Original Article

Methacholine bronchial provocation test for assessment of bronchial hyperresponsiveness in preschool children

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Background: Bronchial hyperresponsiveness (BHR) has not been extensively performed in preschool children, possibly because of the difficulty in cooperating with the tests. We sought to determine the usefulness and safety of methacholine bronchial provocation test (MCh-BPT) for BHR assessment in preschool children.

Methods: We recruited 252 preschool children (190 healthy and 62 with wheezing) who underwent MCh-BPT at baseline. MCh-BPT was re-scheduled in case initial attempts failed. Forced expiratory volumes in 0.5 ($FEV_{0.5}$), 0.75 ($FEV_{0.75}$) and one second (FEV_1) were measured. We recorded the provocative dose causing 15% (PD_{15}) or 20% reduction (PD_{20}) in $FEV_{0.5}$, $FEV_{0.75}$ and FEV_1, thus allowing for comparison of the diagnostic value of PD_{15} and PD_{20}.

Results: A total of 209 children [156 (82.1%) healthy, 53 (85.5%) with wheezing] successfully completed MCh-BPT. Compared with healthy children, a significantly greater proportion of children with wheezing had measurable $PD_{15}FEV_{0.5}$, $PD_{15}FEV_{0.75}$ and $PD_{15}FEV_1$ ($P<0.01$), and $PD_{20}FEV_{0.5}$, $PD_{20}FEV_{0.75}$ and $PD_{20}FEV_1$ ($P<0.05$). The sensitivity was 92.5% and 94.3% for $PD_{20}FEV_1$, and $PD_{15}FEV_1$ and the specificity was 93.6% and 93.6% respectively, for discriminating asthmatic from healthy children.

Conclusions: Most preschool children successfully and safely complete MCh-BPT, with higher success rate in larger age group. $PD_{20}FEV_{0.5}$ and $PD_{20}FEV_{0.75}$ can be surrogates of $PD_{20}FEV_1$ among children whose expiration lasted for less than one second. PD_{15} has a good diagnostic value as PD_{20} for diagnosing of BHR in preschool children, which are also more suitable for children five years old or elder.

Keywords: Preschool children; bronchial hyperresponsiveness (BHR); bronchial provocation test; methacholine; safety

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Introduction

Asthma is a chronic airway inflammation disease resulting from interactions among genetic factors, environmental pollution, and allergen sensitization (1-3). The prevalence of asthma is estimated to be 300 million globally (1) and still increasing (1,2). There has been a rapid increase in the

prevalence of asthma among children in China, possibly because of severe air pollution, and altered environmental exposures and dietary patterns (2-5). The cumulative prevalence of asthma in children under 14 years of age has increased from 1.90% in 1990 (4) and 1.09% in 2000 (5) to 3.02% in 2010 (6). Moreover, approximately 30% of asthmatic children have been underdiagnosed in urban areas of China (5,6). In clinical, the diagnosis of asthma relies heavily on clinical history, however, the measurement of bronchial hyperresponsiveness (BHR), a cardinal pathophysiologic feature (7), with practical approaches such as bronchial provocation test (BPT) (8), is helpful for the diagnosis and differential diagnosis of asthma, especially for those with uncertain history.

Forced expiratory volume in one second (FEV_1) has been the "gold standard" for determining BHR in children aged five years or greater (1). Nevertheless, BPT with spirometry may be challenging among preschool children because of their developmental characteristics (limited ability to comprehend and cooperate), which reduced the reliability of results. Understanding the feasibility of BPT in preschool children (with uncertain history, particularly those with chronic cough and wheezing) may be of practical value (e.g., for differential diagnosis of asthma) (9). Despite the publication of European Respiratory Society/American Thoracic Society recommendations of spirometry (7), no guidelines for BPT specifically targeting at preschool children have been drafted.

We hypothesized that methacholine bronchial provocation test (MCh-BPT) would be safe and feasible in preschool children. To this end, we conducted MCh-BPT in healthy children and preschool children who previously had wheezing, we also evaluated the optimal parameter for determining the positive response for diagnosing BHR.

Methods

Subjects

Children aged 4–6 years were recruited from a kindergarten located in Guangzhou and the pediatric outpatient department of The First Affiliated Hospital of Guangzhou Medical University. All subjects were categorized into healthy children or wheezing children, according to the history of recurrent wheezing. All subjects had normal height and weight (±2 standard deviations) (10), had no history of other severe cardiopulmonary diseases, and had no acute airway infection within 4 weeks. Healthy children had no recurrent wheezing diseases, no prior exposure to noxious gases or dust; had normal chest physical examination findings. Children with wheezing had either a history two or more episodes of wheezing (without upper or lower airway infection), or a clinical diagnosis of asthma (1,11). We excluded subjects with baseline FEV_1 <60% predicted, and those who failed to complete MCh-BPT. Subjects that under allergen immunology were also excluded. Inhaled short-acting and long-acting β2 receptor agonist were withheld for at least 8 and 24 hours respectively, before any bronchial challenge test. Antihistamines and leukotrienes receptor antagonists were withheld for at least 3 days prior to the study.

