Small airway function in children with mild to moderate asthmatic symptoms

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ARTICLE INFO

Article history:

Received for publication May 26, 2018.

Received in revised form July 16, 2018.

Accepted for publication July 22, 2018.

ABSTRACT

Background: Clinical significance of small airway obstruction in mild pediatric asthma is unclear.

Objective: To evaluate small airway properties in children with mild to moderate asthmatic symptoms and the association of small airway function with asthma control and exercise-induced bronchoconstriction (EIB).

Methods: Children (5–10 years old) with recurrent wheezing ($n = 42$) or persistent troublesome cough ($n = 16$) and healthy controls ($n = 19$) performed impulse oscillometry (IOS), spirometry, and a multiple-breath nitrogen washout (MBNW) test. Exhaled nitric oxide (NO) was measured at multiple flow rates to determine alveolar NO concentration (Ca_{LV}). Asthma control was evaluated with the Childhood Asthma Control Test (C-ACT), short-acting β_2 -agonist (SABA) use within the past month, and asthma exacerbations within the past year.

Results: IOS, spirometry, and exhaled NO indexes that are related to small airway function differed between children with recurrent wheezing and healthy controls, whereas only forced expiratory flow at 25% to 75% of the forced vital capacity was associated with persistent cough. The MBNW indexes showed no difference between the groups. Among symptomatic children, conducting airway ventilation inhomogeneity and Ca_{LV} were associated with asthma exacerbations ($P = .03$ and $P = .002$, respectively), and lung clearance index and Ca_{LV} were associated with EIB ($P = .04$ and $P = .004$, respectively). None of the proposed small airway indexes was associated with the C-ACT score or SABA use.

Conclusion: Subtle changes were observed in the proposed small airway indexes of IOS, spirometry, and exhaled NO among children with mild to moderate recurrent wheezing. Small airway dysfunction, expressed as ventilation inhomogeneity indexes and Ca_{LV} , was also associated with asthma exacerbations and EIB.

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Introduction

Bronchial biopsies and postmortem studies have demonstrated the involvement of small airway inflammation in asthma,^{1,2} supported by clinical studies on the association between small airway dysfunction and clinical expression of asthma.^{3,4} In children, abnormalities in the proposed small airway indexes have been related to uncontrolled or severe asthma,^{5–7} but their potential to distinguish milder disease changes remains debated.^{8,9} It is plausible to hypothesize small airways to be involved to some extent in most patients with asthma,¹⁰ even in children. Early involvement of small airways has also been recognized as a risk factor for future chronic obstructive pulmonary disease.¹¹ Furthermore, with the emergence of small-particle aerosols

that reach the lung periphery, accurate measures for small airways are necessitated to identify and follow up patients who might benefit from this treatment. However, conventional lung function measures, such as forced expiratory volume in 1 second (FEV_1), mainly reflect large airways, and no gold standard measures for small airways exist. Potential methods for assessing small airway properties include impulse oscillometry (IOS), spirometry, multiple-breath washout (MBW) tests, and extended exhaled nitric oxide (NO) analysis.

IOS measures respiratory resistance and reactance by analyzing responses to pulse signals of different frequencies. High-frequency resistance values, such as resistance at 20 Hz (R_{20}), are considered to reflect large airway resistive properties, whereas lower frequencies, such as resistance at 5 Hz (R_5), reflect the whole respiratory system.¹² Small airway obstruction is thought to result in frequency dependence of resistance and thus increased difference between R_5 and R_{20} (R_{5-20}).⁵ Negative reactance values reflect the capacitive properties of the lung periphery, and thus reactance at 5 Hz (X_5) and area under the reactance curve (AX) are considered to reflect small airway function.^{5,12}

During forced expiration, small airway obstruction is postulated to contribute to airflow limitation during the middle and end phases of

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Disclosures: Authors have nothing to disclose.

Funding Sources: The study was funded by the Emil Aaltonen Foundation, Foundation for Pediatric Research, Ida Montin's Foundation, The Finnish Allergy Research Foundation, The Finnish Medical Foundation, The Research Foundation of the Pulmonary Diseases, and Väinö and Laina Kivi's Foundation.

the forced vital capacity (FVC). This results in diminished midexpiratory flow rates, without significant effects on FEV₁, which mainly reflects large airway function at the initial part of forced expiration.¹³ Midexpiratory flow rates can be evaluated by calculating the forced expiratory flow between 25% and 75% of the FVC (FEF_{25%–75%}), which is considered to reflect the medium- and small airway function.

