLC  
26 and RV were determined from the measurements of TGV and slow vital capacity.  
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### 31 Spirometry

32 Repeated spirometry efforts were performed until 3 acceptable maximal manoeuvres were  
33 obtained. Traces showing evidence of incomplete expiration, cough, air leak or glottic  
34 closure, were rejected. Acceptable repeatability was obtained when the difference  
35 between the largest and next largest manoeuvres was  $\leq 100$  ml or 5% depending on which  
36 was the larger value.  
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41 The recorded result for FEV<sub>1</sub> and FVC was the highest achieved across all technically correct  
42 efforts, with the ratio calculated from this. Forced expiratory volume in 1 second (FEV<sub>1</sub>),  
43 FEV<sub>1</sub>/FVC (forced vital capacity) and reduction in forced expiratory flow at 25-75% of the  
44 FVC (FEF<sub>25-75</sub>) were expressed as z-scores as described by Quanjer *et al.*<sup>18</sup>  
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### 48 MRI

49 Subjects were scanned using a 1.5T whole body MRI system (GE HDx, Milwaukee, WI)  
50 equipped for hyperpolarised <sup>3</sup>He imaging. <sup>3</sup>He was polarised on site to around 25% using  
51 rubidium spin-exchange apparatus<sup>19</sup> (GE Healthcare, Amersham, UK) under a regulatory  
52 approved license from the UK MHRA.  
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### **<sup>3</sup>He Ventilation MRI**

Subjects were positioned in a <sup>3</sup>He quadrature transmit-receive vest coil (Medical Advances, USA or Clinical MR Solutions, USA depending on size). Hyperpolarized <sup>3</sup>He dose was determined empirically according to the subject's predicted functional residual capacity (FRC).<sup>20</sup> A gas mixture of equal parts <sup>3</sup>He and N<sub>2</sub> was inhaled from functional residual capacity (FRC) and ventilation images were acquired during breath-hold. Breathing control was strictly implemented by thorough training and instruction from a lung physiologist. The specified volume of hyperpolarised gas was delivered to the subject from a Tedlar bag of known volume and subjects inhaled from FRC. A nose clip was used to best ensure that all delivered gas was inhaled into the lungs. A 2D coronal spoiled gradient echo sequence was used for imaging with parameters: full lung coverage,  $\theta=8^\circ$ , field of view = 30 - 40cm depending on subject size, in-plane matrix = 128 x 102, slice thickness = 10 mm, TE / TR = 1.1 / 3.6 ms, bandwidth = 63 kHz and sequential phase encode ordering. With the subject in the same position within the <sup>3</sup>He coil, <sup>1</sup>H images of the same imaging volume were acquired using the scanner body coil after inhalation from FRC of an equivalent volume of air to the gas volume inhaled for <sup>3</sup>He MRI. A 2D coronal steady state free precession sequence was used for imaging with  $\theta = 50^\circ$ , field of view = 30 - 40cm depending on subject size, in-plane matrix = 256 x 204, slice thickness = 10 mm, TE / TR = 0.9 / 2.9ms and bandwidth = 250kHz.

<sup>3</sup>He ventilation images were evaluated for the presence of any abnormal ventilation heterogeneity by a radiologist with six years' experience of clinical paediatric chest imaging (DH), blinded to other results. <sup>1</sup>H body-coil images were registered to the <sup>3</sup>He ventilation images to correct for any changes in position or lung inflation between the helium and proton image acquisitions. Lung un-ventilated volume percentage (UVP) was calculated by manual segmentation of the <sup>3</sup>He images overlaid on the registered <sup>1</sup>H images. A signal threshold was applied to delineate ventilated and un-ventilated voxels. The whole lung ventilated volume (VV) was calculated by summing all ventilated voxels and multiplying by the voxel volume. An outline was manually drawn to estimate total lung volume (TLV), and the whole lung un-ventilated volume percentage<sup>21</sup> was calculated as  $UVP = (1 - VV/TLV) \times 100$ . To measure ventilation heterogeneity, maps of coefficient of variation (CV) were calculated as standard deviation / mean for an in-plane kernel of 3 x 3 pixels. This was calculated for each voxel of the lung and the outcome measure mean CV is the whole lung mean of these coefficient of variation values. If image signal to noise (SNR) ratio was less than 20 no CV analysis was performed as low SNR results in increased CV independent of ventilation heterogeneity.

### **<sup>1</sup>H Anatomical MRI**

Subjects were repositioned into a <sup>1</sup>H 8-element chest receiver array. A volume of air equal to the total gas volume inhaled for <sup>3</sup>He MRI was inhaled from FRC, and anatomical <sup>1</sup>H images were acquired during breath-hold. A 2D coronal steady state free precession sequence was used for imaging with parameters: full lung coverage,  $\theta = 50^\circ$ , field of view =

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3 30 - 40cm depending on subject size, in-plane matrix = 256 x 204, slice thickness = 10 mm,  
4 TE / TR = 0.9 / 2.9 ms and bandwidth = 250 kHz. This steady state sequence was previously  
5 shown to be sensitive to mucus, effusion and other early structural changes in CF lungs.<sup>22</sup>  
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8 The images were evaluated by an experienced paediatric chest radiologist (DH) for the  
9 presence of any morphologic abnormalities including bronchial wall thickening, mucus  
10 plugging, bronchiectasis, consolidation and atelectasis,<sup>23</sup> blinded to other results.  
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14 The MRI scanner room time for the <sup>1</sup>H and <sup>3</sup>He imaging reported in this study - namely fast  
15 anatomical MRI and a HP <sup>3</sup>He breath-hold ventilation scan - takes between 10-15 minutes in  
16 total, comparable to the imaging time of CT.  
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### 19 **Computed Tomography (CT)**