The study protocol was approved by the ethics committee of The First Affiliated Hospital of Guangzhou Medical University, and parents signed informed consent.

Spirometry

Spirometry was performed with MasterScope spirometer (Carefusion Co. Ltd., Hoechberg, Germany) by experienced technicians according to international guidelines (7). Baseline spirometry was performed for 3–8 times (at 1-min intervals) to obtain at least two technically acceptable curves. Reference values were derived from the equation by Zhang *et al.* (12).

MCh-BPT

MCh-BPT with doubling (for children with wheezing) and quadrupling (for healthy children) provocative concentrations of methacholine was performed with Masterscreen system (DeVilbiss 646 nebulizer, power: 160 μL/min, Carefusion Co. Ltd., Hoechberg, Germany) by using Yan's and Zhong's approach of deep inhalation (13,14). The initial and final dose of MCh (Sigma-Aldrich Co, St Louis, Mo) was 0.39 and 12.80 μmol, respectively (*Table S1*). Repeated inhalation challenges were performed at 1-minute intervals. These procedures were terminated when FEV_1 reduced by 20% or the final dosing was accomplished (*Figure S1*). 200–400 mcg salbutamol was administered via a spacer (Volumatic, Allen & Hanbury's, UK) to relieve discomfort among subjects whose FEV_1 fall exceeded 20%. The cumulative doses of MCh causing $FEV_{0.5}$, $FEV_{0.75}$ and FEV_1 to decrease by 15% ($PD_{15}FEV_{0.5}$, $PD_{15}FEV_{0.75}$, $PD_{15}FEV_1$) and 20% ($PD_{20}FEV_{0.5}$, $PD_{20}FEV_{0.75}$ and $PD_{20}FEV_1$), causing peak expiratory flow (PEF) to decrease by 20% ($PD_{20}PEF$), and causing maximal mid-

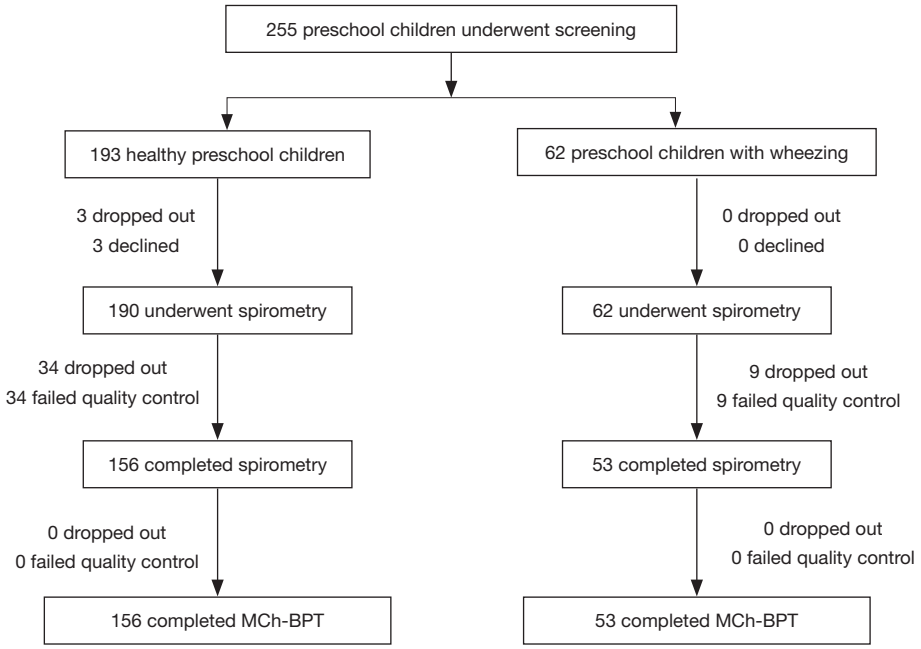

Figure 1 Subject enrollment. Mch-BPT, methacholine bronchial provocation test.

expiratory flow (MMEF) to decrease by 35% (PD_{35}MMEF) were calculated.

