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## Effect of lowering Methacholine Challenge Test cutoff in children

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### ABSTRACT

**Background:** The diagnosis of asthma is based on clinical judgment, history of personal or familial atopy, and testing, typically with a methacholine challenge test (MCT). Guidelines suggest a provocation concentration that caused a decrease in forced expiratory volume in 1 second of 20% (PC<sub>20</sub>) cutoff of 4 mg/mL for a positive test result.

**Objective:** To investigate the effect of lowering the MCT PC<sub>20</sub> cutoff from 8 to 4 mg/mL on the number of positive test results and the distribution of test results.

**Methods:** A retrospective study was conducted at the Montreal Children's Hospital from January 1, 2006, through June 31, 2012, on patients referred by nonrespiratory physicians. A 2-minute tidal breathing dosing protocol was used, and the PC<sub>20</sub> was calculated by linear interpolation.

**Results:** A total of 748 patients were tested using spirometry. A total of 134 (17.9%) had a negative MCT result, and 614 (81.1%) responded at 8 mg/mL or less. A total of 570 patients (92.8% of respondents) responded at a dose of 4 mg/mL or higher (median PC<sub>20</sub> of 0.47 mg/mL), with the remainder (7.2% of respondents) responding at a dose between 4 and 8 mg/mL (median PC<sub>20</sub> of 6.37 mg/mL). There was no difference in the number of positive test results between the sexes, regardless of cutoff. The sensitivity of MCT was 82.1% at a cutoff of 8 mg/mL and 76.2% at 4 mg/mL. With a pretest likelihood of asthma of 75%, the specificity was 71.2%.

**Conclusion:** In a standard pediatric referral population, using a PC<sub>20</sub> cutoff of 4 mg/mL provided a sensitivity of 76.2%, and only excluded 5.8% of all those referred for suspicion of asthma (7.2% of all test results were ≤8 mg/mL). This finding suggests that a PC<sub>20</sub> of 4 mg/mL can reasonably be used as a cutoff for a positive MCT result in children.

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### Introduction

The diagnosis of asthma is based on clinical judgment, history of personal or familial atopy, and testing, typically with a methacholine challenge test (MCT). The MCT measures airway hyperresponsiveness, and standards have been published.<sup>1,2</sup> The test assesses the provocation concentration that caused a decrease in forced expiratory volume in 1 second (FEV<sub>1</sub>) of 20% (PC<sub>20</sub>).<sup>3</sup> Moderate to severe bronchial hyperreactivity is defined as a PC<sub>20</sub> less than 1 mg/mL, mild bronchial hyperreactivity as a PC<sub>20</sub> of 1.0 to 4.0 mg/mL, and borderline bronchial hyperreactivity as a PC<sub>20</sub> of 4.0 to 16.0 mg/mL.<sup>1</sup>

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The American Thoracic Society use data from adult studies to construct a receiver operating curve, taking into account pretest probability of asthma, to estimate the likelihood of the patient having asthma based on the PC<sub>20</sub>. A PC<sub>20</sub> of 8 mg/mL decreased on the diagonal, suggesting that a value of 8 mg/mL did not aid in making the diagnosis, and the guidelines suggested using a cutoff of 4 mg/mL for a positive test result. Despite this, many pediatric studies continue to use cutoffs of 4 to 16 mg/mL.<sup>2–5</sup>

Two studies have looked at cutoff values in children. The first study used physician assessments for pretest probability of asthma.<sup>6</sup> However, these physicians were allergists or allergy trainees, who performed an extensive structured history and physical examination related to personal and familial history of allergy and atopy, use of asthma medications, and prior diagnosis of asthma, and a structured physical examination. These authors found that a cutoff of 4 mg/mL was moderately sensitive in girls, regardless of whether there was a history of atopy (71%) or not (77%), and moderately sensitive in boys with a history of atopy (67%). However, 52% of the children defined as healthy (ie, no history or physical examination suggestive of asthma or rhinitis and negative skin prick test result) had a methacholine response of

