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Assessing direct and indirect airway hyperresponsiveness in children using impulse oscillometry

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ABSTRACT

Background: Airway hyperresponsiveness (AHR) is a hallmark of asthma but its assessment is usually restricted to older children who are capable of performing the maneuvers involved in spirometry. In younger children, a feasible option to perform the lung function measurement is impulse oscillometry (IOS), which requires less cooperation.

Objective: To evaluate whether assessment of AHR by IOS could differentiate children with various obstructive symptoms from one another.

Methods: One hundred twenty-one children (median age 6.0 years, range 3.7–8.1 years) were examined: 31 with probable asthma characterized by current troublesome lung symptoms, 61 with a history of early wheezing disorder (recurrent wheezing ≤24 months of age), 15 with a history of bronchopulmonary dysplasia, and 14 healthy controls. Indirect AHR was assessed by exercise and mannitol challenge tests, and direct AHR was assessed with methacholine using IOS. AHR to exercise was defined as an increase of at least 40% in respiratory resistance at 5 Hz. In the mannitol and methacholine challenges, the dose causing an increase of 40% in respiratory resistance at 5 Hz was calculated.

Results: AHR to exercise was good at differentiating children with current troublesome lung symptoms from those in the other groups ($P < .001$). AHR to methacholine separated children with current troublesome lung symptoms, early wheezing disorder, and bronchopulmonary dysplasia from the controls ($P < .001$), whereas the mannitol test did not distinguish among the study groups ($P = .209$).

Conclusion: The methacholine and exercise challenge tests with IOS identify children with probable asthma characterized by troublesome lung symptoms and therefore may represent a practical aid in the evaluation of AHR in young children.

Introduction

Wheezing disorders in early childhood are common, but the underlying conditions are heterogeneous. A classification according to the temporal pattern of wheeze into episodic or multiple-trigger wheeze has been proposed.[1] The terms *transient, persistent,* and *late-onset* wheeze are used retrospectively to distinguish wheezing phenotypes subdivided by duration of symptoms.[1] Most young children cease to wheeze before school age,[2] but those who continue wheezing seem to have lung symptoms and diminished lung function later in life.[2–4] Asthma diagnosis in young children is still based on clinical symptoms. Quantitative assessment of troublesome lung symptoms has been suggested to be a good predictor of asthma in young children, and relying on the symptom of wheeze may lead to undertreatment.[5] Lung function deterioration in childhood also may be caused by bronchopulmonary dysplasia (BPD), which is a disease of prematurely born infants but may cause obstructive lung symptoms after infancy.[6]

Airway hyperresponsiveness (AHR) related to asthma may begin in infancy and can be assessed by direct and indirect bronchial challenges.[7–9] Methacholine acts directly on smooth muscle muscarinic receptors,[10] and challenge with methacholine is generally considered sensitive but not specific for asthma.[11] AHR to methacholine in childhood has been postulated to predict permanent asthma,[12] and asymptomatic AHR may be the first sign of a process leading to asthma.[13]

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Indirect methods, such as exercise and mannitol challenge, induce airway limitation by releasing mediators and neurotransmitters from inflammatory, epithelial, and neural cells in the bronchus.[9] Exercise-induced bronchoconstriction (EIB) can be assessed with impulse oscillometry (IOS) in children and adults.[14–16] The correlation between the direct and indirect methods is only modest.[9] The mannitol challenge is currently commercially available[17,18] and has been evaluated in children,[19,20] but as far as the authors are aware, there are no published data on the performance of the mannitol provocation test as assessed with IOS in preschool children.