20 Low dose inspiratory and ultra-low dose expiratory non-contrast volume CT images were  
21 acquired from patients only, following the protocol of Loeve and colleagues.<sup>24</sup> All  
22 participants were scanned on a GE Lightspeed VCT 64 CT scanner (GE Healthcare,  
23 Milwaukee, WI, USA) in the supine position. Similar breathing instructions were given to  
24 each child. Tube voltage was 80 kV for children weighing less than 35 kg and 100 kV for  
25 those weighing 35 kg or more. End-inspiratory scans were performed with a modulating  
26 tube current, limited to a maximum of 150 mA (auto mAs, GE) but end-expiratory scans  
27 were performed at a fixed current of 25 mA, and as a result were lower dose. A gantry  
28 rotation time of 0.6 s was used with a pitch of 1.375 and 5 mm collimation (retrospectively  
29 reconstructed to 2.5 mm slice thickness with a 1.25 mm increment).  
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#### 35 *Volume Control*

36 In our experience, we have found that training the patient with the inspiratory and  
37 expiratory manoeuvres used here, before entering the CT and MR scanners, is as effective  
38 as spirometric control and we have previously validated this with pneumotachograph  
39 experiments in the scanner.  
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43 CT images were scored according to the system proposed by Brody and colleagues,<sup>25</sup> for  
44 bronchiectasis, mucus plugging, peri-bronchial thickening, parenchymal opacities and air  
45 trapping, by two radiologists, each with six years' experience of chest imaging (DH and AJS),  
46 blinded to other results. Images were scored independently and then any differences  
47 resolved by consensus. Intra-class correlation and Bland-Altman analysis was used to assess  
48 inter-observer variability between the two radiologists before and after consensus. The  
49 reported CT Brody scores are the mean of the scores from the two observers. The CT gas  
50 trapping score reported is a component of the Brody score,<sup>25</sup> which was extracted for  
51 correlation analysis with other measures.  
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## Statistical Analysis

Statistical analysis was performed using GraphPad Prism (San Diego, USA). Data were tested for normality using the D'Agostino and Pearson omnibus normality test and for difference between variances of the healthy control and CF patient groups. To test quantitative metrics for significant differences between the healthy control and CF patient groups, if data were normal with equal variances a 2-tailed t-test was performed, if data were normal with unequal variances a 2-tailed t-test with Welch's correction was performed, and if data were not normal a 2-tailed Wilcoxon signed rank test was performed. Paired tests were used to reflect the age-matching of data. Pearson's correlations were carried out for normal data and Spearman's correlations were performed where data were not normal.

Receiver-operator characteristic (ROC) curve analysis was performed using GraphPad Prism to compare sensitivity and specificity of different lung function assessments. Sensitivity was defined as the presence of an abnormal measurement (i.e. not within the normal range for that measurement) in the presence of the diagnosis of CF. This assumes that all patients should have some abnormality in at least one measurement, which may not be true in this population with very mild clinical expression of CF, and therefore provides a conservative estimation of sensitivity of the lung assessments. Where confidence intervals for ROC analysis exceeded 1 (or 100%) these have been rounded to 1.

Significance level was set at  $p=0.05$ .

## RESULTS

### Recruitment

Nineteen patients with CF and ten age-matched healthy controls were assessed. Healthy controls were recruited from friends and families of members of staff involved in the study, and one healthy control was a sibling of a CF patient taking part in the study. 150 patients were in care of the SCH Pediatric CF clinic at the time of the study. 43 of these fulfilled the inclusion criteria (normal FEV<sub>1</sub>, no respiratory exacerbation). 23 of these patients were approached to participate, 1 patient did not want to take part as they did not want to miss school, 1 mother thought it might upset her 6 year old so did not want to take part, and 1 patient wanted to take part but we were unable to contact the family. 1 patient dropped out following the CT scan due to emigration of the family. <sup>1</sup>H MRI was not performed on one of the remaining 19 CF patients, and plethysmography could not be performed adequately in one additional CF patient and one control. The study response rate was therefore 87 % and study completion rate was 95%.

