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One-year evolution and variability in multiple-breath washout indices in children and young adults with primary ciliary dyskinesia

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ABSTRACT

Background and objective: Cross-sectional and longer-term studies have demonstrated abnormal yet stable multiple-breath inert gas washout (MBW) indices in patients with primary ciliary dyskinesia (PCD). This study aimed to assess the intermediate term evolution and the between-occasion variability of MBW indices in PCD over 1 year.

Methods: Children and young adults with a confirmed diagnosis of PCD were included in this single-centre, prospective, observational, longitudinal study. Over 1 year, nitrogen (N_2) MBW and spirometry were performed at three occasions during ordinary scheduled outpatient visits. Trends and variability in lung clearance index (LCI), moment ratios, normalized N_2 concentration at six lung volume turnovers, and regional ventilation inhomogeneity indices of the conducting and intra-acinar airways (S_{cond}*V_T and S_{acin}*V_T) were analysed using linear mixed models.

Results: Forty-two patients, aged 6–29 years (median: 15.4), performed 116 N_2 MBW test occasions and 96.6% were technically acceptable. A minimal, although significant, increase in LCI over 1 year (mean: 0.51 units, 95% CI: 0.12–0.91, $p = 0.01$) was found; while, all other N_2 MBW indices and FEV_1 remained unchanged. A moderate correlation was observed between LCI and FEV_1 ($r = -0.47$, $p = 0.0001$). The limits of agreement between tests 1 year apart were for LCI: −1.96 to 2.98; S_{cond}*V_T: ± 0.039; S_{acin}*V_T: −0.108 to 0.128.

Conclusions: Children and young adults with PCD managed at a specialist centre showed slightly, but significant, increasing LCI and otherwise unchanged ventilation inhomogeneity indices and dynamic volumes over the intermediate term of 1 year. Estimates of the variability of N_2 MBW indices may inform sample size calculations of future randomized controlled trials.

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KEYWORDS
Lung clearance index; lung function; moment ratio; ventilation inhomogeneity

Introduction

The genetic, respiratory disease primary ciliary dyskinesia (PCD) results from immotile or dyskinetic cilia, causing defective mucociliary clearance. Accumulation of mucus, bacteria, and debris in the airways gives rise to recurrent respiratory tract infections, which may progress to chronic infection and bronchiectasis [1–4]. PCD causes progressive loss of lung function [5–7]. The aim of PCD management is to maintain normal lung function and prevent decline as long as possible [4]. The routine procedure for monitoring pulmonary function in school-aged children and adults with PCD is spirometry [1,3]. However, spirometric measures of flow and volume are primarily influenced by airway resistance of the larger airways, which makes spirometry mostly sensitive for detecting proximal airway disease [8]. Cross-sectional studies of ventilation inhomogeneity, measured by multiple-breath inert gas washout (MBW), in PCD have demonstrated abnormal values [9–13], even in patients with normal spirometric values [9,12] – reflecting peripheral airway involvement. The lung clearance index (LCI) and the moment ratios (MRs), derived from MBW, are measures of the overall ventilation distribution inhomogeneity in the lungs, of which the LCI is by far the most commonly reported [8]. In recent years, several studies [13–18] have investigated the possibilities of shortening test duration without loss of sensitivity. A study from our group [18] suggested the normalized concentration of nitrogen (N_2) when six lung volume turnovers have been reached (Cn@TO6) as a possible endpoint for abbreviation of the MBW test in school-aged children with PCD. Besides measures of overall ventilation inhomogeneity, the MBW may provide insight into the

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mechanisms behind the ventilation inhomogeneity. Thus, the indices S_{cond}*V_T and S_{acin}*V_T are thought to reflect the ventilation inhomogeneity arising in the small conducting and acinar airways, respectively [8]. In a cohort of 23 children and adolescents with PCD, S_{cond}*V_T and S_{acin}*V_T were abnormal in 96% and 78%, respectively [9].

