Lung function testing and inflammation markers for wheezing preschool children: A systematic review for the EAACI Clinical Practice Recommendations on Diagnostics of Preschool Wheeze

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**Abstract**

Preschool wheeze is highly prevalent; 30-50 % of children have wheezed at least once before age six. Wheezing is not a disorder; it is a symptom of obstruction in the airways, and it is essential to identify the correct diagnosis behind this symptom. An increasing number of studies provide evidence for novel diagnostic tools for monitoring and predicting asthma in the pediatric population. Several techniques are available to measure airway obstruction and airway inflammation, including spirometry, impulse oscillometry, whole-body plethysmography, bronchial hyperresponsiveness test, multiple breath washout test, measurements of exhaled NO, and analyses of various other biomarkers.
This systematic review will cover the main lung function and biomarker techniques used in preschool children to diagnose and monitor airway obstruction and shed light on promising new techniques used in research for measuring airway inflammation. The difficulty of measuring lung function and the lack of large randomized controlled trials makes it difficult to establish guidelines for monitoring asthma in preschool children. If applicable, measuring FEV1 using spirometry is considered useful. For those unable to perform spirometry, whole-body plethysmography and IOS may be useful. Bronchial reversibility to beta2-agonist and hyperresponsiveness test with running exercise challenge may improve the sensitivity of these tests.

Key Message
This systematic review covers the main lung function and biomarker techniques used in preschool children to diagnose and monitor asthma.

To objectively measure lung function and airway inflammation markers is clinically important to avoid over and under treatment of preschool wheeze and asthma.

Introduction
Preschool wheeze is highly prevalent; almost 30-50% of children have wheezed at least once in the first six years of life, and up to 30% of children aged 1-6 years in Europe and the USA have wheezed in the preceding six months. (1, 2) Wheezing in preschool children can be falsely diagnosed as pneumonia, and vice versa, because definitions of these conditions overlap, leading to under- and overtreatment. Wheezing is a symptom manifested by a continuous whistling sound during breathing, suggesting a narrowing or obstruction in parts of the airways. It is essential to identify the correct diagnosis behind this symptom.

Preschool children less than six years of age with wheeze have higher asthma morbidity than any other age group. (3) Lung function measurement is an essential tool in the differential diagnosis of preschool wheezing, although reliable lung function measurements are challenging in this age group. These children have a short attention span, and complete collaboration is required. Several techniques are available to measure airways obstruction, lung volumes, and airway inflammation. They include: (i) Spirometry with or without bronchoprovocation tests, (ii) measuring respiratory resistance and reactance with impulse oscillometry (IOS), whole-body
plethysmography, (iii) multiple breath washout (MBW) test to measure lung clearance index (LCI), and measuring non-invasive biomarkers of inflammation like (iv) fractional exhaled nitric oxide (FeNO), (v) sputum eosinophilia, (vi) exhaled breath condensates, and (vii) volatile organic compounds. However, their role and usefulness in routine clinical practice to diagnose, monitor, and guide therapy are unclear in young children. Currently, clinical guidelines (American Thoracic Society (ATS), European Respiratory Society (ERS), National Asthma Education and Prevention Program (NAEPP) and Global Initiative for Asthma (GINA), NICE, and BTS/SIGN) recommend mainly spirometry for diagnosing and monitoring asthma. At the same time, the other tests are used for research purposes.

We aimed to systematically review all the existing techniques available for measuring lung function and airway inflammation in preschool children to assess their potential and clinical value in the routine diagnostics and monitoring of airway obstruction.

**Search strategy and selection criteria**

This review aimed to summarize the clinical evidence for the use of all available lung function tests in wheezy preschoolers. A systematic literature search was performed in October 2019 across the MEDLINE, EMBASE, and Cochrane Library databases to identify articles with clinical evidence on various lung function techniques from 2000 onwards.

Our goal was to identify all clinically relevant studies that could address these tests' applicability in the population of preschool wheezers (6 years or younger) and their potential and clinical value in the routine diagnostics and monitoring of airway obstruction for future EAACI recommendations. Therefore, we included in the analysis only diagnostic papers with both mechanistic data and clinical data from pediatric studies. A hand search of eligible articles and author expertise were used to supplement the included articles.