### Lung function outcomes

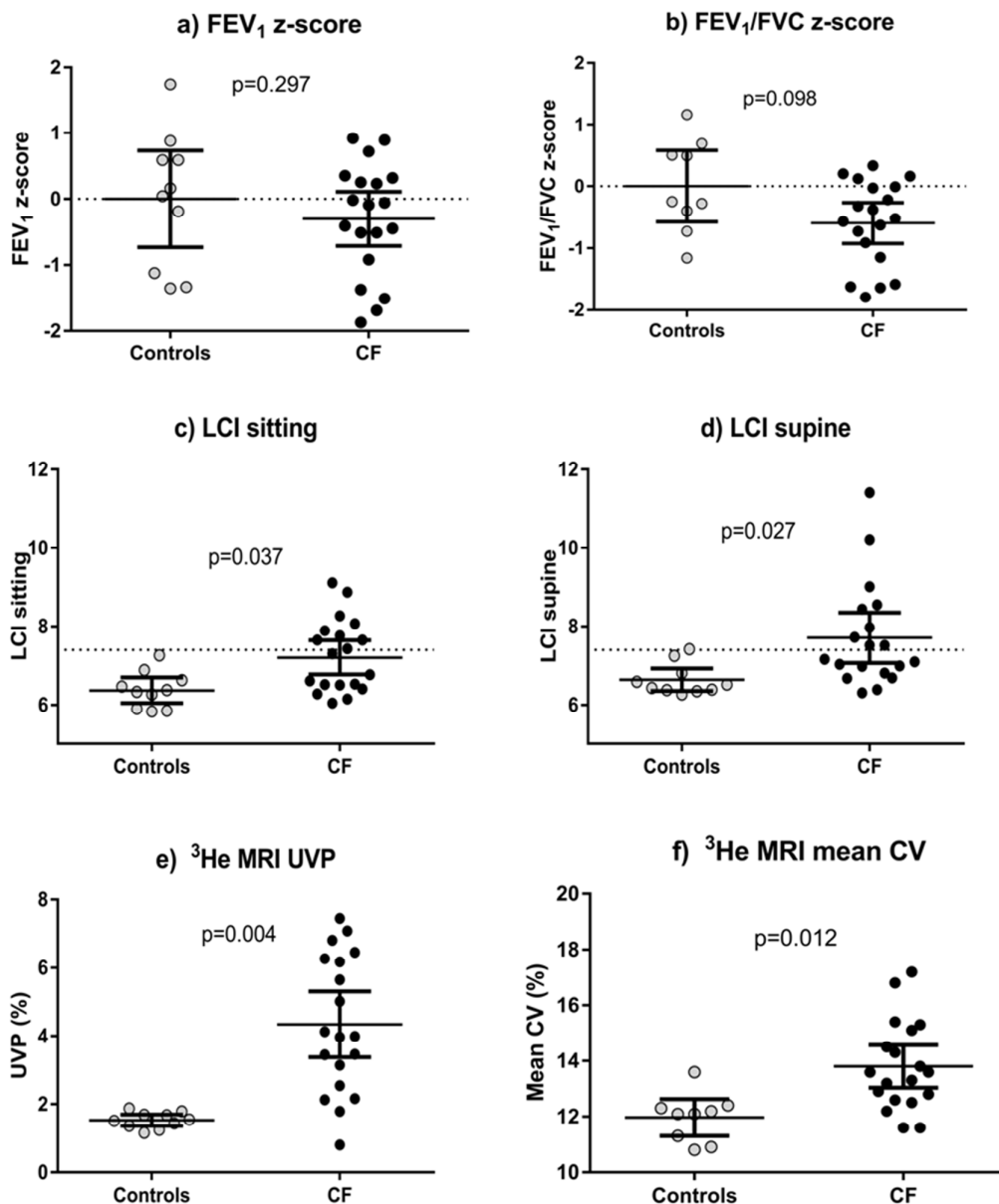
Demographic, lung function, and quantitative imaging data are presented in Table 1.

There were no significant differences between controls and CF patients for age, weight, height or body mass index (BMI). CF patients had early-stage lung disease and all patients had FEV<sub>1</sub> and FEV<sub>1</sub>/FVC z-scores greater than the lower limit of normal. There were no significant differences between groups for FEV<sub>1</sub> z-score (Figure 2a), FEV<sub>1</sub>/FVC z score (Figure 2b), FEF<sub>25-75</sub> z-score or RV/TLC. Both sitting and supine LCI were significantly elevated in the CF patient group when compared to healthy controls (Figure 2c-d) (sitting LCI; mean difference (md) = 0.82, confidence interval (CI) 0.06 – 1.58, p=0.037), though overall CF patient group mean LCI sitting was below the upper limit of normal<sup>14</sup>.

Measurement	Healthy Controls	CF Patients	p-value
Male : Female	4 : 6	10 : 9	
Age (years)	11.3 (2.8)	10.9 (2.5)	0.461
Height z-score	0.830 (0.910)	0.005 (0.951)	0.351
Weight z-score	0.707 (1.222)	0.256 (0.699)	0.625
Body Mass Index z-score	0.265 (1.195)	0.324 (0.864)	0.857
FEV <sub>1</sub> z-score	0.001 (1.022)	-0.302 (0.851)	0.297
FEV <sub>1</sub> /FVC z-score	0.002 (0.749)	-0.594 (0.686)	0.098
FEF <sub>25-75</sub> z-score	-0.07 (1.05)	-0.68 (0.78)	0.447
RV/TLC (%)	25.0 (5.9)	26.6 (4.5)	0.298
LCI sitting	6.4 (0.5)	7.3 (0.9)	<b>0.037</b>
LCI supine	6.6 (0.4)	7.7 (1.3)	<b>0.027</b>
<sup>3</sup> He MRI UVP (%)	1.53 (0.24)	4.34 (2.00)	<b>0.004</b>
<sup>3</sup> He MRI mean CV (%)	11.97 (0.87)	13.81 (1.59)	<b>0.012</b>
CT Brody score	-	12.5 (0.0 - 56.3)	
CT hyperinflation score	-	3.0 (0.0 - 15.0)	

**Table 1 – Comparison of demographic, lung function and imaging data in healthy controls and subjects with cystic fibrosis.**

Values given as mean (standard deviation) except for CT Brody score and CT hyperinflation score (non-parametric data), which are given as median (range). CT was not performed in healthy controls. P-values represent paired t-tests or Wilcoxon signed rank tests of CF subjects compared to healthy controls.



