Multiple breath washout in primary ciliary dyskinesia: a systematic review of the literature

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Multiple breath washout is a useful monitoring tool for lung function and shows higher sensitivity that spirometry in primary ciliary dyskinesia https://bit.ly/4jj5P7C

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Abstract

Primary ciliary dyskinesia (PCD) is a heterogeneous multiorgan genetic disease characterised by motile cilia impairment that primarily affects the respiratory system. Multiple breath washout (MBW) is an emerging pulmonary function test. Its main outcome, the lung clearance index (LCI), is a valuable sensitive measure in obstructive lung disease, especially in cystic fibrosis. The potential value of MBW as a monitoring tool for patients with PCD is not well known. This systematic review summarises all articles published by the end of 2022 reporting MBW data in patients with PCD and compares MBW parameters to spirometry and chest imaging findings. We searched PubMed, Embase and Scopus for original studies with MBW measurements in patients with PCD. 14 studies were included in the analysis with a total number of 398 patients. The mean/median LCI ranged from 7.98 to 11.8, whereas mean/median forced expiratory volume in 1 s (FEV_1) z-score ranged from −1.98 to −0.5. The LCI was abnormally increased in all studies, whereas only two studies had abnormally decreased FEV_1. The LCI also had a stronger correlation with chest computed tomography and magnetic resonance imaging results, compared to FEV_1. In conclusion, this review shows that the LCI is abnormally high in PCD from the preschool age up to adulthood. MBW appears to be more sensitive than spirometry in identifying pulmonary function impairment at the early stages of disease. These findings support the use of the LCI in daily clinical practice and provide evidence of using it as an outcome measure in upcoming clinical trials for patients with PCD.

Introduction

Primary ciliary dyskinesia (PCD) is a rare, heterogeneous genetic disease with an estimated prevalence of 1 in 7500 individuals and is generally considered an underdiagnosed condition [1–3]. It is characterised by structural and functional abnormalities of motile cilia in the airway epithelium, but can also affect the ciliated epithelia of the nervous and reproductive systems, involve retinal impairment and congenital heart disease, as well as manifest with *situs inversus*, a condition of left–right asymmetry in the internal organs [4]. The dysfunction of respiratory cilia leads to impaired airway mucociliary clearance, recurrent respiratory tract infections and the development of bronchiectasis [5], all of which may contribute to an inhomogeneous distribution of ventilation in the lungs.

Pulmonary function is a major predictor of lung morbidity in patients with chronic respiratory diseases [6]. Pulmonary function tests provide valuable information for diagnosis, disease progression assessment and treatment response evaluation; thus, they constitute first-line monitoring tools for chronic respiratory diseases [7]. Spirometry is considered as the gold-standard pulmonary function test. However, its sensitivity is limited at the early stages of small airway diseases because it provides information that

predominantly reflects large airway function. In addition, it requires forced breathing manoeuvres, which limit its use in young children and noncooperative patients. Recently, multiple breath washout (MBW) has been increasingly used as an additional pulmonary function test [8]. The major principle of the test is the measurement of the clearance of an inert tracer gas by the lungs across multiple serial breaths. Either an exogenous, *e.g.* sulfur hexafluoride (SF_6), or an endogenous, *e.g.* nitrogen (N_2), inert gas can be used. In the first case, the inert gas must be first washed-in and then washed-out by breathing room air; whereas, in the second case, the inert gas is washed-out by breathing 100% oxygen [8, 9].

The lung clearance index (LCI) is the most common outcome measure of MBW, reflecting global ventilation inhomogeneity. It represents the number of turnovers of the functional residual capacity (FRC), *i.e.* lung volumes equal to FRC, that should be exhaled to washout the tracer gas to 2.5% of its initial concentration. Other MBW indices are the convection-dependent inhomogeneity index (S_{cond}) and the diffusion convection-interaction-dependent inhomogeneity index (S_{acin}), which reflect conducting and acinar airway heterogeneity respectively, the LCI_5 (LCI at 5% of the initial concentration), the normalised nitrogen concentration at six lung volume turnovers (Cn@TO6) and the moment ratios (moment ratio of first to zeroth moment of MBW (M_1/M_0) and moment ratio of second to zeroth moment of MBW (M_2/M_0)) [9]. However, their clinical utility has so far been limited [10]. The measuring technique, the tracer gas used, as well as hardware and software issues, are known factors that affect MBW outcomes [11–17]. Therefore, those parameters should be specified in studies involving MBW measurements. LCI values have been shown to be age-dependent [18, 19], with infants having higher LCI values compared to older children. This can be mainly attributed to ongoing lung development and rapid alveolarisation during this period, as well as possibly to technical factors [20–23].

Multiple studies have shown the utility of MBW in the investigation and monitoring of cystic fibrosis (CF)-related lung disease, as the LCI has been proven to be a sensitive pulmonary functional parameter at the early stages of disease [21, 24–26]. It is also an established noninvasive marker for treatment response and has been used as a primary outcome measure in interventional studies [27–30]. Furthermore, MBW has been identified as a sensitive measure of disease severity that correlates with clinical assessment tools in patients with non-CF, non-PCD bronchiectasis [31]. Over the last decade, an increasing number of studies have assessed the use of MBW to monitor lung impairment and treatment effects in PCD.

We aimed to systematically review all studies published up until the end of 2022 that included MBW data from both paediatric and adult patients with PCD, and to compare MBW and spirometric parameters in an attempt to investigate the potential value of MBW as a monitoring tool in PCD.

Materials and methods

The review followed the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) and SWiM (Synthesis Without Integration of Meta-analysis) reporting guidelines and was registered with the PROSPERO registry of systematic reviews (CRD42022313494).

Search strategy

We searched PubMed, Embase and Scopus up until 31 December 2022, with no time restrictions, for studies that included MBW performance in patients with PCD. After trying different search keys, we selected those that most concisely led to the largest number of results in order to include virtually all relevant studies, as shown below. To indirectly reduce any potential effects of publication bias, abstracts published in conference proceedings were also included in the screening procedure.

