

## ORIGINAL ARTICLE

## Ventilation inhomogeneity in children with primary ciliary dyskinesia

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## ABSTRACT

**Background** The lung clearance index (LCI) derived from the multiple breath inert gas washout (MBW) test reflects global ventilation distribution inhomogeneity. It is more sensitive than forced expiratory volume in 1 s (FEV<sub>1</sub>) for detecting abnormal airway function and correlates closely with structural lung damage in children with cystic fibrosis, which shares features with primary ciliary dyskinesia (PCD). Normalised phase III slope indices  $S_{\text{cond}}$  and  $S_{\text{acin}}$  reflect function of the small conducting and acinar airways, respectively. The involvement of the peripheral airways assessed by MBW tests has not been previously described in PCD.

**Methods** A cross-sectional MBW study was performed in 27 children and adolescents with verified PCD, all clinically stable and able to perform lung function tests. LCI,  $S_{\text{cond}}$  (n=23) and  $S_{\text{acin}}$  (n=23) were derived from MBW using a mass spectrometer and sulfur hexafluoride as inert marker gas. MBW indices were compared with present age, age at diagnosis and spirometry findings, and were related to published normative values.

**Results** LCI,  $S_{\text{cond}}$  and  $S_{\text{acin}}$  were abnormal in 85%, 96% and 78% of patients with PCD and in 81%, 93% and 79%, respectively, of 13/27 subjects with normal FEV<sub>1</sub>. LCI and  $S_{\text{acin}}$  correlated significantly while  $S_{\text{cond}}$  did not correlate with any other lung function parameters. None of the lung function measurements correlated with age or age at diagnosis.

**Conclusions** PCD is characterised by marked peripheral airway dysfunction. MBW seems promising in the early detection of lung damage, even in young patients with PCD. The relationship of MBW indices to the outcome of long-term disease and their role in the management of PCD need to be assessed.

## INTRODUCTION

Primary ciliary dyskinesia (PCD) is a rare congenital disease characterised by defective ciliary function, which leads to impaired mucociliary clearance and consequently to recurrent and chronic upper and lower airway infections.<sup>1 2</sup> Patients with PCD most often present with persistent rhinitis and chronic productive cough, but the heterogeneous nature of the disease makes early diagnosis difficult.<sup>3</sup> A recently published longitudinal study from our centre suggested that PCD is a disease which seriously compromises lung function already at preschool age with a highly variable course of forced expiratory volume in 1 s (FEV<sub>1</sub>) and forced vital capacity (FVC) even after early diagnosis.<sup>4</sup>

However, traditional spirometry is mostly sensitive in detecting proximal airway disease, which may have limitations as early lung damage in PCD may be of peripheral origin. This is supported by a case report in which two of three patients with PCD who had infant pulmonary lung function performed demonstrated values suggestive of primary pathology in smaller peripheral airways.<sup>5</sup>

PCD lung disease shares several features with cystic fibrosis (CF): clinical findings include chronic productive cough and, although less frequent, colonisation by *Pseudomonas aeruginosa* (PA),<sup>6</sup> and radiological changes include bronchiectasis, mucus plugging and peribronchial thickening.<sup>7</sup> However, although data on early structural changes in PCD lung disease are lacking, it is reasonable to suggest that PCD may resemble CF in which initial lung damage has been shown to start in the peripheral airways.<sup>8</sup> In CF, spirometry has traditionally been used to monitor lung function, but several studies have shown spirometry to be insensitive in tracking early progressive lung disease.<sup>9–12</sup> The lung clearance index (LCI), derived from a multiple breath inert gas washout test (MBW), is a measure of global ventilation inhomogeneity (VI) and small airway dysfunction. LCI has been shown to detect lung damage in CF more readily than other pulmonary function tests<sup>9 13</sup> and to be predictive of subsequent lung function when measured at preschool age.<sup>10</sup> In addition, determination of concentration normalised phase III slope ( $Sn_{\text{III}}$ ) indices allows assessment of VI arising in the small conductive airway zone ( $S_{\text{cond}}$ ) and more peripherally close to or within the acinar airway zone ( $S_{\text{acin}}$ ).<sup>14</sup> Peripheral airway function assessed by LCI,  $S_{\text{cond}}$  and  $S_{\text{acin}}$  in children and adolescents with PCD has not been previously reported.

The aim of our study was to assess peripheral airway function in children and adolescents with PCD using MBW and to compare the findings with spirometry. We hypothesised that PCD lung disease is characterised by marked peripheral dysfunction, and that abnormal ventilation distribution is a frequent finding despite normal spirometry. Some of the study results have been previously reported in abstract form.<sup>15</sup>

## METHODS

## Design of study

This was a cross-sectional prospective study. All patients had lung function and MBW tests performed at their routine annual review at the National Danish PCD Center. Management is according to previous publications from the same centre.<sup>4</sup>

## Study patients

Patients with a diagnosis of PCD aged  $\leq 18$  years were eligible for the study. All patients had a consistent history of symptoms characteristic of PCD,<sup>16 17</sup> and basic tests to rule out CF and immunodeficiency were performed. Nasal nitric oxide measurement (nNO) was used as a preliminary screening test, although without necessarily excluding patients with a high suspicion of PCD.<sup>18</sup> Furthermore, functional studies on ciliary beat pattern and frequency analysis using video recording and electron microscopy (EM) analysis of ciliary ultrastructure were key diagnostic tests.<sup>1</sup> Functional studies were performed twice, at least one month apart. Patients had to be considered in a stable clinical condition on the day of MBW measurement.

## Measurements

### MBW

Tidal breathing sulfur hexafluoride ( $\text{SF}_6$ ) washout was performed in all patients using a mass spectrometer (AMIS 2000, Innovision, Odense, Denmark) for gas analysis, as previously described.<sup>13</sup> The LCI and the concentration normalised slope III indices ( $S_{\text{acin}}$  and  $S_{\text{cond}}$ ) were calculated. LCI was calculated as the number of lung volume turnovers (TO; ie, the cumulative expired volume divided by the functional residual capacity, FRC) needed to lower the end-tidal tracer gas concentration to less than 1/40th of the starting concentration.<sup>13</sup> The mean LCI result from three MBW measurements in each patient was used for analysis. The concentration normalised slope of phase III ( $\text{Sn}_{\text{III}}$ ) for each subsequent breath during MBW was determined to calculate  $S_{\text{cond}}$  and  $S_{\text{acin}}$ . The phase III slope was converted to  $\text{Sn}_{\text{III}}$  by dividing the slope by the mean gas concentration over the slope to allow for gas dilution. The  $\text{Sn}_{\text{III}}$  was further multiplied by tidal volume (VT) giving the  $\text{Sn}_{\text{III}} \times \text{VT}$  in order to account for inter-individual differences in lung size and breathing pattern.<sup>19</sup> The  $\text{Sn}_{\text{III}} \times \text{VT}$  was used in all subsequent analyses and is henceforth referred to as  $\text{Sn}_{\text{III}}$  in this paper. For determination of  $S_{\text{cond}}$  and  $S_{\text{acin}}$ ,  $\text{Sn}_{\text{III}}$  and TO values for each subsequent breath from the three washouts were first averaged. For each breath,  $\text{Sn}_{\text{III}}$  was then plotted against the corresponding TO value.  $S_{\text{cond}}$  was defined as the rate of  $\text{Sn}_{\text{III}}$  increase between TO 1.5 and 6.0.  $S_{\text{acin}}$  was defined as the first breath  $\text{Sn}_{\text{III}}$  value minus the convection-dependent inhomogeneity contribution to this value (ie,  $S_{\text{cond}} \times \text{TO}$  for the first breath). MBW results from this study were compared with data previously collected in healthy Swedish schoolchildren obtained using identical equipment, software and procedures.<sup>13 20</sup> The authors performing and calculating the MBW tests have undergone training and been under continual supervision by the Swedish laboratory. Further technical details and reference values of the MBW technique are provided in the online supplement and elsewhere.<sup>20</sup>

