

Concordance Between Bronchial Hyperresponsiveness, Fractional Exhaled Nitric Oxide, and Asthma Control in Children

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Summary. Background: Previous studies on association between level of asthma control, markers of airway inflammation and the degree of bronchial hyperresponsiveness (BHR) have yielded conflicting results. Our aim was to determine the presence and severity of BHR and the concordance between BHR, asthma control, and fractional exhaled nitric oxide (FeNO) in children with asthma on therapy. Methods: In this cross-sectional observational study, children (aged 6–18 years) with asthma on British Thoracic Society (BTS) treatment steps 2 or 3, underwent comprehensive assessment of their asthma control (clinical assessment, spirometry, asthma control test [ACT], Pediatric Asthma Quality of Life Questionnaire [PAQLQ]), measurement of FeNO and BHR (using mannitol dry powder bronchial challenge test [MCT], Aridol™, Pharmaxis, Australia). Results: Fifty-seven children (63% male) were studied. Twenty-seven children were on BTS treatment step 2 and 30 were on step 3. Overall, 25 out of 57 (43.8%) children had positive MCT. Of note, 9 out of 27 (33.3%) children with clinically controlled asthma had positive MCT. Analyses of pair-wise agreement between MCT (positive or negative), FeNO (>25 or ≤25 ppb) and clinical assessment of asthma control (controlled or partially controlled/uncontrolled) showed poor agreement between these measures. Conclusions: A substantial proportion of children with asthma have persistent BHR despite good clinical control. The concordance between clinical assessment of asthma control, BHR and FeNO was observed to be poor. Our findings raise concerns in the context of emerging evidence for the role of bronchoconstriction in inducing epithelial stress that may drive airway remodeling in asthma. **Pediatr Pulmonol.**

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INTRODUCTION

A substantial proportion of children continue to have suboptimal control of their asthma while on therapy.¹ The step-wise approach to asthma management recommended by the current guidelines^{2–4} rely on clinical assessment of asthma control based on symptoms. There is uncertainty regarding the optimal criteria for defining asthma control and monitoring response to therapy. Objective assessment of airway inflammation or bronchial hyperresponsiveness (BHR), the two key features of asthma pathophysiology, is currently not part of routine therapy decision-making process. Emerging evidence suggests the plausible role of bronchoconstriction and the resultant mechanical forces in the development of airway remodeling,^{5–7} even in the absence of airway inflammation. Persistent BHR may be contributory to longitudinal accelerated decline in lung function, despite asthma being clinically well controlled on therapy. Hence, it may be argued that along with

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Conflicts of interest: None.

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controlling inflammation, prevention of bronchoconstriction and normalizing BHR should be an important aim of asthma management. Given the diverse asthma endophenotypes,^{8,9} differential clinical response to different add-on medications,^{10,11} and the discordance between the clinical expression of asthma and the two key pathophysiologic features of asthma (airway inflammation and BHR),^{12,13} objective and comprehensive assessment of asthma control may be helpful in guiding individualized treatment decisions at selected stages in the step-wise asthma treatment algorithm.

Studies on adult asthma patients suggest that airway inflammation and bronchial hyperresponsiveness persist in most patients despite good clinical control of asthma,^{13,14} as assessed using current guidelines.^{2,4} Previous studies on association between level of asthma control, markers of airway inflammation such as fractional exhaled nitric oxide (FeNO), and the degree of BHR have yielded conflicting results. Data in children are limited. In this cross-sectional observational study, we aimed to determine the presence and severity of BHR; and the concordance between asthma control, BHR and FeNO, in children with asthma on therapy.

MATERIALS AND METHODS

In this cross-sectional study, from January 2013 to September 2013, we recruited children (aged 6–18 years) who were being followed up at KK Women's and Children's Hospital Singapore, under the Singapore National Asthma Program. Diagnosis of asthma was based on criteria recommended by the GINA² and the US National Asthma Education and Prevention Program.⁴ Inclusion criteria were (i) physician diagnosed asthma; (ii) previous demonstration of an increase of in FEV₁ of $\geq 12\%$ following inhalation of bronchodilator (salbutamol 400 μg); (iii) treatment with inhaled corticosteroids (ICS) for more than 1 year; (iv) current treatment with British Thoracic Society (BTS) treatment step 2 (medium dose inhaled corticosteroid [ICS] monotherapy [400 μg Beclomethasone Dipropionate [BDP] equivalent]) or BTS treatment step 3 (on high dose ICS [>400 to ≤ 800 μg Beclomethasone Dipropionate (BDP) equivalent] or combination therapy [ICS 400 μg BDP equivalent] in addition to a non-ICS controller therapy LABA [Long Acting beta-agonist] or LTRA [Leukotriene-receptor antagonist])³; (v) ability to perform reproducible spirometry according to the American Thoracic Society (ATS)/European Respiratory Society (ERS) criteria¹⁵; (vi) non-smoker status; and (vii) willingness of parent/legal guardian to provide informed consent. Children with chronic or active lung disease other than asthma and those with a history of asthma exacerbation requiring systemic corticosteroids within a 4 week period prior to recruitment were excluded. The study was approved