The intermediate term longitudinal trends and natural variability in MBW indices in PCD have not been previously presented. Estimates of the between-occasion variability of MBW indices are essential for designing interventional studies utilizing the indices as outcome measures.

The overall aim of this study was to assess the intermediate term evolution and between-occasion variability of N_2 MBW indices in PCD patients based on repeated measurements over 1 year. The primary objective was to examine if the LCI changed over 1 year, and the secondary objective if MRs, Cn@TO6, S_{cond}*V_T and S_{acin}*V_T changed. The trends in the forced expiratory volume in 1 s (FEV_1), and the FEV_1/forced vital capacity (FVC) were simultaneously evaluated for comparison.

Materials and methods

Study design

This was a single-centre, prospective, observational, longitudinal study of PCD patients. Study procedures were performed on three random visits within 1 year when patients attended the outpatient clinic for scheduled routine visits.

Participants

School-aged children and young adults (aged >5 to <30 years) with a confirmed diagnosis of PCD were eligible for participation in the study and were recruited from the national PCD centre in Copenhagen, Denmark. Participants had to be clinically stable at the baseline visit to be included. Thereafter, study procedures were performed at the patients' routine clinic visits, regardless of clinical status. Ongoing symptoms of upper and lower respiratory tract exacerbation and antibiotic therapy at the test occasions were recorded in the medical records and in the case report forms. Participants were considered to have exacerbation at a test occasion if they reported ongoing worsening respiratory symptoms, which led to the start of systemic antibiotic treatment within 1 week before or at the visit.

Patients were considered to have chronic *Pseudomonas aeruginosa* infection on a test occasion if *P. aeruginosa* was cultured in ≥50% of the mucus samples from the past year provided at least four annual samples were filed.

Details of diagnosis and clinical management can be found in the Supplementary material.

Study procedures

N_2 MBW

The commercially available EXHALYZER® D and the associated software SPIROWARE® version $3.1.6_{ext}$ (ECO MEDICS AG, Duernten, Switzerland) were utilized for the N_2 MBW measurements and calculation of the N_2 MBW indices. The N_2 MBW was performed according to the European Respiratory Society/American Thoracic Society consensus statement [19]. The means of the N_2 MBW indices from intentionally three and at least two technically acceptable measurements performed at each test occasion were reported in absolute values. The N_2 MBW indices of overall ventilation inhomogeneity evaluated in the study were LCI, M_1/M_0 and M_2/M_0 (ratio of first to zeroth and second to zeroth moment of the washout, respectively) [20], and Cn@TO6. Ventilation inhomogeneity thought to arise in the small conductive and acinar airway zones were assessed by the concentration normalized phase III slope indices S_{cond}*V_T and S_{acin}*V_T (normalized phase III slope indices multiplied by tidal volume to account for differences in lung size and breathing pattern) [19].

Results of the N_2 MBW tests were unknown for treating physicians, hence did not influence the clinical management.

Spirometry

Dynamic lung volumes were measured by spirometry (JAEGER MasterScreen, CareFusion, Hoechberg, Germany) according to European Respiratory Society/American Thoracic Society standards [21]. The percent predicted values and *z*-scores were calculated using all-ages prediction equations for spirometry from the Global Lung Function Initiative [22].

Microbiological analysis of mucus

Mucus samples for microbiological analysis were collected at all test occasions, preferably as sputum samples obtained by expectoration. Laryngeal suction was used for patients, who could not expectorate due to their young age or low sputum production. Mucus samples were included in the data analysis if they originated within 1 week before or after the test occasion, and the mucus

samples were considered positive for bacteria if the culture was positive, regardless of the microscopy results.

Statistical analysis

Patient characteristics were presented by medians (ranges) or numbers and percentages of total, as appropriate. Pulmonary function variables were presented by means (SD). For each pulmonary function variable, a linear mixed model was applied to assess whether there was a trend over time. The models included time on study as a continuous fixed effect. To account for the repeated measures design, the models included patient as a random effect. The analyses were adjusted for age at inclusion and age at diagnosis as fixed effects. Due to a nonlinear effect of age at diagnosis for some of the outcome variables, age at diagnosis was modelled using B-splines [23]. The model is described in more detail in the Supplementary material.

