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Inert gas washout: background and application in various lung diseases

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Summary

Multiple breath inert gas washout (MBW) is a lung function technique to measure ventilation inhomogeneity. The technique was developed more than 60 years ago, but not much used for many decades. Technical improvements, easy protocols and higher sensitivity compared with standard lung function tests in some disease groups have led to a recent renaissance of MBW.

The lung clearance index (LCI) is a common measure derived from MBW tests, and offers information on lung pathology complementary to that from conventional lung function tests such as spirometry. The LCI measures the overall degree of pulmonary ventilation inhomogeneity. There are other MBW-derived parameters, which describe more regional airway ventilation and enable specific information on conductive or acinar ventilation inhomogeneity. How this specific ventilation distribution is exactly related to different disease processes has not entirely been examined yet.

MBW measurements are performed during tidal breathing, making this technique attractive for children, even young children and infants. These benefits and the additional physiological information on ventilation inhomogeneity early in the course of lung diseases have led to increasing research activities and clinical application of MBW, especially in paediatric lung diseases such as cystic fibrosis (CF). In these patients, LCI detects early airway damage and enables monitoring of disease progression and treatment response. Guidelines for the standardisation of the MBW technique were recently published. These guidelines will, hopefully, increase comparability of LCI data obtained in different centres or intervention trials in children and adults.

In this non-systematic review article, we provide an overview of recent developments in MBW, with a special focus on children. We first explain the physiological and technical background to this technique with a short explanation of several methodological aspects that are important for understanding the principle behind the technique and enable high quality measurements. We then provide examples of MBW application in different lung diseases of children and adults, with regards to both clinical application and research activities. Lastly, we report on ongoing clinical trials using MBW as outcome and give an outlook on possible future developments.

Key words: lung clearance index, multiple breath washout, paediatric, pulmonary function tests, ventilation inhomogeneity

Introduction

For decades, conventional spirometry has been the standard technique to assess the degree of airway obstruction in most chronic lung diseases, including cystic fibrosis (CF), asthma, and chronic lung disease of prematurity. However, there is mounting evidence that, because of its underlying physiological principle, spirometry is insensitive for the assessment of peripheral airway involvement and for the assessment of ventilation distribution. This resulted in an increased interest in gas dilution techniques, in particular multiple breath-washout (MBW), for the assessment of small airway function, i.e. efficient, homogeneous ventilation distribution [1, 2].

MBW was first described more than 60 years ago by Ward S. Fowler [3]. In his pioneering work of 1952, he compared nitrogen clearance from single breath washouts between healthy subjects and patients with cardiopulmonary disease, to assess the degree of uneven alveolar gas dilution [3]. However, the technique was little appreciated until gas

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ABBREVIATIONS	
AHR	airway hyperresponsiveness
BTPS	body temperature, pressure, saturated with water
CT	computed tomography
CF	cystic fibrosis
CFTR	cystic fibrosis transmembrane conductance regulator
COPD	chronic obstructive pulmonary disease
DLCO	diffusion capacity for carbon monoxide
FEV$_1$	forced expiratory volume in one second
FVC	forced vital capacity
FRC	functional residual capacity
LCI	lung clearance index
MBW	multiple-breath washout
MR	moment ratio
MRI	magnet resonance imaging
PCD	primary ciliary dyskinesia
Sacin	slope of acinar airways
Scond	slope of conducting airways

analysers and computers were further developed to improve automated analysis of gas and volume signals during measurements [4, 5]. Today, the technique is returning to "prime time", especially in the paediatric pulmonology community. Recently, an international workshop reviewed current literature on the monitoring of preschool lung disease. Besides detailed recommendations for technical standards and measurement procedures, this report suggested MBW as a promising tool in preschool children with CF, highlighting its importance [6].

In this non-systematic review article, we provide an overview of recent developments in MBW. Pubmed and the North American and European clinical trial registries were searched. Search terms were: CF, lung clearance index, lung function test, lung disease, and washout. We specifically focused on literature in children. We first explain the physiological and technical background to this technique with a short explanation of several methodological aspects important for understanding the principle behind the technique and enable high quality measurements. We then provide examples of MBW application in different lung diseases of children and adults, with regards to both clinical application and research activities. Lastly, we report on ongoing clinical trials using MBW as outcome and give an outlook on possible future developments.

Physiological background, mechanisms of ventilation inhomogeneity

The main function of the human lung is to homogeneously ventilate the lungs, enabling efficient gas exchange. During fetal lung development, the lungs grow from proximal to distal by a continuous division of the airways, which later form the unique structure of the bronchial tree. The bronchial tree consists on average of 23 bronchial generations, but gas exchange only occurs in approximately the last 9 generations. The bronchial tree resembles a self-similar, so-called fractal structure, enabling efficient gas transport. Normal ventilation distribution occurs by convection and diffusion. Three main mechanisms of ventilation inhomogeneity are currently known: (i) convection-dependent inhomogeneity in the conducting airway zone (more proximal airways); (ii) diffusion-limitation related inhomogeneity in the diffusion-dependent airway zone (distal airways, acini); (iii) interaction between convection and diffusion in an intermediate zone at the level of the diffusion-convection front, which is thought to arise at the acinar entrance [7]. The acinar compartment, the alveoli, is separated by a thin tissue layer from a capillary meshwork and forms a large surface for efficient oxygen and carbon dioxide gas exchange.