The differentiation of preschool wheezing phenotypes by current clinical characteristics is difficult and objective methods are needed. Avital et al[7] and Godfrey et al[21] showed in school-age children using spirometry that exercise but not methacholine challenge distinguished between children with asthma and those with other chronic lung disorders. Most young children, however, cannot perform these maneuvers but can satisfactorily perform IOS, which requires less cooperation.[15,22] The aim of this study was to investigate whether AHR to different agonists could be tested using IOS and whether these tests could distinguish various patient groups of young children.[15,22]

Methods

Patients

In total, 121 children participated in the study. The age of the study children was 3 to 8 years at the time of the study. Thirty-one of these children were referred to the Skin and Allergy Clinic of Helsinki University Central Hospital because of probable asthma owing to current troublesome lung symptoms (TLS). These children had symptoms such as chronic cough and recurrent wheezing, implying probable asthma and often labeled in many general practitioner–driven communities as physician-diagnosed asthma. According to Finnish guidelines, the preschool children are referred to a pediatrician or a pediatric respiratory physician to confirm the asthma diagnosis by objective lung function assessment. The mean duration of the symptoms was 11 months (range 2–36). Twelve of these children (39%) had wheezing, 6 (19%) had respiratory symptoms during exercise, and 13 (42%) had a troublesome cough. They were eligible to participate in the study if the inclusion and exclusion criteria were met; the inclusion criteria were (1) age 4 to 6 years, (2) asthmatic symptoms (exercise-induced symptoms: wheezing, cough, dyspnea), and (3) a satisfactorily completed free running test; the exclusion criteria were (1) earlier asthma diagnosis, (2) earlier regular asthma medication over 6 months, and (3) inhaled corticosteroids during the previous 2 months.

Fifteen age-matched children who had BPD during the neonatal period were recruited from the Children's Hospital of Helsinki University Central Hospital. BPD was diagnosed according to current international guidelines during the neonatal period.[23] The mean gestational age of children with BPD was 27 weeks (range 25–30), the mean birth weight was 940 g (475–1,350) g, the mean duration of ventilator therapy was 26 days (1–77), the mean duration of oxygen therapy was 98 days (30–240), and the mean number of surfactant boluses was 2 (1–6). If any asthma control medication was in use, it was discontinued for 1 month before the lung function tests.

Sixty-one children were recruited for the follow-up study at the age of 3 to 8 years from a previous study that evaluated the effect of montelukast on respiratory symptoms and lung function in wheezy infants.[24] These children had persistent or recurrent wheeze and/or dyspnea at 6 to 24 months of age with at least 1 episode being diagnosed by a physician (denoted as having early wheeze [EW]). Overall, 83% of these children wheezed and/or had dyspnea during and separate from viral infections (multiple-trigger wheeze) at the time of the previous study. If the patient was using asthma control medication at the time of the present study, the medication was stopped 4 weeks before the lung function measurements were performed.

Fourteen healthy age-matched children were recruited as control subjects. They had participated in a study that evaluated the effect of adenoidectomy on respiratory function.[25] The inclusion criteria were no signs or symptoms of atopic disease or asthma and successfully performed free running test and methacholine challenge.

Lung function tests were performed in a random order within a 2-week period. Exercise, methacholine, and mannitol tests were performed on separate days.

The study was approved by the ethics committee of the Helsinki University Central Hospital (337/13/03/03/2008). Written informed consent was obtained from the parents of the children.

Lung Function Tests

Lung function tests were performed using IOS. Respiratory resistance at 5 Hz (Rrs5) was determined as described previously.[15,16,26]

Exercise Challenge

The exercise challenge was performed as an outdoor free running test.[15] The children were urged to run for 6 to 8 minutes at a suitable exercise level such that their heart rate was kept at approximately 85% to 90% of their estimated maximum heart rate. Every child reached this heart rate level. Lung function was measured by IOS at baseline and repeated at 1, 5, and 10 minutes after the exercise. An increase of 40% in Rrs5 was considered a positive test result.[15] The children received salbutamol at a dose of 0.3 mg, administered by a Babyhaler (Allen & Hansburys, Ltd, London, United Kingdom), and lung function measurements were repeated 15 minutes after the inhalation.

