Ventilation inhomogeneity in children with primary ciliary dyskinesia

Kent Green,1 Frederik F Buchvald,1 June Kehlet Marthin,1 Birgitte Hanel,1 Per M Gustafsson,2 Kim Gjerme Nielsen1

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 (FEV1) 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 Scond and Sacin reflect function of the small conducting and acinar airways, respectively. The involvement of the peripherical 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, Scond (n=23) and Sacin (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, Scond and Sacin were abnormal in 85%, 96% and 78% of patients with PCD and in 81%, 93% and 79%, respectively, of 13/27 subjects with normal FEV1. LCI and Sacin correlated significantly while Scond 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.1,2 Patients with PCD most often present with persistent rhinitis and chronic productive cough, but the heterogeneous nature of the disease makes early diagnosis difficult.3 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 (FEV1) and forced vital capacity (FVC) even after early diagnosis.4 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.5

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),6 and radiological changes include bronchiectasis, mucus plugging and peribronchial thickening.7 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.8 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.9–12 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 tests9–13 and to be predictive of subsequent lung function when measured at preschool age.10 In addition, determination of concentration normalised phase III slope (SIII) indices allows assessment of VI arising in the small conductive airway zone (Scond) and more peripherally close to or within the acinar airway zone (Sacin).14 Peripheral airway function assessed by LCI, Scond and Sacin 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.15

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.4
Paediatric lung disease

Study patients
Patients with a diagnosis of PCD aged ≤18 years were eligible for the study. All patients had a consistent history of symptoms characteristic of PCD,16 17 and basic tests to rule out CF and immunodeficiency were performed. Nasal nitric oxide measurement (nNO) was used as a preliminary screen, test, although without necessarily excluding patients with a high suspicion of PCD.18 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.1 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 (SF₆) washout was performed in all patients using a mass spectrometer (AMIS 2000, Innovision, Odense, Denmark) for gas analysis, as previously described.13 The LCI and the concentration normalised slope III indices (S_{acin} and S_{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.15 The mean LCI result from three MBW measurements in each patient was used for analysis. The concentration normalised slope of phase III (S_{III}) for each subsequent breath during MBW was determined to calculate S_{cond} and S_{acin}. The phase III slope was converted to SnIII by dividing the slope by the mean gas concentration over the slope to allow for gas dilution. The S_{III} was further multiplied by tidal volume (VT) giving the SnIII = VT in order to account for inter-individual differences in lung size and breathing pattern.19 The S_{III} VT was used in all subsequent analyses and is henceforth referred to as SnIII in this paper. For determination of S_{cond} and S_{acin}, S_{III} and TO values for each subsequent breath from the three washouts were first averaged. For each breath, SnIII was then plotted against the corresponding TO value. S_{cond} was defined as the rate of SnIII increase between TO 1.5 and 6.0. S_{acin} was defined as the first breath SnIII value minus the convection-dependent inhomogeneity contribution to this value (ie, S_{cond} × 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.15 20 The authors performing and statistical analyses 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.20

Spirometry
Spirometry was performed according to ATS/ERS standards21 and FEV₁, FVC, forced expiratory flow at 25–75% of FVC (FEF₂₅–₇₅%) and FEV₁/FVC ratio were recorded. The recently published ‘all ages’ reference equations were used.22 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.22 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 V9.2 (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. SnIII 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,15 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: LCI=9.48 (2.20) vs 6.53 (0.43), ULN=7.17; S_{cond}=0.076 (0.024) vs 0.018 (0.006), ULN=0.030; and S_{acin}=0.236 (0.115) vs 0.086 (0.025), ULN=0.135. LCI was above the ULN in 84% of patients (23/27), while 96% (22/23) and 78% (18/25) had abnormal S_{cond} and S_{acin} respectively.

