Lung clearance index: assessment and utility in children with asthma

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ABSTRACT There is increasing evidence that ventilation heterogeneity and small airway disease are significant factors in asthma, with evidence suggesting that the small airways are involved from an early stage in childhood asthma. Spirometry is commonly used to monitor lung function in asthmatics; however, it is not sensitive to small airway disease. There has been renewed interest in multibreath washout (MBW) tests, with recognition of the lung clearance index (LCI) as a global index of abnormality in gas mixing of the lungs that therefore also reflects small airway disease. This review summarises the technical and practical aspects of the MBW/LCI in children, and the differences between commercially available equipment. Children with severe asthma are more likely to have an abnormal LCI, whereas most children with mild-to-moderate asthma have an LCI within the normal range, but slightly higher than age-matched healthy controls. Monitoring children with asthma with MBW alongside standard spirometry may provide useful additional information.

Introduction

Asthma is the most common chronic respiratory disease in children and is characterised by airway inflammation and bronchial hyperresponsiveness. Histopathology studies and *in vivo* transbronchial biopsies provide evidence of inflammation in central and peripheral airways [1, 2]. The presence of remodelling is demonstrated by an increased volume of the submucosal bronchial vessels, due to congestion and thickening of the smooth muscle layer [3–7]. The airways can be divided into the conducting and respiratory zones. The conducting airways consist of the first 15 airway generations, up to and including the terminal bronchioles, and are not involved in gas exchange. The respiratory, or acinar, airways comprise the respiratory bronchioles and all more distal airways. Alveoli are found throughout the respiratory zone and hence, this is where gas exchange takes place. The airways can also be divided into large and small airways, with the small airways being those with a diameter <2 mm. Therefore, the large airways are entirely conducting, and small airways (which begin at approximately airway generation eight) include some conducting and all the acinar airways [8]. Most of the structural changes in asthma have been described in the large airways but the role of small airway disease in asthma is increasingly recognised [9, 10]. Small airway involvement in asthma has been shown in lung tissue following surgical

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resection, biopsy and autopsy [3, 11, 12]. These findings suggest more extensive inflammation and remodelling in small or peripheral airways compared with central airways. Knowing the extent of large and small airway involvement is clinically relevant when aiming to achieve symptom control, reduce airway hyperresponsiveness and assess response to treatment [13].

Conventionally, asthmatic airway disease is monitored using spirometry. This measures the airflow limitation at the airway level with the highest resistance but cannot pinpoint the site of major airflow obstruction. In recent years there has been renewed interest in multibreath washout (MBW) tests, with the lung clearance index (LCI) becoming recognised as an index reflecting global lung function, including small airway disease, in conditions such as cystic fibrosis (CF) [14–17]. MBW tests have been found to be useful in the early detection of lung structural alterations and are more sensitive than conventional lung function tests [18, 19]. Studies have shown that airway function in children with asthma is frequently characterised by increased ventilatory inhomogeneity even when spirometry is normal [9, 20–22], confirming that spirometry is neither sensitive nor specific to small airway flow obstruction, unless gross changes are present [9, 23].

The site of ventilatory heterogeneity can be divided into abnormalities occurring predominantly in the acinar or the conducting airways. There is evidence of this in adults with COPD [24] and asthma [25], and indices of acinar and conductive airway ventilatory inhomogeneity are becoming a research focus [26, 27].

We know that there is a small but significant subset of children with severe asthma that are unresponsive to high-dose inhaled corticosteroid (ICS) treatment, and one potential mechanism for this may be disease located in the small airways that may not be reached by conventional inhaled therapies. This is an often overlooked aspect in asthma management that may have therapeutic implications.

Although monitoring with MBW may be useful in certain diseases, including CF and COPD [13, 15, 24, 28–32], the role of MBW in asthma is still unclear. The aims of the current review are as follows. 1) Summarise the technical and practical aspects of the MBW/LCI in children, the practicalities of testing, and the differences between commercially available equipment. 2) Review the findings of MBW testing in children with asthma and discuss potential implications for clinical practice. 3) Provide recommendations for future work.

Multibreath washout tests

Method and derivation of LCI

MBW requires that the participant maintains a leak-free seal while breathing through equipment to measure inspired and expired volumes and gas concentrations. A steady breathing pattern without extreme changes in rate or depth is all that is required and, since there is no need of respiratory manoeuvres requiring active cooperation, the technique can be applied in infants during sleep [17].