### Spirometry

Spirometry was performed according to ATS/ERS standards<sup>21</sup> and  $\text{FEV}_1$ , FVC, forced expiratory flow at 25–75% of FVC ( $\text{FEF}_{25-75}$ ) and  $\text{FEV}_1/\text{FVC}$  ratio were recorded. The recently published ‘all ages’ reference equations were used.<sup>22</sup> Abnormal lung function was defined as z-scores  $< -1.96$ .

### Statistical analysis

Lung function was expressed as z-scores, which were calculated as (measured value – predicted value)/RSD from the reference population.<sup>22</sup> The upper limit of normal (ULN) was defined as the predicted mean plus 1.96 RSD for MBW variables and the lower limit of normal (LLN) as predicted mean minus 1.96 RSD for spirometry variables. MBW parameters were correlated to

spirometry parameters, age and age at diagnosis using a linear regression model. A p value of  $< 0.05$  was accepted as statistically significant. SAS V.9.2 (SAS Institute) was used for statistical analyses.

## RESULTS

Twenty-seven patients with PCD from the National Danish PCD cohort were included in the study; all patients had LCI measurements performed.  $\text{Sn}_{\text{III}}$  indices could not be calculated in four patients owing to irregular breathing patterns. In two patients spirometry was performed on a separate day because of technical problems and the dataset closest in time to the date of the MBW test (5 weeks later) was used instead. Both patients were clinically stable on the day spirometry was performed, and spirometry showed stable measurements over time.

Three patients did not have a conclusive abnormal ciliary beat pattern and frequency during the investigation. One had clinical signs and symptoms of PCD, situs inversus, hydrocephalus, repeated abnormal pulmonary radioaerosol mucociliary clearance tests,<sup>23</sup> borderline abnormal nNO, low exhaled NO measurements  $< 5$  ppb, but conflicting functional studies not certain of abnormal beat pattern and an EM without classical ultrastructural defects. Two other patients did not have a conclusive ciliary function test as they both refused to participate in further functional studies after the initial one: one patient had classical clinical PCD, extremely low nNO and classical abnormal EM; the other had classical clinical PCD and an abnormal EM and very low nNO. In addition, the latter had a brother with PCD with identical EM presentation and immotile cilia on functional studies. Demographic and diagnostic characteristics are shown in table 1.

Lung function results are summarised in table 2. Mean (SD) absolute values of MBW variables in patients with PCD were all markedly abnormal compared with normal reference values:  $\text{LCI}=9.48$  (2.20) vs 6.33 (0.43),  $\text{ULN}=7.17$ ;  $S_{\text{cond}}=0.076$  (0.024) vs 0.018 (0.006),  $\text{ULN}=0.030$ ; and  $S_{\text{acin}}=0.236$  (0.115) vs 0.086 (0.025),  $\text{ULN}=0.135$ . LCI was above the ULN in 84% of patients (23/27), while 96% (22/23) and 78% (18/23) had abnormal  $S_{\text{cond}}$  and  $S_{\text{acin}}$ , respectively.

The relationships between LCI and the  $\text{Sn}_{\text{III}}$  indices  $S_{\text{acin}}$  and  $S_{\text{cond}}$  are shown in figure 1A and B. LCI correlated to  $S_{\text{acin}}$  ( $R^2=0.45$ ;  $p<0.001$ ) but not to  $S_{\text{cond}}$ .  $S_{\text{cond}}$  peaked at an LCI of about 10 z-scores, subsequently decreasing with increase in disease severity (as measured by LCI).  $S_{\text{acin}}$  and  $S_{\text{cond}}$  did not correlate (figure E1 in online supplement).

Mean values of spirometry parameters across the cohort were within or close to normal limits; 52% (14/27) had abnormal  $\text{FEV}_1$  and 15% (4/27) had abnormal FVC.

LCI did not show a statistically significant correlation with either  $\text{FEV}_1$  or FVC. LCI,  $S_{\text{cond}}$  and  $S_{\text{acin}}$  were abnormal in 81% (13/16), 93% (13/14) and 79% (11/14), respectively, among the 13/27 patients with normal  $\text{FEV}_1$  (figure 2A–C). Normal LCI excluded the presence of abnormal  $\text{FEV}_1$ , with the exception of a marginally reduced  $\text{FEV}_1$  ( $-2.1$  z-scores) in one patient. In addition, this patient had  $S_{\text{acin}}$  within the normal range while  $S_{\text{cond}}$  was elevated at more than 10 z-scores. When relating  $\text{Sn}_{\text{III}}$  indices to spirometry parameters, neither  $S_{\text{acin}}$  nor  $S_{\text{cond}}$  correlated with  $\text{FEV}_1$  or FVC.

The inclusion of spirometry results performed for two patients on a different date from the MBW did not have any effect on the statistical analysis with regard to  $\text{FEV}_1$  and FVC. Correlations between lung function parameters are summarised in table E1 in the online supplement.

**Table 1** Demographic and diagnostic characteristics of study population

<b>Demographics</b>	
Number	27
Female/male (n)	17/10
Age, years	11.3 (6.3–18.5)
Age at diagnosis, years	2.2 (0.04–9.8)
Weight, kg	36.5 (16.1–61.6)
Height, cm	145 (114–182)
<b>Bacterial colonisation (n)</b>	
Chronic: <i>P aeruginosa</i>	1
Intermittent: <i>P aeruginosa</i>	2
Intermittent: <i>A xylosoxidans</i>	1
<b>Diagnostic tests (n)</b>	
Typical clinical symptoms	27
Nasal nitric oxide performed	26
Low level	26
Ciliary beat pattern and frequency analysis performed	27
Normal frequency, asynchrony	6*
Low frequency, asynchrony	10*
Immotility	10*
Normal	1*
Inconclusive	2*
Electron microscopy performed	21
<b>Ultrastructural defect</b>	
Outer dynein arm (ODA)	6
Inner dynein arm (IDA)	1
ODA+IDA	2
Radial spoke defect + IDA	5
Transposition defect	1
Periphery microtubuli defect	5
Normal	1

Demographic data were not normally distributed so are presented as median (range). Diagnostic characteristics are presented as n.