For children with wheezing, failure of test was defined as inability to complete spirometry. MCh-BPT would be rescheduled one week thereafter in case the initial MCh-BPT (but not spirometry) was unsuccessful. No further attempts were made in case two consecutive MCh-BPT could not be completed successfully. For healthy children who tested positive to the initial MCh-BPT, they were invited for repeated MCh-BPT at one-month interval. BHR could be confirmed among healthy children who tested positive (FEV_1 fall exceeded 20%) on both occasions. However, among children with wheezing, BHR was diagnosed if they tested positive at either occasion.

Statistical analysis

Statistical analysis was performed by using the SPSS 16.0 software package (SPSS Inc., Chicago, Illinois, USA). Data were expressed as mean ± standard deviation for normal distribution or otherwise median (interquartile range) for non-normal distribution. Independent *t*-test or Mann-Whitney U test was used to compare the difference between the two groups. The chi-square test was used to compare the categorical variables. P<0.05 was considered as statistically significant.

Results

Baseline characteristics

A total of 252 (190 healthy and 62 wheezing) children aged 4–6 years were enrolled. The recruitment flow chart is shown in *Figure 1*. There is no significantly different of height and weight between healthy children and those with wheezing (P>0.05). The demographic characteristics and history of atopy are shown in *Table 1*.

Success rate of spirometry

The success rate of performing spirometry in healthy children was 73.2% (139/190) for the initial visit and 82.1% (156/190) for two visits. The success rate of the 4-year-old group was significantly lower than that of 5-year-old group and 6-year-old group (68.0% *vs.* 86.7% *vs.* 96.4%, both P<0.05). The mean duration of expiration in healthy children was 1.74 s, with seven (4.5%) children whose expiration lasted for no more than one second. In children

Table 1 Characteristics in different age groups

Groups	Parameter	Healthy children		Children with wheezing		P value
		No. (%)	Measured values	No. (%)	Measured values	
All age groups	Height (cm)	156 (100.0)	110.6±6.1	53 (100.0)	112.5±7.0	0.060
	Weight (kg)	156 (100.0)	19.6±3.2	53 (100.0)	20.0±4.2	0.430
	BMI (kg/m^2)	156 (100.0)	15.9±1.6	53 (100.0)	15.6±2.2	0.236
	Food or drug allergy (No., %)	6 (3.9)	–	7 (13.2)	–	0.015
	Upper respiratory tract infection per year	7 (4.5)	–	15 (28.3)	–	<0.001
	Rhinitis (No., %)	5 (3.2)	–	9 (17.0)	–	0.001
	Atopy (No., %)	5 (3.2)	–	4 (7.6)	–	0.180
4 years	Height (cm)	51 (32.7)	104.7±4.2	14 (26.4)	106.0±4.3	0.289
	Weight (kg)	51 (32.7)	17.4±1.8	14 (26.4)	17.2±1.6	0.742
	BMI (kg/m^2)	51 (32.7)	15.9±1.3	14 (26.4)	15.3±0.9	0.117
	Food or drug allergy (No., %)	3 (5.9)	–	2 (14.3)	–	0.230
	Upper respiratory tract infection per year	3 (5.9)	–	5 (35.7)	–	0.003
	Rhinitis (No., %)	2 (3.9)	–	2 (14.3)	–	0.156
	Atopy (No., %)	2 (3.9)	–	0 (0.0)	–	0.455
5 years	Height (cm)	52 (33.3)	111.2±4.2	22 (41.5)	112.7±5.1	0.191
	Weight (kg)	52 (33.3)	19.6±2.5	22 (41.5)	20.9±4.9	0.134
	BMI (kg/m^2)	52 (33.3)	15.8±1.3	22 (41.5)	16.3±2.8	0.272
	Food or drug allergy (No., %)	2 (3.8)	–	3 (13.6)	–	0.128
	Upper respiratory tract infection per year	2 (3.8)	–	6 (27.3)	–	0.003
	Rhinitis (No., %)	1 (1.9)	–	4 (18.2)	–	0.011
	Atopy (No., %)	2 (3.8)	–	2 (9.1)	–	0.365
6 years	Height (cm)	53 (34.0)	115.7±3.8	17 (32.1)	118.3±6.3	0.043
	Weight (kg)	53 (34.0)	21.7±3.6	17 (32.1)	21.0±3.8	0.496
	BMI (kg/m^2)	53 (34.0)	16.1±2.1	17 (32.1)	14.9±1.7	0.029
	Food or drug allergy (No., %)	1 (1.9)	–	2 (11.8)	–	0.082
	Upper respiratory tract infection per year	2 (3.8)	–	4 (23.5)	–	0.012
	Rhinitis (No., %)	2 (3.8)	–	3 (17.7)	–	0.055
	Atopy (No., %)	1 (1.9)	–	2 (11.8)	–	0.082

The data are shown as mean ± standard deviation for normal distribution or median (interquartile range) for non-normal distribution. The percentages were calculated based on the number of different ages groups of healthy children and wheezing children.

with wheezing, the success rate of spirometry and MCh-BPT was 85.5% (73.7% *vs.* 88.0% *vs.* 94.4% in 4-, 5- and 6-year-old groups, respectively), without significant differences between healthy and wheezing groups (P>0.05) (*Table 2*).

