Different cutoff values of methacholine bronchial provocation test depending on age in children with asthma

Eun Lee, Young-Ho Kim, Seungbong Han, Song-I Yang, Young-Ho Jung, Ju-Hee Seo, Hyo-Bin Kim, So Yeon Lee, Ji-Won Kwon, Soo-Jong Hong

Seoul, Korea

Background: **Bronchial hyperresponsiveness (BHR) is a fundamental pathophysiological characteristic of asthma. Although several factors such as airway caliber can affect BHR, no study has established age-dependent cutoff values of BHR to methacholine for the diagnosis of asthma in children. We investigated the cutoff values of the methacholine challenge test (MCT) in the diagnosis of asthma according to age.**

Methods: **A total of 2383 individuals aged from 6 to 15 years old were included in this study. MCTs using the five-breath technique were performed in 350 children with suspected asthma based on symptoms by pediatric allergists and in 2033 healthy children from a general population-based cohort. We determined the provocative concentration of methacholine producing a 20% decrease in forced expiratory volume in 1 second from baseline (PC_{20}). A modified Korean version of the International Study of Asthma and Allergies in Childhood questionnaire was used to distinguish asthmatics and healthy subjects. Receiver-operator characteristic curve analysis was used to assess the cutoff value of PC_{20} for the diagnosis of asthma.**

Results: **Cutoff values of methacholine PC_{20}, which provided the best combination of diagnostic sensitivity and specificity, showed an increasing pattern with age: 5.8, 9.1, 11.8, 12.6, 14.9, 21.7, 23.3, 21.1, 21.1, and 24.6 mg/mL at ages 6, 7, 8, 9, 10, 11, 12, 13, 14, and 15 years, respectively.**

Conclusion: **The application of different cutoff values of methacholine PC_{20} depending on age might be a practical modification for the diagnosis of asthma in children and adolescents with asthmatic symptoms.**

World J Pediatr 2017;13(5):439-445

Key words: **asthma;**
bronchial hyperresponsiveness;
cutoff value;
receiver-operator characteristics

Original article

Introduction

Bronchial hyperresponsiveness (BHR), the exaggerated narrowing of the airways from exposure to various stimuli, and airway inflammation are the two fundamental pathophysiological features of asthma. For this reason, measurement of BHR through bronchial provocation tests is required in the diagnosis of asthma.[1] Assessment of BHR in children complaining of recurrent episodes of wheezing, breathlessness, chest tightness, or paradoxical cough may help to diagnose asthma, especially when reversible airflow is not demonstrated.[2]

Methacholine challenge test (MCT) is a direct bronchoprovocation test to evaluate airway hyperresponsiveness.[3] Methacholine causes airflow limitation resulting from airway smooth muscle contraction via stimulation of muscarinic M3 receptors. According to the American Thoracic Society (ATS) guidelines, >16 mg/mL of methacholine PC_{20}, which means a 20% fall in forced expiratory volume in 1 second (FEV_1) from the baseline value after inhaling methacholine stepwise up to the maximum concentration, is considered for the exclusion of BHR.[3] As various factors such as sex, age, or air pollution can affect the results of BHR,[4] the application of a uniform reference value for MCT as recommended by the ATS in the diagnosis of asthma is done cautiously.

Author Affiliations: Department of Pediatrics, Chonnam National University Hospital, Gwangju, Korea (Lee E); Department of Pediatrics, Gyeongsang National University Changwon Hospital, Changwon, Korea (Kim YH); Department of Applied Statistics, Gachon University, Seongnam, Korea (Han S); Department of Pediatrics, Hallym University Sacred Heart Hospital, Anyang, Korea (Yang SI); Department of Pediatrics, Bundang CHA Medical Center, CHA University School of Medicine, Seongnam, Korea (Jung YH); Department of Pediatrics, Dankook University College of Medicine, Cheonan, Korea (Seo JH); Department of Pediatrics, Inje University Sanggye Paik Hospital, Seoul, Korea (Kim HB); Department of Pediatrics, Childhood Asthma Atopy Center, Research Center for Standardization of Allergic Diseases, Asan Medical Center, University of Ulsan College of Medicine, Seoul, Korea (Lee SY, Hong SJ); Department of Pediatrics, Seoul National University Bundang Hospital, Seongnam, Korea (Kwon JW)

Corresponding Author: Ji-Won Kwon, MD, PhD, Department of Pediatrics, Seoul National University Bundang Hospital, 82, Gumi-ro, 173 beon-gil, Bundang-gu, Songnam-si, Gyeuonggi-do 463-707, Korea (Tel: +82-31-787-7296; Fax: +82-31-787-4054; Email: pedas@snubh.org)

doi: 10.1007/s12519-017-0026-5
Online First March 2017

©Children's Hospital, Zhejiang University School of Medicine, China and Springer-Verlag Berlin Heidelberg 2017. All rights reserved.

