

Methacholine challenge test: Diagnostic characteristics in asthmatic patients receiving controller medications

Kaharu Sumino, MD, MPH,^a Elizabeth A. Sugar, PhD,^b Charles G. Irvin, PhD,^c David A. Kaminsky, MD,^c Dave Shade, JD,^b Christine Y. Wei, MS,^b Janet T. Holbrook, PhD, MPH,^b Robert A. Wise, MD,^d and Mario Castro, MD, MPH,^a for the American Lung Association Asthma Clinical Research Centers* *St Louis, Mo, Baltimore, Md, and Burlington, Vt*

Background: The methacholine challenge test (MCT) is commonly used to assess airway hyperresponsiveness, but the diagnostic characteristics have not been well studied in asthmatic patients receiving controller medications after the use of high-potency inhaled corticosteroids became common.

Objectives: We investigated the ability of the MCT to differentiate participants with a physician's diagnosis of asthma from nonasthmatic participants.

Methods: We conducted a cohort-control study in asthmatic participants (n = 126) who were receiving regular controller medications and nonasthmatic control participants (n = 93) to evaluate the sensitivity and specificity of the MCT.

Results: The overall sensitivity was 77% and the specificity was 96% with a threshold PC₂₀ (the provocative concentration of methacholine that results in a 20% drop in FEV₁) of 8 mg/mL. The sensitivity was significantly lower in white than in African American participants (69% vs 95%, *P* = .015) and higher in atopic compared with nonatopic (82% vs 52%, *P* = .005).

Increasing the PC₂₀ threshold from 8 to 16 mg/mL did not noticeably improve the performance characteristics of the test. African American race, presence of atopy, and lower percent predicted FEV₁ were associated with a positive test result.

Conclusions: The utility of the MCT to rule out a diagnosis of asthma depends on racial and atopic characteristics. Clinicians should take into account the reduced sensitivity of the MCT in white and nonatopic asthmatic patients when using this test for the diagnosis of asthma. (*J Allergy Clin Immunol* 2012;■■■:■■■-■■■.)

Key words: Asthma diagnosis, inhaled corticosteroids, race, atopy

From ^athe Department of Medicine, Division of Pulmonary and Critical Care Medicine, Washington University School of Medicine, St Louis; ^bthe Department of Epidemiology, Johns Hopkins Bloomberg School of Public Health, Baltimore; ^cthe Department of Medicine, Division of Pulmonary Disease and Critical Care Medicine, University of Vermont College of Medicine, Burlington; and ^dthe Department of Medicine, Division of Pulmonary and Critical Care Medicine, Johns Hopkins University School of Medicine, Baltimore.

*A list of American Lung Association–Asthma Clinical Research Center participants is available in Appendix E1 in this article's Online Repository at www.jacionline.org. ClinicalTrials.gov identifier NCT00705341.

Supported by the American Lung Association. Drugs were donated by Methapharm (Provocholine) and GlaxoSmithKline (Ventolin HFA and Flovent). Neither Methapharm nor GlaxoSmithKline played any role in the design, conduct, data analysis, or interpretation of the study.

Disclosure of potential conflict of interest: K. Sumino receives research support from the National Institutes of Health, the US Department of Veterans Affairs, and the American Lung Association. E. A. Sugar receives research support from the American Lung Association, Methapharm, and GlaxoSmithKline. C. G. Irvin is on the speakers' bureau for Merck, has consultant arrangements with Methapharm, and receives research support from the National Institutes of Health and the American Lung Association–Asthma Clinical Research Center. D. A. Kaminsky is on the American Thoracic Society Proficiency Standards Committee. J. T. Holbrook receives research support from the American Lung Association, Methapharm, and GlaxoSmithKline. R. A. Wise has consultant arrangements with and is an advisor for GlaxoSmithKline, Boehringer-Ingelheim, AstraZeneca, Novartis, and Pfizer and receives research support from the American Lung Association, the National Institutes of Health, GlaxoSmithKline, and Boehringer-Ingelheim. M. Castro has consultant arrangements with Asthmatrix; is on the speakers' bureau for Asthmatrix, Genentech, Astra-Zeneca, Merck, and GlaxoSmithKline; is on an Advisory Board for Genentech; receives royalties from Elsevier; and receives research support from Asthmatrix, Amgen, Cephalon/Cephalon, Genentech, MedImmune, Merck, Novartis, the National Institutes of Health, and GlaxoSmithKline. The rest of the authors declare that they have no relevant conflicts of interest.

Received for publication August 23, 2011; revised February 15, 2012; accepted for publication February 21, 2012.

Corresponding author: Kaharu Sumino, MD, MPH, Washington University School of Medicine, 660 Euclid Ave, Campus Box 8052, St Louis, MO 63110. E-mail: ksumino@dom.wustl.edu.