Methacholine Challenge

A dosimetric bronchial provocation test modified to be appropriate for preschool children was applied.[27] After baseline measurements of Rrs5, increasing doses of methacholine chloride were administered using an automatic, inhalation-synchronized dosimeter (Spira Electro 2, Spira Respiratory Care Centre, Ltd, Hämeenlinna, Finland) connected to a calibrated nebulizer (Salter Labs 8900, Arvin, California). By calculating the number of breaths with nebulized methacholine, a rapid dosage scheme with 5 cumulative dose steps was applied, with Rrs5 being remeasured 90 seconds after each dose inhalation. The procedure was continued until a 40% increase in Rrs5 was observed or the maximum dose of methacholine had been administered. The provocative dose of methacholine causing a 40% decrease in Rrs5 (PD_{40}Rrs5) was determined from the dose–response curves.[28–30] A PD_{40}Rrs5 lower than 400 μg was considered a positive test result. Based on data by Schulze et al,[31] this cutoff would correspond approximately to the provocative dose of methacholine causing a 20% decrease in forced expiratory volume in 1 second lower than 1 mg,[31] which in turn is indicative of significantly increased AHR[32] and predictive of active asthma during follow-up.[13]

Mannitol Challenge

The mannitol challenge was performed with dry powdered mannitol (Aridol, Pharmaxis, Ltd, Frenchs Forest, New South Wales, Australia). Mannitol is supplied in a kit form and contains 1 empty capsule, 2 5-mg capsules, 2 10-mg capsules, 2 20-mg capsules, and

Table 1
Characteristics of children in the study

	All	TLS	EW	BPD	Controls
Subjects, n	121	31	61	15	14
Boys, n (%)	74 (61)	18 (58)	50 (82)	3 (20)	3 (21)
Girls, n (%)	47 (39)	13 (42)	11 (18)	12 (80)	11 (79)
Age (y), median (range)	6.0 (3.7–8.1)	5.3 (3.7–6.8)	6.0 (5.6–8.1)	6.2 (5.3–7.4)	5.2 (4.7–7.2)
Height (cm), median (range)	116 (97–153)	112 (97–128)	118 (103–153)	115 (103–127)	114 (105–129)
Atopy, n (%)	46 (38)	25 (81)	19 (31)	1 (7)	1 (7)
Current medication, n (%)	23 (19)	0	21 (34)	2 (13)	0
Seasonal medication, n (%)	10 (8)	0	10 (16)	0	0
Parental asthma, n (%)	47 (39)	14 (45)	28 (46)	5 (33)	2 (14)

Abbreviations: BPD, bronchopulmonary dysplasia; EW, early wheezing; TLS, current troublesome lung symptoms.

18 40-mg capsules. Patients inhaled the encapsuled mannitol through the Osmohaler inhaler (Pharmaxis, Ltd) in cumulative doses followed by holding the breath for 5 seconds. Before the challenge test, the inhalation technique was checked and peak inspiratory flow through the inhaler was measured. Lung function was assessed by IOS with 3 consecutive measurements at baseline and 60 seconds after each mannitol dose (placebo, 5, 10, 20, 40, 80, 160, 160, 160 mg). The challenge was discontinued if the resistance (Rrs5) increased by at least 40% from the baseline measurement or the cumulative dose of 635 mg was administered.[19,28,29] The provocative dose of mannitol causing a 40% increase in Rrs5 was calculated by linear interpolation. A positive challenge was defined as a PD_{40}Rrs5 no higher than 635 mg based on established interpretation in adults.[17]

Fraction of Exhaled Nitric Oxide Measurements

Measurements of fraction of exhaled nitric oxide (FeNO) were performed with the stationary chemiluminescence-based analyzer NIOX (Aerocrine AB, Solna, Sweden) according to American Thoracic Society recommendations.[33] The analyzer was calibrated according to the manufacturer's specifications. Children were seated, without a nose clip, and were asked to fill their lungs completely with NO-free air and then to exhale, with a mean and instantaneous flow of 50 ± 5 mL/s, for at least 6 seconds. Consecutive, acceptable paired measurements of FeNO were recorded, and the repeatability and mean results were calculated. The device contains a dynamic flow control that maintains a constant rate during exhalation.

Atopy

Atopy was screened by skin prick tests for the aeroallergens birch, timothy grass, meadow fescue, mugwort, *Cladosporium herbarum,* cat, dog, horse, cow, and house dust mite and for egg, milk, fish, wheat, shrimp, and peanut.

Skin prick test positivity was defined as a wheal with a diameter of at least 3 mm against at least 1 of the tested allergens.