Although spirometry plays a key role in the diagnosis and monitoring of asthma, inconclusive spirometry results require further objective measures, including BHR, for the diagnosis of asthma.[2] When the parameters of spirometry do not satisfy the criteria for the diagnosis of asthma when asthma is clinically suspected, application of more practical cutoff values of MCT may be helpful for a more accurate diagnosis of asthma. Although several studies have shown a normal distribution of BHR to methacholine challenge in a general population[5,6] and suggested a new diagnostic value of MCT in asthmatic adults,[7,8] there have been no studies to determine the cutoff value of MCT for age-dependent diagnosis of asthma in children with asthmatic symptoms. The primary aim of this study was to evaluate the cutoff value of methacholine PC_{20} indicative of asthma according to age in children with a diagnostic suspicion of asthma.

Methods

Study population

A total of 2383 subjects aged from 6 to 15 years old were included in this study. From January 2009 to December 2012, 350 children visited the Childhood Atopy Asthma Center at Asan Medical Center complaining of asthmatic symptoms such as recurrent episodes of wheezing, breathlessness, chest tightness, and paradoxical cough, and they were diagnosed with asthma by pediatric allergists. As a control group, 2033 healthy children were included from a general population-based cohort, who were from the Study for Standardization in Allergic Diseases in Seoul with no asthma symptoms, diagnosis, or asthma treatment. MCTs were performed for all subjects. Written consent was obtained from all parents or guardians, and the study was approved by the Institutional Review Board of Asan Medical Center, Ulsan University College of Medicine, Seoul, Korea.

Methacholine challenge test

Subjects were excluded from this study if they had a history of upper or lower respiratory tract infection during the three weeks prior to MCT or inhaled corticosteroid therapy at least four weeks before the MCT. All subjects avoided bronchodilator treatment for at least 8-24 hours as appropriate according to the ATS guidelines.[3] In addition, coffee, tea, cola drinks, and chocolate, which can decrease BHR, were avoided on the day of MCT by all of the participants.[3]

MCTs were based on the modified five-breath dosimeter technique and a dosing schedule using methacholine concentrations of 0.625, 1.25, 2.5, 5, 10, and 25 mg/mL.[3] Fresh preparations of methacholine were provided to accurately produce these concentrations. The test ended when a fall in FEV_1 values to ≥20% of the baseline value was achieved or when the highest concentration of methacholine was inhaled. Children completing MCTs without a 20% decrease in FEV_1 were assigned a value of 50.0 mg/mL. PC_{20} was calculated by interpolating between two adjacent data points when the FEV_1 decreased by ≥20%.

Skin prick test (SPT) and measurement of total serum immunoglobulin E (IgE) levels

SPT was performed for the most common aeroallergens (Allergopharma GmbH & Co., Reinbek, Germany) including the 13 most common inhalant allergens (*Dermatophagoides pteronyssinus, Dermatophagoides farinae,* dog dander, cat epithelium, cockroach, *Alternaria alternata, Aspergillus fumigatus,* a grass pollen mixture, tree mixture, ragweed, mugwort, alder, and oak) and 4 food allergens (peanut, egg white, cow's milk, and soybean).[9] Histamine (10 mg/mL) was used as the positive control, and normal saline was used as the negative control. A mean wheal size measured larger than 3 mm after 15 minutes and those caused by histamine were considered positive. Total serum IgE levels were measured using the Immunocap-CAP 1000 system (Aloka, Tokyo, Japan). The lowest detection limit of total serum IgE was 2 kU/L.

Questionnaire

A modified version of the International Study of Asthma and Allergies in Childhood written questionnaire was used for this study. Parents or guardians of the children included in the study completed the questionnaire. The questionnaire consisted of several sections including 1) general characteristics, including the patient's name, sex, date of birth, height, and weight; 2) history of symptoms related to atopic dermatitis, allergic rhinitis, and asthma; and 3) the individual's environmental factors associated with the development of allergic diseases.[10] The questionnaire for the inclusion in the healthy group was as follows: "Have you been diagnosed with asthma at any time by a physician?"; "Have you experienced attacks of chest wheezing 12 months before enrollment in this study?", and "Have you been treated for your asthma symptoms in the 12 months before enrollment in this study?".