MBW measures ventilation distribution homogeneity by analyzing the washout pattern of an inert marker gas. Lung clearance index (LCI) is postulated to reflect the overall ventilation inhomogeneity secondary to small airway disease.¹⁴ The washout pattern over several breaths aims to separate convection-dependent ventilation inhomogeneity in the small conducting airways (Scond) and diffusion-convection-dependent inhomogeneity in the acinar structures (Sacin).¹⁴

Airway inflammation enhances production of NO in the bronchial epithelium.¹⁵ At low flow rates, such as 50 mL/s, the fractional exhaled NO (FENO) mainly reflects NO from the central airways.¹⁶ With increasing flow rates, contribution of proximal airways decreases and FENO represents alveolar NO.¹⁶ Applications with multiple exhalation flow rates have been created to separate alveolar NO concentration (CALV) from bronchial NO flux (JNO).¹⁵

Our primary aim was to investigate whether small airway dysfunction is present in young children with mild to moderate asthmatic symptoms by using different indicators of small airway involvement: IOS, spirometry, multiple-breath nitrogen washout (MBNW) test, and extended FENO measurement. The secondary aim was to evaluate the association of these measurements with asthma control and exercise-induced bronchoconstriction (EIB). Finally, considering their proposed association with small airway function, we hypothesized that the small airway indexes of the compared methods would be associated with each other.

Methods

The study was approved by Helsinki University Hospital (IAS16 ASA04 0116) and Helsinki University Hospital Research Ethics Committee (390/13/03/2015). Parents of all participating children provided written consent, and depending on literacy, the children contributed an oral or written consent.

Study Participants

The symptomatic children (n=58) aged 5 to 10 years were recruited from patients who were referred to the Skin and Allergy Hospital of the Helsinki University Hospital between April 2016 and May 2017 for symptoms suggestive of asthma. Inclusion criteria were (1) symptoms suggestive of asthma (≥3 episodes of wheezing or

troublesome cough lasting continuously for ≥6 weeks without indications of alternative differential diagnoses), (2) no other chronic or acute diseases of the respiratory system, and (3) acceptable lung function measurements at the first visit. At the first visit, the children first performed IOS and then spirometry measurements, combined with an exercise challenge test. MBNW and extended FENO measurements were performed at the second visit within 14 days. The parents of 65 symptomatic children provided informed consent for study participation. Thereafter, 4 children were excluded because of symptoms of a respiratory tract infection before the second visit; 2 children failed to deliver the research questionnaires, and 1 child later refused blood sampling.

Nineteen healthy controls aged 5 to 10 years were recruited through an announcement that was targeted to the personnel of the hospital from which the patients were recruited. Inclusion criteria were (1) no symptoms or diagnosis of acute or chronic respiratory disease and (2) no systemic disease with possible direct or indirect respiratory effects. All the healthy controls who filled the inclusion criteria were included in the study. Healthy children performed MBNW, extended FENO, IOS, and spirometry measurements in the aforementioned order during one visit.

All children were free of respiratory tract infections for at least 2 weeks. Use of possible asthma-control medications ceased at least 4 weeks before the lung function measurements according to national practice guidelines to minimize their possible confounding effect on lung function. Parents filled out a questionnaire on the child's family history, health status, and symptoms. Asthma control was evaluated with the Childhood Asthma Control Test (C-ACT) (a score of ≤19 indicated inadequate asthma control¹⁷), use of short-acting β₂-agonist medication (SABA) within the past month, and asthma exacerbations (episodes of shortness of breath or wheezing) within the past year.

Impulse Oscillometry

IOS (Masterscreen IOS, Carefusion, Hoechberg, Germany) measurements were performed as described earlier,¹⁸ according to international recommendations.¹⁹ During all lung function measurements, the children were in a sitting position and breathed through a mouthpiece using a nose clip. To minimize pressure losses in IOS, cheeks were supported by the hands of the investigator. The measurement was accepted when 3 or more regular tidal breathing patterns lasting 20 seconds or longer without signs of artefacts, such as apnea, speaking, swallowing, or air leak, were obtained. Table 1 summarizes the postulated small airway indexes of the different measurements used in the study and their hypothetical physiologic determinants.