Statistical Methods

The normal distribution was tested by the Shapiro-Wilk test. Owing to non-normally distributed data, nonparametric tests were used. Kruskal-Wallis and Mann-Whitney tests were used to compare continuous data among groups. Bivariate correlations were made by the Spearman correlation test. Receiver operating characteristic curves were applied to assess whether methacholine and mannitol tests could identify EIB. Children with BDP were excluded from the receiver operating characteristic analysis. Binary logistic regression analysis was performed to identify possible explanatory factors. Exercise, methacholine, and mannitol challenges were included in the model as dependent variables, and atopy, parental smoking, and FeNO level were included as covariates. Data were analyzed using SPSS 19.0 (SPSS, Inc, Chicago, Illinois).

Results

The baseline characteristics of the study children are presented in Table 1. All 121 study children performed the exercise and methacholine tests. The mannitol challenge was performed by 97 children and FeNO level was assessed in 114 children. The comparison of all AHR tests and FeNO *z* scores is presented in Table 2.

Baseline Lung Function

The baseline Rrs5 *z* score was significantly higher in children with BPD compared with other children (Table 2). Differences among the other groups were not statistically significant.

Exercise Test

The exercise test was completed by all children who participated in the study, and 27 tests (22%) showed positive results. Nineteen children in the TLS group (61%), 6 in the EW group (10%), 2 in the BPD group (13%), and 0 in the control group showed a positive exercise test result (Table 2). The median exercise-induced increase in Rrs5 was higher in children with TLS (62%)

Table 2
Comparisons of baseline Rrs5, airway hyperresponsiveness tests, and FeNO

	TLS (n = 31)	EW (n = 61)	BPD (n = 15)	Controls (n = 14)	*P* value
Baseline Rrs5 (*z* score), median (range)	0.04 (−1.8 to 2.8)	0.33 (−1.8 to 3.5)	1.5 (−1.4 to 6.2)	0.02 (−1.6 to 2.4)	.023
Exercise test result positive, n (%)	19 (61)	6 (10)	2 (13)	0	
Exercise (% increase in Rrs5), median (range)	62 (−13 to 159)	19 (−16 to 111)	23 (−12 to 53)	9 (6–20)	<.001
Methacholine test result positive, n (%)	24 (77)	47 (77)	10 (67)	3 (21)	
Methacholine PD_{40}Rrs5 (mg), median (range)	0.09 (0.02–2.01)	0.19 (0.02–2.06)	0.21 (0.02–2.11)	0.59 (0.02–2.06)	.001
Mannitol test result positive, n (%)	3 (10)	4 (7)	3 (20)		
Mannitol PD_{40}Rrs5 (mg), median (range)	635 (30–635)	635 (104–635)	635 (133–635)		.209
FeNO (*z* score), median (range)	2.5 (−0.5 to 5.3)	1 (−1.2 to 3.8)	0.6 (−0.9 to 2.1)	0.6 (−1.2 to 2.3)	<.001

Abbreviations: BPD, bronchopulmonary dysplasia; EW, early wheezing; FeNO, fraction of exhaled nitric oxide; PD_{40}Rrs5, provocative dose of methacholine causing 40% increase in respiratory resistance at 5 Hz; Rrs5, respiratory resistance at 5 Hz; TLS, current troublesome lung symptoms.