PubMed search terms

((“primary ciliary dyskinesia”[Title/Abstract]) OR (“kartagener”[Title/Abstract]) OR (“ciliary motility disorders”[Title/Abstract]) OR (“motile cilia disorders”[Title/Abstract]) OR (“ciliopathies”[Title/Abstract])) AND ((“lung function”[Title/Abstract]) OR (“respiratory function”[Title/Abstract]) OR (“pulmonary function”[Title/Abstract]) OR (“lung volume”[Title/Abstract]) OR (“moment ratio”[Title/Abstract]) OR (“washout”[Title/Abstract]) OR (“lung clearance index”[Title/Abstract]) OR (“multiple breath washout indices”[Title/Abstract]) OR (“multiple breath washout”[Title/Abstract]) OR (“multiple breath washout outcomes”[Title/Abstract]) OR (“multiple breath washout indices”[Title/Abstract]) OR (“lower airway clinical outcome parameters”[Title/Abstract]) OR (“ventilation inhomogeneity”[Title/Abstract]) OR (“forced vital capacity”[Title/Abstract]) OR (“forced expiratory volume”[Title/Abstract]) OR (“FEV”[Title/Abstract]) OR (“FEV1”[Title/Abstract]) OR (“spirometry”[Title/Abstract]) OR (“follow-up studies”[Title/Abstract]) OR (“respiratory function tests”[Title/Abstract])).

Embase search terms

("primary ciliary dyskinesia":ab,ti OR "kartagener":ab,ti OR "ciliary motility disorders":ab,ti OR "motile cilia disorders":ab,ti OR "ciliopathies":ab,ti) AND ("lung function":ab,ti OR "respiratory function":ab,ti OR "pulmonary function":ab,ti OR "lung volume":ab,ti OR "moment ratio":ab,ti OR "washout":ab,ti OR "lung clearance index":ab,ti OR "multiple breath washout indices":ab,ti OR "multiple breath washout":ab,ti OR "multiple breath washout outcomes":ab,ti OR "multiple breath washout indices":ab,ti OR "lower airway clinical outcome parameters":ab,ti OR "ventilation inhomogeneity":ab,ti OR "forced vital capacity":ab,ti OR "forced expiratory volume":ab,ti OR "FEV":ab,ti OR "FEV1":ab,ti OR "spirometry":ab,ti OR "follow-up studies":ab,ti OR "respiratory function tests":ab,ti).

Scopus search terms

(TITLE-ABS-KEY("primary ciliary dyskinesia") OR TITLE-ABS-KEY("kartagener") OR TITLE-ABS-KEY ("ciliary motility disorders") OR TITLE-ABS-KEY("motile cilia disorders") OR TITLE-ABS-KEY ("ciliopathies")) AND (TITLE-ABS-KEY("lung function") OR TITLE-ABS-KEY("respiratory function") OR TITLE-ABS-KEY("pulmonary function") OR TITLE-ABS-KEY("lung volume") OR TITLE-ABS-KEY ("moment ratio") OR TITLE-ABS-KEY("washout") OR TITLE-ABS-KEY("lung clearance index") OR TITLE-ABS-KEY("multiple breath washout indices") OR TITLE-ABS-KEY("multiple breath washout") OR TITLE-ABS-KEY("multiple breath washout outcomes") OR TITLE-ABS-KEY("multiple breath washout indexes") OR TITLE-ABS-KEY("lower airway clinical outcome parameters") OR TITLE-ABS-KEY ("ventilation inhomogeneity") OR TITLE-ABS-KEY("forced vital capacity") OR TITLE-ABS-KEY("forced expiratory volume") OR TITLE-ABS-KEY("FEV") OR TITLE-ABS-KEY("FEV1") OR TITLE-ABS-KEY ("spirometry") OR TITLE-ABS-KEY("follow-up studies") OR TITLE-ABS-KEY("respiratory function tests")).

Study selection

The study selection process included three main steps. In the first step, the title and abstract of each result were independently screened by two reviewers (A.M. Matthaiou and A. Demetropoulou) supervised by a third reviewer (P. Anagnostopoulou) and, in case of disagreement, the third reviewer additionally provided input to come to a decision. Studies reporting MBW parameters in patients with PCD were selected to be assessed for eligibility. We excluded duplicate records, studies that were not relevant and/or not original, as well as those that did not include quantitative data of MBW parameters in patients with PCD. In the second step, after reviewing the full text of the selected studies, the three reviewers further excluded those without quantitative MBW data in patients with PCD, even if this was not apparent from the title or abstract, as well as studies that included patients without a confirmed diagnosis of PCD in order to minimise the indirectness of our systematic review.

In the third step, the three reviewers identified clusters of the eligible studies that originated from the same research group and potentially included overlapping patient populations, based on the list of authors, the country of origin and the study centre. We included multiple studies per each research group in the analysis, with the condition that there was no temporal overlap between MBW measurements, even in possibly overlapping patient populations. This condition was confirmed either by checking the dates of MBW measurements, if mentioned in the manuscript, or by contacting the authors. In cases where overlap of MBW measurements could not be excluded, we included the study with the largest patient population size from each research group in the analysis.

Data extraction and analysis

For each study included in the analysis, data were independently collected by the three reviewers. We extracted the following data: 1) publication-related data (list of authors, country of origin, journal of publication and year of publication); 2) study-related data (study design, centre of study and year of study); 3) population-related data (population size, sex and age of participants, body mass index (BMI), nasal nitric oxide, and airway *Pseudomonas aeruginosa* colonisation); 4) MBW technical information (equipment, tracer gas and acquisition-analysis software); 5) MBW data (test feasibility, LCI, S_{cond} or S_{cond} normalised for tidal volume (V_T), S_{acin} or S_{acin} normalised for V_T, and FRC); 6) spirometric data (forced expiratory volume in 1 s (FEV_1), forced vital capacity (FVC), FEV_1/FVC and forced expiratory flow between 25% and 75% of vital capacity ($FEF_{25–75\%}$)). The selected studies were further grouped according to the inclusion of additional data (chest imaging indices, genetics and airway histopathological properties) and their correlations with MBW and spirometry data were recorded.