Statistical analysis

Methacholine PC_{20} and total serum IgE levels were log-transformed before the statistical analysis. Data were expressed as mean±standard deviation. Statistical comparisons between groups and within groups were made by unpaired Student's *t* tests or Chi-square analysis as appropriate. Receiver-operator characteristic (ROC) curves were generated to determine the sensitivity

Table 1. General characteristics of the study population

Variables	Suspected asthmatic group	Healthy group	*P* value
Subject numbers	350	2033	
Gender (male), *n*/total (%)	241/350 (68.9)	914/2033 (45.0)	<0.001
Age (y), mean±SD	8.70±2.62	12.73±3.12	<0.001
BMI (kg/m^2), mean±SD	17.87±9.53	19.29±14.18	<0.001
Methacholine PC_{20} (mg/mL), mean (95% CI)	7.19 (8.03-8.36)	21.78 (21.48-22.07)	<0.001
Dose response slope, mean±SD	1.16±0.19	0.75±0.13	<0.001
FEV_1, % predicted, mean±SD	88.84±17.02 (*n*=286)	97.62±11.64 (*n*=1995)	<0.001
FEV_1/FVC, % predicted, mean±SD	99.13±8.80	93.45±5.45	<0.001
Normal lung function, *n*/total (%) (FEV_1, % predicted ≥80%)	78/286 (27.3)	121/1995 (6.1)	<0.001
≥1 positivity on skin prick test, *n*/total (%)	299/347 (86.2)	835/1997 (41.8)	<0.001
Total serum IgE (kU/L), geometric mean±SD	266.60±3.76 (*n*=344)	86.55±3.77 (*n*=1934)	<0.001
Serum eosinophil (%), mean±SD	6.49±4.46 (*n*=348)	2.81±2.23 (*n*=1956)	<0.001
Parental history of allergic diseases (AD, AR, or asthma), *n*/total (%)	195/346 (56.4)	743/1874 (39.6)	<0.001

AD: atopic dermatitis; AR: allergic rhinitis; BMI: body mass index; CI: confidence interval; FEV_1: forced expiratory volume in 1 second; FVC: forced vital capacity; IgE: immunoglobulin E; PC_{20}: a provocative concentration causing a 20% decrease in FEV_1; SD: standard deviation.

and specificity of methacholine PC_{20} for discriminating children in this study with or without asthma. All data analyses were performed using the SPSS statistical package version 21.0 for Windows (IBM Corp., Armonk, NY). Differences were considered significant when the *P* value was less than 0.05.

Results

Characteristics of the study population

The characteristics of the study population are listed in Table 1. The ratios of boys to girls in the children with asthma and the healthy children groups were 231:109 (2.21) and 914/1119 (0.82), respectively. The age distribution of the asthmatic children is indicated in Table 2. There was a bias towards greater recruitment at younger ages in the asthmatic children. The mean values of methacholine PC_{20} showed an increase with age in both groups (Fig. 1).

Prevalence of bronchial hyperresponsiveness

A BHR defined as PC_{20} ≤16mg/mL was present in 17.5% (355/2033) of children from a healthy general population without any reported asthma symptoms or asthma diagnosis and in 84.0% (294/350) of the asthmatic children. When we applied the cutoff levels from this study, 78.9% (276/350) of children in the suspected asthma group displayed a positive BHR versus 12.4% (253/2033) of the healthy children with no asthma diagnosis or symptoms.

Age-dependent cutoff values of methacholine PC_{20} for the diagnosis of asthma

Cutoff values of methacholine PC_{20}, which provided the best combination of diagnostic sensitivity and specificity, showed an increasing pattern with age as follows: 5.8, 9.1, 11.8, 12.6, 14.9, 21.7, 23.3, 21.1, 21.1, and 24.6 mg/mL at age 6, 7, 8, 9, 10, 11, 12, 13, 14, and 15 years old, respectively (Table 3). Depending on age, the area under the ROC curves (AUC) for the diagnosis of asthma ranged from 0.81 to 0.92 (Fig. 2). Cutoff levels for the diagnosis of asthma in the suspected asthma group obtained in this study were associated with very high negative predictive values (89.7%-99.5%).

Table 2. Distribution of subjects' numbers stratified by age into suspected asthma and healthy groups

Age (y)	Suspected asthma group *n* (%)	Healthy group *n* (%)	Total, *n* (%)
6	84 (24.0)	251 (12.3)	335 (14.1)
7	70 (20.0)	188 (9.2)	258 (10.8)
8	52 (14.9)	174 (8.6)	226 (9.5)
9	30 (8.6)	180 (8.9)	210 (8.8)
10	31 (8.9)	172 (8.5)	203 (8.5)
11	24 (6.9)	161 (7.9)	185 (7.8)
12	15 (4.3)	171 (8.4)	186 (7.8)
13	18 (5.1)	191 (9.4)	209 (8.8)
14	13 (3.7)	203 (10.0)	216 (9.1)
15	13 (3.7)	342 (16.8)	355 (14.9)
Total	350 (100.0)	2033 (100.0)	2383 (100.0)