MBW tests assess ventilation distribution inhomogeneity during tidal breathing by examining inert gas clearance over a series of relaxed breaths. The inert gas can be any gas that is neither absorbed nor excreted to any significant extent during the period of washout. Commonly used gases include sulphur hexafluoride (SF_6) or helium, which must first be inspired (or washed-in) to achieve a stable baseline concentration. An alternative is to use nitrogen, normally resident in the lungs, and record the nitrogen washout from the point at which the participant was switched to breathing from a nitrogen-free source.

By convention, washout is deemed to be complete when the concentration of the tracer gas has fallen to 1/40 of the starting concentration. Historically, the reason for this is related to the accuracy of early nitrogen analysers.

Once washout is complete, different indices of ventilation inhomogeneity can be produced, with LCI being regarded as the most robust and sensitive [18, 33]. LCI is the cumulative expired volume (CEV), the total sum of gas expired during the washout, divided by functional residual capacity (FRC). The CEV requires correction for the apparatus dead space. FRC is determined during washout as follows:

$$FRC = V_{tracer}/(C_{tracer(init)} - C_{tracer(end)})$$

where V_{tracer} is the volume of tracer gas breathed out during washout, and $C_{tracer(init)}$ and $C_{tracer(end)}$ are the end-tidal concentrations of tracer gas at the beginning and end of the washout, respectively. Since this technique for measuring FRC quantifies the volume of gas in the lungs that is in communication with the exterior *via* the mouth (or nose), it approximates FRC as measured by standard gas dilution techniques, such as helium dilution.

LCI is then calculated as:

$$LCI = CEV/FRC$$

In simple terms, LCI represents a measure of the number of times the resting or end-tidal lung volume has to be "turned over" to clear the tracer gas from the lungs. Strictly speaking, LCI is an index but is often described in "turnovers". In the presence of ventilation inhomogeneity there is an increased number of turnovers before the tracer gas has been emptied from the lung [17, 18]. Although the LCI normal range can differ slightly depending on the choice of gas, in healthy children LCI has a normal range of approximately six to seven turnovers, which is largely independent of age, sex, height and weight through to adolescence [13, 15, 17, 29, 30, 34, 35]. During adult life, LCI increases gradually between 20 and 80 years of age [36].

LCI has been shown to be a reproducible and repeatable measure of ventilation inhomogeneity in children [13, 37]. It is more sensitive than standard spirometry in detecting early airways disease and demonstrating the clinical benefit of new interventions in the follow-up of patients with chronic diseases, principally CF [15].

Detailed analysis of MBW

The use of MBW and LCI to provide an index of overall ventilation inhomogeneity has increased over recent years for some diseases, especially CF. Detailed breath-by-breath analysis of the washout has enabled differentiation of the predominant site of ventilatory inhomogeneity into conducting or acinar airways. The usual index of ventilatory inhomogeneity in the conducting airways is S_{cond}; S_{acin} is the index of ventilatory inhomogeneity within the acinar airways. The theoretical basis for differentiating S_{cond} and S_{acin} relates to the predominant means of gas transport (convection or diffusion) and a detailed description can be found elsewhere [37]. It should be noted that the physical properties of the tracer gas affect the measured values and, as with LCI, the results of measurements obtained with different gases are not interchangeable [33]. The analysis is based on the plateau phase (the phase III slope) of each expired breath, which is not a steady concentration but shows an increase. When the slopes are normalised for the mean gas concentration of each breath, the normalised slopes tend to increase over the course of the whole washout (figure 1b).

The phase III slope of the first breath of the washout is generated by asymmetry at the acinar level. After the first breath, the extent to which the normalised slopes increase during the washout is a function of inhomogeneity in the conducting airways. Thus, to look at the relative contributions of S_{cond} and S_{acin}, it is necessary to plot the normalised phase III slopes over the course of the washout, then determine S_{cond} from the increase due to the convective flow component. Finally, S_{cond} is subtracted from the normalised slope of the first breath to calculate S_{acin} (figure 2).