In October 2019, we performed a systematic review of the literature with the search strategy as follows: (preschool child OR infant OR toddler OR pre-school OR kindergarten OR nursery) AND (lung function test* OR breath test* OR exhaled breath condensate OR impulse oscillometry OR oscillometry OR nitric oxide OR plethysmography OR spirometry OR respiratory function test* OR volatile organic compounds OR sputum OR FeNO) AND (asthma* OR wheez* OR bronchiolitis OR bronchitis) AND English[Language]. This search strategy was
formulated for PubMed and then adapted for the other two databases. The inclusion criteria were formulated as follows: published, peer-reviewed articles (conference abstracts were excluded after checking if no article was published based on presented results); age group defined as preschool children; outcome defined as asthma diagnosis. Title and abstracts of all identified papers were read independently by two pediatric pulmonologists (VE, WF with >10 years of experience), one allergist (MR >10 years of experience), and one medical student (AA) and cross-checked the selected records. The consensus resolved any discrepancies were reached using the Delphi method.

Out of 5326 articles retrieved initially and six articles found manually, 102 were selected for full-text review performed independently by four researchers (AA, WF, MR, VE). Subsequently, we cross-checked the results, and any disagreement was resolved by discussion. Forty-six of those studies were rejected on the grounds of not matching the inclusion criteria (Figure 1 in ONLINE Supporting Information). Fifty-six peer-reviewed articles describing nine types of tests were included in the final table of evidence, which was split into online tables 1-9, one for each section.

A modified Delphi system was used to achieve consensus. Three rounds of voting and revisions and three round table meetings (Zurich, Gdansk, Warsaw) were conducted to achieve consensus for literature search strategy, article selection and for final statements. The first author reviewed the results from each round and updated the statements based on comments entered by responders. We used an electronic Delphi survey tool to vote for the final draft statements. Each member was asked to rate each statement on a scale of 0 (completely disagree) to 9 (completely agree), with 80% (7 to 9) of being considered a good agreement. A draft of this review was presented to the EAACI headquarters for ExCom committee approval. Comments collected from this process were also used in the development of the final statements.

**Spirometry**

Spirometry is the most frequently used method for monitoring lung function and measuring airway obstruction, while evidence for its applicability to predict asthma in the preschool population is sparse (online Table 1). Well-designed prospective studies are either lacking or show conflicting results. It is recommended to be used for diagnosing and monitoring asthma in patients over five
years of age. Measurements of spirometric indices such as forced expiratory volume in 1 second (FEV1), forced vital capacity (FVC), and FEV1/FVC ratio, are the gold standard (Table 1), but test performance and its repeatability pose some difficulty. Spirometry is recommended for the assessment of asthma severity, control, and response to treatment by several international guidelines (ATS, ERS, NAEPP, GINA, NICE, and BTS/SIGN). Normal values of spirometry are well established for several different nationalities and are usually defined as a standard deviation score or Z-score of > -2 SD. However, preschool children have small absolute lung volumes and large airway size relative to lung volumes compared to older children. Also, they are often unable to exhale more than 1 second. Therefore, forced expiratory volume in 0.5 seconds (FEV0.5), and the indices of the more peripheral airways, such as forced expiratory flow at 50% of FVC (FEF50%), maximal mid expiratory flow (MMEF), and forced expiratory flow at 25-75% (FEF25-75%) and area under the MMEF (A_ex) have been suggested to be more suitable in this age group and may reflect obstruction in the small airways of children with asthma. Nevertheless, the role of these indices in detecting airway obstruction in clinical practice is controversial. For example, FEF50%, MMEF, and FEF25-75% are highly variable since they are effort dependent. Few studies have shown that these are more sensitive than FEV1 in detecting peripheral airway obstruction, while others have shown that FEF25-75% and FEF75% correlate with conventional spirometric indices. Furthermore, airway obstruction is not necessarily permanent in preschool children, and airflow limitation may be mild or absent. Bronchodilator reversibility testing, although not specific, is useful for confirming the diagnosis of asthma. The most widely used definition of a significant bronchodilator response is an improvement in FEV1 greater than 12% and 200 ml (Table 2). Also, adding an exercise challenge test increase the diagnostic value of spirometry. A reduction in FEV1 of at least 10-15% after exercise is anticipated as a sign of exercise-induced bronchoconstriction (Table 2). Thus, normal spirometry results do not rule out mild asthma, and it is essential to monitor changes over time as lung function varies with symptom burden, exacerbations, and treatment with inhaled corticosteroids (ICS).

