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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 cover 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

95 plethysmography, (iii) multiple breath washout (MBW) test to measure lung clearance index
96 (LCI), and measuring non-invasive biomarkers of inflammation like (iv) fractional exhaled nitric
97 oxide (FeNO), (v) sputum eosinophilia, (vi) exhaled breath condensates, and (vii) volatile organic
98 compounds. However, their role and usefulness in routine clinical practice to diagnose, monitor,
99 and guide therapy are unclear in young children. Currently, clinical guidelines (American Thoracic
100 Society (ATS), European Respiratory Society (ERS), National Asthma Education and Prevention
101 Program (NAEPP) and Global Initiative for Asthma (GINA), NICE, and BTS/SIGN) recommend
102 only spirometry for diagnosing and monitoring asthma. At the same time, the other tests are
103 mainly used for research purposes.

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

107

108 **Search strategy and selection criteria**

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

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

119 In October 2019, we performed a systematic review of the literature with the search
120 strategy as follows: (preschool child OR infant OR toddler OR pre-school OR kindergarten OR
121 nursery) AND (lung function test* OR breath test* OR exhaled breath condensate OR impulse
122 oscillometry OR oscillometry OR nitric oxide OR plethysmography OR spirometry OR
123 respiratory function test* OR volatile organic compounds OR sputum OR FeNO) AND (asthma*
124 OR wheez* OR bronchiolitis OR bronchitis) AND English[Language]. This search strategy was

125 formulated for PubMed and then adapted for the other two databases. The inclusion criteria were
126 formulated as follows: published, peer-reviewed articles (conference abstracts were excluded after
127 checking if no article was published based on presented results); age group defined as preschool
128 children; outcome defined as asthma diagnosis. Title and abstracts of all identified papers were
129 read independently by two pediatric pulmonologists (VE, WF with >10 years of experience), one
130 allergist (MR >10 years of experience), and one medical student (AA) and cross-checked the
131 selected records. The consensus resolved any discrepancies were reached using the Delphi
132 method.

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

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

150 **Spirometry**

151 Spirometry is the most frequently used method for monitoring lung function and measuring airway
152 obstruction, while evidence for its applicability to predict asthma in the preschool population is
153 sparse (online Table 1). Well-designed prospective studies are either lacking or show conflicting
154 results. It is recommended to be used for diagnosing and monitoring asthma in patients over five

155 years of age. Measurements of spirometric indices such as forced expiratory volume in 1 second
156 (FEV1), forced vital capacity (FVC), and FEV1/FVC ratio, are the gold standard (Table 1), but
157 test performance and its repeatability pose some difficulty. Spirometry is recommended for the
158 assessment of asthma severity, control, and response to treatment by several international
159 guidelines (ATS, ERS, NAEPP, GINA, NICE, and BTS/SIGN). Normal values of spirometry are
160 well established for several different nationalities and are usually defined as a standard deviation
161 score or Z-score of > -2 SD. However, preschool children have small absolute lung volumes and
162 large airway size relative to lung volumes compared to older children. Also, they are often unable
163 to exhale more than 1 second. Therefore, forced expiratory volume in 0.5 seconds (FEV0.5), and
164 the indices of the more peripheral airways, such as forced expiratory flow at 50% of FVC
165 (FEF_{50%}), maximal mid expiratory flow (MMEF), and forced expiratory flow at 25-75% (FEF_{25-75%})
166 and area under the MMEF (A_{ex}) have been suggested to be more suitable in this age group
167 and may reflect obstruction in the small airways of children with asthma.(4, 5, 142) Nevertheless,
168 the role of these indices in detecting airway obstruction in clinical practice is controversial. For
169 example, FEF_{50%}, MMEF, and FEF_{25-75%} are highly variable since they are effort dependent. Few
170 studies have shown that these are more sensitive than FEV1 in detecting peripheral airway
171 obstruction,(6) while others have shown that FEF_{25-75%} and FEF_{75%} correlate with conventional
172 spirometric indices.(7) Furthermore, airway obstruction is not necessarily permanent in preschool
173 children, and airflow limitation may be mild or absent. Bronchodilator reversibility testing,
174 although not specific, is useful for confirming the diagnosis of asthma. The most widely used
175 definition of a significant bronchodilator response is an improvement in FEV1 greater than 12%
176 and 200 ml (Table 2). Also, adding an exercise challenge test increase the diagnostic value of
177 spirometry. A reduction in FEV1 of at least 10-15% after exercise is anticipated as a sign of
178 exercise-induced bronchoconstriction (Table 2).(8, 9) Thus, normal spirometry results do not rule
179 out mild asthma, and it is essential to monitor changes over time as lung function varies with
180 symptom burden, exacerbations, and treatment with inhaled corticosteroids (ICS).(10)