Comparison of BHR

Of the 156 healthy children who successfully performed spirometry and MCh-BPT, 11 had an FEV_1 decrease for greater than 20% during the initial visit. At reassessment one month thereafter, 10 children (6.4%) had an FEV_1

Table 2 Rate of children who successfully completed spirometry and MCh-BPT in different age groups

Groups	Healthy children (n=190)			Wheezing children (n=62)	
	Once (No., %)	Twice (No., %)	P value	Once (No., %)	Positive to MCh-BPT (No., %)
Spirometry (n=252)					
4–6 years	139/190 (73.2)	156/190 (82.1)	0.04	53 (85.5)	–
4 years (n=94)	41 (54.7)	51 (68.0)	0.09	14 (73.7)	–
5 years (n=85)	47 (78.3)	52 (86.7)	0.23	22 (88.0)	–
6 years (n=73)	51 (92.7)	53 (96.4)	0.37	17 (94.4)	–
MCh-BPT (n=209)					
4–6 years	154 (98.7)	2 (1.3)		53 (100.0)	49 (92.5)
4 years (n=65)	51 (78.5)	0 (0.0)		14 (100.0)	12 (85.7)
5 years (n=74)	51 (68.9)	1 (1.4)		22 (100.0)	21 (95.5)
6 years (n=70)	52 (74.3)	1 (1.4)		17 (100.0)	16 (94.1)

The percentages were calculated based on the number of different ages groups of healthy children and wheezing children. Mch-BPT, methacholine bronchial provocation test.

Table 3 Diagnostic values of $FEV_{0.5}$, $FEV_{0.75}$, PEF, and MMEF, according to FEV_1

Parameter	Wheezing group (n=53)		Healthy group (n=156)		Sensitivity (%)	Specificity (%)	Youden index	Positive predictive value (%)	Negative predictive value (%)
	Positive	Negative	Positive	Negative					
FEV_1	46	7	10	146	86.8	93.6	0.80	82.1	97.2
$FEV_{0.5}$	45	8	10	146	90.0	93.6	0.83	81.8	94.8
$FEV_{0.75}$	45	8	10	146	90.0	93.6	0.83	81.8	94.8
PEF	29	24	12	144	54.7	92.3	0.47	70.7	86.7
MMEF	35	18	14	142	66.0	91.0	0.57	71.4	88.7

$FEV_{0.5}$, forced expiratory volumes in 0.5; $FEV_{0.75}$, forced expiratory volumes in 0.75; FEV_1, forced expiratory volumes in one second; PEF, peak expiratory flow; MMEF, causing maximal mid-expiratory flow.

decrease for greater than 20%. There was no significant difference of the values of $PD_{20}FEV_{0.5}$, $PD_{20}FEV_{0.75}$ and $PD_{20}FEV_1$of the 10 BHR positive children in healthy subject group. Of the 10 children who had an FEV_1 decrease for greater than 20%, eight had a history of allergy, frequent upper respiratory tract infection, eczema, allergic rhinitis, and family asthma history.

Of the 53 children with wheezing who had successfully completed spirometry and MCh-BPT, 49 (92.5%) tested positive to MCh-BPT. The positive rate of MCh-BPT did not differ significantly between different age groups (85.7% in 4-year-old group *vs.* 95.5% in 5-year-old group *vs.* 94.1% in 6-year-old group, P>0.05) (*Table 2*). Although there was no significant difference in the positive rate of MCh-BPT [97.4% (37/38) *vs.* 80.0% (12/15), P>0.05], however, with significant lower $PD_{20}FEV_1$ in children with clinically diagnosed asthma than those with a history of wheezing only children (4.40±3.67 *vs.* 7.87±3.78 μmol, P=0.007).