Analyses of lung function growth during childhood have shown that the lung function trajectory measured by spirometry is different in preschool children developing recurrent wheeze and asthma compared to healthy children. The trajectory seems to be a static character with a constant deficit in airway obstruction both before symptom debut and even after remission of the disease. This makes the child susceptible to develop asthmatic symptoms and intermittent
Children younger than five years are typically unable to cooperate with the forced exhalation procedure required for spirometry. (15) However, it is possible to perform volume-anchored spirometry in research settings, even in infants and neonates. Neonatal spirometry comparable to spirometry in 5-year-olds can be done by raised volume rapid thoracoabdominal compression (RTC) technique during mild sedation with, e.g., oral chloral hydrate. (16) By applying rapid inflation of the squeeze jacket with silicon putty to ensure tightness, the child performs a forced exhalation maneuver during which flows and volumes such as FEV0.5, FVC, and maximal flow at functional residual capacity (VmaxFRC) can be measured with a pneumotachograph with an air-cushion facemask. (17) Longitudinal follow-up analysis of neonatal spirometry assessments done in healthy 4-week-old children have shown that these are predictive of the development of acute bronchiolitis and wheezing before the age of two years (18) and asthma by age 7. (19)

**Summary & outlook / Clinical perspective:** Most promising spirometric indices of airway obstruction in preschool children (>4 years) are FEV1 and FEV1/FVC. Despite performance challenges, current data support spirometry's clinical use in preschool wheeze if test performance is warranted. Additional bronchodilator reversibility testing is useful for confirming the diagnosis of asthma (online Table 1).

**Whole-body plethysmography**

Whole-body plethysmography allows assessing specific airway resistance (sRaw) and functional residual capacity (FRC) as primary measures. Also, lung volumes such as Total Lung Capacity (TLC) and Residual Volume (RV) can be determined (Table 1). Importantly, and in contrast to spirometry, plethysmography is done during tidal breathing and therefore requires less cooperation and can be conducted in children from age three years, (20, 21) with most children being able to complete testing before age 6. (15)

Increased sRaw (cut-off value for sRaw > 1.6) has been demonstrated in preschool children with persistent wheeze compared to healthy children (Table 2). These differences are even more pronounced in children with persistent wheeze currently requiring treatment with ICS to control.

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their symptoms and in children experiencing acute exacerbations requiring oral corticosteroids and/or hospitalization. (15) Similarly, another study showed that sRaw could separate persistent wheezers from healthy children. (22) Interestingly, an early intervention trial with inhaled corticosteroids during the first episodes of wheezing in young children at risk of asthma showed no differences by age 3 in either persistent wheeze or sRaw. (23) Longitudinal studies of lung function growth by plethysmography from age 3 to 13 years (14) and age 3 to 11 years (24) have shown a trajectory of increased sRaw in preschool children developing persistent wheeze/asthma compared to never wheezers. The latter study reported increasing airway obstruction during childhood with an increasing number of exacerbations (24), whereas the former study did not observe such effect from exacerbations even though sRaw was transiently more increased during acute episodes of wheeze. (14)

RV/TLC and RV are indices of air trapping, and RV is a marker of hyperinflation when the value is more than 120% predicted (Table 2). It has been shown that children with severe asthma have more air trapping and hyperinflation compared to children with less severe disease that had normal RV and TLC. (25)

Summary & outlook / Clinical perspective: Whole-body plethysmography can be used for assessing airway obstruction and diagnosing asthma in preschool children (>3 years), but it is not easily accessible. There is a lack of randomized controlled trials showing its usefulness in routine clinical prediction, monitoring, or asthma treatment (online Table 2). (26)

Forced oscillation technique (FOT)

Challenges to perform spirometry in younger children have led to a search for other methods to measure lung function during normal tidal breathing, including the forced oscillation technique (FOT) and the measurement of interrupter resistance (Rint). (4) For the forced oscillation, commercialized devices called the impulse oscillometry (IOS) are available. IOS is a non-invasive technique performed during tidal breathing (Table 1). The child is seated and breathing through a mouthpiece and wearing a nose clip with the cheeks and chin firmly supported and connected via the mouthpiece to a device delivering the forced oscillatory signals with different frequencies (Figure 1). For children 3-5 years, IOS provides a feasible, simple, non-invasive, and effortless method requiring minimal patient cooperation for lung function measurement. (27, 28, 29) There is an abundance of studies (18) published in the last
eight years, demonstrating its applicability in preschool children, including 2971 children (online Table 3). Also, several published reference values are available from healthy preschool children of Caucasian and Asian origin. (4, 30, 31) According to these studies, height was found to be the most influential variable on respiratory system resistance (Rrs) and reactance (Xrs), the components of respiratory impedance (Zrs) (Table 1). The test results are expressed as a percentage of the predicted values or as z-scores.