by the SingHealth Institutional review board. Parent/legal guardian provided informed consent and children >6 years of age provided written assent.

At recruitment, the following details were recorded: age, gender, race, weight (kg), height (cm), BMI (kg/m^2), age at asthma diagnosis, personal history of other atopic disorders and current medications. A physical examination was performed. Inhaler technique was reviewed and advice on inhaler technique was given as appropriate. FeNO was measured before performing spirometry, using NioxMino (Aerocrine AB, Sweden) as per the manufacturer's instructions and in accordance with the ATS/ERS guidelines.¹⁶ Spirometry was done using the Vitalograph Spirotrac 6800 (Vitalograph Inc., Lenexa, KS) with Spirotrac Version 4.31 software in accordance with published ATS/ERS guidelines¹⁵ and response to bronchodilator (Salbutamol 400 μg) was assessed. Skin prick testing to common aeroallergens was performed, if this has not been done previously. A Written Asthma Action Plan (WAAP) was provided to all subjects. All subjects were asked to record their asthma symptoms on a daily basis in a symptom diary. All subjects were provided with a metered-dose inhaler (MDI) of salbutamol (Ventolin HFA, Glaxo Wellcome S.A., Aranda, Spain) for use as specified by the WAAP.

Subjects were reviewed 8 weeks after recruitment. History pertaining to asthma control was obtained and the symptom diary reviewed. A physical examination was performed. Asthma control was assessed as controlled, partially controlled, or uncontrolled based on the following criteria²: (i) partially controlled: if any of the following measure is present (over the preceding 4 week period)—daytime symptoms (cough/wheezing/shortness of breath) $>$ twice/week, any nocturnal awakening due to asthma, need for reliever/rescue treatment $>$ twice/week (excluding reliever taken before exercise) and any limitation of physical activities due to asthma symptoms; (ii) uncontrolled: if there are ≥ 3 features of partially controlled asthma are present; and (iii) controlled: if none of the features of partially controlled asthma are present. An asthma-control day (ACD) was defined as a day during which there was no use of salbutamol for asthma symptoms (excluding the use of salbutamol as pre-exercise treatment), no daytime or night-time asthma symptoms, no limitation of physical activities due to asthma symptoms, and no unscheduled visit to a health care provider for asthma. The proportion of asthma control days was computed based on the number of asthma control days during the preceding 8 weeks. Adherence to medications was assessed by a combination of self reporting, pharmacy records review, inhaler check, and tablet counting, as appropriate. FeNO was measured and the pre-bronchodilator spirometry was done as before. All subjects completed an age appropriate Asthma Control Test (Asthma Control Test [ACT] for children

≥ 12 years¹⁷ and Childhood ACT for children ≤ 11 years¹⁸). The validated Pediatric Asthma Quality of Life Questionnaire (PAQLQ)¹⁹ was administered to all subjects. Subjects underwent the mannitol dry powder bronchial challenge test (MCT) (Aridol™, Pharmaxis, Frenchs Forest, New South Wales, Australia), according to the manufacturer's instructions.²⁰ All subjects withheld medications as per the manufacturer's test instructions.²⁰ In addition, subjects withheld caffeine containing foods and avoided vigorous exercise on the day of the test. The MCT result was expressed as PD₁₅, which was defined as the cumulative provoking dose of mannitol that causes a $\geq 15\%$ fall in FEV₁ from baseline. The test was considered negative if even after inhalation of 635 mg mannitol dry powder, the FEV₁ did not fall by $\geq 15\%$ from baseline. For positive tests, the severity of BHR was assessed as mild (PD₁₅ ≥ 155 to ≤ 635 mg), moderate (PD₁₅ > 35 to < 155 mg), or severe (PD₁₅ ≤ 35 mg).²¹

Statistical Analysis

Statistical analyses were performed using SAS version 9.3 (SAS Institute Inc., Cary, NC). Descriptive statistics were obtained on continuous baseline measures and frequency tables were generated for categorical variables. Unweighted κ statistics with associated 95% confidence interval and *P*-value were used to assess the pair-wise agreement between MCT (positive or negative), FeNO (> 25 or ≤ 25 ppb) and asthma control (controlled or partially controlled/uncontrolled). Sensitivity, specificity, and area under the receiver operating characteristic (ROC) curve were obtained using ROC analysis.

