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Lung function corner

Multiple breath washout: measuring early manifestations of lung pathology

The multiple breath washout (MBW) test measures the efficiency of gas mixing in the lungs and has gained significant interest over the past 20 years. MBW outcomes detect early lung function impairment and peripheral airway pathology, through its main outcome measure lung clearance index (LCI). LCI measures the number of lung turnovers required to washout an inert tracer gas. MBW is performed during normal (tidal) breathing, making it particularly suitable for young children or those who have trouble performing forced manoeuvres. Additionally, research in chronic respiratory disease populations has shown that MBW can detect acute clinically relevant changes before conventional lung function tests, such as spirometry, thus enabling early intervention. The development of technical standards for MBW and commercial devices have allowed MBW to be implemented in clinical research and potentially routine clinical practice. Although studies have summarised clinimetric properties of MBW indices, additional research is required to establish the clinical utility of MBW and, if possible, shorten testing time. Sensitive, feasible measures of early lung function decline will play an important role in early intervention for people living with respiratory diseases.

Educational aim

- To describe the multiple breath washout test, its applications to lung pathology and respiratory disease, as well as directions for future research.

Introduction

Many common obstructive pulmonary diseases originate in the peripheral airways, often manifesting long before symptoms appear or are detected by standard pulmonary function tests (*e.g.* spirometry). The most commonly used spirometric indices, such as forced expiratory volume in 1 s (FEV_1) and forced vital capacity (FVC), predominantly reflect large airway function and therefore miss the opportunity for early detection of damage to the small airways and intervention to prevent long term sequelae [1–3]. While forced expiratory flows (*i.e.* forced expiratory flow at 25–75% of FVC) may be more sensitive to small airway changes than FEV_1 or FVC and can detect differences between groups, they are highly variable
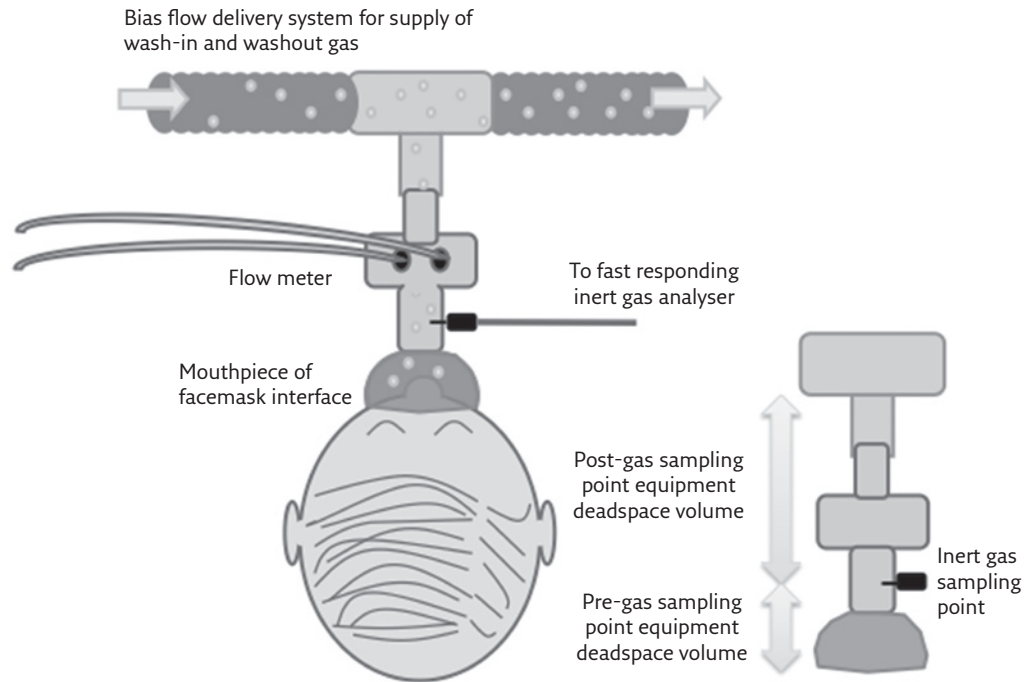
Figure 1 *Schematic illustration of a generic inert gas washout system. Hardware required for washout is relatively simple: a flow meter, a fast-responding inert gas analyser, a gas delivery system, and a patient interface. The equipment-related deadspace volume (V_D) can be divided into pre- and post-gas sampling points. Post-gas sampling point V_D effectively introduces a small rebreathing chamber. Pre-gas sampling point V_D is an extension of anatomical V_D. Reproduced from [10] with permission.*