Both Rrs and Xrs are expressed as a function of oscillation frequency. At high frequencies (>20 Hz), Rrs at 20 Hz is thought to reflect mainly airway resistance in the large airways, whereas at lower frequencies (4-8 Hz), Rrs at 5Hz reflects the whole respiratory system. (27, 32) Small airway disease usually results in the frequency dependence of resistance, i.e. an increased difference between Rrs at 5Hz and 20Hz, although other factors such as upper airway shunting contribute to this phenomenon in children. (33) In the presence of small airway obstruction, the respiratory system’s elastic properties change, resulting in decreased Xrs and increased area under the negative part of the reactance curve (AX). (32)

The studies evaluating the oscillometric technique’s ability to classify lung function in wheezing and healthy children have been contradictory. Depending on the patient selection, both significant differences (34-37) and lack of differences (38, 39) have been reported. Apparent changes in resistance at low frequencies (Rrs at 5Hz) may suggest small airway involvement and predict loss of asthma control. (40, 41)

FOT and IOS have been useful in documenting response to bronchodilators, distinguishing between healthy and wheezing children. (34, 35, 42, 43) In 4-year-old children, IOS was more effective than spirometry to differentiate responses between healthy and asthmatic children. (44) Based on the distribution of bronchodilator responses (5th percentile) in healthy preschool children, an improvement of Rrs at 5 Hz of at least 40 % is considered positive (Table 2). (38, 45, 46) In bronchoprovocation tests (methacholine and exercise) the application of FOT and IOS have also demonstrated increased airway hyperresponsiveness in wheezing children, parallel to the regular spirometry. (47-50)

IOS has also successfully been used in clinical trials evaluating therapeutic responses to ICS in young asthmatic children. (51) However, the role of IOS in monitoring children with preschool wheezing in clinical routine is still unclear. Longitudinal studies evaluating lung function trajectories by the oscillometric technique are few. (37, 52-55) Recent observations,
however, suggest that abnormal IOS (Rrs at 5Hz) in preschool wheezers is associated with low lung function, need for asthma medication, and asthma symptoms in adolescence. (54) However, based on the existing data, the value for asthma prediction in preschoolers seems equivocal. IOS is sensitive to many artefacts, which should be recognized, particularly when young children are studied. (4) Therefore, experienced personnel is a prerequisite for a successful application. Moreover, since spirometry is more widely adopted and studied, interpretation of IOS results may not be straightforward for many clinicians.

Summary & outlook / Clinical perspective: Forced oscillation technique (FOT) may be a useful tool to aid in diagnosing and managing asthma in preschool children (>2 years). It may be particularly useful for children unable to perform spirometry to demonstrate lung function abnormalities, bronchial responsiveness, and bronchodilator response. However, the reference values need to be validated in larger populations. Its applicability in asthma prediction is still controversial (online Table 3). (37, 52-54, 56, 57)

Bronchial hyperresponsiveness

A major characteristic of asthma is the variability of bronchial tone in response to various stimuli. Bronchial hyperresponsiveness may be assessed by bronchial provocation tests with directly stimulating agents such as histamine and methacholine or indirect stimuli such as physical exercise, mannitol, hyperventilation of cold and/or dry air or inhaled allergen. Determination of the provocative concentration or dose causing a 20% reduction in FEV1 is used for provocation tests with histamine and methacholine, although these dose reference values are determined for children over 12 years of age. A bronchoprovocation test with inhaled cumulative doses of mannitol causing a 15% reduction in FEV1 also seems to be safe and feasible in young children. However, it’s role in exploring bronchial hyperresponsiveness in children needs further study. (58) Cold, dry air hyperventilation and airway responsiveness assessment by whole-body plethysmography are feasible and safe in children aged 2 to 5 years, where it can significantly separate children with vs. without asthma-like symptoms. (21) A reduction of at least 10% in FEV1 during exercise is considered a sign of exercise-induced bronchoconstriction in children (ATS guidelines). However, the threshold range varies by 12% in GINA and 8-20% in BTS/SIGN guidelines (Table 2). (9) Running is the preferred exercise, as it is easily standardized using a time of 6-8 minutes, reaching and maintaining an exercise load of 90-95% of maximum heart rate.
Running outside in cold and dry air can increase the sensitivity of the exercise test. The running exercise test gives information about the presence of bronchial constriction during exercise as well as fitness and motor development of the child.

The major disadvantage of bronchial hyperresponsiveness is that the tests take time, up to 1.5 hours depending on the choice of test (oscillometry more time consuming than spirometry), the child’s co-operative skills and trained personnel are required. Therefore, they are not suitable for routine clinical assessment of asthma control in preschool children. However, bronchial hyperresponsiveness assessment may play a role in children with possible exercise limitations, poor symptom perception, or atypical asthma symptoms not responding to treatment.

Summary & outlook / Clinical perspective: Bronchial hyperresponsiveness tests may improve the sensitivity of spirometry, IOS, and plethysmography for diagnosing asthma in preschool children with recurrent wheeze if the quality of baseline measurements is good. Adding exercise challenge tests to children with exercise-induced symptoms, poor symptom perception, or atypical asthma symptoms not responding to treatment may increase the test's diagnostic value.