Based on the linear mixed models, intraclass correlation coefficients (ICC) were calculated, and between-occasion variability was determined as the limits of agreement between two tests of the outcome variable (further details in the Supplementary material). The 95% CI for ICC and limits of agreement were calculated using parametric bootstrap with $B = 1000$ bootstrap samples, parametrically bootstrapping random effects and residual errors [24].

The effect of possible time-dependent predictor variables was assessed by introducing these one by one in the models: positive culture of any bacteria at the test occasion (yes/no); positive culture of *P. aeruginosa* at the test occasion (yes/no); chronic *P. aeruginosa* infection (yes/no); and exacerbation at the test occasion (yes/no).

The correlation between the LCI and the FEV_1 *z*-score was determined from a joint model for the two outcomes by combining the linear mixed models described above and by introducing a correlation between the two random intercepts as well as between the two residual error terms [25].

We assumed missingness to be 'missing at random' (MAR), in which case analyses using linear mixed models are valid [26]. The statistical analyses were performed using R version 3.3.1 (R Foundation for Statistical Computing, Vienna, Austria) and using SAS version 9.4 (SAS Institute Inc., Cary, NC, USA). Two-sided *p*-values <0.05 were considered statistically significant.

Ethics

The study was approved by The Committees on Biomedical Research Ethics of the Capital Region of Denmark (Protocol no.: H-1-2010-042). Informed consent for participation in the study was obtained from all participants or their parents/guardians if subjects were aged <18 years prior to enrolment in the study.

Results

Forty-six patients treated for PCD agreed to participate in the study. Of these, three patients did not fulfil the complete diagnostic inclusion criteria for the study, and one patient did not meet the inclusion criterion of clinical stability at the baseline visit. Six of the 42 included participants withdrew from the study during the follow-up period (four patients due to non-medical reasons and two patients due to infections with intensified treatment burden). Two additional participants did not attend the intermediate test occasion. Patient characteristics are shown in Table 1. There were 116 test occasions performed of which 100 (86.2%) had three and 112 (96.6%) at least two acceptable N_2 MBW measurements. The median (interquartile range) time intervals between the first and the second test occasion and between the first and the third test occasion were 3.8 months (3.1–5.5) and 12.3 months (11.9–13.1), respectively (Figure 1).

Pulmonary function variables are shown in Table 2. The LCI changed minimally but significantly over time (mean increase 0.51, 95% CI: 0.12–0.91, $p = 0.01$), whereas MRs, Cn@TO6, $S_{cond}{}^{*}V_T$, and $S_{acin}{}^{*}V_T$ did not change significantly over the 1-year observation

Table 1. Patient characteristics.

	Baseline visit ($n = 42$)	All visits (No. of visits = 116)
Sex (males/females)	16/26 (38%/62%)	
Age (years)	15.4 (6.5–29.7)	
Age at diagnosis (years)	6.6 (0.0–27.9)	
Body mass index (kg/m^2)	19.8 (13.7–39.0)	
Diagnostics		
Abnormal ciliary beat pattern and/or frequency	42 (100%)	
Abnormal ciliary ultra-structure[a]	35 (83.3%)	
Abnormally low nasal NO production[b]	41 (97.6%)	
Known pathogenic bi-allelic mutations[c]	13 (31.0%)	
Microbiology		
Available mucus samples	31 (73.8%)	93 (80.2%)
Positive culture samples	12 (38.7%)	62 (66.7%)
Haemophilus influenzae	6 (19.4%)	25 (26.8%)
Moraxella catarrhalis	None	7 (7.5%)
Pseudomonas aeruginosa	2 (6.5%)	6 (6.5%)
Streptococcus pneumoniae	2 (6.5%)	13 (14.0%)
Staphylococcus aureus	2 (6.5%)	8 (8.6%)
Other bacteria	None	3 (3.2%)
Chronic *Pseudomonas aeruginosa* infection	3 (7.1%)	6 (5.2%)