Technical background

MBW testing

Besides analysis devices, MBW tests require only a tight facemask or mouthpiece and quiet tidal breathing for 2 to 10 minutes per test, making this technique applicable across all age groups, even infancy. Measurements in infants are made in a supine position during quiet non-rapid-eye-movement sleep (or with sedation), using a face mask. In older children and adults, measurements are usually performed in a sitting position with a mouth-piece and

nose clip. Differences between sitting and supine positions have been described [8] and need to be taken into account when comparing data or in longitudinal studies. To support regular breathing patterns, distraction with videos is recommended for children [9], and visual breathing pattern feedback may be useful for adolescents and adults [10]. Because time for triplicate testing can be demanding in busy outpatient clinics or for patients with advanced lung disease, promising abbreviated protocols have been proposed [11, 12].

Each MBW test consists of a wash-in and a washout phase. Depending on the gas for the MBW test employed, there are in principle two different ways to perform MBW:
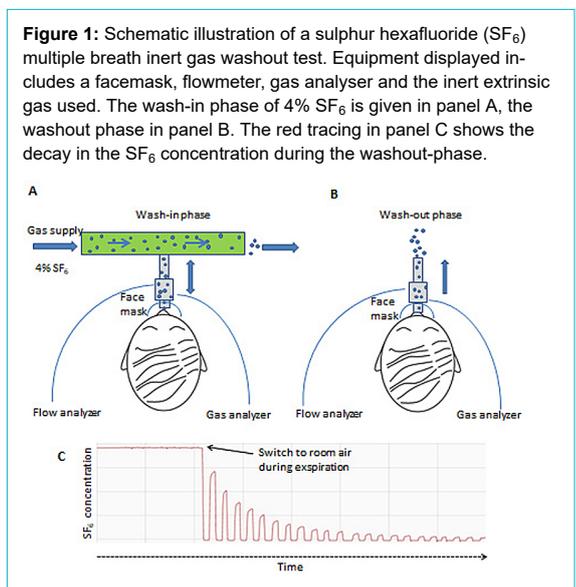
1. When an inert extrinsic gas (4% sulphur hexafluoride; 20% helium) is used, the gas mixture is inspired until an equilibrium is reached; the washout phase (breathing room air) starts from this point of equilibrium.

2. For inert intrinsic gas (nitrogen), no formal wash-in phase is required for the first of the three tests. For nitrogen washout of the airways, 100% oxygen is usually used.

Regardless of the gas used, the washout is stopped when the test gas reaches 1/40th (or 2.5% from the initial starting concentration set to 100%) of the initial gas concentration [7]. This cut-off was recently challenged, in order to improve comparability between different techniques, as the role of nitrogen is not fully understood yet [13]. The typical equipment setup for sulphur hexafluoride MBW is shown in figure 1.

MBW outcomes

Three main parameters are reported from MBW tests: the functional residual capacity (FRC), the lung clearance index (LCI) and moment ratios (MR). The FRC is the volume of air present in the lungs after tidal expiration in ventilated regions of the lungs; LCI and MR are both measures of global ventilation inhomogeneity. Besides LCI and MR, other parameters specifically assessing peripheral airway ventilation can be calculated, as detailed below.

Given that MBW setups measure inert gas concentrations and the cumulative volume required to washout the resting

Figure 1: Schematic illustration of a sulphur hexafluoride (SF$_6$) multiple breath inert gas washout test. Equipment displayed includes a facemask, flowmeter, gas analyser and the inert extrinsic gas used. The wash-in phase of 4% SF$_6$ is given in panel A, the washout phase in panel B. The red tracing in panel C shows the decay in the SF$_6$ concentration during the washout-phase.

lung volume (FRC), the latter can be calculated. The FRC is derived from a ratio: the cumulative expired volume (CEV) of the inert gas over the difference between the end-tidal concentrations of the inert gas (Cet) measured at the start (Cet$_{start}$) and end (Cet$_{end}$) of the washout. The LCI is a volume ratio, net CEV (including all gas fractions) over FRC: LCI = CEV/FRC. An increased ventilation inhomogeneity would thus result in more tidal breaths (greater net CEV) needed to wash out the inert gas, and in an increased LCI. To adjust for lung size, net CEV is divided by FRC to obtain LCI.

MR also quantify ventilation inhomogeneity, but are less commonly used. They have been described in detail elsewhere [4, 14–23]. The advantage of MR over LCI is that they can be weighted to specific parts of the washout curve.

Specific markers for peripheral lung ventilation are the slopes of alveolar phase III (SIII) of the inert gas expirogram. The first SIII value is thought to reflect ventilation inhomogeneity within diffusion-convection-dependent acinar airways (Sacin), whereas the subsequent evolution of SIII values from lung turnover 1.5 to 6.0 is thought to reflect ventilation inhomogeneity within convection-dependent conducting airways (Scond) [7, 24]. Although these indices were derived from numerical lung models [24], recent comparative data from ventilation imaging techniques are reassuring [25, 26].