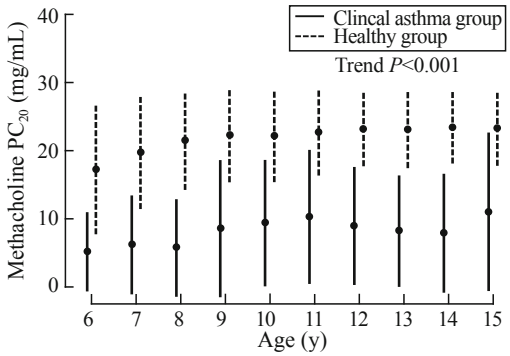

Fig. 1. Distribution of age-dependent methacholine PC_{20} in the clinical asthma and healthy groups without respiratory symptoms. PC_{20}: a provocative concentration causing a 20% decrease in forced expiratory volume in 1 second.

Table 3. Diagnostic sensitivity, specificity, positive and negative predictive values of methacholine PC_{20} for diagnosis of asthma

Age (y)	6	7	8	9	10	11	12	13	14	15
Reference value of methacholine PC_{20} (mg/mL)	5.8	9.1	11.8	12.6	14.9	21.7	23.3	21.1	21.1	24.6
Sensitivity, %	71.4	82.9	84.6	72.4	77.4	75.0	86.7	88.9	92.3	69.2
Specificity, %	83.3	80.9	86.8	86.7	86.0	86.3	87.7	88.5	90.1	88.3
Positive predictive value, %	58.8	61.7	65.7	45.3	50.0	45.0	38.2	42.0	37.5	40.0
Negative predictive value, %	89.7	92.7	95.0	96.2	95.5	95.9	98.7	98.8	99.5	98.5

PC_{20}: a provocative concentration causing a 20% decrease in FEV_1.

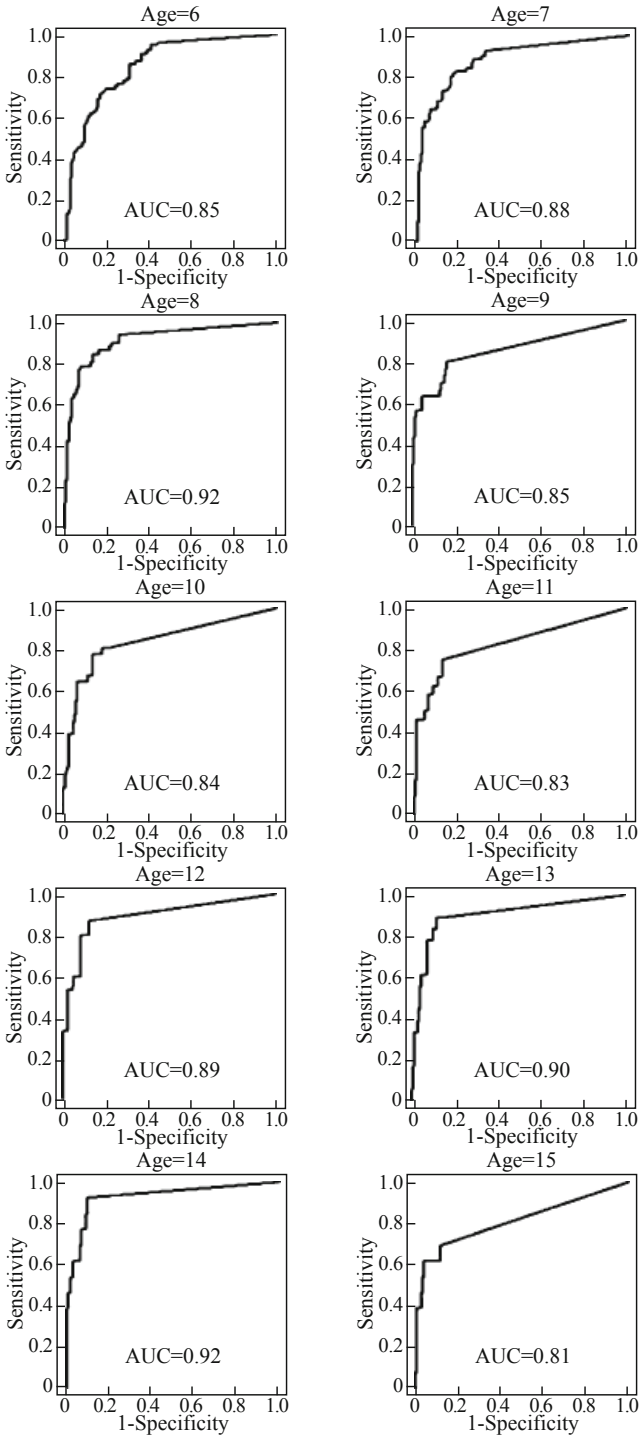

Fig. 2. Receiver-operator characteristic (ROC) curves indicating the sensitivity and specificity of methacholine PC_{20} for the diagnosis of asthma in children stratified by age. AUC: area under the ROC curves; PC_{20}: a provocative concentration causing a 20% decrease in FEV_1.