**Figure 2 – Comparison of lung physiology and ventilation imaging metrics for healthy controls and cystic fibrosis (CF) patients.**

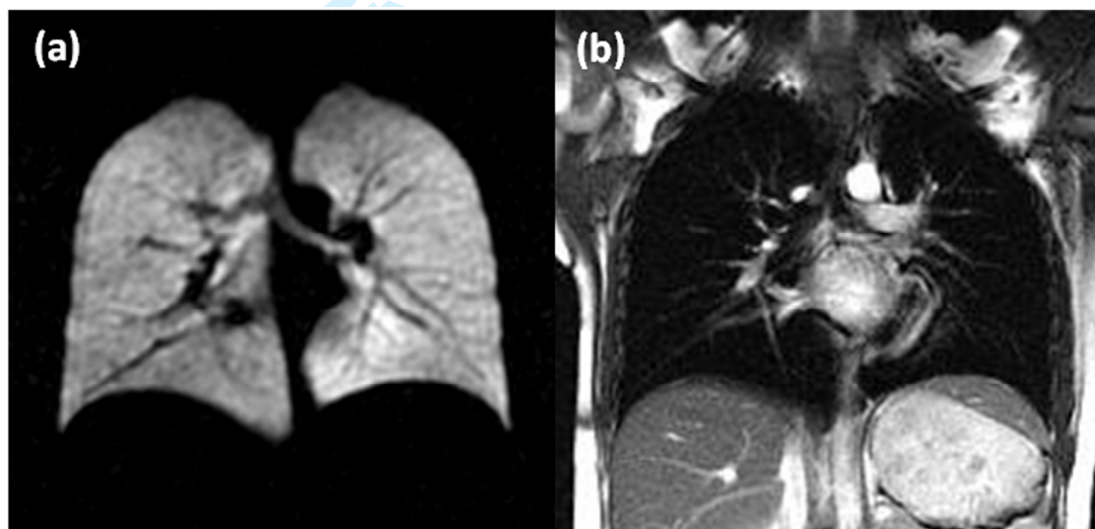
Each point represents a single subject, error bars represent group means and 95% confidence intervals of the mean. (a) FEV<sub>1</sub> z-score and (b) FEV<sub>1</sub>/FVC z-score derived from spirometry. Horizontal dotted line indicates a z-score of zero. (c) Sitting lung clearance index (LCI) and (d) supine LCI from multiple breath washout. Horizontal dotted line represents upper limit of normal for sitting LCI. (e) Un-ventilated lung volume percentage (UVP) and (f) mean coefficient of variation (CV) of ventilation imaging signal, from <sup>3</sup>He MRI. This is the same as Figure 1 in the main letter.



### Imaging outcomes

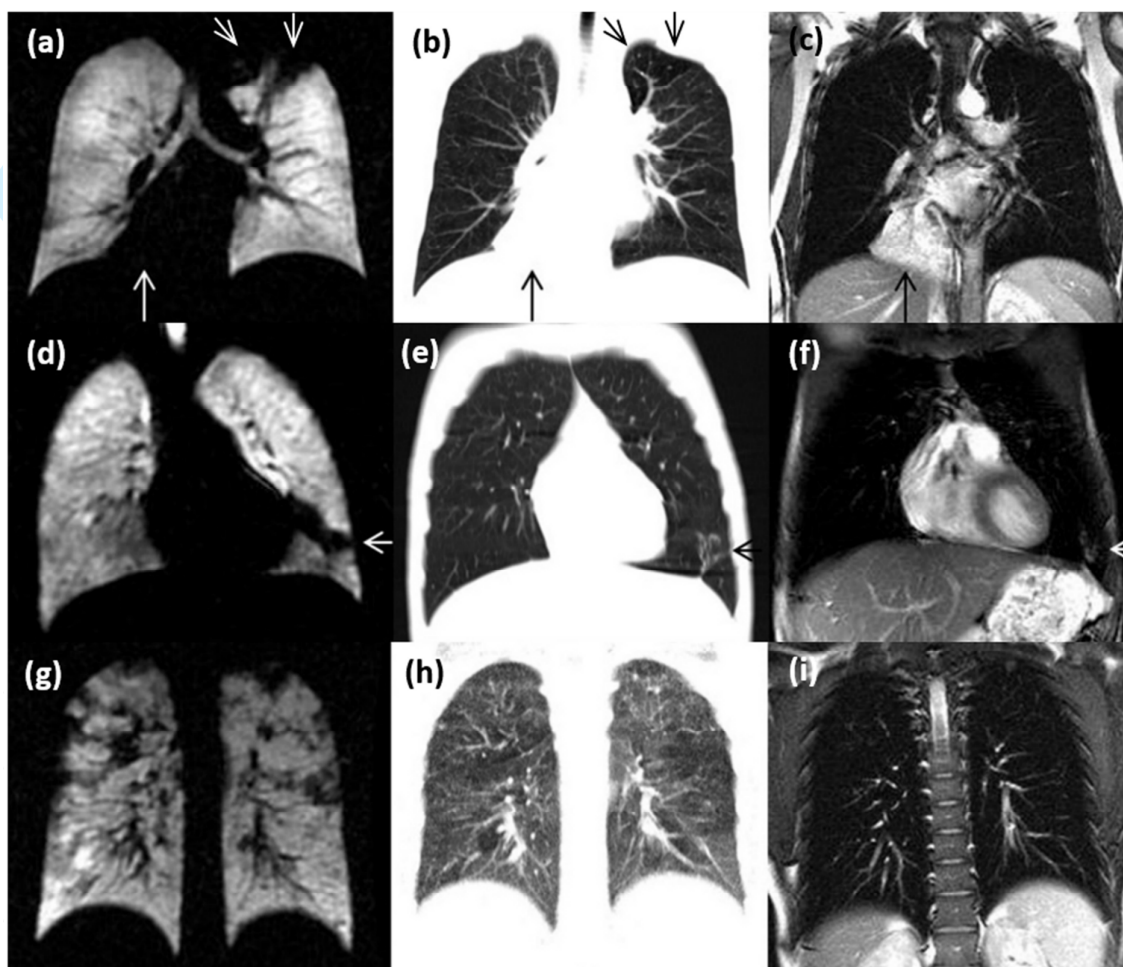
In healthy controls, no ventilation or structural abnormalities were detected on either  $^3\text{He}$  or  $^1\text{H}$  MRI, see Figure 3 for example images.