MBW equipment and analysis procedures

One of the earliest and most recognised systems for MBW is based on a respiratory mass spectrometer, which allows simultaneous measurement of multiple gases at 33 Hz or higher. Drawbacks of the AMIS 2000 (Innovision, Denmark) relate to the custom design, sophisticated maintenance and costs [27]. Other customised systems have been described in detail elsewhere [7, 28]. Currently, there are at least three commercially available devices, which strongly differ in regard to the inert gas used, the gas analyser, analysis algorithms and the age group for which application is recommended (table 1).

Gases for MBW testing

Depending on the choice of gas and setup, derived MBW indices differ substantially [54]. For example, helium is much lighter than sulphur hexafluoride and generates systematically higher LCI values [7]. Furthermore, in subjects with emphysematous diseases, the diffusion equilibrium in the enlarged peripheral airways differs between gases. Other aspects regarding the use of sulphur hexafluoride are its costs and limited availability, since it belongs to the most potent greenhouse gases. Applications using lower sulphur hexafluoride fractions may be more suitable for routine use.

The choice of intrinsic gas (nitrogen) for MBW has the advantage that the oxygen required for washout is widely available and affordable. Another strength is that nitrogen is present in all parts of the lung and this gas, therefore, has great sensitivity to detect abnormality compared with extrinsic gases. Further, the wash-in is with room air and without the need for a tight mouthpiece, making it much easier to apply.

There is clear evidence that breathing patterns may change during MBW. Although in adults fixed 1-L breathing protocols may have some advantages with regards to slope III standardisation and analysis [67], most studies nowadays use free tidal breathing in order to take advantage of the natural breathing pattern. This is especially important in children, as fixed breathing protocols have been shown to influence MBW outcome parameters substantially [68]. Application of 100% oxygen in MBW was shown to alter breathing patterns in infants [22], and sulphur hexafluoride induced transient hypopnoea in preterm and healthy infants [69, 70]. However, in school-age children, MBW indices were not influenced by inhalation of 100% oxygen [71]. The effect of nitrogen back-diffusion from tissue nitrogen also requires further studies.

Table 1: Currently available multiple breath washout devices.

	Eco Medics infants	Eco Medics preschool and older	ndd EasyOne Pro LAB*	Innovision Innocor
Method	SF$_6$	N$_2$	N$_2$	SF$_6$
Flow/volume measurement	Ultrasonic flowmeter;	Ultrasonic flowmeter	Ultrasonic flowmeter	Pneumotachometer
Tracer gas measurement	indirect via molar mass mainstream	Indirect via O$_2$ and CO$_2$ measurement	Indirect via molar mass, O$_2$ and CO$_2$ measurement	Direct via photoacoustic spectroscopy, gas reservoir bag
Gas concentration	4% SF$_6$	100% O$_2$	100% O$_2$	SF$_6$ mixture: 0.1% or 0.2% SF$_6$ (27.6% O$_2$, 0.35% N$_2$O)
Validation studies	Lung model Schmidt [29], Gustafsson [30]	Lung model and *in vivo* Singer [31]	-	*In vivo* Horsley [10] Lung model and *in vivo* Gonem [32]
Methodological studies	Anagnostopoulou [33], Latzin [34]	Jensen [35], Summermatter [36]	-	Horsley [37], Downing [38], Grønbæk [39], Gonem [25], Gonem [32], Nielsen [40], Shawcross [41]
Application in healthy	Fuchs [42], Anagnostopoulou [33], Gray [43] Gray [44]			Downing [38]
Studies in cystic fibrosis	Belessis [45], Simpson [46], Hall [47]	Stanojevic [48], Stahl [49], Amin [50], Ramsey [51]		Davies [52]
Cystic fibrosis and controls		Singer [53], Poncin [54]	Poncin [54]	Downing [38]
Other disease groups	Hulskamp [55], Latzin [56]	Yammine [57], Boon [58], Nyilas [59], Madsen [60], Jarenback [61]	Fuchs [62]	Macleod [63]

CO$_2$ = carbon dioxide; O$_2$ = oxygen; N$_2$ = nitrogen; N$_2$O = nitrogen dioxide; SF$_6$ = sulphur hexafluoride This table is meant to provide a current overview of available setups and does not necessarily represent the full body of existing literature. Please refer to the respective manufacturer for an update on current studies and recent developments. Manufacture of AMIS 2000 (Innovision) has been discontinued (http://www.innovision.dk/Products/AMIS_2000.aspx, accessed on 1 March 2017). * Before 2012, ndd EasyOne Pro was in use for multiple-breath washout measurement using 4% SF$_6$ (MM sidestream) (Fuchs [64], Fuchs [65], Ellemunter [66], Fuchs [62]).

Sample flow and gas analysis

Tidal flow and gases are usually measured within the main path of the respiratory flow (mainstream), or a continuous sample is taken from a capillary (sidestream). Sidestream sample flow (suction) may alter the analyser response and add noise to "small" signals from infants. Flows and integrated volumes have to be further corrected for BTPS (body temperature, pressure, saturated with water) [7]. The gas concentrations can then be measured directly (respiratory mass spectrometry, infrared, etc.) or indirectly (molar mass, cumulative gas fractions, etc.) [31]. Of note, usually the flow and gas signals are not sampled at the same sensor point. Signals, therefore, need to be aligned in time. Poor BTPS correction or signal misalignment can be a source of error in MBW outcomes [7, 36].