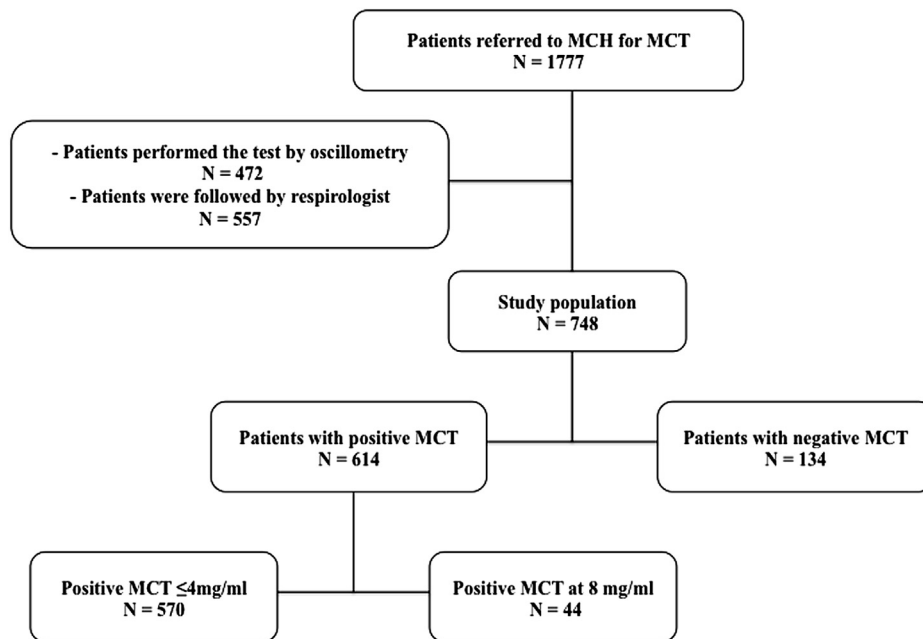


**Figure 1.** Selection of study population. MCT, methacholine challenge test.

8 mg/mL or less. This finding likely contributed to the low reported specificity. The second study evaluated a group of 7-year-olds being followed up because of a high risk of asthma.<sup>7</sup> Physician-diagnosed asthma was made by a single allergist based on symptoms, use of medications, and physical examination findings. These authors found an optimal cutoff of 3 mg/mL, with a sensitivity of 80%, in this population. In their asthmatic population, 92% responded at or below 8 mg/mL, with 11% responding between 4 and 8 mg/mL. In the nonasthmatic population, 73% responded at or below 8 mg/mL, with 15.5% responding between 4 and 8 mg/mL.

Certainly in patients at high risk for asthma, a positive test result is more likely, and in those with allergic rhinitis, a test result between 4 and 8 mg/mL is frequent.<sup>8–13</sup> It is unclear what happens in an unselected population, largely referred from general pediatric and family medicine physicians, rather than a population referred by second-line respiratory specialists. We hypothesized that lowering the threshold of a positive MCT result from 8 to 4 mg/mL would not significantly change the frequency of respondents and nonrespondents. Specifically, we hypothesized that lowering the threshold from 8 to 4 mg/mL would misclassify less than 10% of referrals. The following retrospective study was conducted to investigate the effect of lowering the cutoff from 8 to 4 mg/mL on the sensitivity, number of positive test results, and the distribution of test results in an unselected pediatric population referred to the hospital pulmonary function laboratory by nonrespiratory physicians for MCTs.

## Methods

Study participants consisted of all patients referred by their physicians to the Montreal Children's Hospital for MCTs from January 1, 2006, through June 31, 2012. The request for testing was made without additional information concerning symptoms or severity as to why asthma was suspected in the referred patient. All patients needed to be older than 4 years, to be able to perform spirometry according to American Thoracic Society standards,<sup>1</sup> and to have a baseline FEV<sub>1</sub> of at least 70% predicted.<sup>8,14</sup> The study was approved by the research ethics review committee of the institution. No patient consent was required because the study used retrospective laboratory data with all patient identifiers removed.