and influenced by FVC which makes them difficult to interpret in an individual [4, 5]. The multiple breath washout (MBW) test offers the ability to detect early manifestations of peripheral airway pathology by assessing gas mixing within the lungs, a process that all airways participate in [6–8]. The MBW test was first described in 1952 by Dr Margaret Becklake [9]; however, interest in the technique has only gained significant traction in the past 20 years. Consensus recommendations regarding technical standards for equipment and measurement protocols have been integral to improving the accuracy and consistency of results and the subsequent emergence of robust commercial devices [10]. Validation of MBW equipment is recommended, and the *in vitro* precision of measured functional residual capacity (FRC) for several devices using a double chamber plastic lung model is within 5% across a range of lung volumes and respiratory frequencies [11–14]. The availability of commercial devices has helped facilitate the implementation of MBW into multicentre international research studies [15, 16], and its transition into routine clinical practice [17]. In this review we describe the MBW test, its applications and challenges, and directions for future research.

What is multiple breath washout?

MBW assesses ventilation distribution by measuring how efficiently the lungs clear an inert tracer gas across a series of breaths (figure 1). The lungs have evolved to promote efficient gas mixing, and in healthy individuals, an inhaled gas should distribute evenly throughout the lungs. The distribution of obstructive lung disease is typically patchy in its early stages, and this narrowing/obstruction of peripheral airways leads to uneven and less efficient gas mixing. Therefore, the longer it takes to wash out the tracer gas of interest, the greater the ventilation inhomogeneity present and the less efficient gas mixing is. The "inert" tracer gas must be safe to inhale and not participate in gas exchange. Exogenous gases (*e.g.* sulfur hexafluoride (SF_6) and helium) must be washed-in before they are washed-out by breathing room air, whereas endogenous gases (*e.g.* nitrogen) are washed-out by breathing 100% oxygen (figure 1). One of the advantages of MBW is that it can be performed during tidal (normal) breathing, which makes it ideally suited for infants and young children, as well as some adults, who have trouble performing the forced manoeuvres required for standard pulmonary function tests [17, 18].

The lung clearance index (LCI), the most commonly reported outcome measure from the MBW test, is a global measure of ventilation inhomogeneity. It is calculated as the cumulative expired volume of air exhaled during the washout portion of the test, corrected for a measure of the subject's lung volume (FRC), which is also calculated during the test. In other words, the LCI represents the number of lung volume turnovers (FRCs) required to clear the tracer gas. The change in slope of the normalised nitrogen alveolar plateau

(phase III slope) over a series of breaths can also be reported to distinguish ventilation heterogeneity arising within proximal conducting airways (S_{cond}) from that arising in more distal airways within the region of the lung acinus (S_{acin}) [10, 19].

In research studies the feasibility of MBW is often greater than 80% [20, 21]; whereas when implemented into clinical practice the feasibility drops to 60–70% [8, 22]. The feasibility is also higher in school-age children compared with preschool children; however, with the appropriate training and child-friendly environment, feasibility can be up to 89% in preschool children [23, 24]. The majority of studies, to date, have measured LCI in individuals with normal measures of spirometry, or with mild lung disease. The LCI is more variable in individuals with more advanced lung disease and may not be as useful in those with reduced lung function measured from forced expiratory manoeuvres.

Interpretation of results

As with all pulmonary function tests, MBW must meet the technical requirements of a good quality test before results can be interpreted [10, 25]. In addition, repeatable trials must be obtained to ensure the measured indices reflect the physiology within the lungs; the average of at least 2–3 trials is typically reported. Although some criteria are clear and objective to define, evaluation of the quality of a washout curve requires careful breath-by-breath review. For example, at least three breaths must be present under the target end concentration with no evidence of leaks (*i.e.* the intake of room air causing a change in the tracer gas concentration measured by the system). In the example depicted in figure 2, the washout curve includes several examples of leaks, and the interpretation of the results produced by this washout would be biased and not reflect the true underlying ventilation inhomogeneity within the lungs.