Data are presented as No. (% of total or % of available mucus samples) or median (range).
[a]Demonstrated by transmission electron microscopy or high-resolution immunofluorescence (not including isolated inner dynein arm defects). Three patients without visible abnormalities on routine transmission electron microscopy were known to have the *HYDIN* mutation [27].
[b]Nasal NO production <77 nl/min [28].
[c]Mutational analysis of the entire cohort is ongoing.

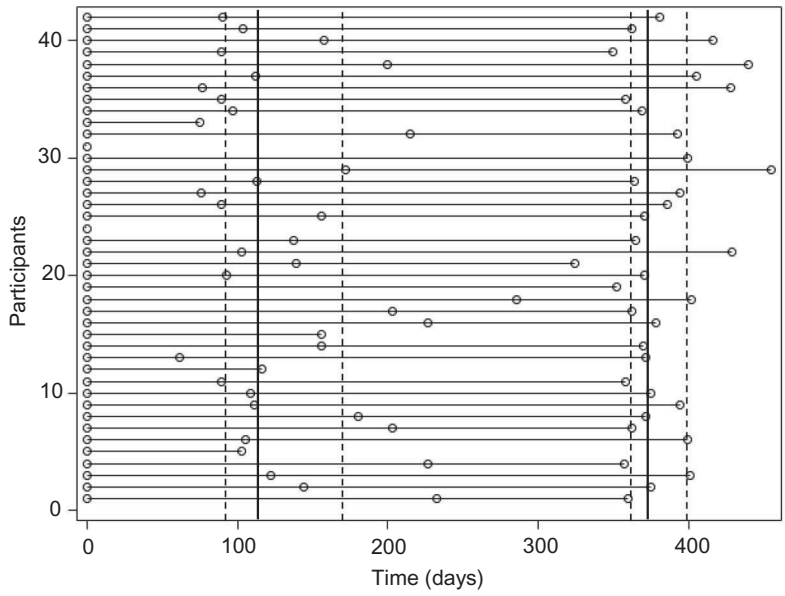

Figure 1. Time intervals between the repeated, longitudinal test occasions. Participants (n = 42) attended three test occasions over a 1-year period during routine outpatient visits. Six of the 42 participants did not complete the follow-up occasions and two participants did not attend the intermediate test occasion. The median time between the baseline and intermediate test occasion and the baseline and last test occasion is indicated with a vertical line (interquartile ranges shown in dashed lines).

Table 2. Pulmonary function variables.

	Baseline visit (n = 42)	Last follow-up visit (n = 36)	Trend
FEV_1% predicted[a]	83.5 (13.7)	85.2 (14.9)	-
FEV_1 z-score[a]	−1.38 (1.15)	−1.24 (1.25)	0.02 (−0.18; 0.22) p = 0.83
FVC % predicted[a]	94.1 (12.4)	95.9 (12.4)	-
FVC z-score[a]	−0.50 (1.05)	−0.35 (1.05)	-
FEV_1/FVC % predicted[a]	88.1 (8.3)	88.1 (9.6)	-
FEV_1/FVC z-score[a]	−1.44 (0.92)	−1.41 (1.06)	0.03 (−0.12; 0.17) p = 0.72
LCI	10.20 (2.11)	10.46 (2.50)	0.51 (0.12; 0.91) p = 0.01
M_1/M_0	2.32 (0.48)	2.34 (0.52)	0.06 (−0.04; 0.16) p = 0.25
M_2/M_0	12.05 (4.87)	12.73 (6.34)	0.90 (−0.25; 2.04) p = 0.12
Cn@TO6	5.70 (1.67)	5.60 (1.78)	0.16 (−0.14; 0.46) p = 0.30
S_{cond}*V_T	0.062 (0.023)	0.062 (0.023)	0.002 (−0.005; 0.008) p = 0.65
S_{acin}*V_T	0.164 (0.101)	0.170 (0.109)	0.011 (−0.007; 0.028) p = 0.22