Technical aspects of MBW

Equipment

The basic requirements are an analyser for the tracer gas, a means of measuring the inspired and expired volumes, and a system for delivering gas mixtures. Where the tracer gas is nitrogen, the gas delivered

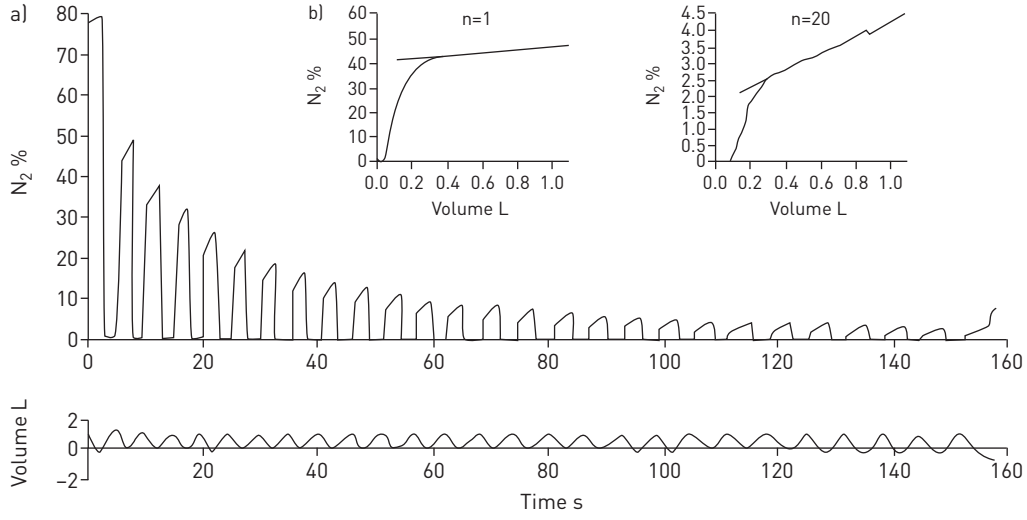

FIGURE 1 a) Nitrogen concentration and volume tracings as a function of time during a multibreath washout test obtained during histamine provocation. b) Illustration of alveolar slope in nitrogen *versus* expired volume for breaths 1 and 20 plotted in an equivalent scaling with respect to mean nitrogen expired concentration of breaths 1 and 20, respectively. Reproduced from [38] with permission from the publisher.

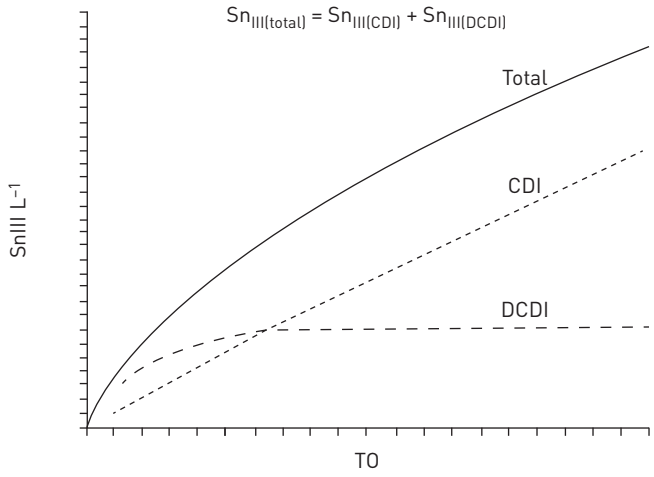

FIGURE 2 Schematic plot of normalised phase III slopes (SnIII) against turnover as washout progresses. The axis TO (turnovers) represents breath number. The solid line (labelled total) represents the combined effect of acinar and conducting inhomogeneity. The slope of the linear portion of this line (S_{cond}; usual index of ventilatory inhomogeneity in the conducting airways) is conventionally calculated from data points between 1.5 and 6 turnovers [39]. The simple dashed line (CDI; convection dependent inhomogeneity) is a transposition of the slope so that it passes through the origin, thereby representing purely the inhomogeneity in the conducting airways. Diffusion–convection dependent inhomogeneity (DCDI) initially contributes more to overall inhomegeneity (rises for first five breaths), but remains unchanged subequently. The index of ventilatory inhomogeneity within the acinar airways (S_{acin}) is calculated by identifying the normalised phase III slope of the first breath and subtracting S_{cond} from this value. Reproduced from [39] with permission from the publisher.

during the washout is usually oxygen, though mixtures of oxygen and argon have also been used [40]. An example set-up of equipment for MBW testing is shown in figure 3.