Furthermore, the breathing pattern should reflect normal tidal breathing. In adult testing, some respiratory function laboratories have used a fixed 1 L tidal volume breathing protocol [27] to ensure clear visualisation of the phase III slope and a more stable breathing pattern. However, in young children this approach is not feasible, and in older children this may lead to significantly different LCI and FRC results [28]. Although extreme deviations from normal tidal breathing may impact LCI and other indices, some degree of variability in breathing pattern is expected and does not necessarily influence outcomes [29]. Further research is needed to identify which quality control criteria impact results significantly. Implementation of clear, objective criteria to evaluate the quality of the washout in real-time will help to increase the confidence with which results can be interpreted and may not require detailed review. These efforts are already well underway [15].
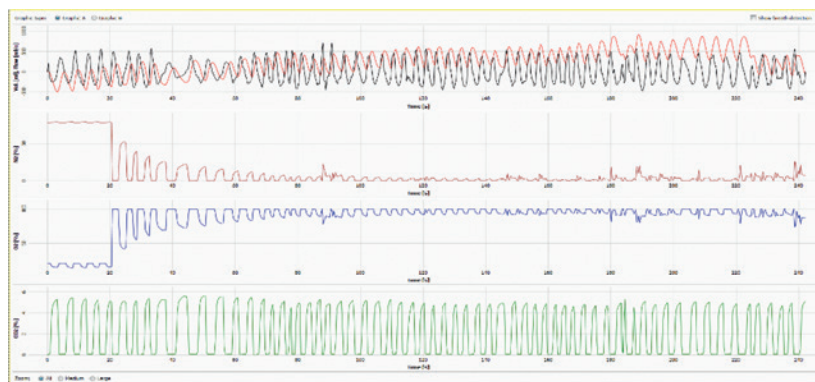

Figure 2 *Example of a washout curve. From top to bottom, the breath-by-breath tracings show the volume/flow (red/black), nitrogen (N_2) concentration (red), oxygen (O_2) concentration (blue), and carbon dioxide (CO_2) concentration (green). In this example there are multiple instances of gas leaks, apparent as increases or spikes in nitrogen concentration, where external nitrogen has been entrained and measured by the system. As the test measures the washout of nitrogen from the lungs, nitrogen contained in room air that enters the system will increase the measured gas concentration and over-estimate the LCI [26].*

How much change in LCI is important?

As with all pulmonary function tests, it is important to monitor changes over time to understand whether changes in lung function are associated with clinically meaningful events. Quantifying the magnitude of change that can be attributed to measurement error and/or biological variability is necessary to be able to distinguish noise from clinically meaningful changes. For the MBW tests, the measurement error of the test can be minimised by appropriate calibration of equipment and test measurement protocols, whereas the biological variability of the test has been shown to be proportional to the degree of impairment (figure 3). In other words, the variability of LCI is higher in individuals with worse LCI. Initially, evidence
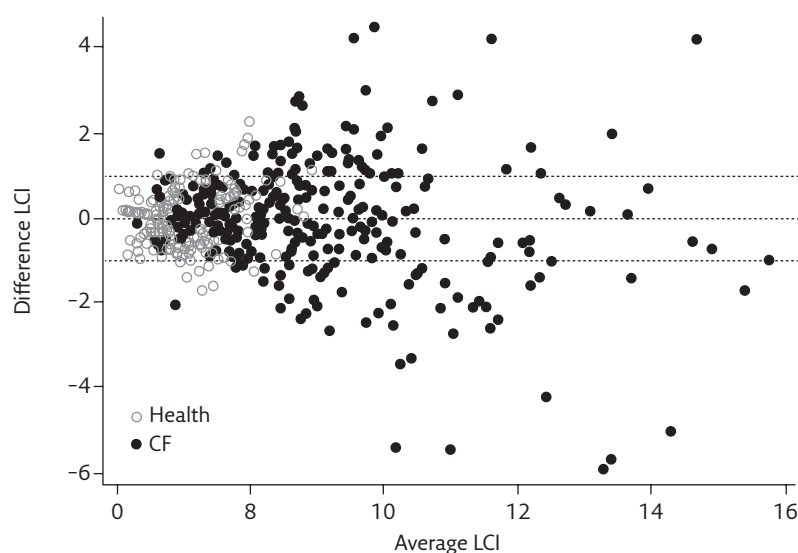

Figure 3 *Bland–Altman plot of the difference in repeated LCI measurements. The difference between LCI measurements is greater at higher LCI values. CF: cystic fibrosis. Reproduced from [31] with permission.*

Self-evaluation questions

1. Can you interpret the LCI value from a single MBW trial?
   a) Yes, provided it is a good quality trial
   b) No, you need to report at least two good trials
   c) Yes, as only one trial can be obtained from participants
2. Can you use LCI_5 (LCI at 5% of the starting gas concentration) instead of $LCI_{2.5}$ (LCI at 2.5% of the starting gas concentration) to define increased ventilation inhomogeneity?
   a) No, the LCI_5 has reduced sensitivity and you may miss important changes
   b) Yes, the LCI_5 and $LCI_{2.5}$ have the same diagnostic characteristics
   c) Yes, the LCI_5 and $LCI_{2.5}$ are interchangeable and provide the same information
3. Which of the following statements is true?
   a) The clinical utility of the MBW test will improve with adaptations to reduce testing time
   b) The MBW test can only be used in infants and young children with CF
   c) The MBW test requires expensive gases that are difficult to purchase

Shortening the testing time?