Impact of dead space on LCI

Dead space roughly consists of two compartments. Technical dead space consists of the volume of MBW hardware (mask, mouthpiece, and tubes) required to transport gases to the sensors. Anatomical and physiological dead spaces are the volume of upper and lower airways, respectively, which transport gases but do not participate in gas exchange. Technical dead space is hardware specific and affects LCI [35, 72]. The impact seems larger in younger children than in adults, and is apparently independent of lung disease. Thus, a small technical dead space of ≤2 ml/kg is recommended [73].

Software for MBW analysis

For analyses of MBW indices, commercial online and offline software is available, and several custom-made software applications exist. Offline data analysis was frequently used in the past, but it has the disadvantage that analysis is time consuming, and this limits its application in clinical settings. Several studies reported an effect on MBW indices of different software and settings [33, 36, 74]. Current commercial setups usually provide recording and analysis software on board. These applications undergo constant development and software updates need to be validated in clinical settings for reliability [75–77].

Normative data for MBW

Depending on the age of subjects and the factors mentioned above (gas, equipment, dead space, software), LCI is usually below 8.5 lung turnovers (LCI units) in healthy subjects. However, normative data for MBW measurements across different age groups are scanty [42]. Some data stem from customised setups [78], which limits their generalisability. Furthermore, data are not only system, but also gas, specific and may even be influenced by the analysis approach. The latter has been shown for both healthy individuals and patients with CF [33, 74]. Two studies reported MBW reference values for infants, recorded with common available equipment from a large Swiss [42] and African population [43] and with measurements conducted at 5 weeks postmenstrual age. Since LCI is thought to decrease throughout infancy and early childhood, then remain constant and increase in the elderly [78], these reference data cannot be applied to other age groups. There are other relevant factors that influence MBW measures, including the posture during tests (supine or seated) [8], the gas choice (sulphur hexafluoride, nitrogen) [35], dead space [34, 72, 79], as well as sedation, which may play a significant role in LCI variation with age.

Limitations of MBW testing, knowledge gaps

Limitations to MBW application relate to technical and physiological aspects. Much effort has been made to improve standardisation of MBW protocols and analyses, but there are still several unanswered questions, as mentioned above. Overcoming these knowledge gaps seems difficult, considering the, at times, poor software transparency [76]. Besides software, there are other aspects that may change MBW indices. There is evidence that interventions prior to MBW testing, such as raised volume rapid thoracoabdominal compression [80] or physiotherapy [81–83], subsequently effect MBW outcomes. Other important aspects have been outlined previously [7].

The impact of repeat LCI measurements on respiratory disease outcomes is largely unknown. Data suggest a clear association with infection burden, structural airway pathology or later pulmonary exacerbation in CF, although it remains unclear what change in LCI should prompt clinicians to intervene. As for most lung function outcome parameters, the beneficial effect of regular measurement of LCI in clinics on disease outcome has not been assessed yet. Recent data may help to establish what would constitute a clinically important change in LCI, at least in preschool children [48].

Single breath washout

There are techniques other than MBW to assess ventilation inhomogeneity: single breath washout tests, with a single or double inert tracer gas mixture. Several studies have used this technique, also in younger children. Single breath washout has been used in CF patients to detect early lung disease [84], assess response to airway clearance [85], and to study the involvement of small airways in patients with mild asthma [86], COPD [87–90], PCD [60, 91] and bronchiolitis obliterans as a complication after lung transplantation [92–94]. SBW may be attractive for clinical settings, since measurements can be completed more quickly than for MBW, and single breath washout can be used during normal tidal breathing or forced manoeuvres. Although acceptable reproducibility of this test has been reported in adults [89] and children [84], reproducibility is lower than with MBW. Several unanswered technical aspects also remain (i.e., impact of breathing pattern) [7, 95, 96], precluding its use for clinical decision making.

Application of MBW in lung diseases

Cystic fibrosis

CF is an inherited life-limiting disease with a mean prevalence of approximately 0.8/10 000 in Europe and the United States [97]. In Switzerland, newborn screening, introduced in 2011, enables early CF diagnosis and follow-up of lung function [77]. CF lung disease is characterised by mucus plugging, chronic infection and inflammation resulting in irreversible lung damage. Treatment advances have resulted in the preservation of normal forced expiratory volume in one second (FEV_1) (>-1.64 z-scores) into young adulthood, but progression of bronchiectasis may be undetected by spirometry [98]. This led to more research into detection of early airway abnormalities in CF patients

with MBW, and several observational studies and clinical trials support its usefulness.

Observational studies

The majority of longitudinal data from infants and children with CF are currently obtained from two large prospective cohorts, the Australian Respiratory Early Surveillance Team for Cystic Fibrosis (AREST-CF) and the London Cystic Fibrosis Collaboration (LCFC), (reviewed in [99]). There is mounting evidence that, compared with healthy controls, LCI is already abnormal shortly after birth in patients with CF [1, 100, 101]. In infancy, LCI is normal in approximately half of the infants. Interestingly, there are infants with normal LCI values in the presence of abnormal forced volume values (as assessed with the raised volume rapid thoracoabdominal compression technique) [102]. Several observational studies "tracked" LCI from preschool to school age [103, 104]. A multicentre study assessed LCI at several time points and was able to identify significant deterioration of LCI in CF over time, which was not detected by spirometry [48]. Another study found that LCI during preschool years was more likely to be abnormal than spirometry, and an abnormal LCI in preschool children predicted both abnormal LCI, and abnormal spirometry at school age [103].