Discussion

In our present study, we identified age-dependent cutoff values of methacholine PC_{20} for the diagnosis of asthma in children and adolescents with a suspicion of asthmatic symptoms that showed a pattern of increase with age. Although ATS guidelines present uniform values of methacholine PC_{20} for both children and adults for the diagnosis of asthma,[3] these guidelines lack consideration of several influencing factors on BHR, such as age-related factors including variations in the airway caliber. Compared to the cutoff values presented in the ATS guidelines, those obtained in our present study were lower in asthmatic children ≤10 years old, but higher in those ≥11 years old. Our findings suggest a different methacholine PC_{20} reference value according to age might represent an improvement for asthma diagnosis in children and adolescents with symptoms suspicious of asthma, rather than the uniform application of a PC_{20} as ≤4.0, ≤8.0, or ≤16.0 mg/mL recommended by the ATS guidelines.[3]

One earlier study on the diagnostic value of methacholine PC_{20} in 106 adult asthmatics reported that the application of a cutoff level of 15 mg/mL PC_{20} instead of 16 mg/mL PC_{20} increases the diagnostic accuracy for asthma.[7] However, no studies have determined the age-appropriate cutoff values of methacholine PC_{20} for the diagnosis of asthma in children suspected with asthma. Our current study is the first attempt to identify age-dependent cutoff values of methacholine PC_{20} for the diagnosis of asthma in children and adolescents.

A number of factors such as age, gender, and baseline airway caliber affect BHR.[11] A previous study investigating the relationship between BHR, sex, age, and atopy in adults aged 18-75 years showed that BHR was positively associated with sex and atopy, but not with age.[4] The lack of a difference in BHR based on age in adults might be attributable, in part, to the completely developed airway caliber in adults. Another study that analyzed the effect of age and severity of asthma on BHR in asthmatic children from 1 to 17 years of age showed no significant age-dependent differences in BHR.[12] Because this study included only asthmatic children requiring therapy, the BHR values in that study were different from the cutoff values for the diagnosis of asthma. One study focused

on the natural history of BHR in childhood asthma and showed that postpubertal female patients had persistent BHR, whereas postpubertal male patients with asthma showed an improvement in BHR with advancing years, improving at about 11 years.[13] Although our study did not evaluate the effect of sex on BHR between children with suspected asthma symptoms and healthy subjects,[13] the increasing pattern of cutoff values of methacholine PC_{20} for the diagnosis of asthma might be partially reflected by the predominance of older male subjects. The combinational effect of sex and age on BHR might be partially attributable to sex differences in the growth, ratio of airway size, lung volume, and hormone levels, especially in adolescents.[14,15] The small airway size relative to lung size observed in adult female patients is associated with higher airway reactivity and sensitivity to methacholine.[16]

In terms of age-dependent hormonal effects on BHR, relative decreases in estrogen and increases in progesterone are known to be associated with increases in BHR.[17-19] Although increases in estrogen levels during early puberty and increases in progesterone levels later in puberty might be associated with age-dependent differences in BHR, an imbalance in sexual hormone levels should also be considered in the interpretation of BHR in male and female adolescents. Further studies are needed to elucidate the effect of hormonal levels on BHR.

When interpreting the results of the methacholine PC_{20}, the pretest probability of asthma including current asthma symptoms should be considered.[3] Subjects in the suspected asthma group were those who visited the allergy and asthma clinic with asthma symptoms and were included by pediatric allergists only when assessed as appropriately possessing asthma symptoms. A higher pretest probability resulting from the aforementioned characteristics of the suspected asthma group might be associated with higher cutoff values in older asthmatic children than those presented by the ATS guidelines. In addition, baseline airway obstruction, quality of the subjects' spirometry maneuvers, and breathing pattern also affect the values of methacholine PC_{20},[3,20,21] therefore, these factors should be considered when interpreting methacholine PC_{20}.

A positive BHR in healthy children might be attributable to the following factors: mild intermittent asthma without detection of asthma symptoms, chest tightness without recognition of the symptom as abnormal, mild BHR due to other causes such as that resulting from a viral respiratory infection, or asymptomatic asthma with the possibility of later asthma development.[11,22-28] A previous study on the prognosis of asymptomatic BHR in childhood showed no association with respiratory symptoms in young adulthood.[29] Consideration of positive BHR in healthy children on the cutoff values in methacholine PC_{20} is beyond the scope of our present report, and further prospective follow-up studies on these issues are needed.