For the main synthesis of the review, LCI data in patients with PCD were evaluated according to the reported upper limit of normal in each study, which was defined either by a parallel group of healthy participants or by published reference values. Investigation of heterogeneity in MBW outcomes was not pre-specified at the initial stage but was qualitatively assessed *post hoc*, based on the variability of MBW

results between studies and measurements in patients with PCD within the normal range. Where available, confidence intervals were evaluated to minimise the possibility of imprecision.

Results

Search results

The initial search produced a total of 1358 results as of 31 December 2022, including duplicates, comprising 528 from Embase, 250 from PubMed and 580 from Scopus. The titles and abstracts of all records were independently screened and 1338 of them, including duplicates, were excluded as not relevant and/or not original studies or research works without reporting MBW data. Of the 20 remaining records, 18 were found eligible for inclusion in the analysis, whereas two studies were excluded due to lack of MBW data, which was not evident during the initial screening, and a lack of a definite diagnosis of PCD in the study participants. Upon further exclusion of studies with potentially overlapping study populations per research group, as stated in the methods section, a total of 14 studies were finally analysed. The flowchart of the selection process is illustrated in figure 1.

Study characteristics

MBW in patients with PCD was first reported in 2013 and, since then, it has been investigated by several research groups across Europe and in North America. From the 14 studies included in the analysis, 12 were cross-sectional and two longitudinal, while all but one were prospective. 12 studies performed MBW using an ultrasonic flowmeter (Exhalyzer® D, Ecomedics AG, Duerten, Switzerland) and N_2 as the tracer gas. Of these, 11 used commercial analysis software (Spiroware®) in various versions, which over time improved the accuracy of the indirect calculation of the N_2 concentration [18], and one used custom-made analysis software. Two studies used a photoacoustic gas analyser (Innocor®, Innovision, Denmark) with SF_6 as the tracer gas. Three studies were multi-centre. MBW was performed according to current

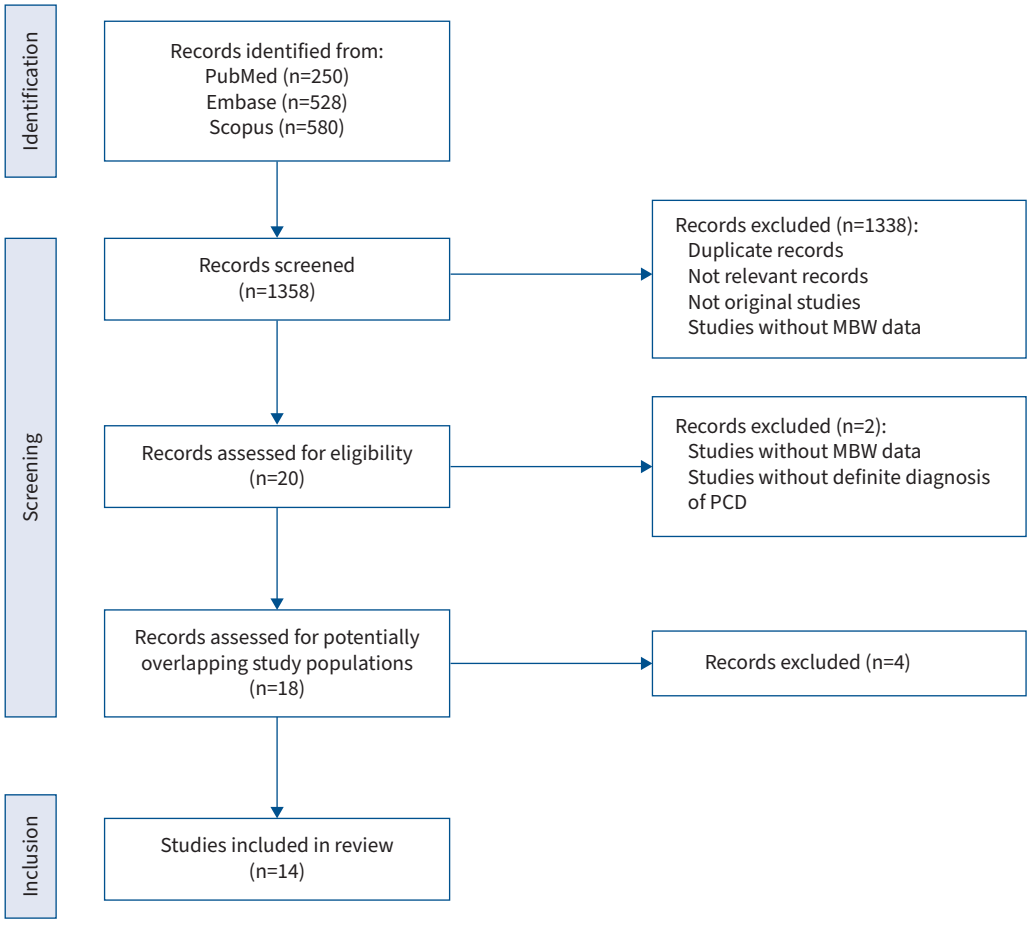

FIGURE 1 PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) flow diagram of the study selection process. MBW: multiple breath washout; PCD: primary ciliary dyskinesia.

recommendations in all studies that took place after the publication date of the relevant consensus statement [9]. Table 1 summarises the characteristics of all 14 studies.

The 14 studies included in the analysis had 398 participants (40% males) in total, with a mean (range) number of 28 (6–69) patients per study, aged from 0.6 to 41 years. BMI was reported in eight studies (n=170 patients) as a mean/median z-score with a weighted mean of −0.26. Airway microbiology data were reported in seven studies (n=254 patients), revealing a weighted mean of 14.4% of the patients to be infected/colonised by *Pseudomonas aeruginosa*. Nasal NO was reported in six studies (n=230 patients) as a median with a weighted mean of 14.4 $nL \cdot min^{-1}$. Table 2 shows the detailed characteristics of the 14 studies included in the analysis.