Patients needed to be free of factors that could increase bronchial hyperreactivity, including current airway infection or use of antibiotics in the previous 2 weeks. Patients were advised to stop taking the following medications before testing: short-acting  $\beta_2$ -agonists for 6 hours, long-acting  $\beta_2$ -agonists and leukotriene receptor antagonists for 24 hours, short-acting antihistamines for 4 days, and long-acting antihistamines for 15 days.

## Methacholine Challenge Test

Spirometry (Jaeger APS; CareFusion Respiratory Care, California) was conducted using a Hans-Rudolph nonbreathing valve (Hand Rudolph Inc. Kansas City Missouri) attached to a Misty-Neb Medication nebulizer set (CareFusion Respiratory Care) with a flow meter at 7 L/min. A 2-minute tidal breathing dosing protocol was used based on Canadian Thoracic Society and American Thoracic Society recommendations. Doubling concentrations of methacholine were delivered, starting with a concentration of 0.25 mg/mL up to 8 mg/mL or a 20% decrease in FEV<sub>1</sub> from baseline. A starting concentration of 0.06 mg/mL was used if (1) the baseline forced expiratory flow between 25% and 75% (FEF<sub>25%-75%</sub>) was equal or less than 50% predicted in patients 8 years or older, (2) the baseline FEF<sub>25%-75%</sub> was equal or less than 65% in patients 6 to 8 years of age, or (3) the patient was younger than 6 years. This was done because lower FEF<sub>25%-75%</sub> values at baseline suggest decreased flows at low lung volumes, due to more narrow airways, and an increased risk for a greater decrease in FEV<sub>1</sub> with methacholine provocation.

At the end of the test, nebulized salbutamol (2.5 mg) was administered. If the FEV<sub>1</sub> did not return to within 5% of the baseline value, spirometry was performed again 5 minutes later. If the FEV<sub>1</sub> was still less than 5% of baseline, the patient was evaluated by a respiratory physician, and a second treatment with salbutamol was administered, after which spirometry was performed again. Oxygen saturation was monitored throughout the challenge and for at least 10 minutes after the challenge and continued until values were greater than 95%.

The PC<sub>20</sub> was calculated using a linear interpolation<sup>15</sup> with the following formula to include all patients who responded at the first dose of methacholine:  $PC_{20} = (20 - R1) (C2 - C1) + C1$

**Table 1**  
Baseline characteristics of the study population

| Characteristic               | Positive MCT result | Negative MCT result |
|------------------------------|---------------------|---------------------|
| No. (%) of patients          | 614 (82.1)          | 134 (17.9)          |
| Age, mean (SD), y            | 11.4 (2.97)         | 12.57 (2.79)        |
| Height, mean (SD), cm        | 149.55 (15.98)      | 156.67 (15.62)      |
| Sex, No. (%)                 |                     |                     |
| Male                         | 271 (44.1)          | 56 (44.1)           |
| Female                       | 343 (55.9)          | 75 (55.9)           |
| Ethnicity (n = 728), No. (%) |                     |                     |
| White                        | 545 (91)            | 119 (92.2)          |
| Black                        | 34 (5.7)            | 3 (2.32)            |
| Asian                        | 11 (1.8)            | 4 (3.1)             |
| East Indian                  | 9 (1.5)             | 3 (2.32)            |

Abbreviation: MCT, methacholine challenge test.

(R2–R1), with C1 indicating methacholine concentration before FEV<sub>1</sub> decreased by 20%, C2 indicating methacholine concentration after FEV<sub>1</sub> decreased by 20%, R1 indicating the percentage decrease in FEV<sub>1</sub> at C1, and R2 indicating the percentage decrease in FEV<sub>1</sub> at C2.