Studies in infants [45], children [53, 102] and adults [105] showed that patients with evidence of a bacterial infection were more likely to have abnormal LCI values. Findings from the longitudinal study AREST-CF suggested that LCI has a more pronounced increase in infants with airway infections compared with those without, and that this increase was long lasting [46]. Several studies used MBW to monitor treatment response to antibiotic therapy in infants and adult patients with CF [49, 106, 107]. A systematic review included data from 176 exacerbations and observed an overall decrease, albeit small (≈-3%), in LCI after antibiotic treatment. The LCI response to therapy was very heterogeneous in CF patients, and is not fully understood [106].

Clinical trials

There is an ongoing debate about whether or not MBW can be used in multicentre trials. The Cystic Fibrosis Foundation Workshop Report from 2015, for example, concluded that lack of knowledge on MBW equipment hampers routine application of MBW in clinical trials [6]. On the other hand, the European CF Society Clinical Trial Network Standardization Committee [108] suggested LCI as an outcome measure, especially in young children and those with mild CF disease.

To date, several single- and multicentre trials in CF patients that used LCI as outcome have been published. Two multicentre interventional studies investigated the treatment effect of drugs modifying the cystic fibrosis transmembrane conductance regulator (CFTR) defect in CF patients. One study enrolled patients aged >6 years, with at least one copy of the rare G551D mutation and normal FEV_1, and assessed the ability of LCI to detect a treatment effect of ivacaftor. Treatment with ivacaftor resulted in significant improvement of LCI compared with placebo [52]. Another study enrolled patients aged 6 to 11 years and homozygous for F508del-CFTR, to assess the treatment effect of combined therapy with ivacaftor and lumacaftor on

LCI. No changes in FEV_1 were observed after 24 weeks of intervention, whereas there was significant improvement in LCI [109].

Two trials examined the usefulness of LCI in infants and preschool children to assess treatment response to hypertonic saline inhalation [50, 110]. In one study, MBW measurements were made before and after treatment with hypertonic saline (twice daily for 48 weeks) in children <6 years (n = 25). LCI decreased (improved) significantly more in the hypertonic saline group, as compared with controls [110]. Of note, the pattern of LCI change with treatment was age dependent: in a subgroup of subjects <1 year of age, LCI was in the normal range at baseline and did not change after treatment [110]. A recent study in older children (mean age 14.0 years, n = 18) investigated the short term effect of hypertonic saline inhalation [50], and found that LCI did not change after 24 hours of treatment [50].

Responsiveness of LCI was assessed in two randomised double-blind placebo-controlled trials in older children. FEV_1 was not systematically affected, whereas LCI improved over a 1-month period after treatment with hypertonic saline [111], and with dornase alfa (Pulmozyme®), an enzymatic agent improving mucus clearance in CF [112].

A cross-sectional study assessed the effect of antibiotic therapy on LCI abnormalities and magnet resonance imaging (MRI) in clinically stable patients, aged 1 to 20 years. LCI and MRI were able to detect an effect of antibiotic treatment of pulmonary exacerbations [49], indicating that these tools can provide useful endpoints for intervention trials.

MBW indices and lung imaging

Several recent studies in children and infants compared the association of LCI with structural airway damage or functional correlates using lung imaging techniques, including computed tomography (CT) and MRI. Studies in infants reported poor association between LCI and bronchiectasis as assessed with CT [47, 51], but a closer correlation was found in preschool- and school-age children [51]. This is not the case for spirometry indices. A strong correlation between LCI and MRI imaging was reported in clinically stable CF patients (age range 2–20 years) [49]. This study further showed that LCI and MRI are able to distinguish disease severity levels, supporting the application of these tools for diagnostic and therapeutic monitoring. Taken together, these data support the concept of LCI as a sensitive measure of structural airway pathology. However, LCI cannot replace lung imaging yet as negative predictive values to exclude bronchiectasis appear too low, especially in younger children. An overview of MBW application in CF lung disease is given in table 2.

Wheezing

Lower respiratory tract infections, or other triggers such as allergen exposure, leading to wheezing episodes in preschool children, are highly prevalent. Some, but not all, wheezers experience worsening lung function [113] and involvement of the small airways, as assessed with MBW and lung biopsy [114]. Functional data from MBW are conflicting. One study measured LCI in 110 infants who required respiratory support during the first days of life. Measurements were performed with sedation, at approx-

imately 5 weeks postmenstrual age. The authors found a higher LCI in infants with less (≤3%) expiratory wheezing, compared with infants with more (>3%) expiratory wheezing [115]. These results were supported in a subsequent study reporting a higher LCI in 40 infants without wheezing compared with 41 wheezing infants [72]. These findings contradict a study in preschool children, which reported an increased LCI in wheezers compared with healthy controls [114], but mean LCI differences were small (6.8 vs 6.6.) and within the normal range. Apparently, the effect of bronchodilator inhalation on LCI is somewhat paradoxical. LCI may increase (worsen) after inhaled salbutamol in controls, but not in wheezing subjects. High variability between MBW tests at baseline and heterogeneous response to bronchodilators questions the use of MBW to distinguish preschool wheezers from healthy controls [116]. To summarise, current data do not support appli-

cation of MBW measurements in infants and children to characterise wheezing or to monitor treatment response.