Multiple breath washout test

The multiple breath washout test (MBW) measures the Lung Clearance Index (LCI), which is the number of times the lung volume has to be turned over to wash out an inert gas such as nitrogen (N2; requires 100% oxygen during washout phase which has effects on infants’ tidal breathing parameters and therefore is used for all age groups except infants) or sulfur hexafluoride (SF6; used for infants) (Table 1). Thus, LCI is an index of inhomogeneity of ventilation, and it has been used to detect early lung damage in children with cystic fibrosis and primary ciliary dyskinesia. The advantages of LCI are the narrow normal range between 6-7 and its independence of age (Table 2). Although LCI has shown to be significantly higher in asthmatic school-age children than healthy controls, there are currently only five studies of LCI in the preschool age group (online Table 4). LCI is not higher in wheezy preschool children with the mild or well-controlled or episodic disease compared to healthy children, whereas significant differences with higher LCI have been reported only for preschool children with a history of severe wheeze exacerbations and children with persistent wheeze. The multiple breath washout technique uses the alveolar slope to determine measures of ventilation inhomogeneity in the acinar (S_{acin}) and conducting (S_{cond}) airway regions (Table 1). Typically, the normal values are calculated

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around 0.033 for $S_{\text{cond}}$ and 0.075 for $S_{\text{acin}}$, and they tend to increase with age. Two studies of 65 children aged 2-6 (63) and 134 children aged 4-6 (22) showed significantly elevated $S_{\text{cond}}$ in children with wheeze vs. healthy controls. However, the lack of extended reference equations to the pediatric population limits the use of this technique.

**Summary & outlook / Clinical perspective:** Due to the paucity of well-designed prospective studies, there is no evidence for the role of multiple breath washout test (MBW) in the routine monitoring of asthma in preschool children (online Table 4). Further studies are needed to assess asthma specific changes in LCI and other MBW indices in preschool children.

**Exhaled biomarkers**

Non-invasive biomarkers of airway inflammation in children with asthma are available, but their role and usefulness in routine clinical practice to monitor and guide therapy are unclear. Fractionated exhaled nitric oxide (FeNO) is used in many tertiary centers, but sputum eosinophilia, exhaled breath condensates, and volatile organic compounds are used primarily in research.

**FeNO**

The best-studied biomarker in asthma is FeNO, a quantitative measure of exhaled nitric oxide, an indirect marker of eosinophilic airway inflammation (Table 1). The measurement of FeNO has been standardized for clinical use in a joint ATS/ERS guideline.(65) FeNO testing with a hand-held device is non-invasive, reproducible, and easy to perform with a success rate of over 70% in children above 5 years.(63)

We identified 25 studies with 10,853 participants, studying FeNO in wheezy preschoolers. There were 11 studies (1455 children) in which a mean value for healthy children was computed (range 5.6–28.7 ppb), and atopic disease was predicted at values higher than 27 ppb (6 studies) (online Table 5). There is no international consensus on using age- and height-adjusted normal values or adjusting for atopy.(66, 67) A high FeNO (> 35 ppb) in children is indicative of eosinophilic inflammation and has been shown to predict asthma relapse and failed ICS reduction (Table 2).(68) A low FeNO (<20 ppb) in symptomatic and steroid-naïve children indicates that eosinophilic inflammation and responsive to ICS is less likely.(68) Values between 20 and 35 ppb
should be interpreted cautiously. Many additional factors such as allergen and tobacco smoke exposure, diurnal variability, atopy status and airway infections associate with changes in FeNO. Thus, small changes in FeNO may clinically be irrelevant for monitoring asthma.

In healthy neonates, elevated FeNO was associated with an increased risk of developing early transient wheeze during preschool age and in infants with recurrent wheezing elevated FeNO predicted decline in lung function and risk of future wheezing. In preschool children, elevated FeNO is associated with current symptoms, atopy, and later asthma. Although ICS has been shown to reduce FeNO in preschool children with wheeze, there are no published preschool data on its utility in monitoring or adjusting asthma treatment or predicting exacerbations. FeNO level is correlated with bronchial hyperresponsiveness during the exercise test. However, FeNO is not useful in clinical practice to predict the need for exercise testing.

Summary & outlook / Clinical perspective: FeNO has been shown to predict allergic asthma among wheezy preschool children (cut-off >35 ppb). Many specialist centers use it to monitor children with difficult-to-treat asthma and as part of differential diagnosis of allergic asthma. This technique may become a tool for diagnosing wheezy preschoolers.