Pulmonary function data

Reported pulmonary function data included the LCI, $S_{cond}/S_{cond} \times V_T$, $S_{acin}/S_{acin} \times V_T$ and FRC for MBW, and FEV_1, FVC, FEV_1/FVC and $FEF_{25–75\%}$ for spirometry, although not all these indices were included in all studies. LCI and FEV_1 data were available in all studies except for one performed in very young children, which did not include spirometry [32].

Reported data were presented as means with standard deviation or 95% confidence interval, as medians with either range or interquartile range (IQR), mean z-scores with standard deviation, median z-scores with either range or IQR, or as mean/median percentages of predicted value. The studies by Pensabene *et al.* [33] and Smith *et al.* [34] reported data for each individual separately. In addition, the studies by Kobbernagel *et al.* [35] and Vandervoort *et al.* [36] included longitudinal data reporting baseline values and their changes on follow-up. Table 3 shows the pulmonary function data in the 14 studies included in the analysis.

TABLE 1 Overall characteristics of studies reporting multiple breath washout (MBW) data in patients with primary ciliary dyskinesia

Study characteristics	Number (%) of studies
Total number of studies	14 (100)
Region of origin	
Europe	13 (92.9)
North America	1 (7.1)
Period of publication	
2013–2015	2 (14.3)
2016–2018	5 (35.7)
2019–2021	4 (28.6)
2022	3 (21.4)
Number of study centres	
Single centre	11 (78.6)
Multiple centres	3 (21.4)
Study design	
Prospective	13 (92.9)
Retrospective	1 (7.1)
Study type	
Cross-sectional	12 (85.7)
Longitudinal	2 (14.3)
MBW equipment/tracer gas	
Exhalyzer® D/N_2	12 (85.7)
Innocor®/SF_6	2 (14.3)
Study size	
⩽20 patients	6 (42.9)
21–40 patients	4 (28.6)
41–60 patients	3 (21.4)
61–80 patients	1 (7.1)
Studies including children and/or adults	
Only children	8 (57.1)
Only adults	0 (0)
Children and adults	6 (42.9)

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TABLE 2 Detailed characteristics of studies reporting multiple breath washout (MBW) data in patients with primary ciliary dyskinesia

First author [reference]	Year of publication	Country of origin	Period of study	Type of study	Design of study	MBW equipment (tracer gas)	Acquisition and analysis MBW software	Patients (male/female)	Age (years)[f]
Madsen [37]	2013	Denmark	NA	Prospective	Cross-sectional	Exhalyzer® D (N_2)	NA	44 (17/27)	14.8 (6.5–29.7)[#]
Boon [42]	2015	Belgium	May 2011–September 2014	Prospective	Cross-sectional	Exhalyzer® D (N_2)	Spiroware® 3.1[§]	38 (13/25)	16.1 (11.1–19.6)[¶]
Green [45]	2016	Denmark	NA	Prospective	Cross-sectional	Exhalyzer® D (N_2)	Spiroware® 3.1.5 and custom software	28 (8/20)	12.4 (10.7–14.6)[¶]
Nyilas [40]	2017	Germany Switzerland	Mar 2013–April 2015	Prospective	Cross-sectional	Exhalyzer® D (N_2)	Spiroware® 3.1.6	49 (19/30)	14.7±6.6[+]
Irving [38]	2018	UK	NA	Prospective	Cross-sectional	Innocor® (SF_6)	NA	69 (25/44)	13 (4–41)[#]
Nyilas [41]	2018	Germany	April 2015–February 2016	Prospective	Cross-sectional	Exhalyzer® D (N_2)	NA	30 (14/16)	13.4 (10.4–17.1)[¶]
Smith [34][f]	2018	UK	NA	Retrospective	Cross-sectional	Innocor® (SF_6)	NA	11 (3/8)	13.3±3.4[+]
Kobbernagel [35]	2019	Denmark	NA	Prospective	Longitudinal	Exhalyzer® D (N_2)	Spiroware® $3.1.6_{ext}$	42 (16/26)	15.4 (6.5–29.7)[#]
Koucký [43]	2019	Czech Republic	NA	Prospective	Cross-sectional	Exhalyzer® D (N_2)	NA	11 (6/5)	7.8 (0.6–15.8)[#]
Kinghorn [44]	2020	USA	March 2017–April 2018	Prospective	Cross-sectional	Exhalyzer® D (N_2)	Spiroware® 3.1.6	17 (7/10)	8.6 (6.1–10)[¶]
Constant [46]	2021	Portugal	December 2018–November 2019	Prospective	Cross-sectional	Exhalyzer® D (N_2)	Spiroware® 3.1	6 (2/4)	13.8 (11.0–19.2)[#]
Pensabene [33]	2022	Italy	July 2021–October 2021	Prospective	Cross-sectional	Exhalyzer® D (N_2)	Spiroware® 3.2.2	12 (6/6)	12 (9.8–14.2)[¶]
Vandervoort [36]	2022	Belgium	June 2020–June 2021	Prospective	Longitudinal	Exhalyzer® D (N_2)	Spiroware® 3.2.1	14 (9/5)	12.8±3.3[+]
Roehmel [32]	2022	Germany Poland	March 2018–March 2020	Prospective	Cross-sectional	Exhalyzer® D (N_2)	Spiroware® 3.2.1	27 (14/13)	4.8±1.5[+]

Methods of MBW: Exhalyzer® D: ultrasonic flowmetry; Innocor®: photoacoustic gas analysis. NA: not available. [#]: Median (range). [¶]: Median (interquartile range). [+]: Mean±SD. [§]: Information provided by Ecomedics AG. [f]: Mean±SD age was calculated based on the reported individual values.