The relationships between LCI and the S_{III} indices S_{acin} and S_{cond} are shown in figure 1A and B. LCI correlated to S_{acin} (R²=0.45; p<0.001) but not to S_{cond}. S_{cond} peaked at an LCI of about 10 z-scores, subsequently decreasing with increase in disease severity (as measured by LCI). S_{acin} and S_{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 FEV₁ and 15% (4/27) had abnormal FVC.

LCI did not show a statistically significant correlation with either FEV₁ or FVC. LCI, S_{cond} and S_{acin} were abnormal in 81% (13/16), 93% (13/14) and 79% (11/14), respectively, among the 13/27 patients with normal FEV₁ (figure 2A–C). Normal LCI excluded the presence of abnormal FEV₁, with the exception of a marginally reduced FEV₁ (−2.1 z-scores) in one patient. In addition, this patient had S_{acin} within the normal range while S_{cond} was elevated at more than 10 z-scores. When relating SnIII indices to spirometry parameters, neither S_{acin} nor S_{cond} correlated with FEV₁ 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 FEV₁ 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

<table>
<thead>
<tr>
<th>Demographics</th>
<th>Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female/male (n)</td>
<td>17/10</td>
</tr>
<tr>
<td>Age, years</td>
<td>11.3 (6.3–18.5)</td>
</tr>
<tr>
<td>Age at diagnosis, years</td>
<td>2.2 (0.04–9.8)</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>36.5 (16.1–61.6)</td>
</tr>
<tr>
<td>Height, cm</td>
<td>145 (114–182)</td>
</tr>
<tr>
<td>Bacterial colonisation (n)</td>
<td></td>
</tr>
<tr>
<td>Chronic: <em>P</em> aeruginosa</td>
<td>1</td>
</tr>
<tr>
<td>Intermittent: <em>P</em> aeruginosa</td>
<td>2</td>
</tr>
<tr>
<td>Intermittent: <em>A</em> xylosodans</td>
<td>1</td>
</tr>
</tbody>
</table>

**Diagnostic tests (n)**

| 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 |
| Ultrastructural defect       |    |
| 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.

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 FEF25–75 and FEV1/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 FEV1, the currently accepted spirometry surrogate marker of disease severity. Abnormal LCI was found in nearly all patients, including those with normal FEV1. Additionally, Scond was abnormal in all but one patient and Sacin 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 FEV1 or FVC. Measures of spirometry were on average all within or close to normal values and half the patients had normal FEV1, 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*. 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.

In a recent large longitudinal study in the Danish PCD cohort published from our centre 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...
versus FEV1 z-scores in 23 patients with PCD. The dashed horizontal
breath inert gas washout; Scond and Sacin, normalised phase III slope
indices (see text for explanation).

lines denote the upper limits of normality for lung clearance index, Scond
monitoring and management of patients with PCD is based on

conclusion that early diagnosis and initiated treatment, even in
a tertiary centre, does not protect against decline in lung func-
tion.4 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 Scond analysis is of limited use in more advanced disease.27
Consistent with this view, we found Scond to be markedly
elevated even in patients with mild disease, as indicated by LCI
or spirometry. In our cross-sectional analysis, Scond reached
a plateau and then declined with higher LCI. Scond 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 ‘patch-
iness’ 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 direc-
tion and the movement of the diffusion front in a proximal
direction will lead to further elevated Sacin. As expected, Sacin
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 compen-
sated for by increased flow through non-flow-limited distal
airways.26

Despite abnormal FEV1, one patient presented with normal
LCI and Sacin but with highly elevated Scond. In children with
asthma, Gustafsson29 has previously shown a more profound
involvement of the small conductive airways, represented by
markedly elevated Scond, in comparison with the other VI
indices. In asthma, Scond 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 Scond
reflects a similar patchiness of disease distribution among lung
units as in asthma. The finding of markedly abnormal Sacin
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 structure30–32 and function30 in
patients with verified PCD. All three patients had abnormal
MBW parameters while FEV1 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, soft-
ware 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