Sputum

Performance and analysis of induced sputum in children is well described and is safe and feasible in over 50% of children over six years of age. There are only two studies with 1252 children, where sputum was collected from preschoolers, but the methodology of its acquisition has not yet been standardized in preschool children. Therefore, no data indicate its applicability in asthma prediction in preschool wheezers.

Exhaled breath condensate

A vast number of inflammatory mediators can be measured in exhaled breath condensate (EBC). It is collected during tidal breathing by cooling exhaled air by contact with a cold surface or condenser. EBC is thus a diluted fluid, and the collected condensate contains volatile (e.g., H$_2$O$_2$) and semi- and non-volatile molecules (proteins and cytokines) carried by respiratory droplets. Its composition with many airway inflammation markers and...
oxidative stress is thought to mirror the airways' inflammatory processes and may relate to asthma control in children. (81, 84) It is a simple, well-tolerated, and safe method and is feasible in children over four years of age. (85) The collection of EBC in younger children may be more difficult due to lower levels of cooperation and smaller amounts of EBC. A systematic review from 2013 of cross-sectional pediatric asthma studies utilizing EBC to compare asthmatic vs. healthy children, (81) different degrees of asthma severity, and acute vs. stable asthma showed that lower pH, markers of increased oxidative stress with elevated H₂O₂ and nitric oxide products, decreased antioxidant glutathione and elevated eicosanoids and Th2 cell cytokines were distinguishing features. However, several methodological issues include cooling temperature, collection time, condenser material, nose clip, saliva trap, resistor, filter, dilution marker, deaeration, assay sensitivity, and reproducibility hamper comparisons and validity of the results. Currently, there are only three longitudinal studies of the utility of EBC in preschoolers, which have shown that EBC cannot identify children progressing from preschool wheezing to asthma (86) or predict asthma exacerbations. (87)

**Summary & outlook / Clinical perspective:** Currently, due to lack of evidence, EBC does not play a role in clinical monitoring or the prediction of asthma in preschool children (online Table 7). (88)

**Volatile organic compounds**

Volatile organic compounds (VOCs) originate from the blood circulation, and they spread from the pulmonary capillary bed into the alveoli and the lungs. (89) They can be analyzed from the exhaled breath using metabolomics approaches (Figure 1). (89) Two different techniques have been used to study exhaled VOCs profiles; 1) gas chromatography with mass spectrometry allowing quantitative/semi-quantitative measurement of specific individual VOCs, and 2) the electronic nose (e-Nose), which contains a panel of semi-selective sensors to discriminate between different biomarker profiles without information of which VOCs are present. (90) The e-Nose is easy to use for children over two years of age, and it does not require experienced personnel, but the technique is limited by the drift of the sensors and lack of methods to calibrate the system. We identified only two studies with 427 children, investigating a possible role of this technique in identifying children at risk of asthma (online Table 8). Preschool children with acute
wheeze have a different VOC profile than children without wheeze (91), and the profile remains
altered even after resolution of symptoms, particularly in children with rhinovirus induced
wheeze. (92) In preschool children with recurrent wheeze, VOC profiles may discriminate between
children developing asthma and those with transient wheeze, improving asthma prediction made
on clinical data alone. (86, 93, 94) However, in other studies, the VOC profiles could not
discriminate between asthmatic and healthy children. (95)

Summary & outlook / Clinical perspective: After methodological improvements, VOC
profiling may help study and monitor preschool asthma phenotypes. It is currently only used in
research settings (online Table 8). (96, 97)

New approaches

Structured light plethysmography is a novel light-based method that measures chest wall
movements during tidal breathing without any physical contact with the patient (Table 1). This
unique method may differentiate airway obstruction, dysfunction or abnormal breathing in
neuromuscular diseases after surgery or lung injury. (98-100) However, the data is still scarce, and
further studies are needed to demonstrate its benefits in preschool wheeze and asthma.

Another promising lung function test for infants is the impedance pneumography. This
method enables continuous measurement of tidal breathing volume curves during sleep using a
chest impedance signal-recording device (Table 1). (101) Airway obstruction in wheezy infants
was associated with reduced variability of the tidal breathing pattern. We identified three studies
with 212 children; however, feasibility data is still scarce, and more studies are needed (online
Table 9). (102-104)

Conclusions

There are three main obstacles to implementing the lung function tests into current
guidelines for managing asthma in preschool children: (i) the difficulty of performance, (ii)
repeatability, and (iii) the lack of large randomized controlled trials. Although quality control
criteria for adults are not applicable for children for physiological reasons, in a center with trained
personnel, lung function testing can be done with high reliability even in children between 3-4

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years of age. If applicable, measuring FEV1 using spirometry is considered useful. For those unable to perform spirometry, whole-body plethysmography and IOS may be useful. Bronchial reversibility to beta2-agonist and hyperresponsiveness tests may improve the sensitivity of these tests. Diagnostic cutoffs have been established for these tests.