To minimize the effects of medications or foods that can decrease BHR such as β-agonists, cola, and chocolate and respiratory infections that can increase BHR,[30,31] we performed MCTs after interrupting β-agonists for at least 8-24 hours, after the patients avoided cola and chocolate on the day of study, and after at least three weeks of any respiratory infection. Although a previous study showed that the results of a 2 minute tidal breathing method were similar to those of the standard dosimeter method in asthmatic adults, another study reported that the tidal breathing method was associated with a higher prevalence of positive methacholine response compared to the dosimeter methods due to exposure to high doses of methacholine aerosol.[20] Therefore, methods of administering the MCT also need to be considered when interpreting methacholine PC_{20}, especially at borderline values.

Since atopy significantly increases the response to methacholine and exercise challenge tests,[32] the cutoff values obtained by our current analyses might be pertinent to atopic asthmatic children, considering the high prevalence of atopy in children with suspected asthma. In the present study, 41.8% of the control group showed atopy on SPTs. In a previous study on the prevalence of allergic sensitization using the ImmunoCAP system, 44.6% showed atopy among 9440 subjects aged 1 year or more from the general population.[33] Although there are differences in participants between the present study and the previous study, asymptomatic sensitization is common and studies on the meaning of asymptomatic sensitization are needed in the future.[34]

Because of the high sensitivity, low specificity, and high negative predictive value of MCTs,[12,35-37] MCT is generally more useful in the exclusion of asthma than the establishment of an asthma diagnosis. The cutoff values obtained in our present study showed a similar high specificity and sensitivity compared to those recommended by the ATS guidelines, which can be considered one of the strengths of age-dependent cutoff values.[3] On the basis of the present findings, the sensitivity and specificity of methacholine PC_{20} was relatively high when age-dependent cutoff values were applied, and even the normal range of BHR (>16 mg/mL PC_{20}) might be more practical for excluding the diagnosis of asthma in children and adolescents.

The differences in the cutoff values of methacholine PC_{20} between children with asthma and adolescents with asthma might be partially attributable to the different components determining the BHR that vary by age.[38]

BHR is associated with airway inflammation in children with asthma. While this reactivity is associated with structural changes, it is only weakly associated with airway inflammation in adolescents with asthma.[38] Although BHR to indirect stimuli reflects airway inflammation better than direct stimuli such as methacholine in asthma,[39] the higher cutoff values suggested in asthmatic adolescents are associated with less airway inflammation on MCTs.

According to the ATS guidelines, the levels of PC_{20} are used to classify asthma severity as follows: $PC_{20}<1.0$ as moderate to severe, $1.0\leq PC_{20}<4.0$ as mild, $4.0\leq PC_{20}\leq 16.0$ mg/mL as borderline BHR.[3] Although $PC_{20}>16$ mg/mL is defined as a normal BHR,[3] cutoff values of methacholine PC_{20} between 21.1 and 24.6 mg/mL have been suggested as levels indicative of asthma in adolescents aged 11 to 15 years. Although a relatively small number of subjects and other factors in the older age group might affect the cutoff values, the lower and upper bounds of the 95% confidence interval of these AUCs, which were more than 0.83, are considered reliable.[40]

MCTs are usually performed to evaluate the likelihood of asthma in patients in whom diagnosis is suggested by current but non-obvious asthma symptoms. To estimate the diagnosis exactly using ROC methods, an accurate diagnosis is essential for analysis.[40] In our present study, experienced pediatric allergists assessed whether the symptoms were truly and accurate for the diagnosis of asthma. Because symptoms suggestive of asthma are diverse and nonspecific in some cases, the cutoff values of methacholine PC_{20} for the diagnosis of asthma suggested in our current study might better reflect the real clinical situation than the uniform application of cutoff values recommended by the ATS guidelines.

This study has a few limitations. Although several factors such as sex, season, and air pollution can affect the results of MCTs,[31,41] we did not consider these factors because of the random timing of hospital visits and the small sample size when our patients were stratified by sex in the group suspected of being asthmatic. Otherwise, we performed the MCTs over an interval of several days in a general healthy population living in a city to minimize the seasonal variations. We assigned a value of 50 mg/mL to subjects who did not respond to methacholine at the highest doses administered; therefore, the distribution of methacholine PC_{20} may not reflect the actual value in relevant subjects, especially in healthy children without respiratory symptoms.

In conclusion, the cutoff values of PC_{20} for the diagnosis of asthma increase with age in children and adolescents. Age-dependent careful interpretation of PC_{20} in MCTs might be more practical for the diagnosis of asthma in children and adolescents with asthma like symptoms, which were assessed as consistent with asthma by pediatric allergists.