The key aspects of system performance have been outlined by the European Respiratory Society/American Thoracic Society (ERS/ATS) consensus statement [33], highlighting the importance of sampling rate and the individual measurements of flow, volume, gas concentration and synchronisation. Fast-responding gas

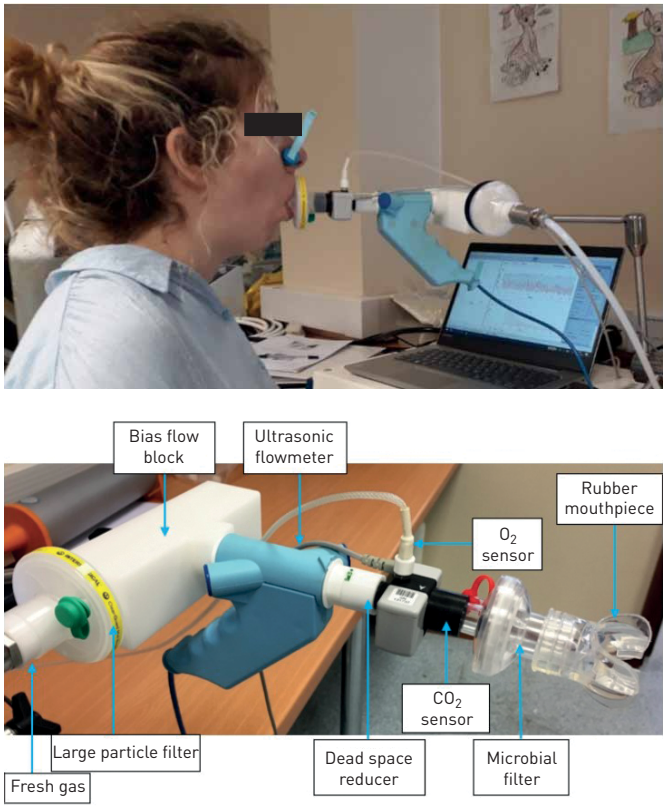

FIGURE 3 An example set-up of equipment for multibreath washout testing.

analysers and a high computer sampling rate are essential to process within-breath changes that, together with standardisation of test performance, are necessary to produce robust results. The requirement for apparatus dead space to be minimised for young children is one factor that means that no single device is suitable for use across the entire paediatric and adult age range [31].

The choice of gas analyser will be determined by the tracer gas, plus considerations of cost and portability. A mass spectrometer can be used to measure any of the commonly used tracer gases; however, they are expensive, and a model used in some previous studies is no longer commercially available. Analysis of SF_6 uses infrared detection or ultrasound techniques, with the latter also able to quantify helium. Technical difficulties associated with nitrogen analysers mean that they are no longer incorporated into commercially available equipment. Instead, a nitrogen signal is derived from oxygen and carbon dioxide, assuming that once oxygen and carbon dioxide are accounted for, the remaining gas is nitrogen. Use of a lung model combined with *in vivo* measurements has shown that a system depending on a derived nitrogen signal reliably measures lung volume and delivers reproducible LCI values, though S_{cond} and S_{acin} were more variable [41].

Currently available commercial devices are at different stages of development with respect to degree of measurement validation, suitability for different age ranges, affordability, regulatory approvals and transparency of results calculation. It is noteworthy that there have been few developments since the publication of the ERS/ATS consensus statement [32]. A summary of some of the key features of available systems is shown in table 1.

Choice of inert gas

The choice of tracer gas used is key, because indices obtained from different gases are not interchangeable. The physical characteristics of the gas, principally gas density, determine the point at which mixing changes from being predominantly due to diffusion to a combination of convection and diffusion, leading to SF_6 generating higher values for LCI than helium, for example.

The differences between tracer gases may be used to an advantage, specifically when washout tests are performed using two gases simultaneously, usually SF_6 and helium [42]. Disease processes predominantly in the acinar region generate greater abnormality in indices obtained using SF_6, whereas those located more proximally, but in the zone of the convection–diffusion front, will have a bigger effect when helium is used. While use of dual tracer gases may provide supplementary information, unfortunately SF_6 is an extremely potent greenhouse gas, with a global warming potential many thousand times that of carbon dioxide.