Currently, neither multiple breath washout nor any of the exhaled biomarkers are useful for routine monitoring of wheezy preschool children, and further research is needed.

The objective assessment of lung function and airway inflammation is clinically essential to avoid over- and under-treatment of preschool wheeze and to monitor difficult-to-treat asthma.

To date, for most methods for measuring lung function, there are no proper randomized clinical controlled trials or longitudinal studies available to establish their role in diagnosing and monitoring asthma in wheezy preschoolers. However, the need for improved diagnostic methods and further research is urgent.

**Figure legends**

**Figure 1.** Lung function tests were identified in the systematic review and applied in preschool children and their main features. Five tests are already in clinical use (upper panel), and three are still in research and development stages (lower panel). Frame colors indicate methods that are: non-invasive (grey), effort-demanding, or semi-invasive (blue).

**Figure 2:** Lung function tests according to the invasiveness and respiratory tract level for obtaining material/data for analysis.

The upper panel includes non-invasive methods: Exhaled breath diagnostics (non-invasive), including the analysis of airborne volatile organic compounds in exhaled breath (VOC) or inflammatory mediators in exhaled breath condensates (EBC), and induced sputum eosinophilia. The lower panel shows an array of current semi-invasive or effort-demanding methods; including regular spirometry (with or without bronchial hyperresponsiveness test), impulse oscillometry (a method based on the forced oscillation technique, FOT), multiple breath washout test (MBW),
whole-body plethysmography (WBP) and quantitative measurement of airway nitric oxide, reflecting airway and tissue eosinophilia (fractionated exhaled nitric oxide, FeNO).
<table>
<thead>
<tr>
<th>Measure attributes</th>
<th>Lung volumes, FVC, FEV1, FEV1/FVC</th>
<th>Respiratory resistance Rs at 5Hz and Rs at 10Hz, reactance Xrs at 5Hz, area of reactance (AX)</th>
<th>Functional Residual Capacity (FRC), specific airway Resistance (sRaw), Residual Volume (RV), Total Lung Capacity (TLC)</th>
<th>Lung Clearance index (LCI), S\textsubscript{acin}, S\textsubscript{cond}</th>
<th>Fractional exhaled nitric oxide (FeNO)</th>
<th>Sputum</th>
<th>Exhaled breath condensate (EBC)</th>
<th>Volatile organic compounds (VOCs)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reference values for preschool children</td>
<td>&gt; 5 yrs, for many nationalities</td>
<td>&gt; 2 yrs</td>
<td>&gt; 2 yrs</td>
<td>Yes, independent of age</td>
<td>no</td>
<td>no</td>
<td>no</td>
<td>Independent of age</td>
</tr>
<tr>
<td>Sensitivity and specificity</td>
<td>good</td>
<td>good</td>
<td>good</td>
<td>poor</td>
<td>poor</td>
<td>?</td>
<td>?</td>
<td>?</td>
</tr>
<tr>
<td>Used for diagnostics</td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
<td>no</td>
<td>no</td>
<td>no</td>
<td>no</td>
<td>no</td>
</tr>
<tr>
<td>Used for monitoring and tapering management of asthma</td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
<td>no</td>
<td>yes</td>
<td>no</td>
<td>no</td>
<td>no</td>
</tr>
<tr>
<td>-----------------------------------------------------</td>
<td>-----</td>
<td>-----</td>
<td>-----</td>
<td>----</td>
<td>-----</td>
<td>----</td>
<td>----</td>
<td>----</td>
</tr>
<tr>
<td>Predicts loss of asthma control</td>
<td>++</td>
<td>+++</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>?</td>
<td>?</td>
<td>+?</td>
</tr>
<tr>
<td>Feasibility/easy to perform</td>
<td>&gt;5 yrs</td>
<td>&gt;3 yrs</td>
<td>&gt; 2 yrs</td>
<td>&gt; 3 yrs</td>
<td>&gt; 5 yrs</td>
<td>&gt; 6 yrs</td>
<td>&gt; 4 yrs</td>
<td>&gt;2 yrs</td>
</tr>
<tr>
<td>Requires specially trained personnel</td>
<td>+</td>
<td>++</td>
<td>+++</td>
<td>+++</td>
<td>+</td>
<td>+</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>Requires expensive equipment</td>
<td>+</td>
<td>++</td>
<td>+++</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>+++</td>
<td>+</td>
</tr>
<tr>
<td>In clinical practice</td>
<td>+++</td>
<td>++</td>
<td>++</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>--</td>
</tr>
</tbody>
</table>