181 Analyses of lung function growth during childhood have shown that the lung function
182 trajectory measured by spirometry is different in preschool children developing recurrent wheeze
183 and asthma compared to healthy children.(11-13) The trajectory seems to be a static character with
184 a constant deficit in airway obstruction both before symptom debut and even after remission of the
185 disease.(14) This makes the child susceptible to develop asthmatic symptoms and intermittent

airway obstruction during exposure to triggers such as respiratory infections. However, the causality of lung function deficit and episodes of wheezing is still not clear.

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 FEV_{0.5}, FVC, and maximal flow at functional residual capacity (V_{max}FRC) 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

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 (R_{int}). (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

249 eight years, demonstrating its applicability in preschool children, including 2971 children (online
250 Table 3). Also, several published reference values are available from healthy preschool children of
251 Caucasian and Asian origin.(4, 30, 31) According to these studies, height was found to be the most
252 influential variable on respiratory system resistance (Rrs) and reactance (Xrs), the components of
253 respiratory impedance (Zrs) (Table 1). The test results are expressed as a percentage of the
254 predicted values or as z-scores.

255 Both Rrs and Xrs are expressed as a function of oscillation frequency. At high frequencies
256 (>20 Hz), Rrs at 20 Hz is thought to reflect mainly airway resistance in the large airways, whereas
257 at lower frequencies (4-8 Hz), Rrs at 5Hz reflects the whole respiratory system.(27, 32) Small
258 airway disease usually results in the frequency dependence of resistance, i.e. an increased
259 difference between Rrs at 5Hz and 20Hz, although other factors such as upper airway shunting
260 contribute to this phenomenon in children.(33) In the presence of small airway obstruction, the
261 respiratory system's elastic properties change, resulting in decreased Xrs and increased area under
262 the negative part of the reactance curve (AX).(32)

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

268 FOT and IOS have been useful in documenting response to bronchodilators,
269 distinguishing between healthy and wheezing children.(34, 35, 42, 43) In 4-year-old children, IOS
270 was more effective than spirometry to differentiate responses between healthy and asthmatic
271 children.(44) Based on the distribution of bronchodilator responses (5th percentile) in healthy
272 preschool children, an improvement of Rrs at 5 Hz of at least 40 % is considered positive (Table
273 2).(38, 45, 46) In bronchoprovocation tests (methacholine and exercise) the application of FOT
274 and IOS have also demonstrated increased airway hyperresponsiveness in wheezing children,
275 parallel to the regular spirometry.(47-50)

276 IOS has also successfully been used in clinical trials evaluating therapeutic responses to
277 ICS in young asthmatic children.(51) However, the role of IOS in monitoring children with
278 preschool wheezing in clinical routine is still unclear. Longitudinal studies evaluating lung
279 function trajectories by the oscillometric technique are few.(37, 52-55) Recent observations,

280 however, suggest that abnormal IOS (Rrs at 5Hz) in preschool wheezers is associated with low
281 lung function, need for asthma medication, and asthma symptoms in adolescence.(54) However,
282 based on the existing data, the value for asthma prediction in preschoolers seems equivocal.