Table 1
Postulated Small Airway Indexes and Their Hypothetical Physiologic Determinants

Index	Unit	Physiologic determinant
IOS		
R5-20	kPa/L/s	Resistive properties of the small airways.
X5	kPa/L/s	Elastic properties of the peripheral lung region.
AX	kPa/L	Integrative measure of the elastic properties of the peripheral lung region.
Spirometry		
FEF _{25%–75%}	L/s	Airflow limitation in the small airways.
FENO		
CALV	ppb	Eosinophilic inflammation in the close to or at the alveolar region.
MBNW		
LCI	—	Total ventilation heterogeneity.
Scond	1/L	Ventilation heterogeneity secondary to small conductive airway dysfunction.
Sacin	1/L	Ventilation heterogeneity secondary to dysfunction in the alveolar region.

Abbreviations: AX, area under the reactance curve; CALV, alveolar nitric oxide concentration; FEF_{25%–75%}, forced expiratory flow between 25% and 75% of the forced vital capacity; FENO, fractional exhaled nitric oxide; IOS, impulse oscillometry; LCI, lung clearance index; MBNW, multiple-breath nitrogen washout test; R5-20, difference between respiratory resistance at 5 and 20 Hz; Sacin, ventilation inhomogeneity in the acinar structures; Scond, ventilation inhomogeneity in the conducting airways; X5, respiratory reactance at 5 Hz.

Table 2
Baseline Characteristics of the Study Children

Characteristic	Healthy (n = 19)	Recurrent wheezing (n = 42)	Persistent Cough (n = 16)	All symptomatic (n = 58)
Boys, No. (%)	7 (37)	26 (62)	10 (63)	36 (62)
Age, median (IQR), y	7.4 (6.2–9.3)	6.9 (6.1–7.8)	7.0 (6.4–8.4)	7.0 (6.2–8.2)
Height, median (IQR), cm	130 (122–139)	124 (118–132)	127 (118–132)	125 (118–132)
Weight, median (IQR), kg	27 (22–30)	25 (21–33)	25 (21–31)	25 (21–32)
Low birth weight, No. (%) ^a	0	0	2 (13)	2 (3)
Prematurity, No. (%) ^b	0	1 (2)	2 (13)	3 (5)
Eosinophilia, No. (%) ^c	6 (32)	24 (57)	8 (50)	32 (55)
Atopy, No. (%) ^d	9 (47)	30 (71)	6 (38)	36 (62)
Parental asthma, No. (%)	6 (32)	14 (33)	3 (19)	17 (29)
Parental smoking, No. (%)	0	14 (33) ^e	7 (44) ^e	21 (36) ^e

Abbreviation: IQR, interquartile range.

^aBirth weight of less than 2,500 g.^bGestational age of less than 36 weeks.^cPeripheral blood eosinophil count of $0.4 \times 10^9/L$ or greater and 4% or greater.^dSerum specific IgE level of 0.35 kU/L or greater or skin prick test wheal diameter of 3 mm or larger for birch, timothy grass, meadow fescue, mugwort, *Cladosporium herbarum*, cat, dog, horse, cow, or *Dermatophagoides pteronyssinus*.^e $P < .05$ compared with the healthy control group using the χ^2 test.

Spirometry

Spirometry (Masterscreen Pneumo, Carefusion) was performed according to international guidelines,²⁰ with 3 or more successful measurements recorded and the highest values of FEV₁ and FVC used in the analyses. Flow indices were derived from the curve with the largest sum of FEV₁ and FVC. During the measurement, the investigator inspected the volume-time and flow-volume tracings and excluded unacceptable measurements.

Multiple-Breath Nitrogen Washout

MBNW (Exhalyzer D, Ecomedics, Duernten, Switzerland) was performed according to international guidelines¹⁴ by analyzing the washout pattern of endogenous nitrogen during tidal breathing of 100% oxygen. During the measurement, the investigator monitored the tracings of tidal volume and marker gas concentrations and rejected measurements with signs of inert gas leaks. A minimum of 2 successful recordings were required, and mean values were used in the analyses.