### Statistical Analysis

Patient baseline characteristics were expressed as mean (SD). The PC<sub>20</sub> values were expressed as mean (SD) and median values. Sex differences for PC<sub>20</sub> values were compared by nonparametric comparison using the Mann-Whitney Test. Sensitivity of different cutoff values of PC<sub>20</sub> was calculated. Sex differences for sensitivities were compared using the Mann-Whitney Test.  $P < .05$  was considered significant. SPSS statistics program (IBM SPSS Statistic, version 20; IBM Corporation, Somers, New York) was used for statistical analysis.

### Results

A total of 1777 patients were referred to the laboratory for testing (Fig 1). A total of 1029 patients were excluded, including 472 who could not properly perform spirometry and who were subsequently evaluated by impulse oscillometry, and 557 who were followed up by a respirologist because our hypothesis was based on an unselected population referred from general pediatric and family medicine physicians.

The remaining 748 patients were included in the analysis. Baseline characteristics are listed in Table 1. A total of 134 patients (17.9%) had a negative MCT result (<20% decrease in FEV<sub>1</sub> at a methacholine concentration of 8 mg/mL).

For the 614 patients who responded at 8 mg/mL or less, the mean PC<sub>20</sub> was 1.18 (1.63) mg/mL, with a median of 0.5 mg/mL. A total of 570 respondents (93%) responded at a dose of 4 mg/mL or less, 551 respondents (89.7%) responded at a dose of 3 mg/mL or less, and 439 respondents (71.5%) responded at a dose of 1 mg/mL or less (Fig 2). Use of a cutoff of 4 mg/mL would only miss 5.8% of all referred patients (7.2% of all positive test results), whereas a cutoff of 3 mg/mL would miss 8.4% of all referred patients.

In those responding at a dose of less than or equal to 4 mg/mL, the mean PC<sub>20</sub> was 0.79 (0.79) mg/mL, with a median of 0.47 mg/mL. For those responding between 4 and 8 mg/mL, the mean PC<sub>20</sub> was 6.23 (1.2) mg/mL, with a median of 6.37 mg/mL.

Of all respondents, 271 were male (44.1%), with a mean PC<sub>20</sub> of 1.11 (1.52) mg/mL and a median of 0.5 mg/mL, whereas 343 (55.9%) were female, with mean PC<sub>20</sub> of 1.23 (1.71) mg/mL and a median of 0.53 mg/mL. The distribution of PC<sub>20</sub> was not different between boys and girls responding at a dose less than or equal to 4 mg/mL and among those responding to a dose between 4 and 8 mg/mL (Mann-Whitney test,  $P > .10$ ) (Fig 2). Similar distributions were also seen using a cutoff of 3 mg/mL (Table 2).

A recent study of adult referrals found that a 10% decrease in FEV<sub>1</sub> (PC<sub>10</sub>) at 1 mg/mL was predictive of a PC<sub>20</sub> of 8 mg/mL or higher.<sup>16</sup> Use of PC<sub>10</sub> at 1 mg/mL as predictive of a positive MCT result at a cutoff of 4 mg/mL or less revealed 96.4% sensitivity, 90% positive predictive value, and 85% negative predictive value. At a cutoff of 8 mg/mL, there was 92.8% sensitivity, 94% positive predictive value, and 69% negative predictive value.

Assuming a diagnosis of asthma by the referring physician, the sensitivity of the MCT was 82.1% at a cutoff of 8 mg/mL, 76.2% at 4 mg/mL, and 73.7% at 3 mg/mL. The sensitivity of the MCT was similar in male and female patients using cutoff points of 3 mg/mL (73.9% vs 72.7%) and 4 mg/mL (76.7% vs 75.1%). With a 50% likelihood of asthma, the sensitivity and specificity of the MCT were 88.2% and 35.6% at a cutoff of 4 mg/mL and 80% and 35.6% at 3 mg/mL, respectively. With a 75% likelihood, the sensitivity and specificity were 92.1% and 71.2% at a cutoff of 4 mg/mL and 88.7% and 71.2% at 3 mg/mL, respectively. Use of a receiver operating curve and a 75% likelihood of asthma, the optimal cutoff (shortest distance from a specificity and sensitivity of 1) was 3 mg/mL. Again, the sensitivity of the MCT did not differ between males and females using either a 3- or 4-mg/mL cutoff.