\*Sum >25 as each patient can have more than one functional abnormality.

There was no significant correlation between any of the lung function parameters and age or age at diagnosis, respectively. Table E2 in the online supplement shows correlations between MBW indices and age and age at diagnosis.

Comparisons between MBW parameters and FEV<sub>25–75</sub> and FEV<sub>1</sub>/FVC ratio are given in the online supplement.

## DISCUSSION

This is the first report presenting data from MBW findings in a cohort of well-characterised children and adolescents with PCD. We found that MBW was more frequently abnormal than FEV<sub>1</sub>, the currently accepted spirometry surrogate marker of

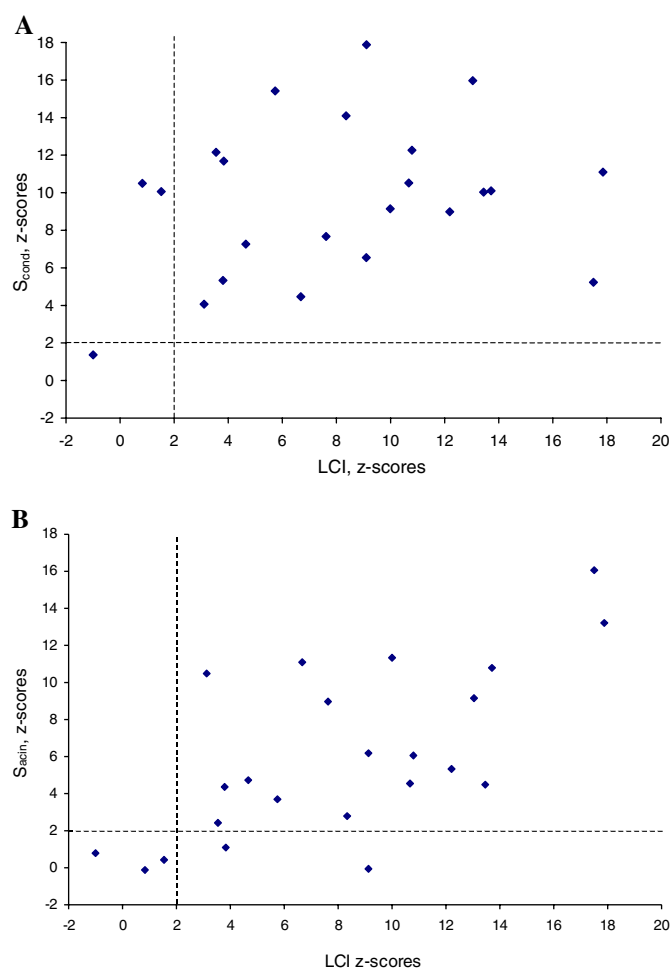
**Table 2** Spirometry and multiple breath inert gas washout (MBW) measurements

Parameter	Mean (SD) z-scores
FEV <sub>1</sub> (n=27)*	−1.59 (1.44)
FVC (n=27)*	−0.71 (1.36)
FEF <sub>25–75</sub> (n=27)*	−2.11 (1.39)
FEV <sub>1</sub> /FVC ratio (n=27)*	−1.40 (1.08)
LCI (n=27)†	7.58 (5.57)
S <sub>cond</sub> (n=23)†	9.64 (4.08)
S <sub>acin</sub> (n=23)†	5.99 (4.60)
FRC <sub>SF6</sub> , ml (n=27)	1649 (603)

\*Spirometry parameters calculated using the British growth reference charts.

†MBW z-scores calculated using Swedish normative data.

FEV<sub>1</sub>, forced expiratory volume in 1 s; FRC, functional residual capacity; FVC, forced vital capacity; FEF<sub>25–75</sub>, forced expiratory flow at 25–75% of FVC; LCI, lung clearance index; S<sub>cond</sub> and S<sub>acin</sub>, normalised phase III slope indices (see text for explanation).

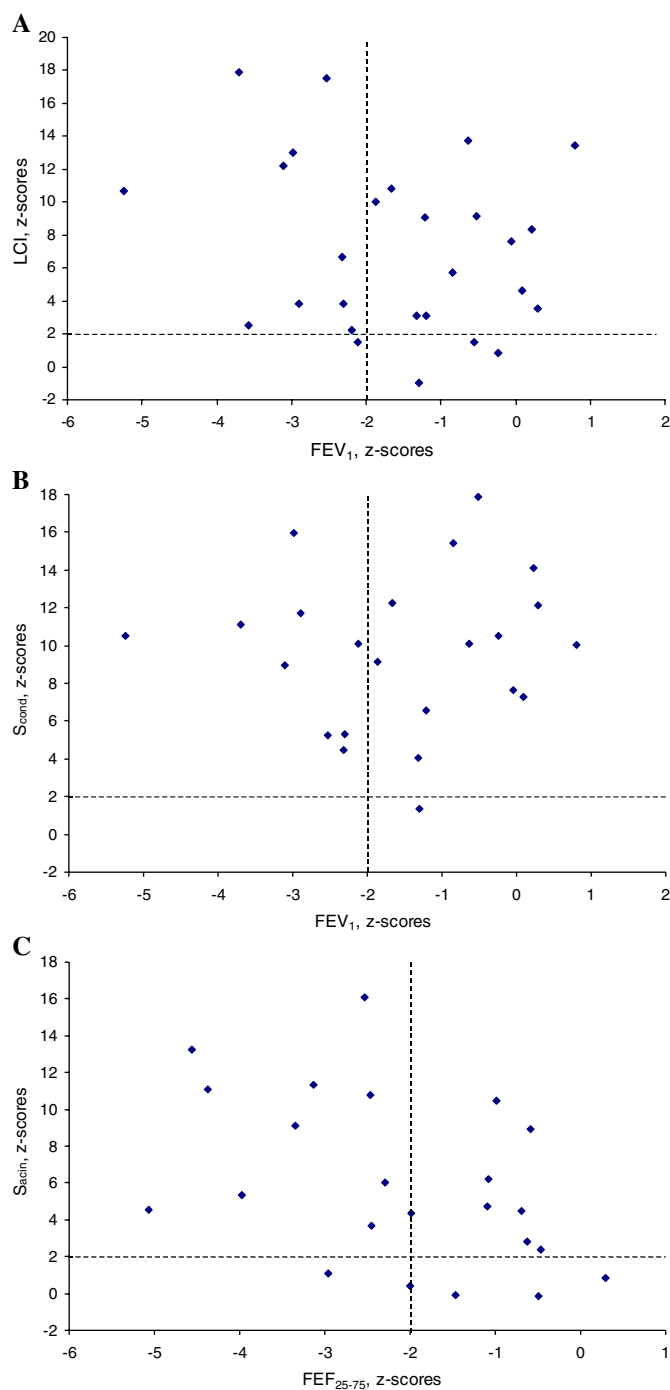


**Figure 1** (A) S<sub>cond</sub> and (B) S<sub>acin</sub> versus LCI z-scores in 23 patients with primary ciliary dyskinesia. The dashed horizontal lines denote the upper limits of normality for LCI (mean plus 1.96 SD) and the dashed vertical lines denote the upper limits of normality (mean plus 1.96 SD) for S<sub>acin</sub> and S<sub>cond</sub>, respectively. LCI, lung clearance index; S<sub>cond</sub> and S<sub>acin</sub>, normalised phase III slope indices (see text for explanation).