$^3\text{He}$  UVP and ventilation heterogeneity measured by mean CV were both significantly increased in the CF patient group when compared to healthy controls (md = 2.77, CI 1.15 – 4.39,  $p=0.004$  and md = 1.62, CI 0.46 – 2.78,  $p=0.012$  respectively) (Table 1, Figure 2e-f). Ventilation heterogeneity and obstruction seen with  $^3\text{He}$  MRI generally corresponded to anatomical abnormalities observed by CT, such as mucus plugging, air trapping, bronchiectasis and atelectasis (Figure 4). However, in several instances regional ventilation heterogeneity was present on  $^3\text{He}$  MRI with no obvious features of structural pathology visible on CT (Figure 5).



**Figure 3 – Lung ventilation hyperpolarised gas MRI and  $^1\text{H}$  MRI in healthy controls**

(a)  $^3\text{He}$  MRI of a representative healthy control subject (age 10 years) showing homogeneous ventilation extending fully to the lung boundaries. The equivalent coronal slice of the  $^1\text{H}$  MRI is shown in 3(b).

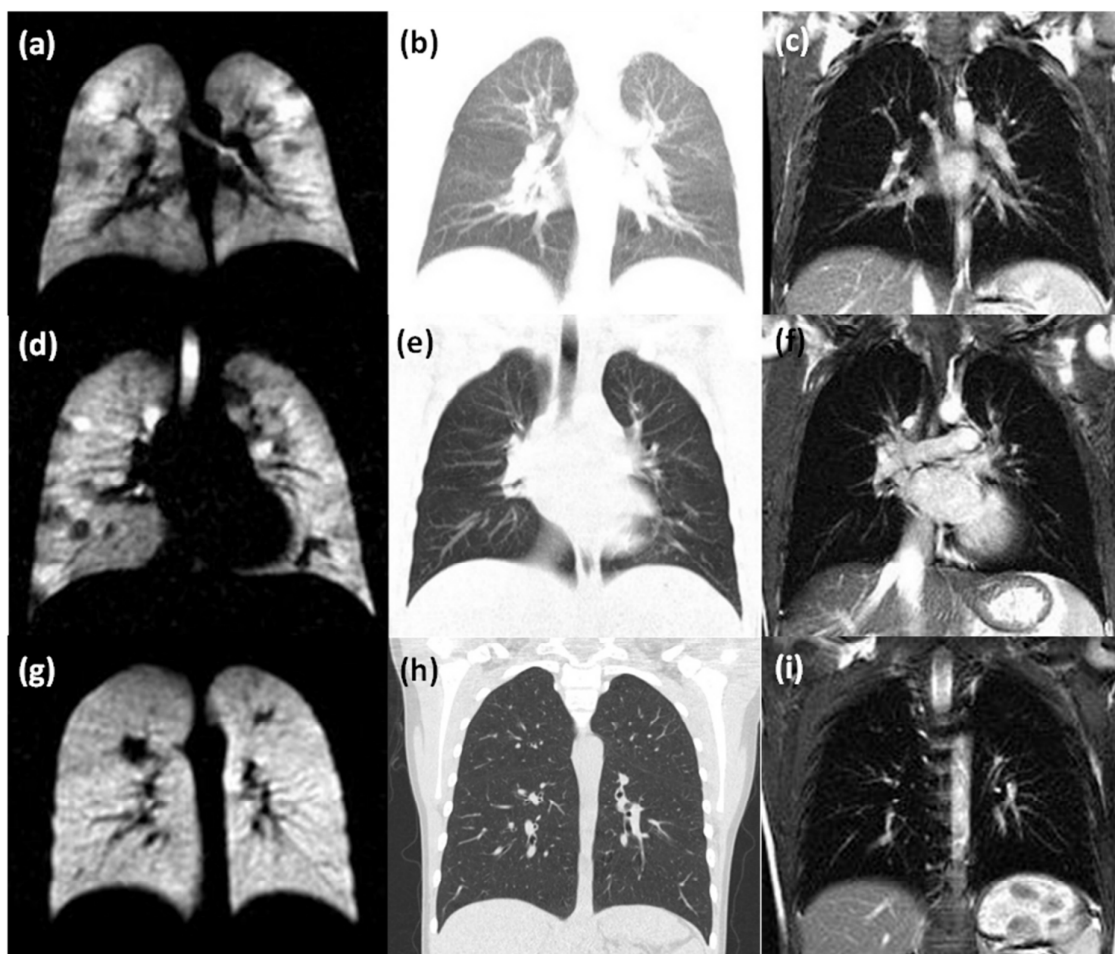


**Figure 4 – Hyperpolarised gas MRI and CT imaging: examples of correspondence in patients with cystic fibrosis (CF).**

Each row represents a single unique CF patient, with equivalent slices from hyperpolarised (HP) gas MRI, CT, and  $^1\text{H}$  MRI in the columns from left to right. (a-c) are scans from a CF patient with arrows indicating segmental collapse visualised by all three imaging modalities.  $^3\text{He}$  ventilation defects in the upper left lung (a) correspond to atelectasis and nodular change on inspiratory CT (b).