Figure 2  (A) LCI z-scores from MBW versus FEV1 in 27 patients with
primary ciliary dyskinesia (PCD). (B) Scond and (C) Sacin, from MBW
versus FEV1 z-scores in 23 patients with PCD. The dashed horizontal
diagonals denote the upper limits of normality for lung clearance index, Scond
and Sacin (mean plus 1.96 SD) and the dashed vertical lines denote the
lower limits of normality (mean minus 1.96 SD) for FEV1. FEV1, forced
expiratory volume in 1 s; LCI, lung clearance index; MBW, multiple
breath inert gas washout; Scond and Sacin, normalised phase III slope
indices (see text for explanation).
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 progression of abnormal lung pathology. Owing to their potential reversibility, MBW tests have been proposed as a marker of lung pathology in PCD. Owing to their potential reversibility, MBW tests have been proposed as a marker of lung pathology 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.

Acknowledgements The authors thank members of the staff at the Danish PCD Center and Pediatric Pulmonary Service at Copenhagen University Hospital and the families who participated in the study.

Funding This study has been funded by The John and Birthe Meyer Foundation, Queen Louise Children's Hospital Research Trust and Aase and Ejnar Danielsen Foundation.

Competing interests None.

Ethics approval Ethics approval was provided by the Danish National Committee on Biomedical Research Ethics.

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

Provenance and peer review Not commissioned; externally peer reviewed.

REFERENCES

Ventilation inhomogeneity in children with primary ciliary dyskinesia

Kent Green¹, Frederik F. Buchvald¹, June K. Marthin¹, Birgitte Hanel¹, Per M. Gustafsson², Kim G. Nielsen¹.

¹Danish PCD Center & Pediatric Pulmonary Service, Department of Pediatrics, Copenhagen University Hospital, Denmark. ²Pediatric Respiratory Physiology Laboratory, Department of Pediatrics, Central Hospital, Skövde, Sweden.

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 (SF₆), 4% helium (He), 21% oxygen (O₂), and balance nitrogen (N₂) was administered using a bias flow applied on the distal port of the PNT. Wash-in was continued until the inspiratory and expiratory SF₆ 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 SF₆ 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 (SF₆, He, CO₂ and O₂) 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 (SF₆) 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 (SF₆) 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 (Sn_{III}) for each subsequent breath during MBW was determined to calculate S_{cond} and S_{acin}. The phase III slope was converted to Sn_{III} by dividing the slope by the mean gas concentration over the slope to allow for gas dilution. The Sn_{III} was further multiplied by tidal volume (VT) giving the Sn_{III} * VT in order to account for interindividual differences in lung size and breathing pattern.[1] The Sn_{III} * VT was used in all subsequent analyses and is henceforth referred to as Sn_{III} in this paper. For determination of S_{cond} and S_{acin}, Sn_{III} and TO values for each subsequent breath from the three washouts were first averaged. For each breath Sn_{III} was then plotted against the corresponding TO value. S_{cond} was defined as the rate of Sn_{III} increase between TO 1.5 and 6.0. S_{acin} was defined as the first breath Sn_{III} 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 (FEF$_{25-75}$), z-scores, in 27 patients with primary ciliary dyskinesia (PCD). b) $S_{\text{cond}}$ from MBW *versus* FEF$_{25-75}$, z-scores, in 23 patients with PCD. c) $S_{\text{acin}}$ from MBW *versus* 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 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 (FEV$_1$/FVC ratio), z-scores, in 25 patients with primary ciliary dyskinesia (PCD). b) $S_{\text{cond}}$ from MBW *versus* FEV$_1$/FVC ratio, z-scores, in 23 patients with PCD. c) $S_{\text{acin}}$ from MBW *versus* FEV$_1$/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 FEV$_1$/FVC ratio.
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. $S_{\text{cond}}$ and $S_{\text{acin}}$: Normalized phase III slope indices (see text for explanation). FEV$_1$: forced expiratory volume in one second. FVC: forced vital capacity. FEF$_{25-75}$: forced expiratory flow at 25%-75% of FVC.