RESULTS

Out of 58 MCT tests done, one child on BTS step treatment 2 could not complete the test because of severe cough and post-tussive vomiting. Demographic and clinical characteristics of the children who completed the study are summarized in Table 1. Baseline FeNO and spirometry measurements are given in Table 2.

Twenty-seven children were on British Thoracic Society (BTS) treatment step 2 (Fluticasone 100 μ g twice a day; Flixotide 100 μ g Accuhaler [GlaxoSmithKline] one blister twice a day) and 30 children were on BTS treatment step 3 (10 children on high dose ICS: Fluticasone 200 μ g twice a day [Flixotide 100 μ g Accuhaler (GlaxoSmithKline) two blisters twice a day]), 10 children on ICS + LABA (Fluticasone 100 μ g + Salmeterol 50 μ g twice a day; Seretide 50/100 Accuhaler [GlaxoSmithKline] one blister twice a day), and 10 on ICS + LTRA (Fluticasone 100 μ g twice a day [Flixotide 100 μ g Accuhaler (GlaxoSmithKline) one blister twice a day] + Montelukast [Singulair, Merck Sharp & Dohme, Kenilworth, NJ, USA] 5 mg [for children < 15 years] or 10 mg [for children > 15 years] once a day). The proportion

TABLE 1—Demographic and Clinical Details

	BTS treatment step 2 (n = 27)	BTS treatment step 3 (n = 30)
Age (year)*	10 (8–13)	10.5 (8–13)
Gender†		
Male	17 (63.0)	19 (63.3)
Female	10 (37.0)	11 (36.7)
Race†		
Chinese	9 (33.3)	10 (33.3)
Malay	11 (40.8)	12 (40.0)
Indian	4 (14.8)	5 (16.7)
Eurasian	3 (11.1)	3 (10.0)
BMI*	19.7 (15.2–22.3)	19.8 (15.2–22.4)
Age (year) at diagnosis*	4 (2–7)	4.5 (2–7)
Eczema†		
Yes	15 (55.6)	16 (53.3)
No	12 (44.4)	14 (46.7)
Allergic Rhinitis†		
Yes	27 (100)	30 (100)
SPT to HDM†		
Positive	25 (92.6)	28 (93.3)
Negative	2 (7.4)	2 (6.7)

BTS, British Thoracic Society; BMI, body mass index; SPT, skin prick test; HDM, house dust mite. Data expressed as *Median (IQR) and †n (%).

of days with adherence to prescribed study medications during the 8 weeks preceding assessment of BHR, FeNO, and asthma control was $\geq 90\%$ for all study subjects.

Results of measures of asthma control and MCT are given in Table 3. The PD₁₅ values of the positive MCT tests are given in Table 4. The Overall, 25 out of 57 (43.8%) children had positive MCT, suggesting persistent BHR. Of note, 9 out of 27 (33.3%) children with clinically controlled asthma (as assessed by GINA criteria),² had positive MCT; suggesting persistent BHR despite apparently good asthma control as assessed by subjective and objective measures used in routine clinical practice. Eleven out of 15 (73.3%) children on BTS treatment step 2; and 6 out of 10 (60%) on BTS treatment step 3, with positive MCT had FeNO < 25 ppb. There was no significant difference ($P = 0.08$) in FeNO between children with negative MCT and those with mild, moderate, or severe BHR (Fig. 1). The results of analyses of pair-wise agreement between MCT (positive or negative), FeNO (> 25 or ≤ 25 ppb) and clinical assessment of asthma control (controlled or partially controlled/uncontrolled) are given in Table 5.