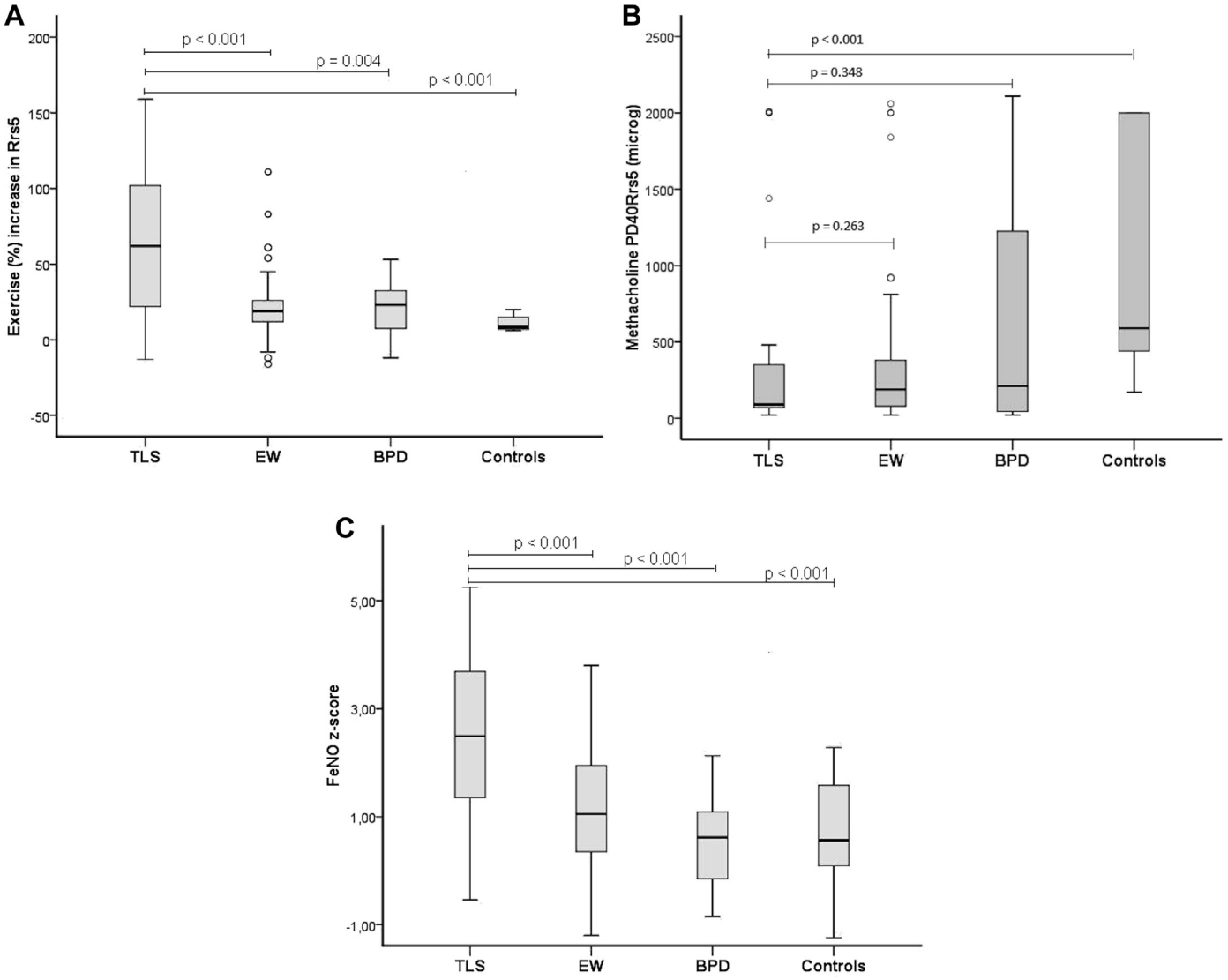

Figure 1. *(A)* Exercise-induced increase in Rrs5 in different patient groups. *(B)* PD_{40}Rrs5 for methacholine challenge in different patient groups. *(C)* FeNO *z* score in children with TLS, EW, and BPD and controls. BPD, bronchopulmonary dysplasia; EW, early wheezing; FeNO, fraction of exhaled nitric oxide; PD_{40}Rrs5, provocative dose of methacholine causing 40% increase in respiratory resistance at 5 Hz; Rrs5, respiratory resistance at 5 Hz; TLS, current troublesome lung symptoms.

compared with the other groups (23% for BPD, 19% for EW, and 9% for controls, $P < .001$; Fig 1A).