disease severity. Abnormal LCI was found in nearly all patients, including those with normal FEV<sub>1</sub>. Additionally, S<sub>cond</sub> was abnormal in all but one patient and S<sub>acin</sub> in more than three-quarters of the patients, implying involvement of small airways even beyond the conducting airway zone. MBW parameters did not correlate with FEV<sub>1</sub> or FVC. Measures of spirometry were on average all within or close to normal values and half the patients had normal FEV<sub>1</sub>, while the indices of VI were considerably elevated. Our findings show that PCD lung disease is characterised by marked peripheral dysfunction which, in most cases, is not detectable by spirometry.

The results of this study are consistent with previous publications showing that MBW is more sensitive than spirometry in detecting pulmonary diseases such as CF.<sup>9 12 13 24 25</sup> To our knowledge, the only other information to date on MBW data in PCD is an abstract by Ives *et al* who investigated adult patients with PCD, thus making direct comparison with our study difficult.<sup>26</sup>

In a recent large longitudinal study in the Danish PCD cohort published from our centre<sup>4</sup> we found a high degree of variation in the course of lung function after diagnosis. This variation could not be linked to age or to the level of lung function (ie, spirometry findings) at the time of diagnosis, with the



**Figure 2** (A) LCI z-scores from MBW versus  $FEV_1$  in 27 patients with primary ciliary dyskinesia (PCD). (B)  $S_{cond}$  and (C)  $S_{acin}$  from MBW versus  $FEV_1$  z-scores in 23 patients with PCD. The dashed horizontal lines denote the upper limits of normality for lung clearance index,  $S_{cond}$  and  $S_{acin}$  (mean plus 1.96 SD) and the dashed vertical lines denote the lower limits of normality (mean minus 1.96 SD) for  $FEV_1$ .  $FEV_1$ , forced expiratory volume in 1 s; LCI, lung clearance index; MBW, multiple breath inert gas washout;  $S_{cond}$  and  $S_{acin}$ , normalised phase III slope indices (see text for explanation).

conclusion that early diagnosis and initiated treatment, even in a tertiary centre, does not protect against decline in lung function.<sup>4</sup> This is in line with the current study where we did not find any relationship between the degree of VI and age at diagnosis of PCD. Possible explanations are that (1) our current monitoring and management of patients with PCD is based on

extrapolation of CF care which may not be sufficient; and (2) changes in spirometry values are unable to detect early lung damage as implied by our study. The latter may cause a delay in intensification of treatment when needed.

Results from studies in children and adults with CF suggest that  $Sn_{III}$  analysis is of limited use in more advanced disease.<sup>27</sup> Consistent with this view, we found  $S_{cond}$  to be markedly elevated even in patients with mild disease, as indicated by LCI or spirometry. In our cross-sectional analysis,  $S_{cond}$  reached a plateau and then declined with higher LCI.  $S_{cond}$  results from differences in specific ventilation and sequential filling and emptying among lung regions sharing branch points in the conducting airway zone. This index thus reflects the 'patchiness' of disease distribution. With increasing disease severity, ventilation of already poorly ventilated lung units will come to an end and inter-regional differences in ventilation non-uniformity will not increase additionally. At the same time, it could be speculated that disease progression in a distal direction and the movement of the diffusion front in a proximal direction will lead to further elevated  $S_{acin}$ . As expected,  $S_{acin}$  was more closely associated with LCI in advanced disease. In severe disease, spirometric lung volumes and forced expiratory flows are also markedly reduced due to gas trapping and because poorly ventilated regions can no longer be compensated for by increased flow through non-flow-limited distal airways.<sup>28</sup>

Despite abnormal  $FEV_1$ , one patient presented with normal LCI and  $S_{acin}$  but with highly elevated  $S_{cond}$ . In children with asthma, Gustafsson<sup>29</sup> has previously shown a more profound involvement of the small conductive airways, represented by markedly elevated  $S_{cond}$ , in comparison with the other VI indices. In asthma,  $S_{cond}$  is thus a more sensitive MBW index than LCI. The present finding could therefore be due to the presence of underlying (undiagnosed) asthma. Further studies in patients with PCD assessing bronchodilator response are consequently warranted. However, we believe that raised  $S_{cond}$  reflects a similar patchiness of disease distribution among lung units as in asthma. The finding of markedly abnormal  $S_{acin}$  values in the majority of the patients with PCD suggests that PCD airway disease generally involves more peripherally located airway generations than asthma and that, in this respect, PCD resembles CF more than asthma.

In our study three patients did not have a conclusive abnormal ciliary beat pattern and frequency. Ciliary beat pattern and frequency and EM analysis play a key role in diagnosis, but PCD is likely to include a small number of phenotypes that may be manifested by subtle or no apparent structural defects and ciliary dysfunction. Consequently, studies have documented the occurrence of normal ciliary structure<sup>30–32</sup> and function<sup>30</sup> in patients with verified PCD. All three patients had abnormal MBW parameters while  $FEV_1$  and FVC were within the normal range (see table E3 in online supplement).

### Limitations of the study

One limitation of our study is the lack of Danish MBW reference material. Instead, we used Swedish normative data as reference which were obtained using exactly the same equipment, software and procedures. In addition, the authors performing and calculating MBW tests have undergone training and have been under continual supervision by the Swedish laboratory in order to affirm the quality of the measurements.

MBW changes in PCD might reflect retained mucus in the airways resulting in VI. If so, MBW measures could improve following coughing, airway clearance manoeuvres or aerobic

exercise. This study would be strengthened with additional information about variability in MBW measures in PCD, such as day-to-day variability, morning versus afternoon variability, before and after controlled coughs or before and after airway clearance manoeuvre. Further studies on these important methodological aspects are needed.

Abnormalities of peripheral airway function might reflect potentially reversible PCD lung pathology. Owing to their sensitivity to peripheral airway dysfunction, MBW tests have the potential to be used to signal the need for and to monitor the effects of early intervention and more aggressive treatment with the aim of preventing irreversible lung damage. As PCD is associated with a progressive and continuous impact on both physical and mental health,<sup>33</sup> identification of children with early lung damage could lead to earlier and more aggressive intervention and, consequently, a better prognosis and quality of life over time.