TABLE 1 Commercially available devices: specifications and characteristics

	Eco Medics AG Exhalyzer D	Innovision Innocor	Ndd EasyOne Pro Lab
Measurement principle	Ultrasonic transit in time	Photoacoustic spectroscopy	Ultrasonic transit in time
Overall design of set-up	Open circuit wash-in and washout design using bias flow	Closed, re-breathing circuit wash-in designed with CO_2 scrubber to prevent CO_2 accumulation Open circuit washout design	Open circuit washout design using bias flow
Inert gas assessed	N_2 and SF_6 MBW options available	SF_6	N_2
Bias flow gas mixture used	Medical air and 100% O_2 required for N_2-based MBW Wash-in $SF_6/O_2/N_2$ mixture for SF_6-based MBW sourced by individual user from local supplier	Wash-in gas available as licensed SF_6 gas mixture supplied by Innovision	Medical air and 100% O_2 required for N_2-based MBW
Application	Adults, children and infants >3 kg	Anyone capable of following instructions from the operator	Adults and children aged >4 years and >18 kg
Additional comments	Semiportable Operator interface through linked laptop Phase III slope analysis for S_{cond} and S_{acin} is integral to the software	Self-contained, portable User interface, LCD touch screen Phase III slope analysis not available	Self-contained, portable Operator interface, LCD touch screen Phase III slope analysis not routinely implemented; research software available

SF_6: sulphur hexafluoride; MBW: multibreath washout; S_{cond}: usual index of ventilatory inhomogeneity in the conducting airways; S_{acin}: index of ventilatory inhomogeneity within the acinar airways.

The advantages of using nitrogen washout include the widespread availability and affordability of 100% oxygen. However, supplying 100% oxygen to some patients (particularly young infants) may impact on distribution of both ventilation and pulmonary blood flow, so caution is warranted. Unlike other tracer gases, nitrogen is present in body tissues as well as in airspaces within the lung. This gives the potential for nitrogen to come out of solution and diffuse into the alveoli, leading to a continuous trace of nitrogen in the exhaled gas [37] and an increase in the total nitrogen exhaled. This, in turn, could lead to an overestimation of FRC. The impact on FRC and therefore LCI will depend on the length of the washout, but may also be impacted by other factors such as body size, body fat content and tissue perfusion [43]. Since there is as yet no robust way of adjusting or correcting for this, no adjustment is made for tissue nitrogen [33].

Test procedure, number of trials and cut-off values

It is mandatory that the operator is properly trained and experienced at working with children. For young children especially, a short practice of breathing through the apparatus may be helpful. Since the tests may take some time, the child should be comfortably seated. Providing a video is a useful distraction. Verbal feedback during testing can be a valuable means of maintaining co-operation. Current recommendations are that three technically acceptable washout tests should be performed [33]. Where the tracer gas is nitrogen, sufficient time needs to elapse between tests for the concentration of nitrogen in the alveoli to return to baseline. The duration of each washout, and the time between consecutive washouts, will therefore depend on the ventilatory efficiency. The time between consecutive washouts should be at least twice as long as the duration of the washout itself [33]. In healthy children, the total time needed to collect three technically acceptable recordings is ∼20 min. If the cut-off value for the end of the test is higher, the test is shortened. Using nitrogen washout to measure LCI in children with CF showed that changing the cut-off value to 1/20 starting concentration rather than 1/40 provided the same discriminative capacity between control participants and patients [44]. Similarly, a cut-off value of 1/20 compared with 1/40, when using SF_6 washout to assess children with asthma and primary ciliary dyskinesia, was not found to decrease the diagnostic performance of the test [45]. This opens the possibility of improved success rates in patients with limited co-operation or where the disease severity means that testing would otherwise take an unrealistically long time.

Acceptability criteria and quality control

The recommended acceptability criteria for MBW testing are explained in detail in the ERS/ATS consensus statement [33]. In summary, for an individual recording to be judged acceptable, the end-expiratory level prior to washout and the tidal volume need to be stable. If an exogenous tracer gas is used, it should be fully equilibrated before washout commences. During washout, respiration should be stable without excessive variation in tidal volume. If phase III slope analysis is to be performed, breath volume should be sufficient to allow for clear identification of the slopes. Critically, there should be no evidence of leaks, such as sudden increases of nitrogen or decreases of exogenous tracer gases or step changes in the end-expiratory level.