Methacholine Challenge

The methacholine test was performed by all study patients, and 84 test results (69%) were positive. The methacholine test result was positive in 24 children (77%) in the TLS group, 47 (77%) in the EW group, 10 (67%) in the BPD group, and 3 (21%) in the control group (Table 2). Methacholine distinguished children with TLS from the controls ($P < .001$), but not from those with EW ($P = .263$) or BPD ($P = .348$; Fig 1B). Pairwise comparisons of the other groups showed the following results: the *P* value was equal to .051 for the BPD vs control group and less than .001 for the EW vs control group. Median PD_{40}Rrs5 was 90 μg for the TLS group, 210 μg for the BPD group, 190 μg for the EW group, and 590 μg for the control group. Children with positive exercise test results (n = 27) showed greater reaction to methacholine than did children with negative exercise test results (n = 94, $P = .004$; Fig 2). The receiver operating characteristic curve for the methacholine challenge to identify children with positive exercise test results is shown in Figure 3. The area under the curve was 0.70 (confidence interval [CI] 0.58–0.82, $P = .02$), which indicates that the agreement in responsiveness to the methacholine and positive exercise tests is fair.

Mannitol Challenge

The mannitol challenge test was performed by 97 children. The mannitol test result was positive in 3 children (10%) in the TLS group, 4 (7%) in the EW group, and 3 (20%) in the BPD group (Table 2). The median peak inspiratory flow through the inhaler before the challenge was 1.2 L/s (range 0.5–3.0), ie, within the specified range optimized for the inhaler. In 9 cases, the challenge could not be completed because of poor cooperation. The median duration of the mannitol challenge was 46 minutes (range 25–66). Ten of the 88 (11%) mannitol challenge results were positive. Cough was experienced by 65 children (73%) during the mannitol challenge.

The PD_{40}Rrs5 with mannitol did not differ among study groups ($P = .209$). The receiver operating characteristic curve for mannitol to identify positive exercise test results is shown in Figure 3. The area under the curve for mannitol was

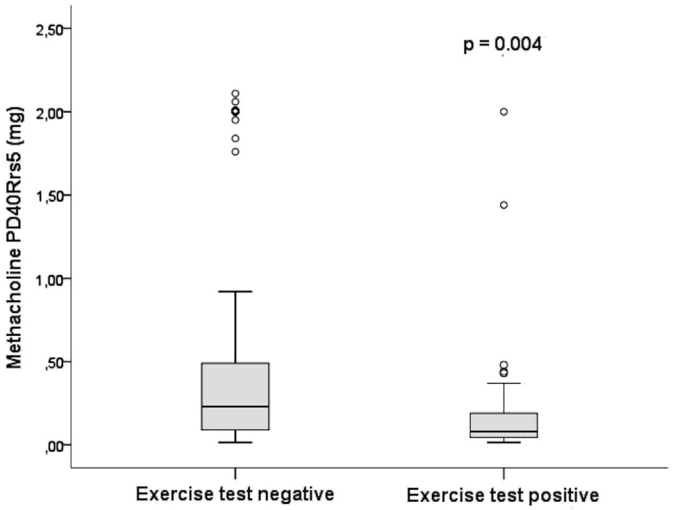

Figure 2. Children with a positive exercise test result were more reactive to methacholine compared with children with a negative exercise test result. $PD_{40}Rrs5$, provocative dose of methacholine causing 40% increase in respiratory resistance at 5 Hz.

0.58 (CI 0.43–0.73, $P = .32$), indicating that the mannitol challenge was unable to identify children with positive exercise test results in this sample.

Association with FeNO and Atopy

Measurement of FeNO was successfully performed in 114 patients. Children in the TLS group had a higher median FeNO *z* score (2.5 SD) than those in the other groups (EW 1.1, BPD 0.6, controls 0.6, $P < .001$; Fig 1C). The FeNO *z* score and exercise-induced increase in Rrs5 (percentage) correlated significantly ($P < .001$, $r = 0.43$). Results of the methacholine or mannitol test