### Discussion

In a standard pediatric population of patient referred from nonrespiratory physicians, we found that most children (92.8%) who respond to a methacholine dose of 8 mg/mL or less will respond at a dose at or below 4 mg/mL. In fact, most responders (71.5%) reacted at a dose of less than or equal to 1 mg/mL, consistent with moderate to severe bronchial hyperreactivity.

Using a dosimeter technique, Joseph-Bowen et al<sup>13</sup> found that a cutoff of 3.9 mg/mL had the best sensitivity and specificity in a birth cohort studied between the ages of 5 and 7 years. However, 60% of those not previously diagnosed as having asthma had a positive response at a dose less than 8 mg/mL. The authors suggested that the delivered dose of methacholine might be too large in smaller patients taking smaller breaths.

Using the same technique as the present study, 2 other studies considered children who underwent a thorough assessment for risk of atopy and asthma before MCTs. In 8-year-olds, a cutoff of 4 mg/mL or less seemed optimal.<sup>6</sup> In 7-year-olds at high risk for asthma, a cutoff of 3 mg/mL seemed optimal. The results of the current study, where patients were unselected and atopy status was unknown, are consistent with these prior studies. Furthermore, they are consistent with the results of the study by Godfrey et al,<sup>17</sup> who combined previous studies to derive a cutoff of 6.6  $\mu$ mol or 3.3 mg/mL to distinguish asthmatic patients from healthy individuals, with a sensitivity of 92% and a specificity of 89%.

However, in the study by Liem et al,<sup>6</sup> significant differences were found in the utility of the MCT, depending on sex and presence of atopy. They found, in girls, that a PC<sub>20</sub> of 4 mg/mL or less was moderately sensitive regardless of whether there was a history of atopy or not. In atopic boys, a PC<sub>20</sub> of 4 mg/mL or less was moderately sensitive. In nonatopic boys, the MCT was not useful for the diagnosis of asthma. They emphasized that atopy was the best predictor for persistent asthma.

Unlike Carlsten et al,<sup>7</sup> the current study did not find any sex differences in the distribution of PC<sub>20</sub> or its sensitivity and specificity. Furthermore, there were no sex differences with respect to sensitivity using the different cutoff points of 3, 4, or 8 mg/mL.

Previous studies found that MCT results could be positive in healthy individuals<sup>17</sup> and in patients with different medical conditions, such as allergic rhinitis, chronic bronchitis, cystic fibrosis, chronic obstructive pulmonary disease, and atopy.<sup>8–11</sup> Nieminen et al<sup>10</sup> similarly found that patients with rhinitis or chronic bronchitis reacted at higher doses of methacholine. Furthermore,

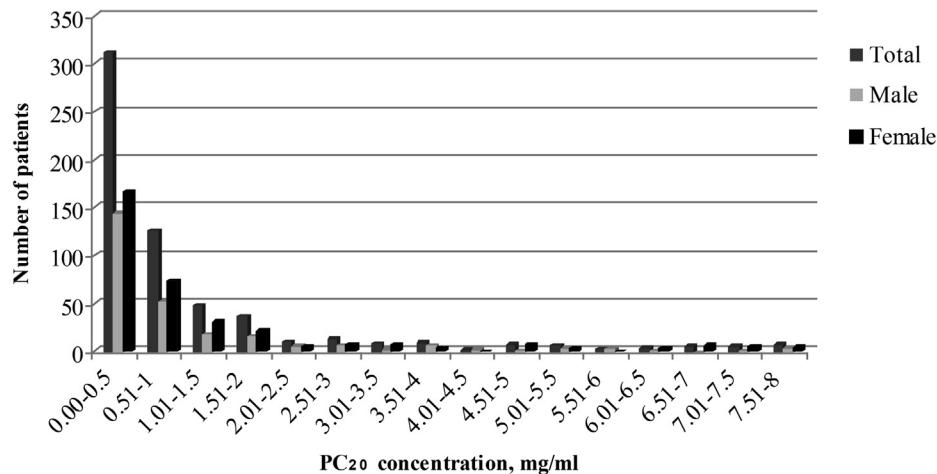


**Figure 2.** Frequency of provocation concentration that caused a decrease in forced expiratory volume in 1 second of 20% (PC<sub>20</sub>) among patients with positive methacholine challenge test (MCT) results.