In (d-f) arrows indicate lingular atelectasis, in a CF patient with normal lung clearance index. The obstruction seen with  $^3\text{He}$  MRI (d) extends more proximally than the atelectasis seen on inspiratory CT (e) and  $^1\text{H}$  MRI (f).

In the final subject,  $^3\text{He}$  MRI ventilation heterogeneity (g) and air trapping on expiratory CT (h) were visible throughout the lungs, especially in the upper lobes. No abnormalities were detected with  $^1\text{H}$  MRI (i).



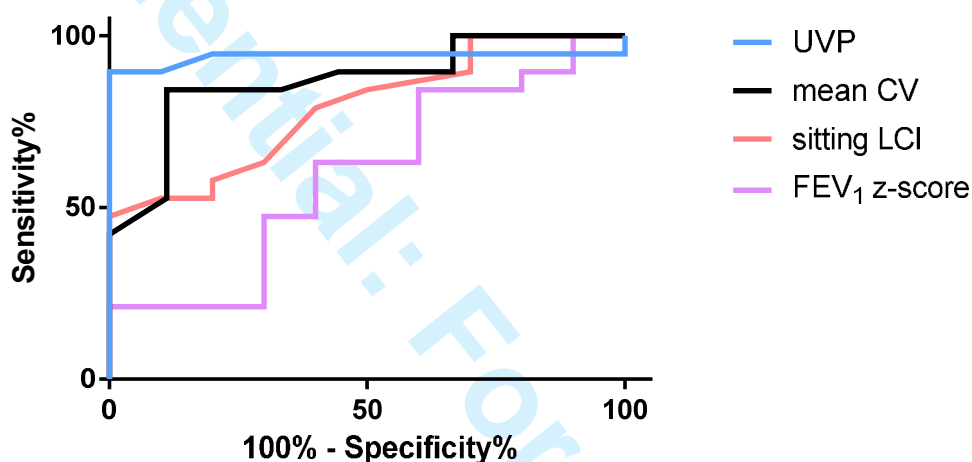
**Figure 5 - Hyperpolarised gas MRI and CT imaging: examples of discordance in patients with cystic fibrosis (CF).**

Each row represents a single unique CF patient, with equivalent slices from hyperpolarised gas MRI, CT, and  $^1\text{H}$  MRI in the columns from left to right. In the first subject (a-c),  $^3\text{He}$  MRI showed heterogeneous ventilation with widely distributed patchy, semi-ventilated defects (a). CT for this slice was normal (expiratory shown in b) and  $^1\text{H}$  MRI (c) was normal throughout. In the second CF patient, small, sub-segmental defects were observed throughout the lungs with  $^3\text{He}$  MRI (d). CT for this slice was normal (expiratory shown in e) but elsewhere showed lingular atelectasis and minimal air-trapping.  $^1\text{H}$  MRI was normal for this slice (f) but showed lingular atelectasis elsewhere. In the final patient, several small ventilation defects were seen on  $^3\text{He}$  MRI (g), whilst CT (inspiratory shown) (h),  $^1\text{H}$  MRI (i), and lung clearance index were all normal.

This figure is also presented as Figure 2 in the main letter.

### Comparison of Different Methods

The presence of visible defects on  $^3\text{He}$  MRI had the greatest sensitivity for detecting evidence of CF airways disease: nine CF patients (47%) had elevated sitting LCI, compared to 17 CF patients (89%) with ventilation abnormalities detected by  $^3\text{He}$  MRI. Receiver operating characteristic (ROC) analysis showed  $^3\text{He}$  MRI metrics to be more accurate than LCI or spirometry, with area under the curve values of 0.94 (CI 0.84 – 1.0) for  $^3\text{He}$  MRI UVP, 0.87 (CI 0.73 – 1.0) for  $^3\text{He}$  MRI mean CV, 0.79 (CI 0.62 – 0.95) for sitting LCI and 0.59 (CI 0.37 – 0.82) for  $\text{FEV}_1$  z-score (Figure 6 and Table 2).



**Figure 6 - Receiver Operating Characteristic Curves for  $\text{FEV}_1$ , sitting LCI and  $^3\text{He}$  MRI metrics**

Receiver operating characteristic (ROC) curves for  $\text{FEV}_1$  z-score (Area Under Curve (AUC) = 0.59 (CI 0.37 – 0.82), purple), sitting LCI (AUC = 0.79 (CI 0.62 – 0.95),  $p < 0.05$ , pink),  $^3\text{He}$  MRI mean CV (AUC = 0.87 (CI 0.73 – 1.01),  $p < 0.01$ , black) and  $^3\text{He}$  MRI UVP (AUC = 0.94 (CI 0.84 – 1.04),  $p < 0.001$ , blue). See also Table 2.

Metric	Area Under Curve	95% Confidence Interval	p-value
$\text{FEV}_1$ z-score	0.59	0.37 - 0.82	0.4089
$\text{FEV}_1/\text{FVC}$ z-score	0.70	0.49 - 0.92	0.0851
Sitting LCI	0.79	0.62 - 0.95	<b>0.0116</b>
Supine LCI	0.82	0.65 - 0.98	<b>0.0059</b>
$^3\text{He}$ MRI UVP (%)	0.94	0.84 - 1.0	<b>0.0001</b>
$^3\text{He}$ MRI mean CV (%)	0.87	0.73 - 1.0	<b>0.0019</b>

**Table 2 - ROC analysis for spirometry, LCI and  $^3\text{He}$  MRI**

Structural abnormalities were detectable in four patients (22%) with  $^1\text{H}$  MRI when compared to 13 patients (68%) with CT (Table 3).  $^1\text{H}$  MRI showed lingular atelectasis in four patients, segmental lobar collapse in one patient and severe bronchiectasis in one patient. However the  $^1\text{H}$  MRI performed here was not sensitive to more subtle changes detected by  $^3\text{He}$  MRI and CT in this group of patients with early CF lung disease. In contrast, only two CF patients (11%) had FEV<sub>1</sub> z-scores below the 5th percentile (-1.87 and -1.69 respectively), and one patient had airflow obstruction as defined by FEV<sub>1</sub>/FVC z-score <-1.64.  $^3\text{He}$  MRI detected abnormality in four patients when CT was normal. LCI was abnormal in only two of these four patients. Two patients with mild variant disease (both Phe508del and R117H heterozygotes with 7T poly-T status and sweat chloride of 28 and 68mmol/L respectively) had no abnormality detected by any technique; these were the only two patients where  $^3\text{He}$  MRI was normal.