Pulmonary function values are presented by means (SD). Trends in the pulmonary function variables over 1 year were analysed with linear mixed models (including data on all 42 participants from the baseline visit and all available follow-up data) and are presented with estimates of the mean difference and 95% CI (from basic statistical model only adjusted for age at inclusion and age at diagnosis).

FEV_1: forced expiratory volume in 1 s; FVC: forced vital capacity; LCI: lung clearance index; M_1/M_0: moment ratio to the first degree; M_2/M_0: moment ratio to the second degree; Cn@TO6: normalized N_2 concentration at six lung volume turnovers; S_{cond}*V_T: regional ventilation inhomogeneity of the conducting airways corrected for tidal volume; S_{acin}*V_T: regional ventilation inhomogeneity of the intra-acinar airways corrected for tidal volume.

[a]Reference values from Quanjer et al. Eur Respir J 2012 [22].

(Figure 2). There was no significant time trend in FEV_1 z-score and FEV_1/FVC z-score over the 1-year period. When mutually adjusted for age at inclusion and age at diagnosis, there was no effect of age at inclusion on any of the pulmonary function parameters, whereas age at diagnosis had a significant effect on M_1/M_0, M_2/M_0, and S_{cond}*V_T. Five participants met the definition of chronic *P. aeruginosa* infection at one or more test occasions, which had a significant effect on M_1/M_0, M_2/M_0, and Cn@TO6. Estimates of the effect of chronic *P. aeruginosa* infection on M_1/M_0, M_2/M_0, and Cn@TO6 were a mean increase (95% CI) of 0.31 (0.02–0.60), 4.15 (0.95–7.35), and 0.92 (0.04–1.80), respectively. The presence of exacerbation at the test