Extended F_{ENO}

F_{ENO} was measured with a chemiluminescence analyzer (CLD 88, Ecomedics) at 4 different flow rates (30, 50, 100, and 200 mL/s) with 2 or more successful measurements required for each flow rate. F_{ENO} was determined at 50 mL/s (F_{ENO50}) according to international recommendations.²¹ A 2-compartment linear model²² with 3 flow rates (100, 200, and either 30 or 50 mL/s for achieving the best goodness of fit [r^2]) was used to calculate J_{NO} and CALV.

Exercise-Induced Bronchoconstriction

The symptomatic children performed an outdoor free-running test with IOS or spirometry as described earlier.²³ EIB was defined as an increase of 35% or more in R5²³ or a decrease of 15% or more in FEV₁²⁴ at 1, 5, or 10 minutes after the exercise.

Eosinophilia and Atopy

Eosinophilia was defined as a peripheral blood eosinophil count of $0.4 \times 10^9/L$ or greater and eosinophils composing 4% or more of leukocytes. Atopy was defined as a serum specific IgE level of 0.35 kU/L or higher or skin prick test wheal diameter of 3 mm or greater to 1 or more inhaled aeroallergens: birch, timothy grass, meadow fescue, mugwort, *Cladosporium herbarum*, cat, dog, horse, cow, and

Dermatophagoides pteronyssinus. Histamine dihydrochloride (10 mg/mL, ALK, Horsholm, Denmark) served as a positive and physiologic saline as a negative control in the skin prick tests.

Statistical Analysis

Power calculations were performed before the study for 80% power and a type I error of 0.05, with the assumption that the mean AX (SD, 0.87 kPa/L)²⁵ in symptomatic children would differ 0.5 kPa/L from healthy controls, and a 20% margin for possible unsuccessful measurements was used. Accordingly, 58 symptomatic children were recruited.

Because of the lack of applicable reference values that would encompass the age range and nationality of the study children, the data were analyzed with height- and sex-adjusted regression analyses. Normality of the variables was evaluated with Kolmogorov-Smirnov or Shapiro-Wilk test. Comparisons between groups were performed with Mann-Whitney *U* test or with logistic regression analyses that were adjusted for relevant baseline factors. Receiver operating characteristic (ROC) curves were adjusted for height and sex by plotting the probability of the height- and sex-adjusted logistic regression model. Associations between the proposed small airway indexes (R5–20, X5, AX, FEF_{25%–75%}, LCI, Scond, Sacin, and CALV) were evaluated using height- and sex-adjusted linear regression analysis. Two-tailed tests with a significance level of .05 were used. The data was analyzed with SPSS statistical software, version 23 (IBM, Armonk, New York).

Results

Baseline characteristics of the healthy and symptomatic children are presented in Table 2. The healthy and symptomatic groups significantly differed regarding only parental smoking. Of the symptomatic children, 14 (24%) used continuous and 8 (14%) used intermittent asthma-control medication; use of these medications ceased 4 weeks or more before the diagnostic lung function measurements.

Technical Success and Reproducibility

All study children performed successful IOS and spirometry measurements. The coherence at 5 Hz was 0.80 or greater in 64 IOS measurements (83%), and coherence at 20 Hz was 0.80 or greater in all measurements. Successful F_{ENO50} measurements were obtained from all except 1 child. F_{ENO} measurements at all flow rates were successfully performed by 19 healthy controls (100%) and 46 symptomatic children (79%), with a median r^2 of