	FEV <sub>1</sub> z-score <-1.96	LCI ≥ 7.4	Visible Abnormality on $^3\text{He}$ MRI	Visible Abnormality on basic $^1\text{H}$ MRI	Abnormality on CT (Brody score>6)
<b>Cystic fibrosis (N=19)</b>	0 (0%)	9 (47%)	17 (89%)	4 (22%)*	13 (68%)
<b>Controls (N=10)</b>	0 (0%)	0 (0%)	0 (0%)	0 (0%)	N/A

**Table 3 – Comparison of sensitivity of lung function and imaging modalities to detection of lung abnormalities in children with cystic fibrosis.**

Data are presented as number of subjects where lung abnormalities were detected (percentage of total number of patients or controls respectively).

\*Total number of patients who underwent  $^1\text{H}$  MRI was 18.

#### **Correlation between Different Measures**

$^3\text{He}$  MRI UVP ( $r=0.61, p=0.008$ ),  $^3\text{He}$  MRI mean CV ( $r=0.52, p=0.026$ ), LCI ( $r=0.47, p=0.047$ ), and CT gas trapping score (0.48,  $p=0.045$ ) all showed moderately significant correlations with RV/TLC, but no significant correlation with other pulmonary function test results. LCI and  $^3\text{He}$  MRI metrics showed moderately significant correlation (LCI sitting and  $^3\text{He}$  MRI UVP  $r=0.55, p=0.015$ ; LCI supine and  $^3\text{He}$  MRI mean CV  $r=0.48, p=0.038$ ).  $^3\text{He}$  MRI UVP correlated significantly with CT Brody score ( $r=0.47, p=0.04$ ) and CT gas trapping score ( $r=0.58,$

p=0.009). There was no significant correlation between LCI and CT scores. The observed correlations are summarised in Table 4.

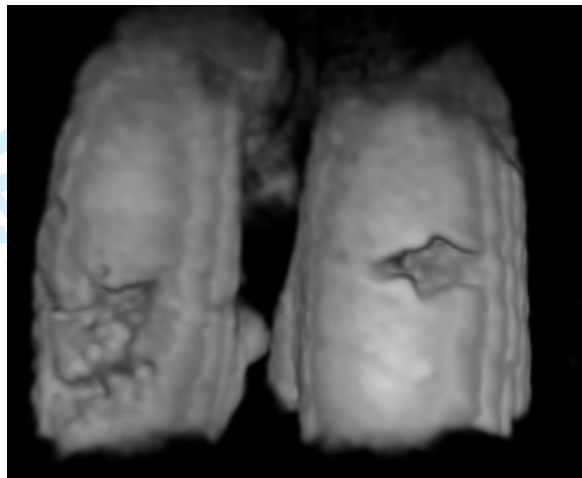
Metric 1	Metric 2	p-value	R value
FEV <sub>1</sub> /FVC z-score	FEF <sub>25-75</sub> z-score	<0.0001	0.78
LCI sitting	LCI supine	<0.0001	0.73
<sup>3</sup> He MRI UVP	RV/TLC	0.008	0.61
<sup>3</sup> He MRI UVP	CT gas trapping	0.009	0.58
<sup>3</sup> He MRI UVP	<sup>3</sup> He mean CV	0.009	0.58
LCI sitting	<sup>3</sup> He MRI UVP	0.015	0.55
FEV <sub>1</sub> z-score	FEF <sub>25-75</sub> z-score	0.019	0.55
<sup>3</sup> He MRI mean CV	RV/TLC	0.026	0.52
FEV <sub>1</sub> z-score	RV/TLC	0.026	-0.52
LCI supine	<sup>3</sup> He mean CV	0.038	0.48
<sup>3</sup> He MRI UVP	CT Brody score	0.044	0.47
CT gas trapping	RV/TLC	0.045	0.48
LCI sitting	RV/TLC	0.046	0.47

**Table 4 - Significant correlations between lung function, LCI, <sup>3</sup>He MRI and CT Quantitative Results**

Correlations were calculated using Pearson's correlation where both sets of data were normally distributed and Spearman's correlation where one or more datasets were not normally distributed. Only correlations where the p value was less than 0.05 are shown. No attempt has been made to adjust for multiple correlations in this summary.

### Three-dimensional imaging of ventilation distribution

Three-dimensional images of lung ventilation can be generated from the two-dimensional slices shown in Figures 3-5. These allow an overview of ventilation distribution in the lungs, and by extension a view of disease distribution. A three-dimensional image rendered from the scans performed for this study is shown in Figure 7. Higher resolution is possible with alternative protocols.