283 IOS is sensitive to many artefacts, which should be recognized, particularly when young
284 children are studied.(4) Therefore, experienced personnel is a prerequisite for a successful
285 application. Moreover, since spirometry is more widely adopted and studied, interpretation of IOS
286 results may not be straightforward for many clinicians.

287

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

294

295 **Bronchial hyperresponsiveness**

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

312 Running outside in cold and dry air can increase the sensitivity of the exercise test. The running
313 exercise test gives information about the presence of bronchial constriction during exercise as well
314 as fitness and motor development of the child.

315 The major disadvantage of bronchial hyperresponsiveness is that the tests take time, up to
316 1.5 hours depending on the choice of test (oscillometry more time consuming than spirometry), the
317 child's co-operative skills and trained personnel are required.(59) Therefore, they are not suitable
318 for routine clinical assessment of asthma control in preschool children.(26) However, bronchial
319 hyperresponsiveness assessment may play a role in children with possible exercise limitations,
320 poor symptom perception, or atypical asthma symptoms not responding to treatment.

321

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

327

328 **Multiple breath washout test**

329 The multiple breath washout test (MBW) measures the Lung Clearance Index (LCI), which is the
330 number of times the lung volume has to be turned over to wash out an inert gas such as nitrogen
331 (N₂; requires 100% oxygen during washout phase which has effects on infants' tidal breathing
332 parameters and therefore is used for all age groups except infants) or sulfur hexafluoride (SF₆;
333 used for infants) (Table 1). Thus, LCI is an index of inhomogeneity of ventilation, and it has been
334 used to detect early lung damage in children with cystic fibrosis and primary ciliary dyskinesia.
335 The advantages of LCI are the narrow normal range between 6-7 and its independence of age
336 (Table 2).(60) Although LCI has shown to be significantly higher in asthmatic school-age children
337 than healthy controls (61), there are currently only five studies of LCI in the preschool age group
338 (online Table 4). LCI is not higher in wheezy preschool children with the mild or well-controlled
339 or episodic disease compared to healthy children,(15, 62, 63) whereas significant differences with
340 higher LCI have been reported only for preschool children with a history of severe wheeze
341 exacerbations(64) and children with persistent wheeze.(22, 62) The multiple breath washout
342 technique uses the alveolar slope to determine measures of ventilation inhomogeneity in the acinar
343 (S_{acin}) and conducting (S_{cond}) airway regions (Table 1). Typically, the normal values are calculated

around 0.033 for S_{cond} and 0.075 for S_{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_{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 handheld 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

375 should be interpreted cautiously.(68) Many additional factors such as allergen and tobacco smoke
376 exposure, diurnal variability, atopy status and airway infections associate with changes in FeNO.
377 Thus, small changes in FeNO may clinically be irrelevant for monitoring asthma.

378 In healthy neonates, elevated FeNO was associated with an increased risk of developing
379 early transient wheeze during preschool age(69) and in infants with recurrent wheezing elevated
380 FeNO predicted decline in lung function and risk of future wheezing.(69-71) In preschool
381 children, elevated FeNO is associated with current symptoms, atopy, and later asthma.(22, 34, 72)
382 Although ICS has been shown to reduce FeNO in preschool children with wheeze, there are no
383 published preschool data on its utility in monitoring or adjusting asthma treatment or predicting
384 exacerbations.(68, 73-76) FeNO level is correlated with bronchial hyperresponsiveness during the
385 exercise test. However, FeNO is not useful in clinical practice to predict the need for exercise
386 testing.(77)

387

388 *Summary & outlook / Clinical perspective:* FeNO has been shown to predict allergic asthma
389 among wheezy preschool children (cut-off >35 ppb). Many specialist centers use it to monitor
390 children with difficult-to-treat asthma and as part of differential diagnosis of allergic asthma. This
391 technique may become a tool for diagnosing wheezy preschoolers (online Table 5).