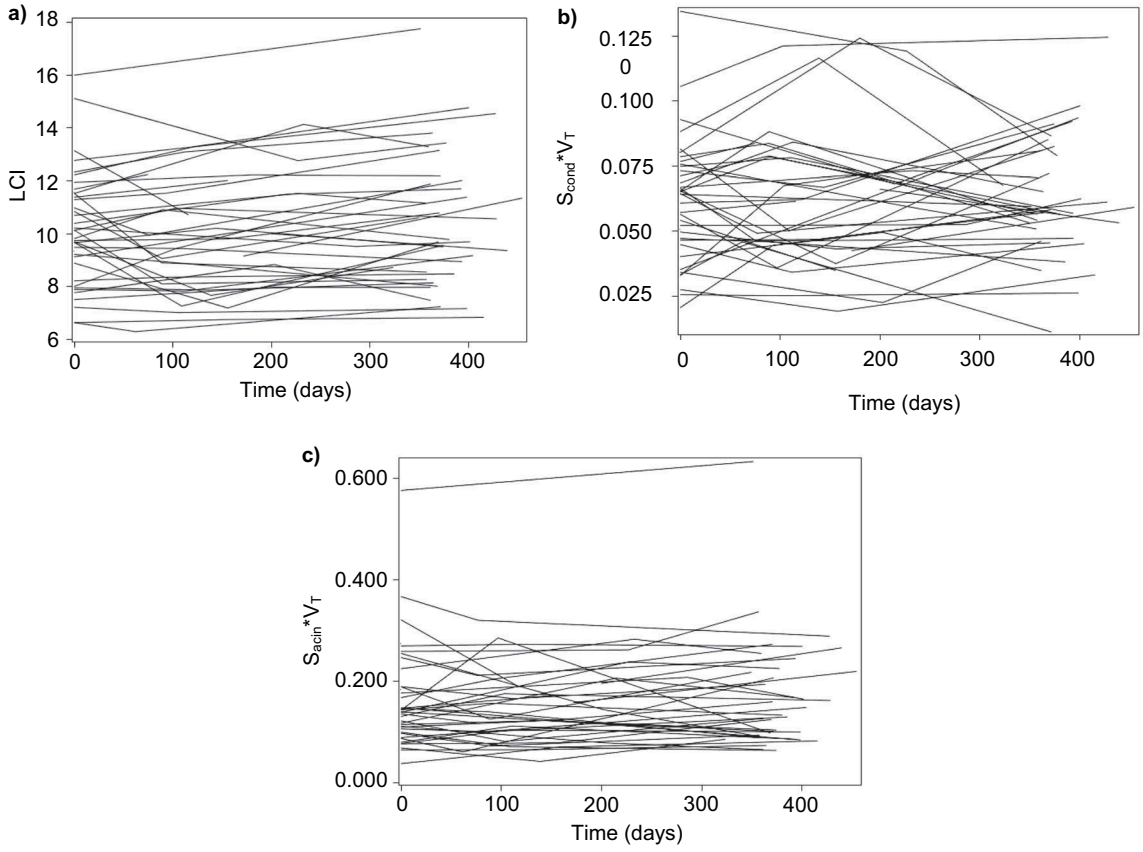

Figure 2. The individual longitudinal trends over 1 year in LCI, S_{cond}*V_T, and S_{acin}*V_T. (a) LCI, (b) S_{cond}*V_T, and (c) S_{acin}*V_T (n = 42). The LCI, S_{cond}*V_T, and S_{acin}*V_T are presented as absolute values. Time is presented in days. LCI: lung clearance index; S_{cond}*V_T: regional ventilation inhomogeneity of the conducting airways corrected for tidal volume; S_{acin}*V_T: regional ventilation inhomogeneity of the intra-acinar airways corrected for tidal volume.

Table 3. Intraclass correlation coefficients and between-occasion variability.

		Between-occasion variability - 1 year between tests		
	Estimated ICC (95% CI)	Estimated mean difference (SD of the difference)	Limits of agreement	(95% CI LL)/(95% CI UL)
FEV_1 *z*-score	0.87 (0.78; 0.92)	0.02 (0.64)	−1.23 to 1.27	(−1.52; −0.94)/(0.99; 1.53)
FEV_1/FVC *z*-score	0.88 (0.80; 0.93)	0.03 (0.46)	−0.87 to 0.93	(−1.09; −0.67)/(0.73; 1.12)
LCI	0.83 (0.71; 0.89)	0.51 (1.26)	−1.96 to 2.98	(−2.49; −1.40)/(2.44; 3.56)
M_1/M_0	0.76 (0.62; 0.85)	0.06 (0.33)	−0.59 to 0.71	(−0.72; −0.43)/(0.55; 0.85)
M_2/M_0	0.77 (0.63; 0.85)	0.90 (3.62)	−6.20 to 8.00	(−7.78; −4.61)/(6.40; 9.72)
Cn@TO6	0.85 (0.74; 0.90)	0.16 (0.95)	−1.70 to 2.02	(−2.10; −1.28)/(1.61; 2.46)
S_{cond}*V_T	0.53 (0.32; 0.68)	0.00 (0.02)	−0.04 to 0.04	(−0.05; −0.03)/(0.03; 0.05)
S_{acin}*V_T	0.86 (0.76; 0.91)	0.01 (0.06)	−0.11 to 0.13	(−0.12; −0.07)/(0.10; 0.15)

Estimates of between-occasion variability were calculated as the limits of agreement between two test occasions with a 1-year interval and are presented with the estimated mean difference in the outcome and SD of the difference, and the calculated limits of agreement with 95% CI on the lower and upper limit of agreement, respectively.