**Table 1. Comparison of different lung function tests used in wheezing preschool children.**

**Abbreviations:**

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area of reactance (AX), exhaled breath condensate (EBC), forced expiratory volume in 1 second (FEV1), forced vital capacity (FVC), FEV1/FVC ratio, fractional exhaled nitric oxide (FeNO), impulse oscillometry (IOS), lung clearance index (LCI), multiple breath washout (MBW), nitric oxide (NO), specific airway resistance (sRaw), total lung capacity (TLC), residual volume (RV), volatile organic compounds (VOCs), years (yrs), + some, ++ intermediate, +++ high, - negative, ? not known
### Table 2. Cutoff-values for different lung function tests in preschool children

<table>
<thead>
<tr>
<th>Test</th>
<th>Criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Spirometry</strong></td>
<td>FVC, FEV1, FEV1/FVC%; less than 80% of predicted values or z-scores &lt; -2 †</td>
</tr>
<tr>
<td></td>
<td>with bronchodilatation FEV1 increase over +12% (and 200 ml)‡</td>
</tr>
<tr>
<td></td>
<td>with exercise test FEV1 decrease at least 10%*</td>
</tr>
<tr>
<td>Whole-body plethysmography</td>
<td>RV over 120% of predicted, sRaw &gt;1.6 ‡</td>
</tr>
<tr>
<td>Impulse oscillometry</td>
<td>Rrs at 5 Hz and Xrs at 5 Hz; Z-score &gt; 2 §</td>
</tr>
<tr>
<td></td>
<td>with bronchodilatation Rrs at 5Hz increase &gt;40% §</td>
</tr>
<tr>
<td>FeNO</td>
<td>&gt;35 ppb ¶</td>
</tr>
<tr>
<td>Multiple breath washout test</td>
<td>LCI &gt; 7 **</td>
</tr>
</tbody>
</table>

† ATS/ERS, GINA, BTS/SIGN guidelines

‡ in preschool children lower thresholds have been suggested to be clinically significant, especially for the minimum of 200 ml is not always required

* range of thresholds used in children varies: -10% in ATS/ERS, 12% in GINA, 8-20% in BTS/SIGN guidelines


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**INUCED SPUMT**
- Cellular analysis: eosinophilia, neutrophilia, mononuclear cells
- Not yet standardised in preschool children

**FRACTIONAL NITRIC OXIDE (FENO)**
- Measures alveolar nitric oxide
- Marker of eosinophilic inflammation
- Marker of current symptoms, atopy
- Possible nasal congestion

**SPIROMETRY**
- Requires patient's cooperation
- Monitors lung function and airway obstruction
- Possible extension to provocation, reversibility, and exercise test

**FORCED OSCILLATION TECHNIQUE (FOT)**
- Easy, non-invasive
- Measures resistance, reactance and impedance

**WHOLE BODY PLETYSIMOMOGRAPHY**
- No patient's cooperation required
- Measures airway obstruction and resistance

**VOLATILE ORGANIC COMPOUNDS (VOC)**
- Originates from blood and spreads from the pulmonary capillary bed into the alveoli
- Biochemical pathways
- Identification of endotypes

**EXHALED BREATHING CONDENSATE (EBC)**
- Collected during tidal breathing
- Dyes, proteins, exocytosed cells
- Marker of airway inflammation and oxidative stress

**MULTIPLE BREATH WASHOUT (MBW)**
- Measures washout of inert respiratory gases and inhomogeneity of ventilation
- Lung Clearing Index (LCI)

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Figure 1
Figure 2
Graphical Abstract

60 peer-reviewed articles

Quality of studies
- Heterogeneous
- Different outcomes
- Paucity of well-designed prospective studies
- No firm conclusions

Barriers
- Difficult to perform in preschool children
- Only spirometry recommended

CLINICALLY APPLICABLE
Cut-off values for abnormal lung function identified
- Spirometry
  - FEV1, FVC <80%
- Impulse oscillometry
  - R at 5Hz, z-score >2
- Whole body plethysmography
  - sRaw >1.6
- Multiple breath washout
  - LCl>7
- Fractionated exhaled nitric oxide (FeNO)
  - Positive cut-off >35 ppb
  - Negative cut-off <20 ppb

Inclusion criteria for systematic review
2000-2020
- preschool children with wheeze
- prediction of asthma
- lung function tests

8 unique types of tests identified
- induced sputum
- volatile organic compounds
- exhaled breath condensate
- fractionated exhaled nitric oxide
- spirometry
- forced oscillation technique
- multiple breath washout
- whole body plethysmography

RELIABLE PREDICTION OF ASTHMA NOT POSSIBLE
References:


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