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TABLE 3 Multiple breath washout (MBW) and spirometric data in studies reporting MBW data in patients with primary ciliary dyskinesia

First author [reference]	$LCI_{2.5}$ upper limit of normal, reference	$LCI_{2.5}$	$S_{cond} \times V_T/(S_{cond})$	$S_{acin} \times V_T/(S_{acin})$	FRC	FEV_1	FVC	FEV_1/FVC	$FEF_{25-75\%}$
Madsen [37]	Age-dependent 1.96 z-score [47]	12.2 (−0.4–28.9)[#]	6.4 (−0.2–14.1)[#]	2.7 (−0.9–17.3)[#]	2.4 (1.2–5.9)[#] 122.7 (78.4–190.6)[¶]	−1.2 (−4.1–1.3)[#]	−0.2 (−2.6–1.5)[#]	−1.4 (−3.0–0.9)[#]	−1.9 (−4.5–1.1)[#]
Boon [42]	8.36[+]	9.48 (8.28–10.92)[§]	0.057 (0.036–0.078)[§]	0.079 (0.034–0.116)[§]	NA	−1.54 (−2.1– −0.43)[f]	NA	−1.52 (−2.2– −1.01)[f]	−1.99 (−2.68– −0.61)[f]
Green [45][##]	7.45[+]	9.51±1.73[¶¶]	NA	NA	NA	−1.3 (−1.7– −0.6)[f]	−0.4 (−1.3–0.5)[f]	−1.4 (−1.9– −0.8)[f]	−1.8 (−2.4– −1.8)[f]
Nyilas [40]	7.85[+]	11±3.6[¶¶]	0.053±0.022[¶¶] 3±2[++]	3.1±4.3[++]	NA	−1.1±1.6[++]	NA	NA	−1.1±1.9[++]
Irving [38]	7.4 [65]	8.44 (5.84–14.98)[§§]	NA	NA	NA	−1.98 (−5.33–0.73)[#]	NA	NA	−2.17 (−5.78–0.55)[#]
Nyilas [41]	7.85 [40]	10.8 (6.6–17.9)[§§]	0.05 (0–0.1)[§§]	0.11 (0.03–0.38)[§§]	NA	−0.5 (−5.7–0.77)[#]	NA	NA	NA
Smith [34][ff]	7.4 [65]	7.98±1.66[¶¶]	0.06±0.02[¶¶]	0.12±0.09[¶¶]	NA	−1.2±1.5[++]	NA	−1.72±1.15[++]	NA
Kobbernagel [35]	NA	10.2±2.11[¶¶]	0.062±0.023[¶¶]	0.164±0.101[¶¶]	NA	−1.38±1.15[++]	−0.5±1.05[++]	−1.44±0.92[++]	NA
Koucký [43]	8.4[+]	11.8 (9.2–14.3)[###]	0.046 (0.024–0.067)[###]	0.191 (0.137–0.244)[###]	117.9 (71.2–164.6)[###]	72 (56.2–87.7)[###]	76.1 (60.3–91.8)[###]	NA	57 (40.3–73.7)[###]
Kinghorn [44]	7.8[+]	8.19 (7.35–11.38)[§]	0.03 (0.02–0.05)[§]	0.11 (0.06–0.23)[§]	NA	−0.62 (−1.57– −0.17)[f]	0.05 (−0.37–0.67)[f]	−1.47 (−1.68– −0.72)[f]	−1.74 (−2.03– −1.04)[f]
Constant [46]	7.5[+]	10.7 (8.6–13.2)[§§]	NA	NA	2.6 (1.6–2.8)[§§]	−1 (−2.8– −0.3)[#]	−1.3 (−2.7–0.2)[#]	0.84 (0.74–0.95)[#]	−1.5 (−2.6–0.2)[#]
Pensabene [33]	7.91 [18]	11.15 (8.07–12.9)[§]	0.074 (0.062–0.084)[§]	0.184 (0.103–0.324)[§]	NA	−1.32 (−2.3– −0.44)[f]	NA	NA	NA
Vandervoort [36][¶¶¶]	7.17 [9, 47]	8.6±1.2[¶¶]	0.069±0.029[¶¶]	NA	2.1±1.2[¶¶]	91.9±11.1[+++]	96.6±9.6[+++]	NA	NA
Roehmel [32]	8.1[+]	9.1 (6.4–13.9)[§§]	NA	NA	NA	NA	NA	NA	NA

$FEF_{25-75\%}$: forced expiratory flow between 25% and 75% of vital capacity; NA: not available; $S_{acin} \times V_T$: acinar ventilation heterogeneity index normalised for tidal volume; $S_{cond} \times V_T$: conductive ventilation heterogeneity index normalised for tidal volume. [#]: Median z-score (range). [¶]: Median percentage of predicted (range). [+]: Lung clearance index at 2.5% of starting concentration ($LCI_{2.5}$) upper limit of normal was determined by measurements in healthy controls. [§]: Median (interquartile range (IQR)). [f]: Median z-score (IQR). [##]: $LCI_{2.5}$ values reported by Spiroware® software were included. [¶¶]: Mean±SD. [++]: Mean±SD z-score. [§§]: Median (range). [ff]: Mean±SD $LCI_{2.5}$, conductive ventilation heterogeneity index (S_{cond}), acinar ventilation heterogeneity index (S_{acin}), forced expiratory volume in 1 s (FEV_1) and FEV_1/forced vital capacity (FVC) were calculated based on the reported individual values. [###]: Mean (95% confidence interval). [¶¶¶]: Mean±SD S_{cond}×tidal volume (V_T) and functional residual capacity (FRC) were calculated based on the individual values shared by the authors upon request. [+++]: Mean±SD percentage of predicted.