Table 3
Baseline Lung Function of the Study Children^a

Index	Healthy (n = 19)	Recurrent wheezing (n = 42)	P value ^b	Persistent cough (n = 16)	P value ^b
IOS					
R5, kPa/L/s	0.58 (0.50 to 0.69)	0.79 (0.73 to 0.91)	<.001	0.79 (0.59 to 1.01)	.02
R20, kPa/L/s	0.45 (0.41 to 0.53)	0.59 (0.53 to 0.64)	<.001	0.58 (0.51 to 0.71)	.006
R5-20, kPa/L/s	0.14 (0.08 to 0.21)	0.21 (0.15 to 0.29)	.047	0.15 (0.08 to 0.32)	.26
X5, kPa/L/s	−0.18 (−0.21 to −0.16)	−0.25 (−0.31 to −0.20)	.01	−0.23 (−0.29 to −0.19)	.07
AX, kPa/L	1.01 (0.49 to 1.87)	1.89 (1.48 to 2.67)	.004	1.95 (0.85 to 2.95)	.06
Spirometry					
FEV ₁ , L	1.67 (1.43 to 2.00)	1.54 (1.27 to 1.71)	.16	1.45 (1.20 to 1.58)	.03
FVC, L	1.88 (1.66 to 2.17)	1.71 (1.40 to 2.08)	.91	1.64 (1.37 to 1.92)	.17
FEV ₁ /FVC	0.89 (0.85 to 0.92)	0.85 (0.81 to 0.91)	.03	0.86 (0.81 to 0.90)	.22
FEF _{25%-75%} , L/s	2.01 (1.63 to 2.55)	1.59 (1.39 to 1.89)	.02	1.65 (1.33 to 1.91)	.04
FENO					
FENO ₅₀ , ppb	8.50 (4.00 to 13.80)	12.90 (7.40 to 23.33)	.07	9.30 (4.00 to 13.90)	.60
JNO, nL/s	0.35 (0.17 to 0.56)	0.67 (0.32 to 0.99)	.08	0.22 (0.13 to 0.52)	.98
CALV, ppb	1.35 (1.16 to 2.83)	2.36 (1.54 to 4.56)	.046	1.44 (0.64 to 2.70)	.74
MBNW					
FRC, L	1.44 (1.09 to 1.57)	1.13 (1.00 to 1.43)	.51	1.18 (1.03 to 1.59)	.61
LCI	6.81 (6.41 to 7.33)	6.87 (6.52 to 7.47)	.46	6.43 (6.08 to 6.55)	.46
Scond, 1/L	0.022 (0.019 to 0.037)	0.035 (0.021 to 0.042)	.49	0.026 (0.020 to 0.034)	.97
Sacin, 1/L	0.095 (0.043 to 0.158)	0.147 (0.049 to 0.192)	.80	0.114 (0.042 to 0.192)	.78

Abbreviations: AX, area under the reactance curve; CALV, alveolar nitric oxide concentration; FEF_{25%-75%}, forced expiratory flow between 25% and 75% of the forced vital capacity; FENO, fractional exhaled nitric oxide; FENO₅₀, FENO at 50 mL/s; FEV₁, forced expiratory volume in 1 second; FRC, functional residual capacity; FVC, forced vital capacity; IOS, impulse oscillometry; Jno, bronchial nitric oxide flux; LCI, lung clearance index; MBNW, multiple-breath nitrogen washout test; R5 and R20, respiratory resistance at 5 and 20 Hz, respectively; R5-20, difference between respiratory resistance at 5 and 20 Hz; Sacin, ventilation inhomogeneity in the acinar structures; Scond, ventilation inhomogeneity in the conducting airways; X5, respiratory reactance at 5 Hz.

^aData presented as median (interquartile range).

^bP value compared with the healthy control group using height- and sex-adjusted logistic regression model.

Figure 1. Height- and sex-adjusted receiver operating characteristic curves for the discriminative properties of respiratory resistance at 5 Hz (R5), difference between respiratory resistance at 5 and 20 Hz (R5-20), respiratory reactance at 5 Hz (X5), and area under the reactance curve (AX) (A), ratio of forced expiratory volume in 1 second to forced vital capacity (FEV₁/FVC) and forced expiratory flow at 25%–75% of the FVC (FEF_{25%-75%}) (B), and alveolar nitric oxide concentration (CALV) (C) between children with recurrent wheezing and healthy controls. AUC indicates area under the curve; CI, confidence interval.

0.82 (interquartile range [IQR], 0.73–0.91). Of the healthy controls, 18 children (95%) and 55 symptomatic children (95%) produced successful MBNW measurements with a median coefficient of variation of 5% (IQR, 2%–10%) for LCI. Successful Scond measurements were achieved in 13 healthy controls (68%) and 40 symptomatic children (69%) and successful Sacin measurements in 14 healthy controls (74%) and 35 symptomatic children (60%). Children with unsuccessful Scond measurements were significantly younger ($P = .01$) than those with successful measurements, but no other differences in baseline factors or other lung function indices were observed in children with unsuccessful Scond or Sacin measurements compared with those with successful measurements.