ICC: intraclass correlation coefficient; LL: lower limit; UL: upper limit; FEV_1: forced expiratory volume in 1 s; FVC: forced vital capacity; LCI: lung clearance index; M_1/M_0: moment ratio to the first degree; M_2/M_0: moment ratio to the second degree; Cn@TO6: normalized N_2 concentration at six lung volume turnovers; S_{cond}*V_T: regional ventilation inhomogeneity of the conducting airways corrected for tidal volume; S_{acin}*V_T: regional ventilation inhomogeneity of the intra-acinar airways corrected for tidal volume.

occasion (6.9% of the occasions) was the only covariate that had a significant effect on FEV_1 *z*-score (mean decrease 0.65, 95% CI: 0.25–1.04, p = 0.002).

All N_2 MBW indices, except S_{cond}*V_T, showed strong ICC ranging from 0.76 to 0.86 (Table 3). The S_{cond}*V_T had a more moderate ICC. Estimates of between-occasion variability for tests 1 year apart are shown in Table 3. The variance parameters for each of the pulmonary function variables can be found in Supplementary Table S1.

Excluding the test occasions where participants had exacerbation from the analysis, did not change whether the time trend in the N_2 MBW indices and spirometric

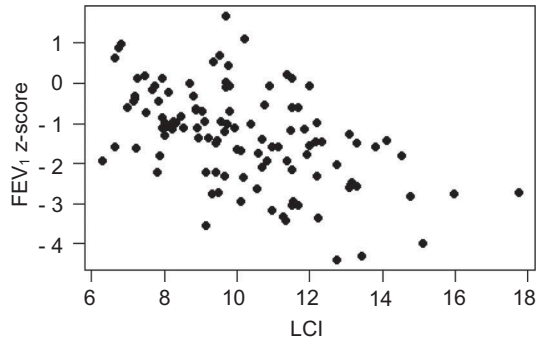

Figure 3. Association between LCI and FEV_1 *z*-score ($r = -0.47$, $p = 0.0001$), data from all test occasions. The LCI is presented as absolute values. LCI: lung clearance index; FEV_1: forced expiratory volume in 1 sec.

lung function parameters was significant or not, and did not narrow the between-occasion variability (Supplementary Table S2).

There was a moderate correlation between the LCI and FEV_1 *z*-score ($r = -0.47$, $p = 0.0001$; Figure 3).

Discussion

This is the first report of longitudinal measurements and variability of N_2 MBW indices in PCD. The LCI increased; whereas, MRs, Cn@TO6, S_{cond}*V_T and S_{acin}*V_T did not change significantly over 1 year in this cohort of children and young adults managed at a national PCD specialist centre. We found a moderate correlation between the LCI and FEV_1. This study provided estimates of the between-occasion variability of N_2 MBW indices, which are important for designing future longitudinal interventional studies of PCD utilizing N_2 MBW indices as outcome measures. For all N_2 MBW outcomes, the ICC indicated strong correlation between tests performed on the same patient, although lower for S_{cond}*V_T than for the other indices.

Contrary to our finding, a pilot study by Irving et al [29], which measured the LCI twice with 5-year interval in 12 PCD patients, found no significant overall change in the LCI. This difference might be because Irving's study group was simply too small to find the rather small increase in LCI, which we demonstrated; another tracer gas was used (sulphur hexafluoride); the patient cohorts might be different with respect to age, infection status, or other disease characteristics; or management practices might be different. The PCD patients participating in the present study were young and the majority diagnosed in their childhood. We found that age at diagnosis had an effect on some of the N_2 MBW indices, though not on the LCI or S_{acin}*V_T, which is consistent with previous cross-sectional studies that did not find a significant association with age at diagnosis [9,12]. The presence of chronic *P. aeruginosa* infection might also affect the N_2 MBW indices towards higher values. However, the proportion of test occasions where participants met the definition of chronic *P. aeruginosa* infection was small. Prolonged infection with *P. aeruginosa* may be less persistent in PCD than in cystic fibrosis [30]. The impact of *P. aeruginosa* infection on pulmonary function in PCD remains unclear, since currently only a few studies have demonstrated significantly more abnormal pulmonary function in PCD patients with *P. aeruginosa* infection compared with patients without [7,31,32]. Recently, two cross-sectional studies in PCD demonstrated relationship between phenotype assessed by lung function and ultrastructural defects and genotype [33,34]. Davis et al. [33] showed that children with central apparatus defects with microtubular disorganization had worse FEV_1 than patients with outer dynein arm or outer- plus inner dynein arm defects and similar when limited to the patients with biallelic mutations in the PCD-associated genes *CCDC39* or *CCDC40* compared with gene mutations associated with outer dynein arm or outer- plus inner dynein arm defects [33], and Irving et al. [34] found more abnormal LCI in PCD patients with microtubular defects compared with patients with dynein arm defects and patients with normal ultrastructure. We were not able to compare the severities of ventilation inhomogeneity between subgroups with different genetic mutations in this study due to the limited number of participants and limited number with known genetic mutations.