Diagnostic value of different parameters

Of the 49 children with wheezing that tested positive to MCh-BPT, 46 subjects had both an expiration of more than one second and an FEV_1 decrease of greater than 20%. The positive rates of $FEV_{0.5}$, $FEV_{0.75}$, PEF and MMEF are shown in *Table 3*. Comparison of the provocative doses revealed that both $FEV_{0.75}$ and $FEV_{0.5}$, but not PEF and MMEF, could be the surrogates of FEV_1 in children with an

Table 4 Comparison of different cumulative doses of MCh for diagnosing BHR

Dosage	Parameter	Healthy group *vs.* wheezing group					Asthmatics or wheezing only				
		No.	Healthy (μmol)	No.	Wheezing (μmol)	P	No.	Asthmatic (μmol)	No.	Wheezing (μmol)	P
PD_{15}	$FEV_{0.5}$	10	5.01±2.55	49	2.35 (2.99)*	0.019	37	2.33±2.32	12	4.25±2.01	0.017
	$FEV_{0.75}$	10	5.18±2.38	48	2.48 (3.19)*	0.014	37	2.57±2.33	11	4.88±2.67	0.008
	FEV_1	10	5.49±2.62	46	2.78 (5.02)*	0.032	36	2.95±2.56	10	5.53±2.78	0.008
	P		0.91		0.552			0.542		0.511	
PD_{20}	$FEV_{0.5}$	10	6.73±3.57	48	3.89 (5.02)*	0.053	37	3.77±3.21	12	6.31±3.0	0.024
	$FEV_{0.75}$	10	6.90±3.46	47	3.75 (6.09)*	0.075	37	3.50 (6.01)*	10	6.30±2.91	0.037
	FEV_1	10	7.97±3.67	46	4.17 (6.54)*	0.036	36	4.50±3.67	10	7.86±4.0	0.016
	P		0.7		0.54			0.712		0.49	

The data are shown as mean ± standard deviation for normal distribution or median (interquartile range) for non-normal distribution. *, non-normal distribution. PD_{15}, provocative dose causing 15% reduction in $FEV_{0.5}$, $FEV_{0.75}$ and FEV_1; PD_{20}, provocative dose causing 20% reduction in $FEV_{0.5}$, $FEV_{0.75}$ and FEV_1. Mch, methacholine; BHR, bronchial hyperresponsiveness; $FEV_{0.5}$, forced expiratory volumes in 0.5; $FEV_{0.75}$, forced expiratory volumes in 0.75; FEV_1, forced expiratory volumes in one second.

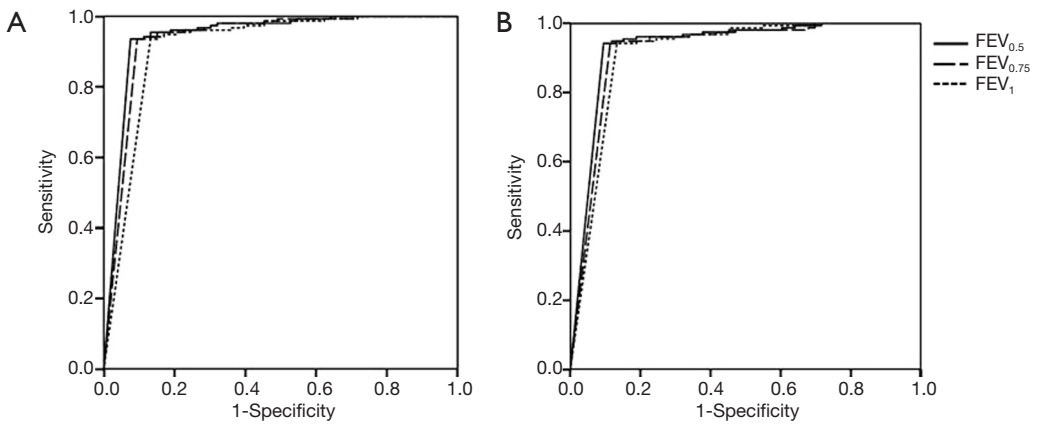

Figure 2 Receive operation characteristic curve of $PD_{15}FEV_{0.5}$, $PD_{15}FEV_{0.75}$, $PD_{15}FEV_1$ (A) and $PD_{20}FEV_{0.5}$, $PD_{20}FEV_{0.75}$ and $PD_{20}FEV_1$ (B) for diagnosing asthma. The area under the curve (AUC) of receiver operating characteristic curves (ROC) of $PD_{15}FEV_{0.5}$, $PD_{15}FEV_{0.75}$, $PD_{15}FEV_1$ and $PD_{20}FEV_{0.5}$, $PD_{20}FEV_{0.75}$ and $PD_{20}FEV_1$ were 0.944 (95% CI: 0.900, 0.988), 0.935 (95% CI: 0.887, 0.984), 0.914 (95% CI: 0.858, 0.970) and 0.932 (95% CI: 0.883, 0.981), 0.922 (95% CI: 0.869, 0.975), 0.914 (95% CI: 0.858, 0.970), respectively. $FEV_{0.5}$, forced expiratory volumes in 0.5; $FEV_{0.75}$, forced expiratory volumes in 0.75; FEV_1, forced expiratory volumes in one second.

expiration of less than one second.