<table>
<thead>
<tr>
<th></th>
<th>LCI</th>
<th>$S_{\text{cond}}$</th>
<th>$S_{\text{acin}}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$S_{\text{cond}}$</td>
<td>$R^2$: 0.06</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>ns</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$S_{\text{acin}}$</td>
<td>$R^2$: 0.45</td>
<td>$R^2$: 0.02</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>$p&lt;0.001$</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>FEV$_1$</td>
<td>$R^2$: 0.05</td>
<td>$R^2$: 0.006</td>
<td>$R^2$: 0.07</td>
</tr>
<tr>
<td></td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>FVC</td>
<td>$R^2$: 0.02</td>
<td>$R^2$: 0.08</td>
<td>$R^2$: 0.00</td>
</tr>
<tr>
<td></td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>FEF$_{25-75}$</td>
<td>$R^2$: 0.22</td>
<td>$R^2$: 0.01</td>
<td>$R^2$: 0.19</td>
</tr>
<tr>
<td></td>
<td>$p=0.010$</td>
<td>ns</td>
<td>$p=0.040$</td>
</tr>
<tr>
<td>FEV$_1$/FVC</td>
<td>$R^2$: 0.38</td>
<td>$R^2$: 0.15</td>
<td>$R^2$: 0.20</td>
</tr>
<tr>
<td></td>
<td>$p&lt;0.001$</td>
<td>ns</td>
<td>$p=0.030$</td>
</tr>
<tr>
<td></td>
<td>Age at diagnosis</td>
<td>Age at diagnosis</td>
<td></td>
</tr>
<tr>
<td>----------------</td>
<td>------------------</td>
<td>------------------</td>
<td></td>
</tr>
<tr>
<td>LCI</td>
<td>R(^2): 0.00</td>
<td>R(^2): 0.09</td>
<td></td>
</tr>
<tr>
<td></td>
<td>ns</td>
<td>ns</td>
<td></td>
</tr>
<tr>
<td>S(_{\text{cond}})</td>
<td>R(^2): 0.002</td>
<td>R(^2): 0.007</td>
<td></td>
</tr>
<tr>
<td></td>
<td>ns</td>
<td>ns</td>
<td></td>
</tr>
<tr>
<td>S(_{\text{acin}})</td>
<td>R(^2): 0.001</td>
<td>R(^2): 0.11</td>
<td></td>
</tr>
<tr>
<td></td>
<td>ns</td>
<td>ns</td>
<td></td>
</tr>
</tbody>
</table>

**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).
<table>
<thead>
<tr>
<th>Patient</th>
<th>LCI, absolute value</th>
<th>LCI, z-scores</th>
<th>Scond, z-scores</th>
<th>Sacin, z-scores</th>
<th>FEV₁, z-scores</th>
<th>FVC, z-scores</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10.25</td>
<td>9.11</td>
<td>6.55</td>
<td>-0.08</td>
<td>-1.21</td>
<td>-0.68</td>
</tr>
<tr>
<td>2</td>
<td>8.80</td>
<td>5.73</td>
<td>15.42</td>
<td>3.67</td>
<td>-0.85</td>
<td>0.94</td>
</tr>
<tr>
<td>3</td>
<td>14.01</td>
<td>17.85</td>
<td>11.10</td>
<td>13.22</td>
<td>-3.70</td>
<td>-0.68</td>
</tr>
</tbody>
</table>

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


**REFERENCES**


Figure E2

A

![Graph A](image)

FEF<sub>25-75</sub>, z-scores

LCI, z-scores

B

![Graph B](image)

S<sub>cond</sub>, z-scores

FEF<sub>25-75</sub>, z-scores
Figure E3

A

B
C

![Graph showing scatter plot of FEV\textsubscript{1}/FVC, z-scores vs. S\textsubscript{acini}, z-scores.](image)