Asthma

Asthma is the most common chronic respiratory disease in children, typically characterised by eosinophilic airway inflammation, airway hyperresponsiveness (AHR) and reversible airway obstruction. AHR is the ability of airways to narrow excessively as a result of provoking stimuli. Whereas current guidelines recommend symptom assessment and spirometry for the diagnosis of asthma [117], their value for monitoring asthma is not clear [118]. Spirometry was rather insensitive in the assessment of peripheral airways, unless severe obstruction was present [119]. LCI in asthmatic subjects was slightly elevated compared with healthy controls, but often within the normal range, and therefore not helpful in the diagnosis of asthma

Table 2: Overview of studies in paediatric patients with cystic fibrosis using multiple-breath washout measurements.

Study description	Patient number	Age at study	Study type	Main findings	Reference*
CF vs controls					
Infants	71	3 months	Cross-sectional	Higher LCI and FRC in infants with CF compared with 71 controls.	Hoo [102]
Preschool / school age / adult	40	2.5–5 years	Cross-sectional	Higher LCI in patients with CF compared with 37 controls.	Aurora [1]
	48	4.09 (0.7) and 7.83 (1.3) years	Prospective	Higher LCI in CF patients at preschool age compared with 45 controls. Preschool LCI predicted abnormal LCI in CF patients at school age.	Aurora [103]
	78	2.5–6 years	Prospective	LCI measurements in CF patients and 70 controls at 1, 3, 6 and 12 months detected lung function deterioration over time in CF patients.	Stanojevic [48]
	60	7.8 (1.3) years	Cross-sectional	Higher LCI in CF patients compared with 60 controls.	Owens [101]
	43	3–18 years	Cross-sectional	Higher LCI and MR in CF patients compared with 28 controls.	Gustafsson [100]
	47	9.5–16.1 years	Cross-sectional	LCI was higher in CF patients compared with 50 controls. Comparison of LCI measured with two different MBW devices revealed poor agreement between different setups.	Poncin [54]
CF with vs without bacterial infection	47	0.32–3.24 years	Cross-sectional	LCI of 25 healthy subjects was lower compared with CF patients without and with bacterial infection. In CF patients, inflammatory markers from BAL correlated with LCI.	Belessis [45]
	78	4–16 years	Prospective	LCI was higher in CF patients compared with 53 controls. LCI strongly correlated with *Pseudomonas aeruginosa* infection.	Singer [53]
	108	0.1–2.5 years	Prospective	LCI measurements at three time points during 2 years revealed a long-lasting increase in LCI after pulmonary infections. *Haemophilus influenzae* infections had a particularly detrimental effect on lung function.	Simpson [46]
	41	8–67 years	Cross-sectional	LCI was higher in CF patients compared with 6 controls. A lower colony count of aerobic/anaerobic bacteria was associated with a higher LCI in CF patients.	O`Neill [105]
Assessment of treatment					
Antibiotic treatment for pulmonary exacerbations			Systematic review of studies published until March 2014	Overall, LCI decreased after antibiotic treatment but individual response was heterogeneous in CF patients.	Sonneveld [106]
	26	1.8–19.9 years	Prospective	LCI was sensitive to detect response to antibiotic therapy for pulmonary exacerbations.	Stahl [49]
Hypertonic saline (7%)	25	1–5 years	Observational	Hypertonic saline twice daily for 48 weeks improved LCI.	Subbarao [110]
	18	7–56 years	Observational	Hypertonic saline 5 times over 24 hours did not improve LCI.	Amin [50]
	19	10.5 (3.1) years	Observational	Hypertonic saline twice daily over 4 weeks improved LCI.	Amin [111]
CFTR regulator ivacaftor	21	8–43 years	Clinical trial	Ivacaftor 150 mg twice daily for 28 days improved LCI.	Davies [52]
CFTR regulators lumacaftor/ ivacaftor combined	58	6–11 years	Clinical trial	Lumacaftor 200 mg + ivacaftor 250 mg twice daily for 24 weeks improved LCI, sweat chloride and nutritional status.	Milla [109]
Dornase alfa (Pulmozyme®)	17	6–18 years	Clinical trial	For weeks 2.5 ml daily Pulmozyme® inhalation improved LCI.	Amin [112]
Lung imaging and MBW					
Magnet resonance imaging	97	0.2–21.1 years	Prospective, cross-sectional	LCI correlated with abnormalities on MRI in infants, toddlers and children with CF.	Stahl [49]
Computed tomography	60	7.8 (1.3) years	Cross-sectional	Abnormal findings on lung CT correlated strongly with increased LCI in CF patients.	Owens [101]
	49	8.7–112.1 weeks	Cross-sectional	Air trapping and bronchiectasis assessed by CT were associated with MR, but not LCI.	Hall [47]
	42, 38, and 39	0–2, 3–6 and 7–16 years	Cross-sectional	Agreement between LCI and bronchiectasis in preschool and school age children, but not infants. Air trapping and LCI was only associated in infants.	Ramsey [51]

BAL = bronchoalveolar lavage; CF = cystic fibrosis; CFTR = cystic fibrosis transmembrane conductance regulator; CT = computed tomography; LCI = lung clearance index; MR = moment ratios; MRI = magnetic resonance imaging This table is meant to provide a current overview on studies in paediatric patients with CF and does not necessarily represent the full body of existing literature. *At the time of publication. Age at study is given as range or mean (standard deviation).