Differences Between Patients and Controls

Baseline lung function of the study children are presented in Table 3. All IOS indexes, as well as FEV₁/FVC, FEF_{25%-75%}, and CALV,

differed between children with recurrent wheezing and healthy controls. Regarding children with persistent cough, only IOS indexes R5 and R20 and spirometry indexes FEV₁ and FEF_{25%-75%} differed from healthy controls. FENO₅₀, JNO, and MBNW indexes showed no difference between the symptomatic children and healthy controls. None of the lung function indexes differed between symptomatic children with or without parental smoking ($P > .05$). However, separate logistic regression analyses were applied to adjust the parameters for atopic status or parental smoking. After atopic status was adjusted for, all associations except for the difference observed in CALV ($P = .07$) between children with recurrent wheezing and healthy controls remained significant. After adjusting for parental smoking, the differences in FEV₁ ($P = .12$) and FEF_{25%-75%} ($P = .09$) between children with persistent cough and healthy controls did not remain significant.

Figure 1 presents the height- and sex-adjusted ROC curves illustrating the discriminatory properties of IOS, spirometry, and CALV between children with recurrent wheezing and healthy controls. All the investigated parameters had an area under the curve (AUC) of

Figure 2. Association between asthma exacerbations within the past year and conducting airway ventilation inhomogeneity (Scond) (A), bronchial nitric oxide flux (JNO) (B), and alveolar nitric oxide concentration (CALV) (C) in the symptomatic children. The boxes represent the interquartile ranges (IQR) with medians, the tails represent 1.5 times the IQR, the circles represent outliers (between 1.5 and 3 times the IQR), and the stars represent extreme outliers (>3 times the IQR). Statistical analyses were performed using the Mann-Whitney U test.

Figure 3. Association between exercise challenge test and lung clearance index (LCI) (A), bronchial nitric oxide flux (JNO) (B), and alveolar nitric oxide concentration (CALV) (C) in the symptomatic children. The boxes represent the interquartile ranges (IQR) with medians, the tails represent 1.5 times the IQR, the circles represent outliers (between 1.5 and 3 times the IQR), and the stars represent extreme outliers (>3 times the IQR). Statistical analyses were performed using the Mann-Whitney U test.

greater than 0.70, with R5 and AX providing the best discriminatory power (AUC of >0.80). The unadjusted AUCs for the lung function indexes were as follows: 0.857 (95% confidence interval [CI], 0.748–0.965; $P < .001$) for R5, 0.705 (95% CI, 0.563–0.846; $P=.01$) for R5–20, 0.777 (95% CI, 0.653–0.901; $P=.001$) for X5, 0.791 (95% CI, 0.667–0.916; $P < .001$) for AX, 0.664 (95% CI, 0.523–0.806; $P=.04$) for FEV₁/FVC, 0.747 (95% CI, 0.614–0.881; $P=.002$) for FEF_{25%–75%}, and 0.686 (95% CI, 0.536–0.836; $P=.03$) for CALV.

Associations With Asthma Control and EIB

C-ACT was acquired from 51 symptomatic children with a median score of 24 (range, 12–27). A C-ACT score of 19 or less was reported by 11 of the 36 children (31%) with recurrent wheezing and none of the 15 children with persistent cough from whom a completed C-ACT questionnaire was received. None of the lung function parameters were associated with the C-ACT score.

Of the symptomatic children, 23 (40%) reported using SABA within the past month, which was associated with increased FENO₅₀ ($P=.007$) and JNO ($P=.03$) but not with the other indexes of interest. The differences in FENO₅₀ but not in JNO remained significant in the height- and sex-adjusted logistic regression analyses ($P=.02$ and $P=.06$, respectively). Sixteen children (28%) required SABAs 1 time per week or less and 7 (12%) 2 times per week or more. Both FENO₅₀ and JNO correlated positively with the increasing use of SABAs ($r_s=0.400$, $P=.004$, and $r_s=0.377$, $P=.02$, respectively).

Among the symptomatic children, asthma exacerbations within the past year were reported as follows: no exacerbations in 13 (22%), 1 to 3 exacerbations in 17 (29%), 4 to 12 exacerbations in 20 (35%), and more than 12 exacerbations in 8 children (14%). Scond, FENO₅₀, JNO, and CALV correlated positively with the increasing number of

exacerbations ($r_s=0.340$, $P=.032$; $r_s=0.457$, $P < .001$; $r_s=0.493$, $P < .001$; and $r_s=0.337$, $P=.02$, respectively). Frequent (≥ 4 per year) exacerbations were associated with increased Scond, FENO₅₀ ($P=.003$), JNO, and CALV in the symptomatic children (Fig 2). These associations remained significant after adjusting for height, sex, and the use of asthma-control medications ($P < .05$).