In the present study, we found a moderate negative correlation between LCI and FEV_1, similar to cross-sectional studies on PCD by Boon et al. [12] and Nyilas et al. [35]. Two previous cross-sectional studies of LCI in children with PCD at our centre did not find a significant correlation between FEV_1 and LCI [9,18], consistent with a study by Irving et al. [11]. These discrepant findings and also the contradictory results on correlations between the LCI and high-resolution computed tomography scores have led to discussions about the usefulness of MBW in PCD [11,12,36]. The fact that the LCI is abnormal in most patients with PCD, including patients with normal FEV_1 [9,12], indicates that MBW does provide additional information to FEV_1 by reflecting peripheral airway disease, which is not fully assessable by spirometry.

A strength of this study is that the longitudinal trends in N_2 MBW indices were based on several follow-up measurements, and that the longitudinal design makes it possible to estimate the ICC and limits of agreements of the pulmonary function outcomes. One limitation is that the study was not conducted in a strict controlled manner since the study procedures were performed as part of routine clinical visits;

therefore, not all patients were clinically stable at the follow-up visits. On the other hand, an observational study like this more truly reflects the natural course of pulmonary function in PCD patients. The presence of exacerbation on the test occasion was included in the statistical models as a covariate and had no significant effect on the N_2 MBW outcomes and excluding the test occasions with exacerbation from the analysis did not alter whether there was a time trend in the indices or change the limits of agreement. The data collected in the current study are not appropriate for investigating the direct impact of acute exacerbations on MBW indices in PCD. Another limitation is the rather small sample size, considering the wide age range. However, PCD is rare, and the present sample size is quite large for a single-centre study within the age range represented. The limits of agreement reported in this study are, for many of the outcomes, subject to some uncertainty due to the limited number of patients. Management and treatment practices in PCD vary, even within Europe [37]; since this is a single-centre study, the estimates of variability may require confirmation in PCD cohorts with different management practices and in multi-centre studies. Moreover, longitudinal trends and between-occasion variability in healthy subjects are warranted.

In conclusion, this is the first study to report intermediate term longitudinal trends and variability of N_2 MBW indices in PCD. Children and young adults with PCD managed at a specialist centre showed overall a minimal but significant increasing LCI and otherwise persistently mild to severe abnormal ventilation inhomogeneity indices over 1 year. The increase in LCI may reflect disease progression and/or natural fluctuations. The N_2 MBW indices seem to be promising clinical outcome measures in PCD, according to their ICC. Estimates of the variability may be useful for interpretation of changes in N_2 MBW indices in routine management of PCD and for future interventional studies utilizing N_2 MBW indices as outcome measures. However, the longitudinal trends in MBW indices need to be confirmed in future studies investigating the trends over longer follow-up periods and in larger patient cohorts including all ages and in healthy subjects, where to our knowledge only cross-sectional data exist. In addition, studies should assess whether time trends in MBW indices may depend on disease severity, infection status, or disease characteristics such as underlying genotype.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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