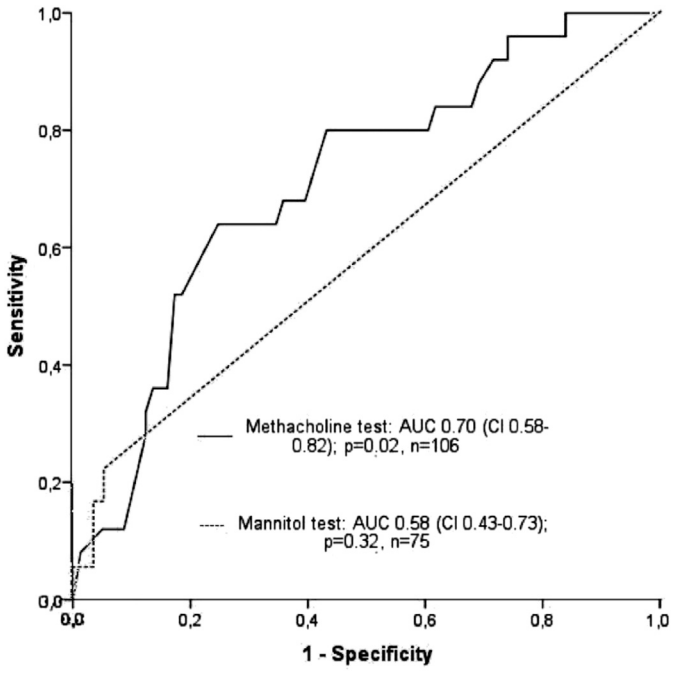

Figure 3. Receiver operating characteristic curves for methacholine and mannitol tests to indicate exercise-induced bronchoconstriction. AUC, area under the curve; CI, confidence interval.

and FeNO level did not correlate significantly ($P = .089$ for methacholine, $P = .06$ for mannitol).

Atopy was found in 46 children (38%). The increase in Rrs5 during the exercise test was greater in atopic children (median 43%) than in nonatopic children (14%, $P < .001$). Atopic children in the TLS group (n = 25) had a greater median Rrs5 increase (69%) after the exercise test than did nonatopic children in the TLS group (n = 6, 17%, $P = .006$). Atopic children were more sensitive to methacholine than were nonatopic children (median 130 vs 250 μg, $P = .02$), but results of the mannitol test did not differ between atopic and nonatopic children ($P = .148$).

Multivariate Analysis

In multivariate logistic regression, the positive skin prick test result (odds ratio 10.2, CI 2.9–36.4, $P < .001$) and higher FeNO level (odds ratio 1.1, CI 1.0–1.2, $P = 0.012$) were associated with a positive exercise test result. Such significant associations were not found with the methacholine or mannitol test.

Discussion

In this study, indirect and direct challenges were compared to measure AHR by using IOS in children with obstructive airway symptoms. IOS was found to be a feasible method for lung function measurement when assessing AHR in young children. The exercise test was able to separate the children with probable asthma having TLS from the children with other conditions. The methacholine challenge distinguished children with TLS from the controls, but the mannitol challenge could not differentiate the patient groups from one another.

The established guidelines for AHR assessment are available for adults and school-age children but not for young children.[34] The lack of convenient lung function measurement techniques in this age group has been one of the difficulties encountered in the assessment. Klug and Bisgaard[35] found good repeatability with IOS in the methacholine provocation test in 2- to 4-year-old children. Oscillometry and spirometry have shown similar sensitivities to detect bronchoconstriction in children.[31,36] The success rate for IOS performance in young children is high,[26,37] and with exercise testing IOS is a feasible method to assess EIB.[15] In the present study, all young children were able to perform acceptable IOS measurements.

The interpretation of AHR tests in young children is ambiguous.[8] Some healthy children also may exhibit reactivity during broncho-provocative tests,[38,39] whereas pharmacologic challenges may fail to identify children with EIB.[40] Avital et al[7] found that all pediatric patients with asthma were responsive to adenosine, methacholine, and exercise challenges. In school-age children, exercise testing could distinguish children with asthma from those with other chronic lung function disorders, whereas the methacholine challenge did not.[7,21] These results are consistent with the present results. Wilson et al[41] were not able to distinguish previous from current wheezers or the degree of clinical severity in 4- to 6-year-old patients with methacholine and hypertonic saline challenges using IOS. In the present study, 69% of children were responsive to methacholine compared with 22% positivity during the exercise test. The methacholine test could differentiate the TLS, EW, and BPD groups from the control group but not the TLS, EW, and BPD groups from one another. In line with previous results, there was a significant correlation between EIB and FeNO, indicating the involvement of eosinophilic inflammation.[9,42] Methacholine acts directly on muscarinic receptors on airway smooth muscle and is less dependent on inflammation.[11] The responsiveness to challenge agonists in children also may depend on age-related factors and an inherent sensitivity for an agent rather than the presence of bronchial inflammation.[38] In the present study, the median