DISCUSSION

In this study, we assessed asthma control, FeNO, and the presence and severity of BHR using an indirect bronchial challenge test, in children with asthma on therapy. We observed persistent BHR in a large proportion of children with asthma on therapy. Of particular note, one-third of children with apparently

TABLE 2—Baseline FeNO and Spirometry Measurements

	BTS treatment step 2 (n = 27)		BTS treatment step 3 (n = 30)	
	Pre-bronchodilator	Post-bronchodilator	Pre-bronchodilator	Post-bronchodilator
FEV ₁ (%)*	81.3 (69.7–87.4)	89.5 (83–98.1)	80.3 (73.8–86.8)	90.4 (82.9–98.2)
FVC (%)*	93 (84.3–101)	94.9 (88–101.1)	89.5 (82.7–98.7)	96.2 (87.4–101.2)
FEV ₁ /FVC (%)*	86.2 (79.5–90.1)	96.1 (90.2–99.0)	86.2 (79.9–90.7)	96.1 (90.2–99.5)
FEF _{25–75} (%)*	54.8 (40.9–64.6)	80.4 (69.1–96.9)	55.8 (44.6–67.1)	80.8 (68.2–97.9)
BDR FEV ₁ (%)*	10 (7.6–11.9)		10.5 (7.8–13.2)	
BDR FEF _{25–75} (%)*	39.2 (24.2–50.4)		33.4 (25.2–39.3)	
FeNO (ppb)*	25.5 (13–63.3)		18.6 (14–34)	

Data expressed as *Median (IQR). BDR, bronchodilator (Salbutamol 400 µg) reversibility.

TABLE 3—Measures of Asthma Control and BHR

	BTS treatment step 2 (n = 27)	BTS treatment step 3 (n = 30)
ACT*	23 (22–25)	23 (22–25)
PAQLQ*	5.73 (4.81–6.6)	6 (5.12–6.92)
ACD (%)*	89.3 (78.6–96.4)	94.6 (89.3–100)
FeNO (ppb)*	15 (10–25)	12.5 (11–25.7)
FEV ₁ (%)*	82.7 (74.6–86.9)	83.7 (78–87.4)
FVC (%)*	93 (87.6–99.1)	91.8 (87.8–98.8)
FEV ₁ /FVC (%)*	87.9 (81.6–92.7)	88.6 (83.8–93.6)
FEF _{25–75} (%)*	60.2 (48.1–68.8)	59 (52.1–75.3)
Asthma control [†] (GINA criteria)		
Controlled	7 (25.9)	20 (66.7)
Partially controlled	19 (70.4)	10 (33.3)
Uncontrolled	1 (3.7)	0 (0)
MCT [†]		
Positive	15 (55.5)	10 (33.3)
Controlled asthma	3	6
Partially controlled asthma	12	4
Negative	12 (44.5)	20 (66.7)
BHR grade (n)		
Mild	11	6
Moderate	4	3
Severe		1

Data expressed as *Median (IQR) and [†]n (%). BHR, bronchial hyperresponsiveness; ACT, asthma control test; PAQLQ, Pediatric Asthma Quality of Life Questionnaire; ACD, proportion of asthma control days in the preceding 8 weeks.

TABLE 4—PD₁₅ Values of Positive MCT

PD ₁₅ (mg)	BTS treatment step 2* (n = 15)	BTS treatment step 3* (n = 10)
35	0 (0)	1 (10)
75	4 (26.7)	3 (30)
155	3 (20)	2 (20)
315	5 (33.3)	4 (40)
475	1 (6.7)	0
635	2 (13.3)	0

*Data expressed as n (%). PD₁₅, cumulative provoking dose of mannitol that causes a ≥15% fall in FEV₁ from baseline; MCT, mannitol challenge test; BTS, British Thoracic Society.

good asthma control had persistent BHR. Analyses of concordance between clinical assessment of asthma control, FeNO, and BHR showed poor agreement between these measures.

Persistent BHR in patients with asthma on therapy has been reported in adult studies. Hanxiang et al. reported persistent BHR (as assessed by methacholine challenge test) in 85% of adult patients (n = 76) with totally controlled asthma.¹³ Muñoz studied 84 adults with well controlled asthma and 78% has persistent BHR characterized by positive methacholine challenge test.¹⁴ Romberg et al. assessed BHR using mannitol challenge test in 74 adults with well controlled asthma and observed positive test results in 81% of subjects.²² Although data in children are limited, persistent BHR has been reported in children with both atopic and non-atopic asthma as well.²³ Our results are consistent with these data. We used the indirect bronchial challenge test using mannitol rather than the more commonly used methacholine challenge test, as a positive response to the former may reflect active airway inflammation more closely.²⁴