In conclusion, our study demonstrates for the first time that MBW measures of peripheral airway function are abnormal in young patients with PCD, being far more frequent findings than abnormal spirometry. The study shows that PCD lung disease is characterised by marked peripheral dysfunction and that MBW is a promising and feasible method for early detection of airway disease in PCD. Further prospective controlled longitudinal studies assessing the utility of MBW in the management of PCD and their importance for long-term outcome are warranted.

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**Competing interests** None.

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**Contributors** All authors contributed to aspects of study design, data collection, data interpretation and manuscript review.

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## **Ventilation inhomogeneity in children with primary ciliary dyskinesia**

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### **On-Line supplement**

#### **Methods**

##### Multiple-breath sulfur hexafluoride washout method.

The studies were done in triplicate in the sitting position. The patients watched a film as distraction or a tidal volume trace on a computer screen and were instructed to keep breathing regular with a tidal volume (VT) between 10–15 ml/kg body weight (bw). They wore a nose clip and breathed through a Fleisch no.1 pneumotachograph (PNT) (Metabo SA, Lausanne, Switzerland) via a mouthpiece, connected to the PNT. A sampling tube from a mass spectrometer was introduced in the middle of the air stream between the mouthpiece and the PNT through a short connecting piece. The post-sample line external dead space was 15 ml. The test consisted of a wash-in phase during which a dry gas mixture containing 4% sulfur hexafluoride (SF<sub>6</sub>), 4% helium (He), 21% oxygen (O<sub>2</sub>), and balance nitrogen (N<sub>2</sub>) was administered using a bias flow applied on the distal port of the PNT. Wash-in was continued until the inspiratory and expiratory SF<sub>6</sub> concentrations were stable and equal, plus another 30 s. At this moment the administration of

inert gas mixture was disconnected by taking away the T-piece during an expiration and the washout phase started, and the patient breathed room air. The washout phase continued until the end-tidal SF<sub>6</sub> concentration was below 0.1% over several breaths (i.e. 1/40th of the starting concentration). The PNT was connected to a differential pressure transducer and the flow signal was demodulated and amplified. The PNT was calibrated with separate calibration constants for inspiratory and expiratory flows using a precision syringe. Recorded inspiratory and expiratory flows and volumes were converted to body temperature and ambient pressure, and saturated with water vapor conditions. Gas concentrations (SF<sub>6</sub>, He, CO<sub>2</sub> and O<sub>2</sub>) were measured at the mouth using a respiratory mass spectrometer (AMIS 2000, Innovision A/S, Odense, Denmark). Sample flow of the mass spectrometer was 20 ml/min and the gas concentration signals were updated at a rate of 33.3 hertz (Hz) and all signals were recorded at 100 Hz by a computer through an 8-channel USB AD-conversion board. The software corrected the flow signal sample-by-sample for changes in dynamic viscosity caused by the variations in gas composition. Gas samples and flow signals were aligned in time using an in-house built device for automated generation of instant gas steps. The same technique is currently used in several other pediatric lung function testing labs worldwide using this set-up and has been produced by one of the co-authors, Dr. Per Gustafsson. One of the two inert tracer gases (SF<sub>6</sub>) was used for the assessments presented in this paper. Helium was included for other assessments of ventilation distribution not presented here. Functional residual capacity (FRC) and lung clearance index (LCI) were calculated as the average value from three technically acceptable runs without evidence of gas leaks or other artifacts during washouts. FRC was determined from the cumulative exhaled marker gas (SF<sub>6</sub>) concentration divided by the differences in end-tidal gas concentration at the start of the washout and the end-tidal concentration at completion of the washout. The number of lung volume turnovers (TO) at each breath during the washout was calculated as the cumulative expired

volume (CEV) corrected for the external dead space (15 ml) up to that breath, divided by the FRC. Only one index of overall ventilation inhomogeneity (the LCI) is given. The LCI was calculated as the number of TO required to lower the end-tidal tracer gas concentration to 1/40th of the starting concentration. An increase in LCI indicates increased global non-uniformity of ventilation distribution. LCI was calculated for each washout, and the mean value of the three recordings in each patient was then calculated.

The concentration normalized slope of phase III ( $S_{nIII}$ ) for each subsequent breath during MBW was determined to calculate  $S_{cond}$  and  $S_{acin}$ . The phase III slope was converted to  $S_{nIII}$  by dividing the slope by the mean gas concentration over the slope to allow for gas dilution. The  $S_{nIII}$  was further multiplied by tidal volume (VT) giving the  $S_{nIII} * VT$  in order to account for inter-individual differences in lung size and breathing pattern.[1] The  $S_{nIII} * VT$  was used in all subsequent analyses and is henceforth referred to as  $S_{nIII}$  in this paper. For determination of  $S_{cond}$  and  $S_{acin}$ ,  $S_{nIII}$  and TO values for each subsequent breath from the three washouts were first averaged. For each breath  $S_{nIII}$  was then plotted against the corresponding TO value.  $S_{cond}$  was defined as the rate of  $S_{nIII}$  increase between TO 1.5 and 6.0.  $S_{acin}$  was defined as the first breath  $S_{nIII}$  value minus the convection-dependent inhomogeneity contribution to this value (i.e.  $S_{cond} * TO$  for the first breath).

#### Swedish normative data used as reference:

Mean, standard deviation (SD) and upper limit of normality (ULN; mean plus 1.96 RSD) for LCI were 6.33, 0.43 and 7.17, respectively.[2] Reference values for  $S_{cond}$  and  $S_{acin}$  were also obtained from the same Swedish laboratory and recently reported in a review paper.[3] Mean, SD and ULN for  $S_{cond}$  were 0.018, 0.006 and 0.030, and for  $S_{acin}$  0.086, 0.025 and 0.135, respectively.

## Additional results and discussion

Seventy per cent of the patients (19/27) showed abnormal  $FEF_{25-75}$  and 37% (10/27) abnormal  $FEV_1/FVC$  ratio. LCI showed a weak but statistically significant correlation with  $FEF_{25-75}$  ( $R^2=0.22$ ;  $p=0.01$ ) and  $FEV_1/FVC$  ratio ( $R^2=0.38$ ;  $p<0.001$ ). Among the patients with normal  $FEF_{25-75}$ , the MBW variables LCI,  $S_{cond}$  and  $S_{acin}$  were abnormal in 77% (10/13), 91% (10/11) and 73% (8/11), respectively. When relating  $Sn_{III}$  indices to spirometry variables,  $S_{acin}$  correlated weakly with both  $FEV_1/FVC$  ratio ( $R^2=0.20$ ;  $p=0.03$ ) and  $FEF_{25-75}$  ( $R^2=0.19$ ;  $p=0.04$ ), while  $S_{cond}$  did not show correlation with any of the spirometry parameters. See text and Figs. E2 and E3 in online supplement for association between MBW indices and  $FEF_{25-75}$  and  $FEV_1/FVC$  ratio, respectively. By including spirometry results performed for two patients on a different date than the MBW, correlation between  $S_{cond}$  and  $FEV_1/FVC$  ratio changed from being statistically significant to insignificant ( $R^2=0.15$ ; 0.07). Among the patients with normal  $FEF_{25-75}$ , LCI,  $S_{cond}$  and  $S_{acin}$  were abnormal in 9/13 (69%), 8/9 (89%) and 7/9 (78%), respectively (see Fig. E1). Among the patients with normal  $FEV_1/FVC$  ratio, LCI,  $S_{cond}$  and  $S_{acin}$  were abnormal in 14/17 (82%), 13/14 (93%) and 10/14 (71%), respectively (see Fig. E2).