The $PD_{15}FEV_{0.5}$, $PD_{15}FEV_{0.75}$ and $PD_{15}FEV_1$ were significantly lower in children with wheezing than those tested positive to MCh-BPT in healthy children [2.35 (2.99) *vs.* 5.01±2.55 μmol for $PD_{15}FEV_{0.5}$, 2.48 (3.19) *vs.* 5.18±2.38 μmol for $PD_{15}FEV_{0.75}$, 2.78 (5.02) *vs.* 5.49±2.62 μmol for $PD_{15}FEV_1$, all P<0.05, *Table 4*]. The area under the curve (AUC) of receiver operating characteristic curves (ROC) of $PD_{15}FEV_{0.5}$, $PD_{15}FEV_{0.75}$, $PD_{15}FEV_1$ and $PD_{20}FEV_{0.5}$, $PD_{20}FEV_{0.75}$ and $PD_{20}FEV_1$ for the diagnosis of BHR was 0.944 (95% CI: 0.900, 0.988), 0.935 (95% CI: 0.887, 0.984), 0.914 (95% CI: 0.858, 0.970) and 0.932 (95% CI: 0.883, 0.981), 0.922 (95% CI: 0.869, 0.975), 0.914 (95% CI: 0.858, 0.970), respectively (*Figure 2*).

The positive rate of MCh-BPT, determined with $PD_{20}FEV_1$, was 6.4% in healthy children and 92.5% in children with wheezing. $PD_{20}FEV_1$ in children with wheezing was significantly lower than that of healthy children [4.17 (6.54) *vs.* 7.97±3.67 μmol, P<0.05], whereas $PD_{20}FEV_{0.5}$ [3.89 (5.02) *vs.* 6.73±3.57 μmol, P>0.05] and

Table 5 Adverse events in different age groups

Groups	Mild wheeze (%)	Pharyngeal itching (%)	Cough (%)	Hoarseness (%)	Sore throat (%)	Shortness of breath (%)	Chest tightness (%)
Healthy children (n=156)	4 (2.6)	7 (4.5)	2 (1.3)	3 (1.9)	1 (0.6)	0 (0.0)	0 (0.0)
4 years (n=51)	1 (2.0)	2 (3.9)	1 (2.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
5 years (n=52)	1 (1.9)	2 (3.9)	1 (1.9)	1 (1.9)	1 (1.9)	0 (0.0)	0 (0.0)
6 years (n=53)	2 (3.8)	3 (5.7)	0 (0.0)	2 (3.8)	0 (0.0)	0 (0.0)	0 (0.0)
Wheezing children (n=53)	22 (41.5)	5 (9.4)	7 (13.2)	0 (0.0)	1 (1.9)	0 (0.0)	1 (1.9)
4 years (n=14)	6 (42.9)	2 (14.3)	2 (14.3)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
5 years (n=22)	9 (40.9)	1 (4.5)	2 (9.1)	0 (0.0)	1 (4.5)	0 (0.0)	1 (4.5)
6 years (n=17)	7 (41.2)	2 (11.8)	3 (17.6)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Total (n=209)	26 (12.4)	12 (5.7)	9 (4.3)	3 (1.4)	2 (1.0)	2 (1.0)	1 (0.5)

$PD_{20}FEV_{0.75}$ [3.75 (6.09) *vs.* 6.90±3.46 μmol, P>0.05] were comparable between the two groups. Among children with wheezing, $PD_{20}FEV_{0.5}$, $PD_{20}FEV_{0.75}$ and $PD_{20}FEV_1$ were significantly lower in children with a clinical diagnosis of asthma than in those who had a history of wheezing but no clinically diagnosed asthma [3.77±3.21 *vs.* 6.31±3.0 μmol, 3.50 (6.01) *vs.* 6.30±2.91 μmol, 4.50±3.67 *vs.* 7.86±4.0 μmol, P<0.05].