MBW data

The mean/median LCI for each study was above the upper limit of normal, ranging from 7.98 to 11.8 turnovers, except in the study by Madsen *et al.* [37], which reported the median z-score instead of the absolute LCI value (table 3), reflecting variable levels of severity of ventilation inhomogeneity. Interestingly, several patients with PCD had normal LCI values. The studies with the lowest mean/median LCI values were those using the Innocor® photoacoustic gas analyser and SF_6 as the tracer gas [34, 38], which is in accordance with previous reports comparing MBW outcomes using different equipment and/or tracer gases [15, 39]. Among the studies with reported data, the mean/median $S_{cond}/S_{cond}\times V_T$ ranged from 0.05 to 0.06 [34, 40, 41] and from 0.03 to 0.074 [33, 35, 36, 42–44], respectively. The mean/median $S_{acin}/S_{acin}\times V_T$ ranged from 0.11 to 0.12 [34, 41] and from 0.079 to 0.191 [33, 35, 42–44], respectively, while the mean/median percentage of the predicted value for FRC ranged from 117.9 to 122.7% [37, 43]. Alternative MBW outcomes were reported in four studies and included LCI_5, M_1/M_0, M_2/M_0 and Cn@TO6 [35, 40, 44, 45] (table 4).

Spirometric data

The mean/median FEV_1 z-scores ranged from −1.98 to −0.5 in 11 studies. The studies by Koucký *et al.* [43] and Vandervoort *et al.* [36] reported the mean percentage of predicted values instead. Among the studies with reported data, the mean/median FVC z-scores ranged from −1.3 to 0.05 [35, 37, 44–46], FEV_1/FVC ranged from −1.72 to 0.84 [34, 35, 37, 42, 44–46] and $FEF_{25–75\%}$ z-scores ranged from −2.17 to −1.1 [37, 38, 40, 42, 44–46] (table 3).

Comparison between MBW and spirometric data

In most studies, there was a notable difference in the degree to which LCI and FEV_1 values were affected. In all studies, the mean/median LCI was increased above the upper limit of normal (+1.96 SD), as defined per study. In the study by Kobbernagel *et al.* [35], no upper limit of normal was reported for the LCI, thus we compared LCI values to previously reported normative values [47]. The mean/median FEV_1 z-scores were within the normal range in 10 out of 11 studies, whereas the mean percentage of predicted FEV_1 was within the normal range in one out of two studies, using 80% as the lower limit of normal. Notably, the upper limit of normal for the LCI was set to exclude the top 2.5% of healthy individuals (+1.96 SD), while the lower limit of normal for FEV_1 was set at −1.64 SD, a common practice for patients at elevated risk for lung disease [7].

Studies with longitudinal MBW and spirometric data

Two studies included longitudinal data for MBW in patients with PCD, as shown in table 5 [35, 36]. Kobbernagel *et al.* [35] were the first to report longitudinal trends of MBW parameters in children and young adult patients over a period of 1 year. They showed that the LCI followed a minimal but statistically significant increase, whereas other ventilation inhomogeneity indices did not change. Vandervoort *et al.* [36] investigated the short-term effect of airway clearance therapy on pulmonary function and found no significant changes in MBW and spirometric parameters in serial assessments over a period of 6 months.

Correlation of MBW data with chest imaging findings

Four studies reported chest imaging data obtained through different imaging modalities and further correlated them with MBW and spirometric parameters in patients with PCD. In the study by Boon *et al.*

TABLE 4 Alternative multiple breath washout (MBW) outcomes in studies reporting MBW data in patients with primary ciliary dyskinesia

First author [reference]	LCI_5	M_1/M_0	M_2/M_0	Cn@TO6
Kobbernagel [35]#	NA	2.32±0.48¶	12.05±4.87¶	5.7±1.67¶
	NA	2.34±0.52¶	12.73±6.34¶	5.6±1.78¶
Green [45]	6.41±0.94¶	NA	NA	5.55±1.6¶
Nyilas [40]	4.4±3.9+	6.4±7.2+	4.6±4.0+	NA
Kinghorn [44]	5.5 (5.31–6.27)§	1.5 (1.45–1.58)§	4.08 (3.9–4.48)§	NA

Cn@TO6: normalised nitrogen concentration at six lung volume turnovers; LCI_5: lung clearance index at the point where the tracer gas is washed-out up to the 5% of its initial concentration; M1/M0: moment ratio of first to zeroth moment of MBW; M2/M0: moment ratio of second to zeroth moment of MBW; NA: not available. #: Longitudinal values in the first (upper row) and the third (lower row) visit of the patients are reported. ¶: Mean±SD. +: Mean±SD z-score. §: Median (interquartile range).

TABLE 5 Longitudinal multiple breath washout (MBW) and spirometric data in studies reporting MBW data in patients with primary ciliary dyskinesia

First author [reference]	Visit	$LCI_{2.5}$	$S_{cond}\times V_T$	$S_{acin}\times V_T$	FRC	FEV_1	FVC	FEV_1/FVC
Kobbernagel [35]	First	10.2±2.11[#]	0.062±0.023[#]	0.164±0.101[#]	NA	−1.38±1.15[¶]	−0.5±1.05[¶]	−1.44±0.92[¶]
	Third	10.46±2.5[#]	0.062±0.023[#]	0.17±0.109[#]	NA	−1.24±1.25[¶]	−0.35±1.05[¶]	−1.41±1.06[¶]
Vandervoort [36][§]	First	8.6±1.2[#]	0.069±0.029[#]	NA	2.1±1.2[#]	91.9±11.1[+]	96.6±9.6[+]	NA
	Second	8.6±1.4[#]	0.065±0.027[#]	NA	2.2±1.1[#]	92.9±9.4 [+]	97.2±9.2[+]	NA

NA: not available; $S_{acin}\times V_T$: acinar ventilation heterogeneity index normalised for tidal volume. [#]: Mean±SD. [¶]: Mean±SD z-score. [+]: Mean±SD percentage of predicted. [§]: Mean±SD conductive ventilation heterogeneity index normalised for tidal volume ($S_{cond}\times V_T$) and functional residual capacity (FRC) at the first visit and mean±SD lung clearance index at 2.5% of starting concentration ($LCI_{2.5}$), $S_{cond}\times V_T$, FRC, forced expiratory volume in 1 s (FEV_1) and forced vital capacity (FVC) at the second visit were calculated based on the individual values shared by the authors upon request.