LCI and  $S_{acin}$  both correlated to spirometry findings of airway obstruction ( $FEF_{25-75}$  and  $FEV_1/FVC$  ratio). In our study, several patients had abnormal  $FEF_{25-75}$ . This has previously shown to correlate poorly with peripheral airway abnormalities[4] and should consequently only be interpreted in case of normal FVC since mid-expiratory flow depends on FVC. In addition,  $FEF_{25-75}$  is highly variable in healthy patients,[4, 5] and abnormal values should, accordingly, be interpreted with caution.

## Figure Legends

### Figure E1.

$S_{\text{cond}}$  versus  $S_{\text{acin}}$ , z-scores, in 23 patients with primary ciliary dyskinesia (PCD). The dashed lines denote upper limits of normality (mean plus 1.96 SD).

### Figure E2.

a) Lung Clearance Index (LCI) from MBW versus forced expiratory flow at 25%-75% of forced vital capacity ( $\text{FEF}_{25-75}$ ), z-scores, in 27 patients with primary ciliary dyskinesia (PCD). b)  $S_{\text{cond}}$  from MBW versus  $\text{FEF}_{25-75}$ , z-scores, in 23 patients with PCD. c)  $S_{\text{acin}}$  from MBW versus  $\text{FEF}_{25-75}$ , z-scores, in 23 patients with PCD. The dashed horizontal lines denote the upper limits of normality for LCI,  $S_{\text{cond}}$  and  $S_{\text{acin}}$  (mean plus 1.96 SD). The dashed vertical lines denote the lower limits of normality (mean minus 1.96 SD) for  $\text{FEF}_{25-75}$ .

### Figure E3.

a) Lung Clearance Index (LCI) from MBW versus ratio between forced expiratory volume in one second and forced vital capacity ( $\text{FEV}_1/\text{FVC}$  ratio), z-scores, in 25 patients with primary ciliary dyskinesia (PCD). b)  $S_{\text{cond}}$  from MBW versus  $\text{FEV}_1/\text{FVC}$  ratio, z-scores, in 23 patients with PCD. c)  $S_{\text{acin}}$  from MBW versus  $\text{FEV}_1/\text{FVC}$  ratio, z-scores, in 23 patients with PCD. The dashed horizontal lines denote the upper limits of normality for LCI,  $S_{\text{cond}}$  and  $S_{\text{acin}}$  (mean plus 1.96 SD). The dashed vertical lines denote the lower limits of normality (mean minus 1.96 SD) for  $\text{FEV}_1/\text{FVC}$  ratio.

	LCI	S <sub>cond</sub>	S <sub>acin</sub>
S <sub>cond</sub>	R <sup>2</sup> : 0.06 ns	-	-
S <sub>acin</sub>	<b>R<sup>2</sup>: 0.45</b> <b>p&lt;0.001</b>	R <sup>2</sup> : 0.02 ns	-
FEV <sub>1</sub>	R <sup>2</sup> : 0.05 ns	R <sup>2</sup> : 0.006 ns	R <sup>2</sup> : 0.07 ns
FVC	R <sup>2</sup> : 0.02 ns	R <sup>2</sup> : 0.08 ns	R <sup>2</sup> : 0.00 ns
FEF <sub>25-75</sub>	<b>R<sup>2</sup>: 0.22</b> <b>p=0.010</b>	R <sup>2</sup> : 0.01 ns	<b>R<sup>2</sup>: 0.19</b> <b>p=0.040</b>
FEV <sub>1</sub> /FVC	<b>R<sup>2</sup>: 0.38</b> <b>p&lt;0.001</b>	R <sup>2</sup> : 0.15 ns	<b>R<sup>2</sup>: 0.20</b> <b>p=0.030</b>

**Table E1: Summary of correlations between indices of MBW and spirometry parameters.**

Correlations were assessed using linear regression. Presented as:  $R^2$ ;  $p$ -value. Correlations with  $p$ -values less than 0.05 are highlighted in bold. LCI: Lung Clearance Index. S<sub>cond</sub> and S<sub>acin</sub>:

Normalized phase III slope indices (see text for explanation). FEV<sub>1</sub>: forced expiratory volume in one second. FVC: forced vital capacity. FEF<sub>25-75</sub>: forced expiratory flow at 25%-75% of FVC.

	Age	Age at diagnosis
LCI	$R^2$ : 0.00 ns	$R^2$ : 0.09 ns
$S_{\text{cond}}$	$R^2$ : 0.002 ns	$R^2$ : 0.007 ns
$S_{\text{acin}}$	$R^2$ : 0.001 ns	$R^2$ : 0.11 ns

**Table E2. Association between MBW indices and age and age at diagnosis.**

Correlations were assessed using linear regression. Presented as:  $R^2$ ; *p-value*. LCI: Lung Clearance Index.  $S_{\text{cond}}$  and  $S_{\text{acin}}$ : Normalized phase III slope indices (see text for explanation)

Patient	LCI, absolute value	LCI, z-scores	S <sub>cond</sub> , z-scores	S <sub>acin</sub> , z-scores	FEV <sub>1</sub> , z-scores	FVC, z-scores
1	10.25	9.11	6.55	-0.08	-1.21	-0.68
2	8.80	5.73	15.42	3.67	-0.85	0.94
3	14.01	17.85	11.10	13.22	-3.70	-0.68

**Table E3: Results of the three patients with less definite diagnosis of PCD.**

LCI: Lung Clearance Index. S<sub>cond</sub> and S<sub>acin</sub>: Normalized phase III slope indices (see text for explanation). FEV<sub>1</sub>: forced expiratory volume in one second. FVC: forced vital capacity.