at the individual level [63, 120, 121]. Few studies have assessed the association between MBW measures and AHR. A study in older asthmatic subjects (age range 59–80 years) found that AHR was predicted by higher Sacin [122], and a study in asthmatic adults (age range 18–66 years) found that AHR was associated with LCI, and that treatment with inhaled corticosteroids decreased the LCI [123]. In adults with severe asthma, inhaled corticosteroid uptitration resulted in a decrease in Scond with no effect on Sacin [124]. The study further reported that those patients with increased ventilation inhomogeneity at baseline had the greatest improvement following inhaled corticosteroid dose uptitration [124]. An effect of bronchodilator response on LCI is still poorly understood. Two studies in children found no effect [63, 121], whereas another study reported that the LCI was elevated in adolescents with asthma and decreased after treatment with nebulised bronchodilator [125]. To summarise, MBW can be useful to explore the exact degree of airway damage and physiological impairment in asthma, but up to now the technique is not established for diagnosis or for monitoring treatment response in asthma.

Chronic lung diseases of prematurity

Disruption of normal lung development because of preterm birth results in complex structural changes in the airways, lung parenchyma and vasculature. This may result in smaller airway calibres and impaired alveolarisation, with a reduced number of alveoli and enlarged airspaces [126]. The functional relevance of disrupted airway development during infancy and thereafter is not fully understood. This can be partly explained by the fact that the degree of airway obstruction was underestimated by spirometry. Recent studies in former preterm infants used other outcomes, including the LCI [55–57, 127–129], FRC [44, 55, 56] and parameters specifically assessing peripheral airway damage (Scond, Sacin) [57]. Results are inconsistent, probably reflecting the heterogeneity of the disease and different measurement techniques. Abnormal values for LCI have been reported in preterm infants during infancy [55], and for former preterm infants at school age [129]. Other studies reported no difference in LCI values in former preterm infants compared with healthy subjects during early infancy [56], at 1 year [44], between 9 and 11 years [130], and between 7 and 13 years [57] of age. The last study further investigated parameters that specifically reflect peripheral airway ventilation. In that study, FEV$_1$ and Scond were often abnormal at school age, whereas Sacin was comparable between former preterm and healthy subjects. A functionally normal alveolar compartment at school age, with functionally abnormal central conducting airways, suggest a disynaptic growth pattern of the lungs in former prematurely born children [57]. Using MBW measurements, studies assessed the development of lung volume. Prematurity was associated with a reduced FRC during early infancy [55] and at 1 year of age [44].

Primary ciliary dyskinesia

Primary ciliary dyskinesia (PCD) is a rare congenital disease characterised by defective ciliary function leading to impaired mucociliary clearance and recurrent chronic upper and lower airway infections [131]. The diagnosis of PCD is challenging because of the heterogeneous nature of the disease and poorly standardised algorithms [131]. Patients with PCD may already have reduced lung function (FEV$_1$, forced vital capacity [FVC]) in the preschool years [132]. As in CF, spirometry may underestimate the full degree of functional impairment in early PCD lung disease [58, 59]. Recent studies reported marked peripheral airway dysfunction, assessed with MBW, in almost all patients with PCD [60, 91, 133]. Another study compared the association between LCI and FEV$_1$ with structural abnormalities, assessed with CT, in 38 patients. The authors reported a higher concordance between LCI and structural abnormalities compared with FEV$_1$ (83 vs 53%), suggesting that LCI measurements may be of clinical relevance in PCD patients [58]. Interestingly, studies using sulphur hexafluoride MBW did not find this association [134]. Overall, PCD lung disease is characterised by marked peripheral airway dysfunction, and MBW may be a promising tool for early detection of airway damage. Compared with CF, MBW data in PCD are scarce. Whether MBW has utility in the management and/or treatment of PCD still needs to be determined in longitudinal studies.

Chronic obstructive pulmonary disease

Chronic obstructive pulmonary disease (COPD) is characterised by a variable combination of airway and parenchymal abnormalities. COPD may partly develop irrespective of tobacco smoking, and much earlier in childhood than previously assumed [135, 136]. The hallmark of COPD is mainly irreversible airflow obstruction resulting from the narrowing of small conducting airways or loss of lung elastic recoil or both. In the diagnosis of airflow obstruction in COPD, simple spirometry is the main diagnostic tool, although it is recognised that none of the derived parameters are sensitive indicators of peripheral airway narrowing [137, 138].

Within a prospective study of subjects who smoked ≥10-pack years, an increase in ventilation inhomogeneity in both the conductive and acinar airways was detected by means of normal spirometry, as indicated by increased Scond and Sacin values. Moreover, both Scond and Sacin were associated with FEV$_1$/FVC values, but the former was also associated with FEV$_1$ and the latter with diffusion capacity for carbon monoxide (DLCO) [139]. Similar results were reported from a cross-sectional study of 57 COPD patients with ≥15-pack years. COPD patients had increased Sacin but not Scond values compared with healthy controls [61], and an association between Sacin and DLCO was also reported [89]. Taken together, these studies suggest that MBW measurements have the potential to diagnose subclinical disease at an earlier stage, separate airway from parenchymal abnormalities, and to further characterise clinical features of advanced COPD. Although good feasibility and reproducibility of washout measurements in COPD patients have been shown [89], there is still further need to validate this method for noninvasive detection of early abnormalities or peripheral or parenchymal sites [140].