When three technically acceptable tests have been recorded, repeatability can be examined. Where a measurement of FRC differs from the median of all three tests by >25%, that test should be rejected. Where the difference between maximum and minimum FRC or LCI exceeds 10% of the mean, the data should be closely examined for acceptability, though not necessarily excluded. Since it is difficult to totally eliminate an element of subjectivity in deciding whether an individual recording is acceptable, the establishment of override centres may contribute to improvement of test quality, reducing interlaboratory variability and facilitating multicentre studies.

The role of washout tests in asthma

Asthma is a complex condition that presents significant therapeutic challenges. Different inflammatory phenotypes characterised by T-helper (Th)2 high, Th2 low and Th17 mediator profiles are now well described [46–48]. Disease severity in asthma is conventionally assessed using spirometry (forced expiratory volume in 1 s (FEV_1)) in conjunction with symptom control and exacerbation frequency. Detection of distal airway pathology, in particular, may be important because peripheral airways are less accessible to inhaled medication, and a different therapeutic approach, including extra-fine particle ICSs or systemic steroid treatment, may be required to treat pathology that is predominantly situated in the peripheral airways. A population study involving a large cohort of adult asthmatics found that patients prescribed an extra-fine particle ICS (<2 µm particle size) were less likely to experience asthma attacks, had better overall asthma control and required a lower prescribed ICS dose to achieve this compared with patients prescribed standard-particle ICSs [49]. We hypothesise that this benefit is due to improved distribution of the inhaled medication into the small airways.

Since spirometry is neither sensitive nor specific at detecting small airways disease unless gross changes are present, the addition of MBW testing should be considered, having regained popularity in recent years as a tool for investigating global airways disease in CF, primary ciliary dyskinesia [50, 51] and non-CF bronchiectasis [52].

LCI in children with asthma

MBW testing in adults with asthma has confirmed that subtle changes in gas trapping are frequently present and more likely to be detected using MBW than spirometry [23]. Few studies using MBW have been conducted in children and these tended to be small observational cohort studies comparing children with asthma to healthy controls. These have shown that children [9, 22] and adolescents with moderately severe asthma have a higher LCI compared with controls, but the LCI usually remains in the normal range. Results were conflicting with respect to the change in LCI following short-acting β_2-agonist (SABA) administration. In one study of adolescents with asthma, LCI following SABAs became indistinguishable from that of healthy controls suggesting that bronchodilation alone corrected ventilation heterogeneity in this group [16, 21]. This was not the case in two other studies involving school-age children and adolescents [9, 22], where LCI in patients with asthma remained high following bronchodilator in comparison with the respective controls, though the absolute differences in LCI between patients and controls were modest. Individual patient data were only reported in one study, precluding assessment of the proportion of asthmatic children with an abnormal LCI. Differences in the tracer gases may also have contributed to the contrasting findings, with one study employing nitrogen [14] and two others using SF_6 [4, 42].

One additional study involving school-age children and adolescents with doctor-diagnosed allergic asthma did report data from individual participants [43]. This study also demonstrated a higher LCI in the asthma group, but only seven out of 47 children with asthma had an abnormal LCI and in these children, FEV_1 was also abnormal. Children with uncontrolled asthma, as defined by an asthma control test score $\leqslant$19, had significantly higher LCIs compared with their well-controlled counterparts, despite no difference in the FEV_1 between these groups. The relationships between LCI and asthma severity and bronchodilator response were not explored [53].

Summarising the findings from paediatric studies, we conclude that children with mild-to-moderate asthma generally have LCIs within the normal range. Asthma diagnosis in children is often difficult, leading to over/under diagnosis [54]. With this in mind, it is unlikely the MBW has a major role in the diagnosis of asthma; however, monitoring the LCI of those with severe or uncontrolled asthma can provide information regarding lung function abnormalities that may be overlooked by spirometry alone. The clinical relevance of the involvement of the peripheral airways in the asthma pathophysiological process requires clarification and this should be further explored, particularly in children with severe and/or uncontrolled asthma despite high-dose preventer inhaler treatment.