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Figure E1

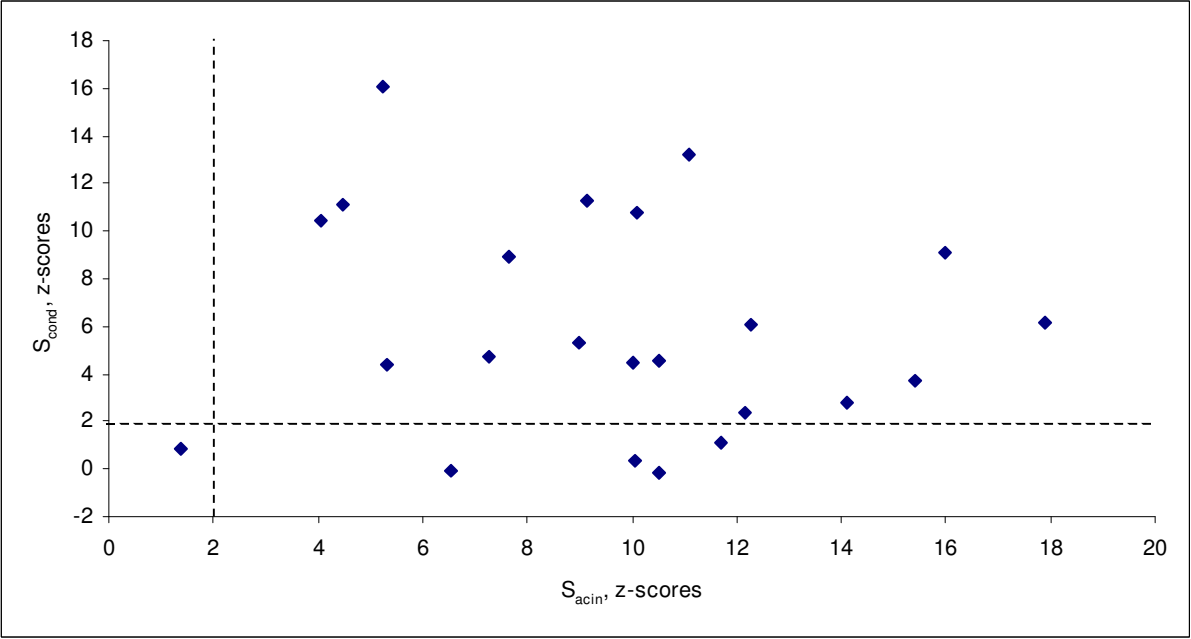
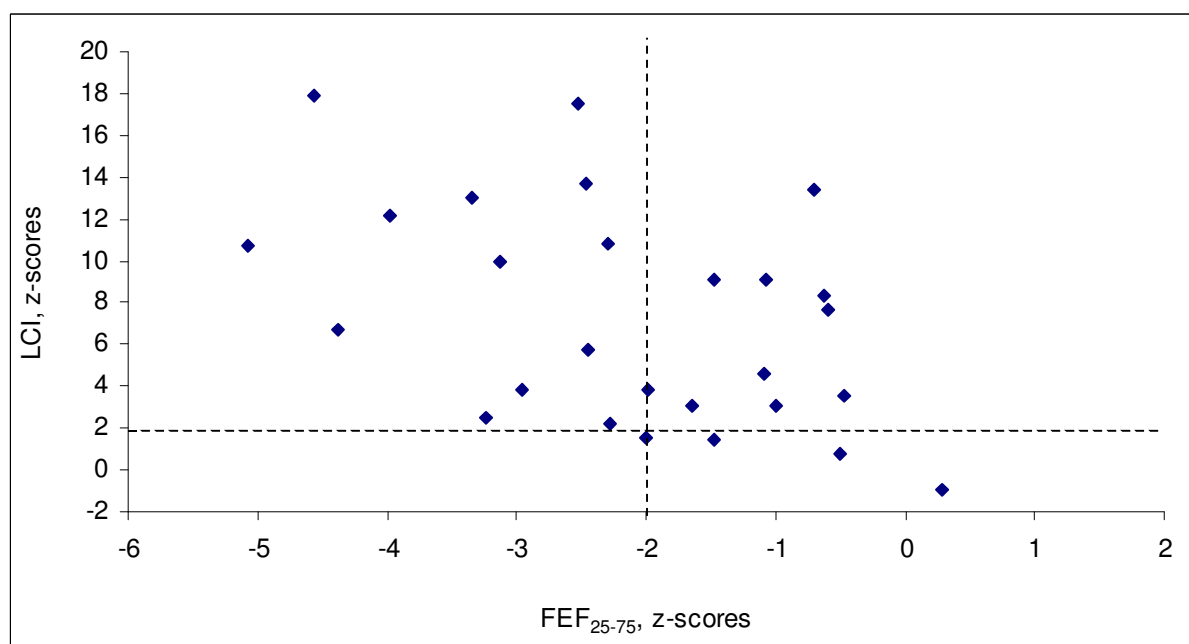
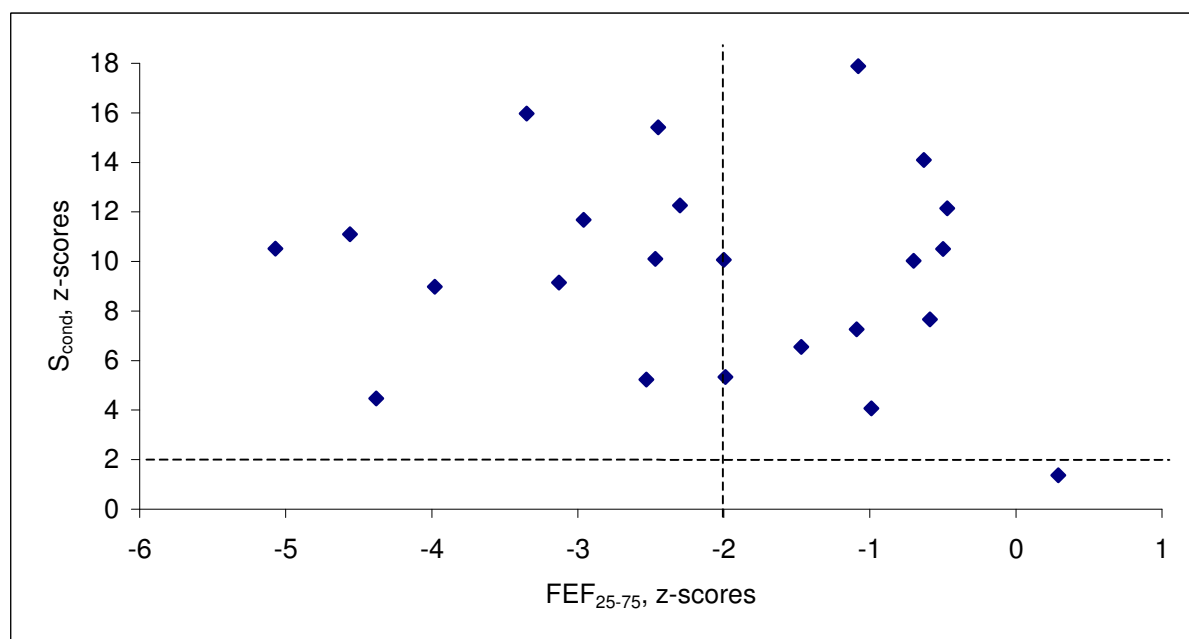