[42], CF computed tomography (CFCT) score was used to quantify chest computed tomography (CT) findings, including severity and extent of bronchiectasis, airway wall thickening, mucus plugging, and parenchymal abnormalities, in 30 patients. The authors found a stronger correlation of CFCT with the LCI compared to FEV_1. Nyilas *et al.* [41] performed functional magnetic resonance imaging (MRI) to assess ventilation impairment in 30 patients. MBW showed better concordance with functional MRI findings compared to spirometry, as more than half of the patients exhibited abnormal lung ventilation on imaging and an abnormally increased LCI values, but normal FEV_1. In the study by Smith *et al.* [34], hyperpolarised gas MRI was used in 11 patients to determine lung ventilation inhomogeneity. Although all patients demonstrated ventilation defects on MRI, LCI and FEV_1 values were abnormally affected only in five and six patients, respectively. However, only the LCI correlated with both ventilation defect percentage and the metric of regional ventilation inhomogeneity [34]. Finally, Pensabene *et al.* [33] performed electrical impedance tomography in 12 patients to visually assess the distribution of lung ventilation and used the global inhomogeneity index (GI_{TOT}) to quantify the inhomogeneity of the distribution of V_T. They found a strong correlation between GI_{TOT} with FEV_1, but only a weak correlation with the LCI [33].

Correlation of MBW data with ciliary ultrastructure, genetics and airway histopathological findings

The association between MBW and spirometric outcomes and ciliary ultrastructural and genetic features in 69 patients with PCD was investigated only in the study by Irving *et al.* [38]. Defects identified by transmission electron microscopy in different ciliary components, attributed to specific involved genes, were better correlated with MBW than with spirometric indices. More specifically, LCI values were significantly worse in 16 patients with "defects in the microtubular arrangement" (involved genes: *RSPH4A*, *CCDC39* and *CCDC40*) compared to 39 patients with outer dynein arm defects with or without inner dynein arm defects (involved genes: *DNAH5*, *ARMC4*, *LRRC6*, *DNAAF3*, *DNAI1*, *DNAAF3*, *DYX1C1*, *ZMYND10*, *CCDC103*, *LRRC6* and *SPAG1*), whereas spirometric indices were similarly abnormal in both groups. In contrast, the LCI, FEV_1 and $FEF_{25–75\%}$ were all significantly worse in patients with "defects in the microtubular arrangement" compared to patients with a normal ciliary ultrastructure (involved genes: *DNAH11*, *HYDIN*, *CCDC103* and *RPGR*). Of note, the group that causes "defects in the microtubular arrangement" included 1) patients with central complex defects (n=4), with two of them carrying mutations in the *RSPH4A* gene (radial spoke head gene), and 2) patients with microtubular disorganization (n=12), with nine of them carrying mutations in the *CCDC39* or *CCDC40* genes [38]. This classification is not in line with the more recent consensus that correlates *RSPH4A* mutations with normal ciliary ultrastructure and *CCDC39*/*CCDC40* mutations with abnormal cilia ultrastructure, based on transmission electron microscopy findings [48]. Furthermore, a significant correlation between the LCI and thickness of bronchial epithelial reticular basement membrane in seven patients with PCD was found in the study by Koucký *et al.* [43].

Studies with MBW feasibility data

The feasibility of performing MBW in patients with PCD was mentioned in half of the studies, while six of them also reported additional data on the success rates of the procedure. Boon *et al.* [42] reported the generally good feasibility and repeatability of the LCI. In two studies including children and adults, all patients were able to perform at least one acceptable MBW measurement [40, 41], while 95.9% and 34.7% of them performed a second and a third valid measurement, respectively [40]. Koucký *et al.* [43] reported the feasibility of MBW in 81.8% and 54.5% of patients according to loose and strict criteria, respectively. In the study by Kinghorn *et al.* [44], a success rate of MBW performance in 75% of school-age children

was observed. Constant *et al.* [46] reported a high success rate of 75% on the first MBW trial in children of school age. Roehmel *et al.* [32] reported high feasibility of MBW measurements in preschool children with PCD, achieving a success rate of 93.1%. Unsuccessful MBW measurements were mainly attributed to leakage and unstable breathing pattern [43, 44].

Discussion

This review revises the current literature on the utility of MBW and how it compares with spirometry in paediatric and adult patients with PCD. Using a systematic search protocol with specific inclusion criteria, we identified 14 studies reporting MBW data in patients diagnosed with PCD. All of them showed abnormally increased mean/median LCI values, starting from infancy up to adulthood. In contrast, in 12 out of 14 studies, the mean/median FEV_1 was within the normal range, *i.e.* z-score >−1.65 or percentage of predicted value >80%. Two studies reported a mild decrease in FEV_1, *i.e.* z-score between −1.65 and −2.5 [7, 38, 43]. Our review suggests that there is growing evidence that the LCI is much more sensitive than FEV_1 to detect early impairment of pulmonary function in patients with PCD.

Among the MBW outcomes, the LCI was the most commonly reported, although alternative MBW outcomes were also measured in several studies. In one study, Cn@TO6 appeared to have similar values to the LCI [45]. Other MBW parameters, such as LCI_5 and moment ratios, requiring less time to be measured than the LCI, were found to be comparably sensitive to detect ventilation inhomogeneity in PCD [40]. In three studies, $S_{cond}\times V_T$ and $S_{acin}\times V_T$ were significantly increased in patients with PCD compared to healthy controls [42–44]. In the studies by Nyilas *et al.* [40, 41], S_{cond} was abnormal in up to 78% of the patients, whereas S_{acin} was abnormal in only half of the patients. Interestingly, the same group previously described three main physiological phenotypes based on MBW parameters and found an over-representation of patients with PCD in phenotypes with either increased S_{cond} alone or increases in both S_{cond} and S_{acin}. Patients classified under the second phenotype showed a higher rate of exacerbations and hospitalisations in the year preceding the measurement [49].