Funding: This research was supported by a fund (2014-ER5602-00) from Research of Korea Centers for Disease Control and Prevention.

Ethical approval: This study was performed according to the guideline of Asan Medical Center, which abides by the Helsinki Declaration on ethical principles for medical research.

Competing interest: The authors declare that they have no competing interests.

Contributors: Hong SJ (Email: sjhong@amc.seoul.kr) and Kwon JW contributed equally to this work as co-corresponding authors. Hong SJ and Kwon JW designed and supervised the execution of the study. Lee E and Han S performed the analyses. Lee E wrote the manuscript. Kim YH, Yang SI, Jung YH, Seo JH, Kim HB, and Lee SY participated in the interpretations.

References

1 van Aalderen WM. Childhood asthma: diagnosis and treatment. Scientifica (Cairo) 2012;2012:674204.
2 Warrier PJ, Bellamkonda P, Stokes JR, Casale TB. Utility and limitations of objective measures of asthma. Ann Allergy Asthma Immunol 2009;102:518-522.
3 Crapo RO, Casaburi R, Coates AL, Enright PL, Hankinson JL, Irvin CG, et al. Guidelines for methacholine and exercise challenge testing-1999. This official statement of the American Thoracic Society was adopted by the ATS Board of Directors, July 1999. Am J Respir Crit Care Med 2000;161:309-329.
4 Trigg CJ, Bennett JB, Tooley M, Sibbald B, D'Souza MF, Davies RJ. A general practice based survey of bronchial hyperresponsiveness and its relation to symptoms, sex, age, atopy, and smoking. Thorax 1990;45:866-872.
5 O'Connor G, Sparrow D, Taylor D, Segal M, Weiss S. Analysis of dose-response curves to methacholine. An approach suitable for population studies. Am Rev Respir Dis 1987;136:1412-1417.
6 Cerveri I, Bruschi C, Zoia MC, Zanon P, Maccarini L, Grassi M, et al. Distribution of bronchial nonspecific reactivity in the general population. Chest 1988;93:26-30.
7 Perpina M, Pellicer C, de Diego A, Compte L, Macian V. Diagnostic value of the bronchial provocation test with methacholine in asthma. A Bayesian analysis approach. Chest 1993;104:149-154.
8 Jayet PY, Schindler C, Kunzli N, Zellweger JP, Brandli O, Perruchoud AP, et al. Reference values for methacholine reactivity (SAPALDIA study). Respir Res 2005;6:131.
9 Kim BJ, Seo JH, Jung YH, Kim HY, Kwon JW, Kim HB, et al. Air pollution interacts with past episodes of bronchiolitis in the development of asthma. Allergy 2013;68:517-523.
10 Kim WK, Kwon JW, Seo JH, Kim HY, Yu J, Kim BJ, et al. Interaction between IL13 genotype and environmental factors in the risk for allergic rhinitis in Korean children. J Allergy Clin Immunol 2012;130:421-426.e5.
11 Hewitt DJ. Interpretation of the "positive" methacholine challenge. Am J Ind Med 2008;51:769-781.
12 Avital A, Noviski N, Bar-Yishay E, Springer C, Levy M, Godfrey S. Nonspecific bronchial reactivity in asthmatic children depends on severity but not on age. Am Rev Respir Dis 1991;144:36-38.
13 Tantisira KG, Colvin R, Tonascia J, Strunk RC, Weiss ST, Fuhlbrigge AL, et al. Airway responsiveness in mild to moderate childhood asthma: sex influences on the natural history. Am J Respir Crit Care Med 2008;178:325-331.
14 Mead J. Dysanapsis in normal lungs assessed by the relationship