392

393 *Sputum*

394 Performance and analysis of induced sputum in children is well described (78) and is safe and
395 feasible in over 50% of children over six years of age (Table 1) (79, 80). There are only two
396 studies with 1252 children, where sputum was collected from preschoolers (online Table 6), but
397 the methodology of its acquisition has not yet been standardized in preschool children. Therefore,
398 no data indicate its applicability in asthma prediction in preschool wheezers.

399

400 *Exhaled breath condensate*

401 A vast number of inflammatory mediators can be measured in exhaled breath condensate (EBC)
402 (Table 1). It is collected during tidal breathing by cooling exhaled air by contact with a cold
403 surface or condenser.(81-83) EBC is thus a diluted fluid, and the collected condensate contains
404 volatile (e.g., H₂O₂) and semi- and non-volatile molecules (proteins and cytokines) carried by
405 respiratory droplets (Figure 2).(83) 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

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

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

474 The objective assessment of lung function and airway inflammation is clinically essential
475 to avoid over- and under-treatment of preschool wheeze and to monitor difficult-to-treat asthma.
476 To date, for most methods for measuring lung function, there are no proper randomized clinical
477 controlled trials or longitudinal studies available to establish their role in diagnosing and
478 monitoring asthma in wheezy preschoolers. However, the need for improved diagnostic methods
479 and further research is urgent.

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484 **Figure legends**

485

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

490

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

493 The upper panel includes non-invasive methods: Exhaled breath diagnostics (non-invasive),
494 including the analysis of airborne volatile organic compounds in exhaled breath (VOC) or
495 inflammatory mediators in exhaled breath condensates (EBC), and induced sputum eosinophilia.
496 The lower panel shows an array of current semi-invasive or effort-demanding methods; including
497 regular spirometry (with or without bronchial hyperresponsiveness test), impulse oscillometry (a
498 method based on the forced oscillation technique, FOT), multiple breath washout test (MBW),

499 whole-body plethysmography (WBP) and quantitative measurement of airway nitric oxide,
500 reflecting airway and tissue eosinophilia (fractionated exhaled nitric oxide, FeNO).
501

	Spirometry	Impulse oscillometry (IOS)	Whole-body plethysmography	Multiple breath washout test (MBW)	Nitric oxide (NO)	Sputum	Exhaled breath condensate (EBC)	Volatile organic compounds (VOCs)
Measure attributes	Lung volumes, FVC, FEV1, FEV1/FVC	Respiratory resistance Rrs at 5Hz and Rrs at 10Hz, reactance Xrs at 5Hz, area of reactance (AX)	Functional Residual Capacity (FRC), specific airway Resistance (sRaw), Residual Volume (RV), Total Lung Capacity (TLC)	Lung Clearance index (LCI), S _{acin} , S _{cond}	Fractional exhaled nitric oxide (FeNO)	eosinophils	Metabolomics and proteomics of inflammation	Biomarker profile
Reference values for preschool children	> 5 yrs, for many nationalities	>2 yrs, for many nationalities	> 2 yrs	Yes, independent of age	no	no	no	Independent of age
Sensitivity and specificity	good	good	good	poor	poor	?	?	?
Used for diagnostics	yes	yes	yes	no	no	no	no	no

Used for monitoring and tapering management of asthma	yes	yes	yes	no	yes	no	no	no
Predicts loss of asthma control	++	+++	+	-	+	?	?	+
Feasibility/easy to perform	>5 yrs	>3 yrs	> 2 yrs	> 3yrs	> 5 yrs	> 6 yrs	> 4 yrs	>2 yrs
Requires specially trained personnel	+	++	+++	+++	+	+	++	+
Requires expensive equipment	+	++	+++	++	+	+	+++	+
In clinical practice	+++	++	++	-	+	-	-	--

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

Abbreviations:

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

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

[†] 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

[‡] ref AARC clinical practice guideline. Body plethysmography. American Association for Respiratory Care. Respir Care. 1994;39(12):1184-90

§ ref 38, 45, 46

¶ ref 68

** ref 60

Figure 1

Figure 2

Graphical Abstract

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