S_{cond} and S_{acin} in children with asthma

S_{cond} and S_{acin} were reported in a small number of studies involving children with asthma [22, 27, 53]. These suggest that S_{cond} is likely to be affected, whereas the data for S_{acin} is inconclusive with only one study showing higher values in children with asthma [53]. LCI was associated with S_{cond} only, suggesting that abnormalities in the conducting rather than the acinar airways determined the higher LCI in the patients with asthma. Neither S_{acin} nor S_{cond} showed significant reversibility post-bronchodilation in this study [49].

A link between airway hyperresponsiveness, LCI and S_{cond} was demonstrated in a separate study in children and adolescents, where LCI and S_{cond} increased significantly in response to a cold air challenge, whereas S_{acin} remained unaffected [27]. In this report, both LCI and S_{cond} values normalised after the administration of a bronchodilator.

Finally, one study reported MBW test results in 35 pre-school children aged 3–6 years with a doctor diagnosis of asthma [26]. No differences were found in LCI or S_{acin} between the wheezy children and healthy controls but S_{cond} was significantly elevated in the asthma group, suggesting the presence of conducting airways pathophysiology early in the disease process. While the studies reported above are generally modest in size, they highlight the potential for detailed analysis of MBW to contribute to knowledge of pathophysiology of asthma.

Ventilation inhomogeneity in adults with asthma

Studies of adults with asthma are not the focus of this review, but several reports have identified the presence of ventilation inhomogeneity in this patient group. A study of 196 adults with asthma managed in primary care showed that LCI was abnormal in 44% of the study population, of whom only half had an

abnormal FEV_1 [55]. Furthermore, measurements of LCI in 18 adults with severe asthma (Global Initiative for Asthma step 4–5) showed a mean (range) LCI of 10.5 (6.3–17.5), which improved in some individuals following bronchodilator, although not to normal levels [56]. This suggests that in adults, greater asthma severity is associated with a higher and abnormal LCI.

Few studies have explored the changes and relevance of S_{cond} and S_{acin} in adults with asthma, although both have been shown to be abnormal [10, 27].

The role of LCI in predicting the response to changes in the dose of ICSs has also been explored [57]. Increased LCI predicted an improvement in symptom control in response to treatment escalation and symptom control was more likely to be lost when treatment was reduced [57]. This suggests a potential application of MBW testing in asthma, whereby indices of ventilatory inhomogeneity could be used to inform the pharmacological management of asthma. There is clearly a need for additional and larger studies on well-phenotyped patients with asthma, linking measures of severity, ventilation heterogeneity and responsiveness to treatment.

Summary and recommendations for future work

Despite a slowly increasing body of published studies relating to ventilation heterogeneity in children with asthma, the research base remains low. The evidence to date suggests that the small airways are involved from an early stage in childhood asthma; however, most children with mild-to-moderate asthma have LCIs that remain within the normal range. It is therefore unlikely that MBW has a major role in the diagnosis of asthma in the future. Children with severe asthma, however, are more likely to have an abnormal LCI.

More studies have been published in adults, but the airways of adults differ from those of children and these differences do not only relate to airway calibre. This limits the extent to which studies in adults can be extrapolated to children. Therefore, studies with a focus on children with asthma are required to investigate the potential role of MBW in their clinical management. In the first instance, the role of MBW monitoring in children with severe and or persistently uncontrolled asthma needs to be explored further. Such studies should include adherence monitoring as this is a major confounder in asthma studies. There may also be a role for MBW in clinical trials conducted in children with severe asthma to quantify the effect of newly available biologicals and ultrafine ICS formulations on global lung function that includes measurements of small airway dysfunction. The association between LCI and S_{cond} abnormalities and airway inflammation also merits further consideration.

To further understand the role clinically, high-quality longitudinal studies examining the progression of MBW parameters over time in asthmatic children would be of benefit. This could be done as part of an annual review as the stability of reference ranges over time make it well suited to longitudinal monitoring.

Conclusion

To date, the following is known: 1) small airway disease and ventilation heterogeneity has been demonstrated in asthmatic children; 2) children with severe or uncontrolled asthma are more likely to have an abnormal LCI; 3) abnormal LCI measurements have been demonstrated in children with normal spirometry; and 4) monitoring MBW alongside spirometry may provide useful additional information and have implications for management.

The following still needs to be done: 1) further studies, particularly longitudinal studies, to examine the utility of MBW in asthmatic children; 2) clinical studies examining treatment response using MBW parameters; and 3) studies focusing on whether MBW can be used to help predict onset of acute episodes in asthmatic children.

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