Previous studies on association between level of asthma control, markers of airway inflammation such

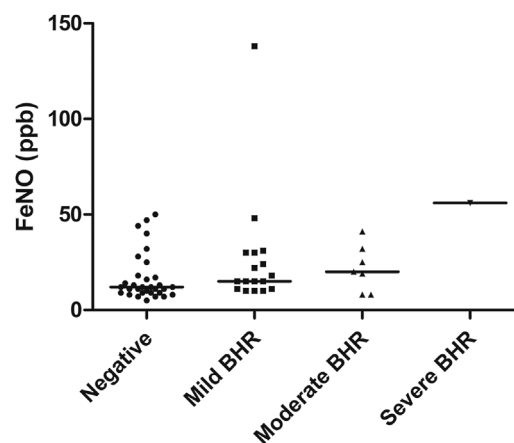


Fig. 1. FeNO values of children with negative MCT and those with mild, moderate, or severe BHR. MCT, mannitol challenge test; BHR, bronchial hyperresponsiveness.

TABLE 5—Agreement Between Asthma Control, FeNO, and MCT

Agreement	κ	95%CI	<i>P</i> -value	Sensitivity	Specificity	AUC
Between MCT and FeNO	0.207	−0.021, 0.435	0.1041	0.320	0.125	0.598
Between MCT and asthma control	−0.201	−0.454, 0.052	0.1825	0.563	0.360	0.601
Between FeNO and asthma control	−0.103	−0.320, 0.113	0.4003	0.440	0.313	0.564

FeNO, fractional exhaled nitric oxide; MCT, mannitol dry powder bronchial challenge test; AUC, area under the curve.

as FeNO and the degree of BHR have yielded conflicting results, both in adults and children. Some adult studies have shown an association between clinical control and markers of airway inflammation,²⁵ as well as between FeNO and BHR.^{26,27} However, these observations could not be replicated by other authors.^{28,29} Results of studies in children have also been inconsistent. Malby Schoos et al. reported a significant linear association between BHR and FeNO in young children, independent of presence and severity of asthma.³⁰ In a study of 59 asthmatic children, Perzanowski et al. observed a significant correlation between FeNO and BHR in the steroid-naïve group, but not in the steroid-treated group.³¹ Motomura et al. studied 267 children with asthma and observed that the association between markers of inflammation and BHR in children may be influenced by the age and growth stage of children.³² However, many previous studies in children with asthma have reported poor concordance between clinical asthma control and FeNO³³; and between FeNO and BHR,^{34,35} and our results are consistent with these observations. In this study, we used a FeNO cut off of <25 ppb to define low FeNO, as approximately half of our study subjects were >12 years of age. It appears that treatment with steroids,³¹ presence of atopy,²³ and longitudinal age and asthma related changes in the airways³² may be factors contributory to the discordance.

The main limitations of our study include the small sample size and the cross-sectional design. Moreover, we did not assess markers of airway inflammation other than FeNO. Despite these limitations, our findings of persistent BHR in the presence of apparently good clinical control, in a substantial proportion of asthmatic children on therapy, raises concerns in the context of emerging evidence that suggests a plausible role of bronchoconstriction and the resultant mechanical forces in the development of airway remodeling in asthma.^{5–7,36,37} Available evidence suggests that persistent BHR is associated with accelerated decline in lung function and reduced maximal level of lung function.^{38–40} Evidence from long-term studies showing the lack of modifying effect of ICS on the natural history and lung function decline in children with asthma, calls for focusing on normalizing BHR in addition to controlling inflammation in order to improve long-term outcomes. Unraveling the complex relationship between persistent BHR, asthma endotypes, and treatment response; and its

implications for long-term outcomes, pose further challenges for future studies.

In summary, our data show that a substantial proportion of children with asthma have persistent BHR despite good clinical control. We have also shown that there is poor concordance between symptom-based assessment of asthma control, FeNO, and BHR, in children with asthma on therapy. Careful clinical review remains the cornerstone of assessment of asthma control in routine practice. However, our findings suggest that asthma control as assessed by a composite outcome measure—one that includes bronchodilator reversibility, markers of airway inflammation, and BHR, in addition to measures of asthma control used in routine clinical practice, may be helpful in guiding individualized treatment decisions at selected stages in the step-wise asthma treatment algorithm. It may be desirable to use such a composite outcome measure in interventional studies as well.

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