Other disease groups

In addition to the application of MBW in patients with CF and other common lung diseases, recent studies have applied this technique in less frequent lung disease, of which some studies will be reported.

Bronchiolitis obliterans is a small airway disease in which chronic inflammation results in fibrotic remodelling of the peripheral airways. It can occur, for example, after exposure to toxic substances or following allogeneic haematopoietic stem cell transplantation. Early detection of bronchiolitis obliterans is important in order to prevent later lung damage. One study reported abnormal washout indices before a decrease in FEV_1 was observed [141], and measurements may even be used to detect different severity grades of bronchiolitis obliterans, as recently reported [142]. In patients undergoing haematopoietic stem cell transplantation, a study of 33 clinically stable recipients provided some evidence that measurements of MBW were more sensitive than spirometry to detect bronchiolitis obliterans at an early stage [143].

Alpha-1-antitrypsin deficiency is a genetically inherited disorder resulting in emphysematous lung changes. Within a heterogeneous group of 193 patients with alpha-1-antitrypsin deficiency (age range 4–79 years), LCI was higher in patients than in controls. Furthermore, LCI was found to be abnormal in patients with normal spirometry measures, indicating that LCI identifies lung disease related to alpha-1-antitrypsin deficiency earlier than spirometry, comparable to the chronic lung diseases discussed earlier in this article [62].

Bronchiolitis is a common respiratory tract infection during early childhood. A recent study included 29 infants (mean age 3.7 months) with bronchiolitis and reported an increased LCI compared with controls [144]. Another prospective study enrolled infants below <1 year of age hospitalised with bronchiolitis due to respiratory syncytial virus infection and reported differences in FEV_1, but not in LCI, between patients and controls at the age of 18 years [145].

MBW in ongoing clinical trials

Currently, there are more than 50 ongoing studies using MBW indices as outcome measures listed in the North American [146] and European [147] clinical trials registries. Most of these trials are in patients with CF or asthma, but some are in less common lung diseases. We present a selection of trials we consider interesting, although it is not a comprehensive overview.

The first trial using LCI as primary outcome in CF patients (aged 6–11 years) homozygous for the F508del mutation after treatment with combined therapy of lumacaftor/ivacaftor (Orkambi®) twice daily over 24 weeks is still ongoing [148]. The study assesses efficacy and safety of the drug, as well as treatment effects on LCI. Two further trials in children are investigating the treatment effect of 8 weeks of ivacaftor. One trial is already ongoing, aiming to recruit 50 children aged 3–5 years with different CFTR mutations to assess changes in LCI [149], and another trial will study the efficacy of ivacaftor in subjects >6 years old, focusing on a specific CFTR mutation [150].

One trial is being conducted in PCD patients within the BESTCILIA (Better Experimental Screening and Treatment for primary CILIAry dyskinesia) study. This is a European multicentre, double-blind, randomised, placebo-controlled trial with the aim to determine the efficacy of azithromycin maintenance therapy for 6 months on respiratory exacerbations in patients with PCD. This interventional trial is currently ongoing and aims to recruit 125 PCD patients, aged 7–50 years old. Besides the quality-of-life assessment, one of the main outcomes is the LCI [151]. This trial will hopefully help elucidate whether or not maintenance therapy with azithromycin has beneficial effects in patients with PCD.

Several trials assess treatment response to inhaled glucocorticoids, betamimetics or hypertonic saline in adult [152] and paediatric patients with asthma [153] and CF [154]. For example, in patients with CF aged 6–18 years of age, a randomised controlled trial is assessing the short term effects (within 24 hours post-dose) of hypertonic saline inhalation on LCI [154].

There is an observational, prospective study recruiting children with CF and asthma, but interestingly, also children with bronchiolitis obliterans and sickle cell anaemia [155]. This trial will validate the MBW device for different lung diseases in Canada, where it is not currently licensed yet. This study may also provide insights into the application of MBW in pulmonary complications in children with sickle cell disease, which has not yet been investigated.

Little is currently known of the involvement of small airways in patients with interstitial lung disease. One trial currently underway will perform MBW in adult patients with different manifestations of interstitial lung disease (Wegner's Disease, idiopathic pulmonary fibrosis, sarcoidosis) to further understand the complex pathology and heterogeneous presentation of these diseases [156].

Conclusion

MBW has regained interest in recent years. Reasons for this renaissance relate to technical and physiological considerations. The technique is sufficiently sensitive for the early detection of lung function impairment often arising in small peripheral airways. MBW provides information that cannot currently be obtained by other lung function tests or chest imaging. MBW is especially attractive for young patients, as measurements require minimal cooperation. There is mounting evidence that the LCI is useful for assessing the extent and progression of lung disease, as well as treatment response, in patients with CF. LCI is already broadly applied in clinical care of CF patients, yet a number of unresolved questions remain. For example, there are currently poorly defined upper limits of normal for LCI values and its natural variability over time. Further, different software settings and device setups may have an effect on LCI values. Application of MBW tests in other chronic lung diseases may be attractive for research, whereas the impact on clinical management and respiratory disease outcomes require further studies.

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