Of the 55 symptomatic children (95%) who performed the exercise challenge test, 20 (36%) had significant EIB. LCI, FENO₅₀ ($P=.007$), JNO, and CALV were associated with EIB in the symptomatic children (Fig 3). All associations remained significant in the height- and sex-adjusted logistic regression analysis ($P < .05$).

Associations Between Proposed Small Airway Indexes

Significant associations were observed between all the IOS indexes (R5–20, X5, and AX) and FEF_{25%–75%} ($P \leq .001$ in each comparison), as well as between Scond and CALV ($P=.04$) but not regarding other investigated indexes.

Discussion

Despite the increasing clinical and research interest toward small airways, their role in pediatric asthma is poorly understood. We found subtle but significant differences between 5- to 10-year-old children with mild to moderate asthmatic symptoms and healthy controls regarding IOS, spirometry, and FENO indexes related to small airway function, which suggests that small airway dysfunction is present even in mild asthma at this age group. Most of these differences were observed only in children with recurrent wheezing but not in those with persistent cough without wheezing episodes. None of the MBNW indexes revealed differences in either of the symptomatic subgroups when compared

with healthy controls. Among the symptomatic children, Scnd and CALV were associated with asthma exacerbations within the past year, and LCI and CALV were associated with EIB. However, none of the investigated parameters was associated with C-ACT, and only bronchial NO measures were related to SABA use within the past month.

In children, the postulated small airway IOS indexes have been found superior to spirometry in distinguishing asthmatic symptoms and disease control.^{3,5,6} Results so far have been ambiguous, however.²⁶ We found differences in R5-20, X5, and AX, which are proposed to reflect small airway properties, between children with recurrent wheezing and healthy controls, suggesting that these indexes might be sensitive in detecting early disease changes of the peripheral airways. Contrary to R5 and R20, however, R5-20, X5, and AX were not associated with persistent cough. Although persistent troublesome cough is associated with later asthma,²⁷ the symptom can result from a wide variety of causes²⁸ and is thus less specific for airway obstruction than recurrent wheezing episodes.²⁹ Therefore, it is possible that this patient subgroup included children with symptoms that resulted from other causes than asthma, blunting the discriminatory power of the investigated indexes. Another possible explanation is that this subgroup of children with very mild symptoms presented with too subtle or nonexistent changes in the peripheral airway region and thus remained undetected with the investigated methods.

On the basis of previous studies, FEF_{25%-75%} appears superior to FEV₁ in detecting different clinical features of pediatric asthma, such as symptoms and disease control.^{30,31} Accordingly, in our study FEF_{25%-75%} was the most sensitive spirometric parameter for distinguishing between healthy and symptomatic children and, interestingly, the only proposed small airway index that differed from healthy controls in both symptomatic subgroups. However, FEF_{25%-75%} is not specific to small airway obstruction¹³ and, therefore, does not confirm the presence of small airway impairment in these individuals.

Increased ventilation inhomogeneity, especially in the conducting airways, has been reported in patients with asthma with well-controlled and mild symptoms when compared with healthy controls.³²⁻³⁴ These changes have predominantly been subtle and within normal ranges, however.^{33,35} Other studies have found differences restricted to severe subgroups.⁴ We detected no differences in MBNW indexes between healthy and symptomatic children. Regarding Sacin and Scnd, strict technical requirements reduced the number of successful measurements, decreasing the power in the statistical analyses, however. In accordance with previous reports,^{34,36} we found a weak association between LCI and EIB. In addition, we found an association between Scnd and asthma exacerbations within the past year. Therefore, MBNW might offer information on the disease activity, which is supported by the concurrently observed increases in the FENO indexes, especially CALV. In line with this, multiple-trigger wheezers present with conducting airway inhomogeneity.³⁷ However, the clinical significance of MBNW in pediatric asthma warrants further investigation.