PD_{40}Rrs5 value for methacholine was lower than 400 μg in the TLS, EW, and BPD groups. This cutoff value was chosen based on previous data of children with asthma who underwent challenge testing with methacholine, and changes in IOS and spirometry were compared.[31] In children, the cutoff in PD_{40}Rrs5 lower than 400 μg seems to correspond approximately to the provocative dose of methacholine causing a 20% decrease in forced expiratory volume in 1 second lower than 1 mg, which in turn indicates significantly increased AHR[32] and has been shown to be predictive of active asthma during follow-up.[13] However, the data of clinically useful cutoff values for challenge tests with pharmacologic agents in young children are still limited and may need to be re-evaluated in future studies.

The mannitol test has shown a high diagnostic specificity for asthma,[43] consistent results with other broncho-provocative tests,[17,19,38] and a good ability to identify EIB[20] in school-age children and adults. The forced oscillation technique has provided good sensitivity and repeatability to measure AHR with a mannitol challenge in adults.[28] As far as the authors are aware, there are no previous studies using a mannitol challenge with IOS in preschool children. In this study, the mannitol test could not differentiate the patient groups. Nine of 97 mannitol challenges could not be completed because of difficulties in inhaling the test agonist. For a child still not at school, inhaling a total of 19 capsules separately may be too many. Cough was experienced by 73% of children during the mannitol challenge, and the children considered this rather discomforting. To ensure the correct inhalation technique, the peak inspiratory flow through the inhaler before the challenge was measured and all children surpassed the test. The repeated inhalations may impair inspiratory effort in young children, and all the powder may not have reached the lower airways.

In the present study, children with TLS had current obstructive lung symptoms with probable asthma (physician-diagnosed asthma). The exercise challenge could distinguish these children from the others, which agrees with the concept of indirect challenge tests being highly specific for asthma.[9] The atopy prevalence was high in the TLS group, and the FeNO level was higher in this group than in other study groups, indicating active inflammation of the bronchial mucosa. Conversely, the methacholine challenge was not able to differentiate children with TLS from children with other lung disorders, which is in agreement with the findings from older children.[7] In the EW group, the children started to display symptoms during (viral wheezers) and separate from (multiple-trigger wheezers) viral episodes and received asthma control medication in early childhood (6–24 months of age). The multiple-trigger wheezers had pronounced AHR at infancy.[44] At the time of the present study, however, 66% of these children were not using daily asthma control medication. They were less responsive to the AHR challenges than children with TLS. It is well known that recovery from early lung symptoms commonly occurs and a patient can move from one phenotype to another during the preschool years.[1,2] The present findings support this concept.

Bronchopulmonary dysplasia is associated with decreased lung function and AHR later in childhood and early adulthood,[6,45] but little is known about BPD and AHR at preschool age. In line with previous results, the baseline Rrs5 was higher in children with BPD than in the other children.[6,45,46] In the present study, no significant differences in EIB were detected between the BPD and control groups. This is likely due to a lack of eosinophilic bronchial inflammation, which is considered an important origin of EIB in allergic asthma. Instead, children with BPD were more reactive to methacholine than the control children, although the difference did not reach statistical significance as in a previous study.[6,45,47] The BPD group was small, and improvements in neonatal intensive care in recent years may have influenced the AHR values in these children.

The present study demonstrates that methacholine and exercise challenges with IOS can be used to identify AHR in young children. Exercise testing distinguished well between children with probable asthma (TLS) and children with other conditions, and the methacholine test differentiated children with TLS, EW, and BPD from the controls. The mannitol challenge did not discern among the study groups, and thus the results do not favor the use of mannitol challenge in the assessment of AHR in young children. Further studies are needed to understand the implication of AHR in young children and to assess reliable cutoff limits for pharmacologic agents in this age group.

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