Figure. E2

**A**



**B**



C

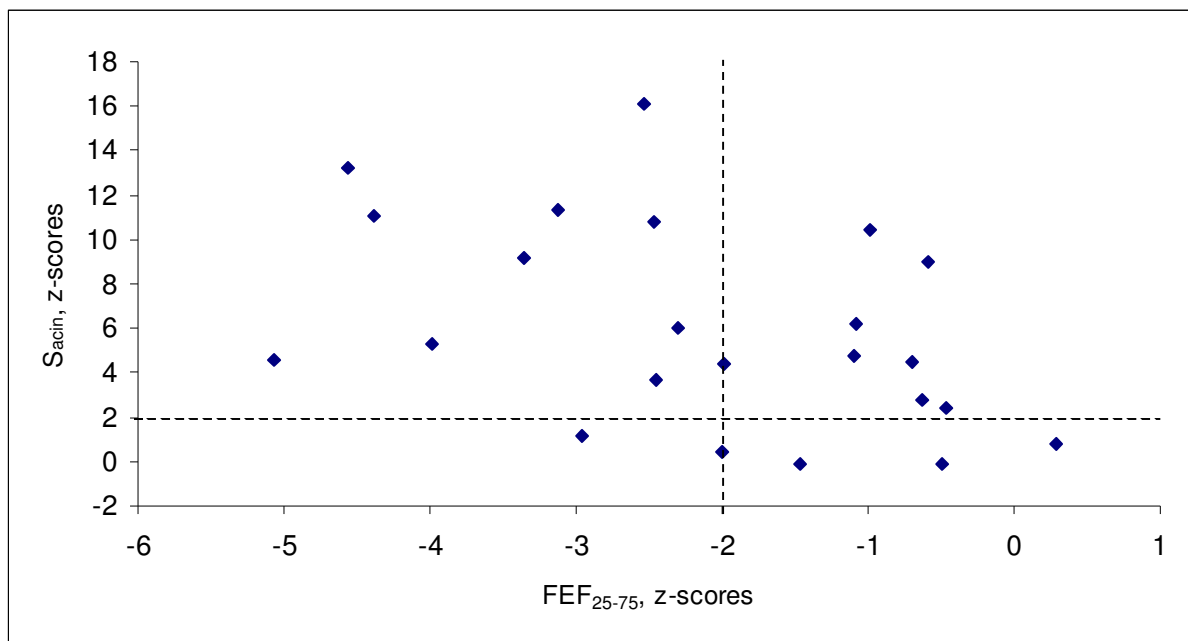
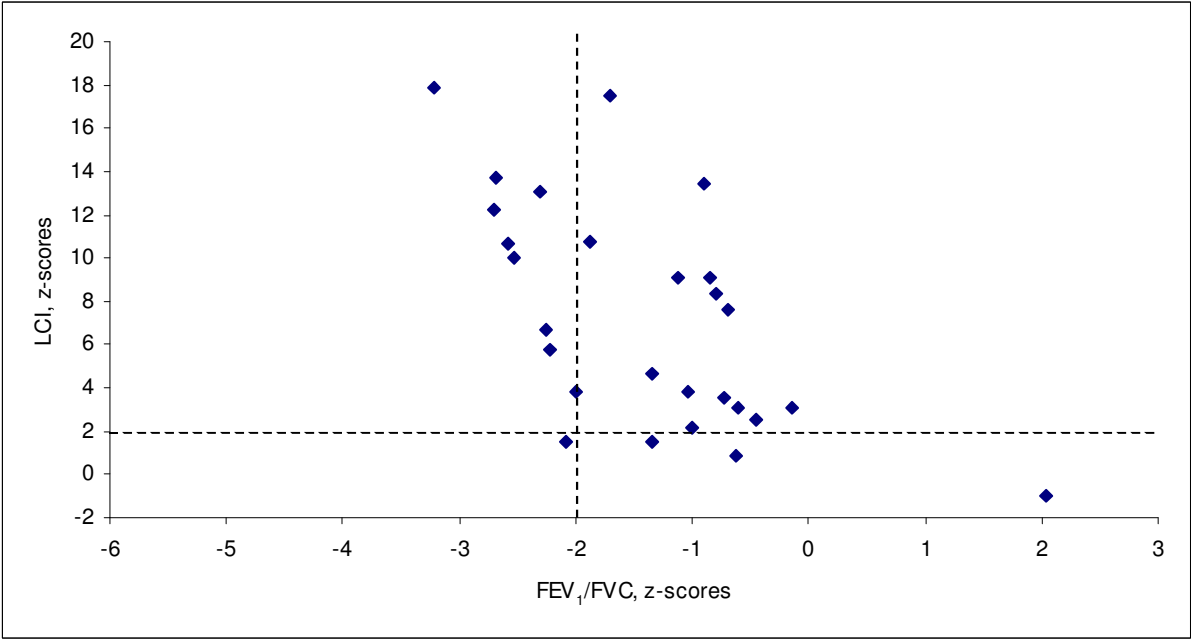
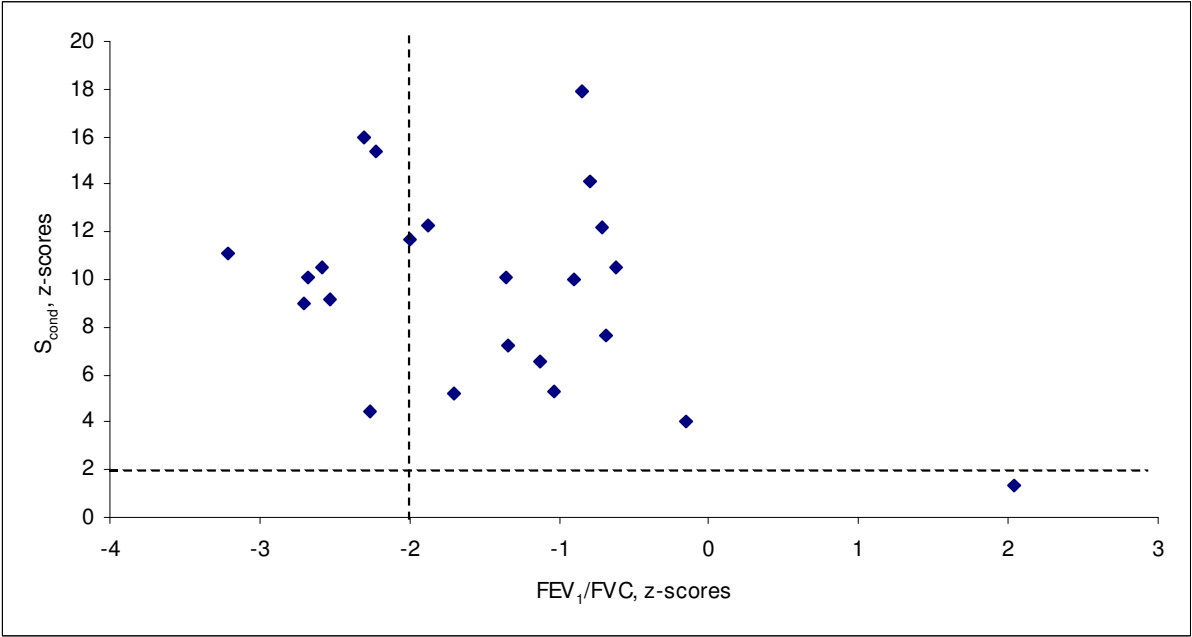


Figure E3

A



B



C