Increased CALV has predominantly been associated with severe or uncontrolled asthma⁷ and has been observed as less affected in mild disease presentations.^{7,9,15,34,38} Accordingly, we found that of the investigated indexes, CALV had the strongest associations with asthma exacerbations and EIB, indicating that the parameter might be useful in reflecting disease control. In addition, in line with the proposed small airway IOS indexes, CALV differed from healthy controls only in children with recurrent wheezing but not in those with isolated cough. FENO measures are, however, influenced by several confounding factors,³⁹ limiting their diagnostic potential. We also found that after adjusting for atopic status, the difference in CALV between healthy and wheezing children did not remain significant.

Studies on the association between small airway dysfunction and asthma control questionnaires are conflicting and mainly restricted to adults.^{4,26,40} We found no association between the investigated

lung function indexes and C-ACT scores. However, only 22% of the study participants reported an uncontrolled disease on the C-ACT, which complicates segregation based on the questionnaire. However, we observed a significant association between asthma exacerbations and Scnd and CALV, supporting that peripheral airway dysfunction and inflammation might be associated with an uncontrolled disease.^{6,7} We also detected an association between EIB and LCI as well as CALV, supporting previous reports that small airway dysfunction might be related to airway hyperresponsiveness.^{31,34,36}

Functional features related to small airway dysfunction are peripheral ventilation heterogeneity, air trapping, and premature airway closure. All available techniques measure these changes indirectly, and their potential to assess small airways relies on theoretical models that do not elucidate the specific lung region or the physiologic determinants they reflect. Studies on the associations among different methods are scarce and mainly restricted to older children and adults. In accordance with our findings, the postulated small airway IOS indexes have been found to correlate with midexpiratory flow spirometric parameters,⁴¹ whereas CALV seems unrelated to IOS and spirometry.⁴⁰ However, CALV has been found to correlate with Scnd and Sacin in children with asthma.³⁴ We found an association between CALV and Scnd but not with Sacin. In addition, IOS and MBNW indexes were unrelated, which is in accordance with previous reports in adults that have found weak or nonexistent associations between IOS and MBW at baseline.⁴² Although temporal variability of airway function is characteristic for asthma, we postulated that 1 to 14 days between the measurements would not have a significant effect on lung function because no exacerbations or interventions occurred between the measurements.^{43,44}

The present study has some limitations regarding the sample size and characteristics. First, because power calculations were performed based on AX, it is possible that the study was underpowered for some of the other parameters, especially Sacin and Scnd, which did not show differences between healthy and symptomatic children. Second, our patient population consisted of children with mild to moderate symptoms in the early disease stage. This setting aimed to minimize the confounding effects of asthma-control medication and a more generalized airway obstruction. It is, however, possible that not all the symptomatic children present with chronic asthma, especially those with isolated cough. Because of challenges in the differential diagnostics of persistent cough,²⁸ the discriminatory analyses were performed for both symptomatic subgroups separately, and the ROC analyses only included children with recurrent wheezing. Third, the control group was relatively small and could not be completely matched with symptomatic children with regard to age and sex. Therefore, the statistical analyses were adjusted for height and sex. The lung function measurements of 19 healthy controls, however, resulted in a power that was sufficient to detect statistically significant differences in the postulated small airway indexes of IOS and spirometry, as well as CALV when compared with children with recurrent wheezing.

In conclusion, we found subtle but significant differences in the proposed small airway IOS, spirometry, and FENO indexes between children with recurrent wheezing and healthy controls, suggesting that early disease changes in the distal airways might be present in young children with mild asthmatic symptoms, mainly restricted to recurrent wheezing. In addition, ventilation inhomogeneity indexes and alveolar inflammation were associated with asthma exacerbations and EIB. The presence of changes in the proposed small airway indexes that were not detected with FEV₁ highlights the additional value of these measures when assessing children with asthmatic symptoms. Therefore, they might be used as additional measures in the follow-up of children who present with small airway abnormalities. Additional studies are needed to clarify whether children who present with abnormalities in the proposed small airway indexes benefit from treatment with novel therapies that target the small

airways and which of the available measures are optimal for identifying and following up these patients. Of the compared methods, IOS provided the best discriminatory properties between healthy and symptomatic children, whereas CALV had the strongest associations with asthma exacerbations and EIB. The lack of associations between most of the proposed small airway indexes suggests that their physiologic determinants may be different.

Acknowledgments

We express our gratitude for the skillful work of Research Nurse Anssi Koivuselkä in performing the lung function measurements and Professor Seppo Sarna for his statistical consultation.

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