between maximal flow, static recoil, and vital capacity. Am Rev Respir Dis 1980;121:339-342.
15 Hibbert M, Lannigan A, Raven J, Landau L, Phelan P. Gender differences in lung growth. Pediatr Pulmonol 1995;19:129-134.
16 Parker AL, Abu-Hijleh M, McCool FD. Ratio between forced expiratory flow between 25% and 75% of vital capacity and FVC is a determinant of airway reactivity and sensitivity to methacholine. Chest 2003;124:63-69.
17 Tan KS, McFarlane LC, Lipworth BJ. Loss of normal cyclical beta 2 adrenoceptor regulation and increased premenstrual responsiveness to adenosine monophosphate in stable female asthmatic patients. Thorax 1997;52:608-611.
18 Lieberman D, Kopernic G, Porath A, Levitas E, Lazer S, Heimer D. Influence of estrogen replacement therapy on airway reactivity. Respiration 1995;62:205-208.
19 Tan KS, McFarlane LC, Lipworth BJ. Paradoxical down-regulation and desensitization of beta2-adrenoceptors by exogenous progesterone in female asthmatics. Chest 1997;111:847-851.
20 Cockcroft DW, Davis BE, Todd DC, Smycniuk AJ. Methacholine challenge: comparison of two methods. Chest 2005;127:839-844.
21 Cockcroft DW, Davis BE. The bronchoprotective effect of inhaling methacholine by using total lung capacity inspirations has a marked influence on the interpretation of the test result. J Allergy Clin Immunol 2006;117:1244-1248.
22 Salome CM, Xuan W, Gray EJ, Belooussova E, Peat JK. Perception of airway narrowing in a general population sample. Eur Respir J 1997;10:1052-1058.
23 Hopp RJ, Townley RG, Biven RE, Bewtra AK, Nair NM. The presence of airway reactivity before the development of asthma. Am Rev Respir Dis 1990;141:2-8.
24 Jansen DF, Timens W, Kraan J, Rijcken B, Postma DS. (A) symptomatic bronchial hyper-responsiveness and asthma. Respir Med 1997;91:121-134.
25 Zhong NS, Chen RC, Yang MO, Wu ZY, Zheng JP, Li YF. Is asymptomatic bronchial hyperresponsiveness an indication of potential asthma? A two-year follow-up of young students with bronchial hyperresponsiveness. Chest 1992;102:1104-1109.
26 Laprise C, Boulet LP. Asymptomatic airway hyperresponsiveness: a three-year follow-up. Am J Respir Crit Care Med 1997;156:403-409.
27 Pin I, Radford S, Kolendowicz R, Jennings B, Denburg JA, Hargreave FE, et al. Airway inflammation in symptomatic and asymptomatic children with methacholine hyperresponsiveness. Eur Respir J 1993;6:1249-1256.
28 Brand PL. Airway inflammation in symptomatic and asymptomatic children with methacholine hyperresponsiveness. Eur Respir J 1994;7:829.
29 de Gooijer A, Brand PL, Gerritsen J, Koeter GH, Postma DS, Knol K. Changes in respiratory symptoms and airway hyperresponsiveness after 27 years in a population-based sample of school children. Eur Respir J 1993;6:848-854.
30 Henderson JC, O'Connell F, Fuller RW. Decrease of histamine induced bronchoconstriction by caffeine in mild asthma. Thorax 1993;48:824-826.
31 Cheung D, Dick EC, Timmers MC, de Klerk EP, Spaan WJ, Sterk PJ. Rhinovirus inhalation causes long-lasting excessive airway narrowing in response to methacholine in asthmatic subjects *in vivo*. Am J Respir Crit Care Med 1995;152:1490-1496.
32 Clough JB, Hutchinson SA, Williams JD, Holgate ST. Airway response to exercise and methacholine in children with respiratory symptoms. Arch Dis Child 1991;66:579-583.
33 Salo PM, Arbes SJ Jr, Jaramillo R, Calatroni A, Weir CH, Sever ML, et al. Prevalence of allergic sensitization in the United States: results from the National Health and Nutrition Examination Survey (NHANES) 2005-2006. J Allergy Clin Immunol 2014;134:350-359.
34 Bodtger U. Prognostic value of asymptomatic skin sensitization to aeroallergens. Curr Opin Allergy Clin Immunol 2004;4:5-10.
35 Godfrey S, Springer C, Noviski N, Maayan C, Avital A. Exercise but not methacholine differentiates asthma from chronic lung disease in children. Thorax 1991;46:488-492.
36 Avital A, Springer C, Bar-Yishay E, Godfrey S. Adenosine, methacholine, and exercise challenges in children with asthma or paediatric chronic obstructive pulmonary disease. Thorax 1995;50:511-516.
37 Gilbert R, Auchincloss JH Jr. Post-test probability of asthma following methacholine challenge. Chest 1990;97:562-565.
38 Motomura C, Odajima H, Tezuka J, Murakami Y, Moriyasu Y, Kando N, et al. Effect of age on relationship between exhaled nitric oxide and airway hyperresponsiveness in asthmatic children. Chest 2009;136:519-525.
39 Anderson SD. Provocative challenges to help diagnose and monitor asthma: exercise, methacholine, adenosine, and mannitol. Curr Opin Pulm Med 2008;14:39-45.
40 Zou KH, O'Malley AJ, Mauri L. Receiver-operating characteristic analysis for evaluating diagnostic tests and predictive models. Circulation 2007;115:654-657.
41 Fruchter O, Yigla M. Seasonal variability of the methacholine challenge test. J Asthma 2009;46:951-954.

Received December 9, 2015
Accepted after revision March 30, 2016