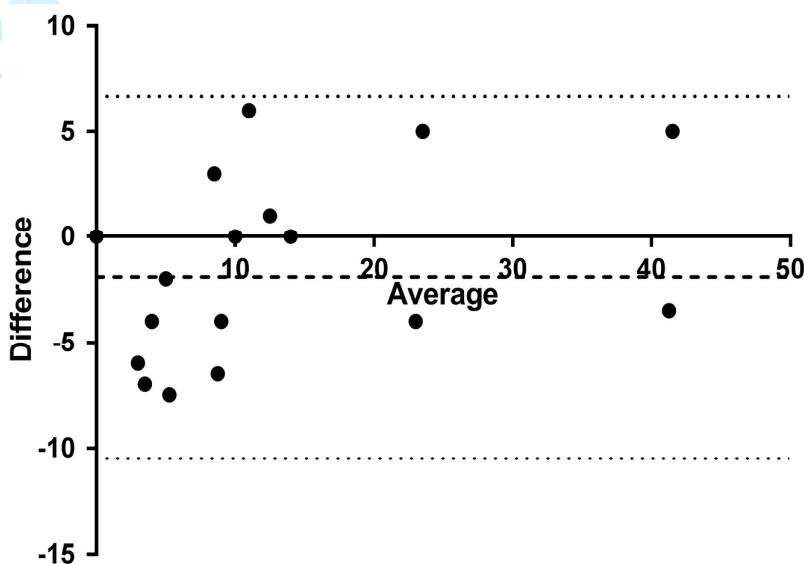


**Figure 7 – Three-dimensional rendering of ventilation distribution in a child with cystic fibrosis**

Image was generated from 10mm slices performed as part of protocol described in the methods.

### Computed Tomography scoring

Intra-class correlation between the CT scores of the two radiologists was  $r=0.76$ ,  $p<0.01$  before consensus and  $r=0.93$ ,  $p<0.01$  after consensus was achieved, demonstrating a high level of agreement. Agreement between scorers is illustrated in Figure 8. A mean difference between scorers of  $-1.9$  was found, with standard deviation of  $4.4$ . This is similar to, if not better than, previous reports of Bland-Altman analysis between CT raters<sup>26</sup>. As previously reported, agreement tends to be least good in those with the lowest scores<sup>26,27</sup>.



**Figure 8. Bland-Altman plot comparing Brody scores of both radiologists after consensus.**

A mean difference of  $-1.9$  with a standard deviation of  $4.4$  was found. The dashed line represents the mean difference and dotted lines 95% confidence intervals.

### DISCUSSION

This is the first study to combine the powerful functional technique of ventilation imaging using HP gas MRI with assessments of structural lung disease from CT and the more scalable assessment of ventilation heterogeneity provided by MBW. Although both  $^3\text{He}$  MRI and MBW measure aspects of ventilation heterogeneity in the lungs, the detailed spatial information provided by ventilation-MRI meant that abnormalities in ventilation distribution were visible even when these were insufficient to affect the LCI. Likewise, ventilation defects were visible in some cases in the absence of structural abnormalities on CT (e.g. Figure 5), which may be due to the inherent sensitivity of ventilation imaging to small airways obstruction that cannot be explicitly resolved on CT.



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3 These are important findings for our understanding of how CF lung disease develops and  
4 how we interpret lung function data. In this cohort of CF patients, specifically selected to  
5 represent those with the mildest airways disease, LCI was abnormal in almost half of all  
6 subjects (47%), in keeping with prior observations<sup>1-5</sup>. Ventilation-MRI however was more  
7 sensitive than both LCI and CT and detected ventilation defects in all but two patients (89%),  
8 both of whom had genetic variants that may not be associated with CF lung disease until  
9 adulthood. The great advantage of ventilation-MRI is that it offers detailed regional  
10 information about the nature and distribution of ventilation defects. Thus in patients with  
11 early disease we predominantly detected patchy ventilation defects distributed throughout  
12 the lung, with larger focal defects in areas where there was already evidence of structural  
13 damage on CT or <sup>1</sup>H MRI. In contrast, MBW follows an averaged washout signal from the  
14 lungs, so that mild ventilation heterogeneity is inevitably masked, and differentiation of the  
15 signal into regional or anatomical lung compartments is at best speculative<sup>6</sup>.

20  
21 The <sup>1</sup>H MRI protocol deployed here was used to determine lung boundaries and was not  
22 optimised for structural imaging. Alternative CT scoring systems and protocols also exist that  
23 may offer additional sensitivity<sup>7</sup>. Finally, we did not perform gadolinium contrast enhanced  
24 imaging, as we felt this would deter children from participating. Even given these limitations  
25 however, it is clear that ventilation-MRI is a powerful tool for detecting early lung changes.  
26 The superior sensitivity and detail of information provided by ventilation-MRI also offers the  
27 prospect that this measurement will allow an earlier or more detailed radiation-free  
28 appreciation of the response to novel therapies than even LCI.<sup>8</sup> The advent of high quality  
29 HP gas ventilation-MR imaging using the cheaper and readily available <sup>129</sup>Xe isotope means  
30 that the technology is now much more readily clinically deployed.<sup>9</sup> The ventilation-MR  
31 images presented here are thus exemplars of what may become routine assessments in  
32 detecting early disease and treatment effects.

37  
38 In conclusion, in this population of CF children with very mild lung disease, we have shown  
39 that ventilation-MRI is highly sensitive to detecting the consequences of airway disease.  
40 Even patients with apparently pristine lungs by all current physiology and imaging standards  
41 have evidence of ventilation-MRI abnormalities. HP gas MRI provides detailed regional  
42 information about disease severity and physiological impairment.  
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This supplement represents the full study report, which it was not possible to present in the limited manuscript, summarising the study highlights, recommended by Thorax.

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