Several studies explored the feasibility of MBW testing in patients with PCD demonstrating very high success rates, similar to those observed in other patient groups and healthy individuals [50, 51]. The studies that included very young patients are of particular interest, as spirometry performance is limited in preschool age children. For preschoolers, MBW appears to be a reliable and feasible pulmonary function test and can further serve as a primary outcome measure in clinical studies [52]. In addition, promising studies including infants with PCD confirm high feasibility rates of MBW measurements in the PCD group [44, 53], similar to previous reports in CF [54, 55]. Infant-specific reference values for both SF_6 MBW [22, 56] and N_2 MBW will further enhance the routine use of MBW in this age group.

The superiority of the LCI over spirometric indices in detecting early lung disease is well documented in CF. In this context, the LCI is used routinely for monitoring [21, 57] and serves as a primary outcome measure in clinical trials [29, 30, 58, 59]. The LCI has also been found to be abnormal in other chronic obstructive lung diseases, such as non-CF, non-PCD bronchiectasis [31]. A recent systematic review of pulmonary function tests in PCD, focusing solely on paediatric patients, showed that LCI abnormalities appear earlier than in spirometry [60]. Our review also systematically collected data from studies in the adult PCD population and found that the LCI remains abnormal in later life. Further studies including more adult patients with PCD are needed to describe LCI changes with aging and disease progression.

Our review confirms that there is limited data on the alterations of MBW parameters over time in patients with PCD. The existing data in the literature are controversial in relation to the value of longitudinal assessment of MBW in patients with PCD. Vandervoort *et al.* [36] observed no significant alterations in LCI values over a 6-month period despite the use of chest physiotherapy before testing. On the other hand, Kobbernagel *et al.* [35] reported a minimal but significant increase in LCI values over 1 year, while other ventilation inhomogeneity indices remained unchanged. This suggests that such alterations either reflect disease progression or represent natural fluctuations. These data support the need for longitudinal studies on the natural history of lung disease in PCD using MBW as a monitoring tool.

Chest imaging findings in PCD were generally better associated with MBW parameters than spirometric indices. Compared to FEV_1, the LCI was found to have a stronger correlation with bronchiectatic changes, airway wall thickening and mucus plugging on chest CT. It also demonstrated a better correlation with lung ventilation impairment, as revealed by functional MRI and hyperpolarised gas MRI [34, 41, 42]. Thus, MBW appears to be a more sensitive tool than spirometry to identify structural changes of the airways in PCD. However, in a pilot study with a limited number of patients, FEV_1 showed a better correlation, with an inhomogeneity index calculated by electrical impedance tomography recordings,

compared to the LCI [33]. A possible explanation for this difference could be that CT and MRI provide imaging of the whole lung and may better match global changes in ventilation as measured by the LCI. In contrast, electrical impedance tomography images are localised in one transversal level of the lung, usually in the midline of the chest cavity, and may correlate more closely with large airway function. Further studies are needed to shed further light on these interesting associations.

Interestingly, MBW parameters were found to be associated with airway histopathological and ciliary ultrastructural findings in PCD. Abnormal LCI values were associated with bronchial epithelial reticular basement membrane thickening in PCD. This particular structure–function relationship was also seen in CF but was absent in asthma [43]. Furthermore, worse LCI values were found in patients with microtubular defects compared to those with dynein arm defects, which is in accordance with previous reports showing a more severe clinical phenotype in those patients [61], although the precise underlying pathways linking ciliary ultrastructure to clinical outcomes remain unknown [38]. More extended studies examining the correlation between genotype and MBW characteristics are needed to explain these associations [62].

This review has several strengths. We performed a detailed search of the technical details of the pulmonary function testing in the included studies and highlight differences in MBW acquisition, specifically in terms of 1) tracer gas used (N_2 or SF_6), 2) hardware, and 3) software versions. Due to the lack of universal reference values at the time the measurements were performed [63], interpretation of the results for each study was carefully conducted. On the other hand, the above findings should be interpreted in the light of several limitations. First, we followed a common methodological process for the initial screening of the existing literature, which may have overlooked publications that did not include the use of MBW in the title or the abstract. As such, a clinical trial on azithromycin use in patients with PCD included MBW measurements among the measured outcomes. After 6 months of azithromycin or placebo use, no differences were detected in the LCI [64]. In addition, the abovementioned differences in MBW performance, tracer gases and analysis software used [11–13], the large age variability of the participants, and the low number of existing studies, did not allow for data collation in order to perform a meta-analysis. As software issues have become more apparent recently, the software versions used for MBW were not reported in five studies performed in earlier years. Furthermore, the lack of specific diagnostic data in most of the studies prevented us from safely drawing any conclusions on their correlations with the MBW findings. Finally, besides the LCI and FEV_1, not all MBW and spirometric indices were reported in all studies, so other parameters of pulmonary function could not be directly compared.

In conclusion, although spirometry has traditionally been the gold standard for the assessment of pulmonary function, MBW is gaining ground as a practical and feasible method that quantifies ventilation inhomogeneity in PCD, starting from early childhood. This systematic review identifies the LCI as being superior to FEV_1, as it constitutes a more sensitive tool to detect pulmonary function impairment in children and adults with PCD. Given that the recently published Global Lung Initiative reference values promise easy data interpretation [63], the above findings support the use of MBW as a routine clinical tool in daily clinical practice and provide evidence for using the LCI as an outcome measure in upcoming clinical trials for patients with PCD.

Points for clinical practice

- MBW is a reliable and feasible pulmonary function test in both children, including preschoolers, and adults with PCD.
- The LCI is a more sensitive marker for the early detection of pulmonary functional impairment in PCD compared to FEV_1.
- Chest imaging findings, including bronchiectatic changes, are better associated with the LCI than spirometric indices in PCD.

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