The biggest barrier to implementation of the MBW into the busier environment of routine clinical care is the time required to complete the test and obtain 2–3 good quality curves. There is strong evidence in support of using earlier cut-offs, namely LCI_5 at 1/20th of the starting concentration. The earlier cut-point can save considerable time (~30–40%) and produces similar results in terms of repeatability, diagnostic characteristics, and detecting significant treatment effects [33–35]. Yet there is some concern that shortening the washout may reduce the sensitivity to detect lung function impairment [36]. LCI_5 (*i.e.* LCI at 1/20th of the starting concentration) has currently not been widely used in clinical care or research and future work is needed to define the clinimetric properties of LCI_5 to aid clinical interpretation and implementation.

What's next?

The feasibility for measuring MBW in childhood, including infants and preschool children, has been repeatedly demonstrated for different MBW devices and tracer gases across both the clinical and research environment. Most of the research has focused on the paediatric CF population. As the majority of preschool-age children with CF have an elevated LCI [22, 24, 37], further standardisation efforts to develop robust equipment and define testing protocols for infants are necessary, and currently ongoing [10, 38].

While the vast majority of MBW research to date has been in the CF population, increasingly MBW is also being used in other obstructive lung diseases. For example, MBW outcomes have been used in adults to detect the early damage from cigarette smoking [39], bronchiectasis [16], COPD [40] and early post-transplant bronchiolitis obliterans syndrome (BOS) [41, 42]. Ventilation inhomogeneity is present in asthma (both mild and uncontrolled) [43–45]. More recently a study of symptomatic military deployers was able to detect airway injury due to exposure to high concentrations of particulate matter from sandstorms, diesel combustion, and burning waste using MBW [46]. Cumulatively, these studies highlight the potential for MBW to be used as a screening tool to detect early peripheral airway injury (table 1).

A number of challenges remain before recommendations can be made to use MBW as part of routine clinical care. Although normative reference ranges, as well as within- and between-test reproducibility limits have been published [31, 32, 47–49], the minimal clinically important difference remains to be determined, and we need to better understand how often measurements should be performed, and for which conditions MBW has demonstrable clinical utility. There are

suggested a 1-unit change in LCI was considered meaningful, and studies were designed to look at whether clinical care could be guided when a 1-unit change in LCI was observed [30]. However, since variability is proportional to the measured value, a 1-unit change may underestimate variability in those with worse LCI values, and therefore overestimate meaningful changes, whilst missing important changes in those with values closer to the normal range. For example, a 1-unit change in an individual with an LCI of 15 represents a 6.6% change, whereas a 1-unit change in an individual with an LCI of 7 represents a 14% change. Therefore, reporting relative change in LCI may be a solution to this, with changes greater than 15% considered outside variability observed in health and stable cystic fibrosis (CF) [31, 32]. Further studies are needed to verify these cut-off points, or to develop anchor-based approaches that are tied to clinical end-points.

Table 1 *Summary of potential utility across disease groups*

Disease group	Use
Healthy	Assess airway function in individuals too young to perform spirometry
Asthma	Assess ventilation inhomogeneity Assess asthma control
CF	Detect early lung damage Tracking longitudinal changes in lung function Assessing response to treatment
Bronchiectasis	Detect early peripheral airway involvement
COPD	Detect early peripheral airway involvement
BOS	Detection of early post-transplant BOS

several commercial MBW devices now available, each using different gases and/or sensors. Despite standards for equipment and software and validation studies, results from different devices/gases are not interchangeable [50]. Currently, reference equations for healthy populations need to be derived for each device separately. There is a European Respiratory Society task force underway to develop standardised reference equations. The development of these equations, along with further adaptations to software and testing protocols, will aid incorporation into clinical practice.

Conclusions

There is accumulating evidence that the peripheral airways play an important role in several obstructive lung diseases. In addition, the origins of many chronic respiratory diseases lie in early life. Sensitive, feasible measures of lung function to detect peripheral airway changes in childhood and early adulthood, such as MBW, are likely to play an important role in future screening and early intervention strategies.

Key points

- The MBW test detects peripheral airway narrowing/obstruction by measuring the efficiency of gas mixing in the lungs.

- Standard pulmonary function tests, such as spirometry, predominantly reflect large airway function and therefore are less sensitive to early signs of lung function impairment in the peripheral airways.

- MBW has evolved in the past 20 years, and now has validated commercial equipment, detailed technical standards, and established clinimetric properties.

- Although the MBW test has been implemented into international clinical trials, further work is required to better understand its clinical utility.

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Conflict of interest

None declared.

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Suggested answers

1. b.
2. a.
3. a.

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