Laprise et al<sup>18</sup> conducted a cohort study that found that asymptomatic individuals could have a PC<sub>20</sub> greater than 4 mg/mL at baseline, with the possibility that some of them could develop asthma in the future. Accordingly, patients with test results greater than 4 mg/mL could have an alternative diagnosis to asthma or develop asthma in the future.

Reducing the test to stop at 4 mg/mL would save time. Other protocols have been proposed to reduce testing time. Cockcroft et al<sup>19</sup> proposed that the protocol could be significantly shortened based on baseline lung function, asthma medication, and clinical features. More recently Segel et al<sup>16</sup> suggested from their study of a young military male adult population with symptoms suggestive of asthma and normal spirometry, a history of asthma, or first-degree relative with asthma that a decrease of 10% or more in FEV<sub>1</sub> at a methacholine dose of 1 mg/mL could reasonably be considered a positive test result with a positive predictive value of 97%, a negative predictive value of 91%, a specificity of 98%, and a sensitivity of 86%. Similarly in our unselected pediatric population at a cutoff 4 mg/mL or less, a PC<sub>10</sub> at 1 mg/mL had high sensitivity and positive predictive value (90%).<sup>20</sup> The nature and severity of symptoms in the patients of the current analysis were not provided at the time of request for testing.

Our study was purposefully limited to patients referred by their primary physician to reflect a general referral population. Our study is limited in that the clinical history of the patients and their risk of having atopic disease, asthma, or other conditions that could affect MCTs were not evaluated. Further, the MCT was not performed at a

concentration greater than 8 mg/mL (ie, 16 mg/mL) to be able to classify patients as truly having negative results.

The present results support that a cutoff of 4 mg/mL or less would identify most patient suspected of having asthma and exclude only a small percentage (5.8%) of the population suspected of having asthma. Furthermore, there does not appear to be a need for sex-specific cutoffs. A 10% decrease in FEV<sub>1</sub> at a concentration of 10 mg/mL indicates that the test could be further shortened.

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**Table 2**  
Distribution of PC<sub>20</sub> according to sex

| PC <sub>20</sub>           | No. (%) of patients | Mean (SD), mg/mL | Median, mg/mL |
|----------------------------|---------------------|------------------|---------------|
| PC <sub>20</sub> ≤ 4 mg/mL |                     |                  |               |
| Male                       | 253 (93.35)         | 0.77 (0.8)       | 0.46          |
| Female                     | 314 (91.54)         | 0.77 (0.73)      | 0.48          |
| PC <sub>20</sub> > 4 mg/mL |                     |                  |               |
| Male                       | 18 (6.64)           | 5.83 (1.42)      | 5.49          |
| Female                     | 29 (8.45)           | 6.25 (1.2)       | 6.57          |
| PC <sub>20</sub> ≤ 3 mg/mL |                     |                  |               |
| Male                       | 244 (90)            | 0.67 (0.62)      | 0.43          |
| Female                     | 304 (88.6)          | 0.69 (0.58)      | 0.36          |
| PC <sub>20</sub> > 3 mg/mL |                     |                  |               |
| Male                       | 27 (9.96)           | 5.05 (1.61)      | 4.6           |
| Female                     | 39 (11.37)          | 5.49 (1.68)      | 5.33          |

Abbreviation: PC<sub>20</sub>, provocation concentration that caused a decrease in forced expiratory volume in 1 second of 20%.

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