Adverse effects

Adverse events happened during the MCh-BPT included transient wheezing, cough, pharyngeal itching, hoarseness, sore throat, shortness of breath, chest tightness, which were mild and comparable in different age groups (*Table 5*). Mild wheezing and cough were significantly more common in children with wheezing than in healthy children (41.5% *vs.* 2.6%, 13.2% *vs.* 1.3%, P<0.01), whereas the incidence of sore throat, hoarseness and chest tightness was comparable between the two groups (all P>0.05). After challenge, 200–400 mcg inhaled salbutamol had been given through a spacer to the children whose FEV_1 decreased more than 20% from baseline, or self-reported chest-tightness wheezing, or wheezing by auscultation. Ten minutes after the inhalation of salbutamol, FEV_1 recovered to the baseline and the symptoms relieved in all of the children with adverse events. There was no serious adverse event requiring continuous nebulization with bronchodilators and/or corticosteroids.

Discussion

This study revealed that preschool children who were able to perform spirometry could successfully complete MCh-BPT, with tolerable and minor adverse events. $PD_{20}FEV_1$ remains to be an optimal parameter for assessing BHR with MCh-BPT in preschool children. For children with expiration for less than one second, both $PD_{20}FEV_{0.5}$ and $PD_{20}FEV_{0.75}$ can be surrogates of $PD_{20}FEV_1$.

Currently, $PD_{20}FEV_1$ is extensively recommended as a positive indicator of bronchial provocation tests (including MCh-BPT) in elderly children and adults (15,16). However, some studies have reported that the quality control standards for adults might not be suitable to directly extrapolate to preschool children (6,17,18). The diagnosis of asthma among children aged 5 years or younger has been challenging because of the risks of misdiagnosis. For instance, children without asthma may often experience intermittent respiratory symptoms such as wheezing and cough (19,20). Therefore, ascertainment of the feasibility of spirometry and MCh-BPT for assessment of BHR, and the safety and diagnostic value of MCh-BPT among preschool children may provide valuable insights to clinical management of diseases with BHR (particularly asthma).

We found that the sensitivity and specificity were both 90.0% and 93.6% for $PD_{20}FEV_{0.5}$ and $PD_{20}FEV_{0.75}$, which did not differ significantly from that of $PD_{20}FEV_1$. However, the sensitivity and the specificity were only 71.9% and 66.7% for $PD_{20}PEF$, and 77.97% and 66.67% for $PD_{35}MMEF$. Our findings indicated that in preschool children, for those with an expiratory duration of less than one second, both $PD_{20}FEV_{0.5}$ and $PD_{20}FEV_{0.75}$, but not $PD_{20}PEF$ or $PD_{35}MMEF$, could serve as the surrogates for $PD_{20}FEV_1$ for assessment of BHR. Bentur *et al.* (21) reported that when $PD_{20}FEV_1$ was applied as the main parameter for reflecting BHR, it would be more feasible,

safer, and require less provocative agents to be inhaled than assessment with the symptoms or the breath sounds of wheezing in preschool children. $PD_{20}FEV_1$ was significantly lower in children with wheezing than in healthy children, whereas $PD_{20}FEV_{0.5}$ and $PD_{20}FEV_{0.75}$ were comparable between the two groups, indicating that $PD_{20}FEV_1$ might be preferable to $PD_{20}FEV_{0.5}$ and $PD_{20}FEV_{0.75}$ for evaluation of BHR in preschool children.

Noteworthily, healthy children who tested positive to MCh-BPT displayed a significantly higher incidence of exposure or sensitive to allergens, frequent upper airway infections, and allergic rhinitis than those with negative results ($P<0.01$). It is likely that drugs, food allergens, frequent upper airway infection history, and allergic rhinitis may have contributed to the development of, or exacerbated, BHR. A family history of asthma has been identified as a major risk factor for asthma in children (22,23), whereas asymptomatic BHR may predispose to asthma (24). The mechanisms for the BHR among some of the healthy children warranted further studies.

Methacholine has been the most common provocative agent for BPT in clinical practice. There are international guidelines that offered detailed instructions to perform MCh-BPT (25). However, the safety of MCh-BPT in preschool children is unclear. We have previously reported that (26) the use of histamine BPT for diagnosing BHR in children aged 11–14 years was safe, with no serious adverse events having been reported. There are also literature reports (27) documenting the use of MCh-BPT in infants and toddlers with tidal breathing method, the results of which showed minor safety concerns. Kivastik *et al.* (28) have confirmed the safety of MCh-BPT with tidal breathing methods and tripling dose increment, by using air flow blocking technology to measure respiratory resistance in children aged 3 to 6 years old. These pieces of evidence have reaffirmed the safety of MCh-BPT to be applied among preschool children.

Some children developed pharyngeal itching, sore throat, hoarse cough, cough, chest tightness, and mild wheezing. However, all these symptoms were ameliorated through throat gargling, water ingestion, having a rest, or inhalation of salbutamol for 200 μg within 10–30 minutes. No serious adverse event was reported, indicating that MCh-BPT in preschool children was safe. The frequency of cough and mild wheezing was significantly higher in children with wheezing than in healthy children. In children with wheezing, the incidence of transient wheezing during MCh-BPT was higher in those with abnormal lung function.

This study assessed BHR in preschool children and confirmed that the methacholine bronchial provocation test was safe and applicable in children aged 4–6 years. Similar with toddlers and adults, some preschool children may have asymptomatic BHR. Whether this would require further medical evaluation or dynamic follow-up merits further investigations. However, some limitations should be considered. First, the sample size and single-center study design might have limited the validity of our conclusion. Moreover, we did not include 3-year-old children, some of whom might also be capable of performing the MCh-BPT.

In summary, although most preschool children in this study can successfully and safely complete MCh-BPT for the diagnosis of BHR in preschool children, however higher success rate was found in larger age groups. $PD_{20}FEV_1$ remains an optimal indicator of BHR for MCh-BPT, both $PD_{20}FEV_{0.5}$ and $PD_{20}FEV_{0.75}$ could be surrogate endpoints in preschool children, particularly those with expiration of less than one second. PD_{15} has a good diagnostic value as PD_{20} for diagnosing of BHR in preschool children.

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Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study protocol was approved by the ethics committee of The First Affiliated Hospital of Guangzhou Medical University, and parents signed informed consent.

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Supplementary

Table S1 Work flow of MCh-BPT

Steps	Regular (doubling dose increase)		Simplified (quadrupling dose increase)		Cumulative dose (μmol)
	Concentration (%)	Inhalation times	Concentration (%)	No. of inhalation	
1	0.30	1	–	–	0.05
2	0.30	1	0.60	1	0.1
3	0.60	1	–	–	0.2
4	0.60	2	0.60	3	0.4
5	2.50	1	–	–	0.8
6	2.50	2	2.50	3	1.6
7	2.50	4	–	–	3.2
8	5.00	4	5.00	6	6.4
9	5.00	8	5.00	8	12.8

MCh inhalation with Yan's method with 4-fold dose increase for healthy subjects and during the provocation test if FEV_1 decrease less than 10%, quadrupling dose increase was used, if FEV_1 decrease >10% but <20%, then doubling dose increase was applied. Mch-BPT, methacholine bronchial provocation test. FEV_1, forced expiratory volumes in one second.

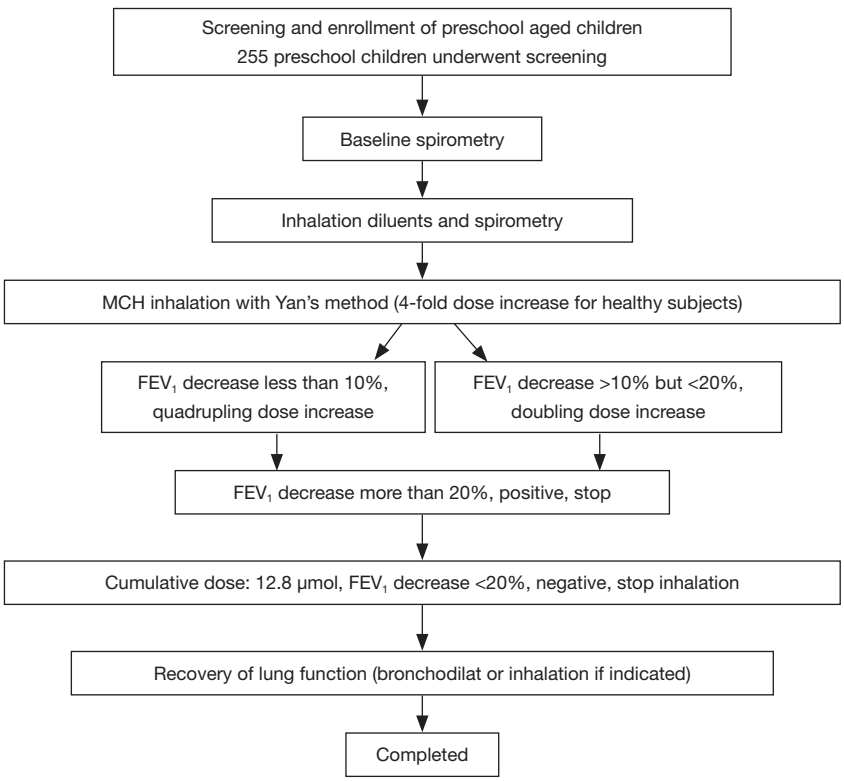

Figure S1 Procedure of MCh-BPT. Mch-BPT, methacholine bronchial provocation test. FEV_1, forced expiratory volumes in one second.