0091-6749/\$36.00

© 2012 American Academy of Allergy, Asthma & Immunology
doi:10.1016/j.jaci.2012.02.025

The methacholine challenge test (MCT) is widely used to evaluate airway hyperresponsiveness (AHR) in patients with unexplained dyspnea when the diagnosis of asthma is uncertain. Asthmatic patients usually demonstrate a large decrease in lung function to an inhaled concentration of methacholine that causes little or no change in those without asthma. In clinical studies in asthmatic patients, PC₂₀ (the provocative concentration of methacholine that results in a 20% drop in FEV₁) is often used as one of the inclusion criteria or as an outcome measure.¹⁻⁴

Previous clinical studies have reported a wide range of values for the sensitivity of the MCT for detecting asthma.⁵⁻¹⁴ However, based mainly on selected older clinical trials,^{5,6,9} this test is often interpreted by clinicians to have a high sensitivity and negative predictive value. The American Thoracic Society (ATS) guidelines on MCT state that “if the prior probability of asthma is 30-70% and the PC₂₀ is >16 mg/mL, it may be stated with a high degree of confidence that the patient does not currently have asthma.”¹⁵ Hence a negative MCT result has been generally interpreted as indicating that it is unlikely that a patient with unexplained respiratory symptoms has asthma. However, in previous studies evaluating the ability of the MCT to diagnose asthma, many patients were not receiving asthma controller medications.

We have observed that many potential participants evaluated for American Lung Association–Asthma Clinical Research Center clinical trials were disqualified because their PC₂₀ was greater than 16 mg/mL, despite having a clinical history of physician-diagnosed asthma and receiving asthma treatment. We

Abbreviations used

AHR: Airway hyperresponsiveness
 ATS: American Thoracic Society
 ICS: Inhaled corticosteroid
 MCT: Methacholine challenge test

hypothesized that these results might be false-negative MCTs due to the increase in the use of high-potency inhaled corticosteroids (ICSs) in the treatment of asthma in recent years, since the dissemination of the National Asthma Education Prevention Program guidelines (Expert Panel Report 2)¹⁶ because high-potency ICSs can reduce AHR.^{17,18} Therefore we investigated the ability of the MCT to differentiate nonasthmatic participants from participants with physician-diagnosed asthma receiving controller treatment.

METHODS

Study design and participant selection

We conducted a cross-sectional, cohort-control study of asthmatic patients and nonasthmatic control participants to measure the sensitivity and specificity of the MCT. The study was conducted at 15 centers in the American Lung Association–Asthma Clinical Research Centers from December 2008 until May 2010. Asthmatic and nonasthmatic participants completed prebronchodilator spirometry, MCTs, allergy skin testing, and the Asthma Control Questionnaire.¹⁹

Inclusion criteria for asthmatic participants were as follows: age 12 to 69 years; physician-diagnosed stable asthma; current treatment for asthma within the preceding 12 months with regular use of asthma controller medications (regular use of ICSs, leukotriene receptor modifiers, or both); no asthma exacerbation during the prior 4 weeks; and prebronchodilator FEV₁ of 70% of predicted value or greater. For women of childbearing potential, inclusion criteria also included that they were not pregnant and not lactating. Study staff had no knowledge of the results of any prior MCTs. Exclusion criteria for asthmatic participants were use of only as-needed short-acting bronchodilator, chronic oral steroid therapy, and oral corticosteroid use within the past 4 weeks. Inclusion criteria for healthy control participants were age 12 to 69 years and overall good health. We excluded participants with conditions that are associated with AHR, which include a history within the previous 5 years of self-reported, physician-diagnosed allergic disease (allergic rhinitis, hay fever, or atopic dermatitis); asthma, chronic nasal or sinus disease; or other chronic lung diseases and respiratory tract infections within the past 4 weeks. Both asthmatic and healthy control participants were excluded if they had chronic diseases that, in the opinion of the investigator, would prevent participation in the trial and if there was a history of cigarette smoking within the past 5 years or greater than 10 pack years total. Both asthmatic and healthy control participants were recruited from those who responded to advertisements for a research study in the community at each study site.

The study was approved by the institutional review board at each participating center, and all participants provided written informed consent. The protocol was approved by a Data and Safety Monitoring Board appointed by the American Lung Association. The Data and Safety Monitoring Board reviewed the results and adverse events throughout the course of the study. The trial was registered at www.clinicaltrials.gov (NCT00705341).

MCT methods

We used the 5-breath dosimeter method according to the published guidelines of the ATS¹⁵ using a stand-alone dosimeter (Koko dosimeter; nSpire Health, Longmont, Colo). Briefly, participants inhaled increasing concentrations of methacholine by using a nebulizer with an electronically controlled valve that allowed a controlled amount of material to be delivered during the course of 5 inspiratory capacity breaths from functional residual

capacity to total lung capacity. This 5-breath procedure was repeated with increasing concentrations of methacholine until the FEV₁ decreased by at least 20% from the baseline value or until the highest concentration of methacholine (32 mg/mL) had been delivered and the PC₂₀ was calculated. Provocative methacholine, which was donated by Methapharm (Brantford, Ontario, Canada) was diluted to the desired concentration with 0.9% normal saline containing 0.4% phenol as a preservative. The test sequence included 11 steps: diluent only and 0.03125, 0.0625, 0.125, 0.25, 0.5, 1.0, 2.0, 4.0, 8.0, 16.0, and 32.0 mg/mL. All medications and foods that might interact with methacholine were held before the test, including short-acting bronchodilators (6 hours), long-acting bronchodilators (24 hours), leukotriene modifiers (24 hours), antihistamine (48 hours), and theophylline (48 hours). Participants were allowed to continue their ICSs as prescribed.

Quality control measures were implemented centrally by the data-coordinating center, including calibration of each nebulizer cup to deliver 9 μ L $\pm$ 10% per 0.6-second actuation and certification of the technicians who performed the MCT. Certification required submission and review of 5 acceptable tests.

Statistical analysis

An MCT result was considered positive if the PC₂₀ was less than or equal to 8 mg/mL. A cohort of 140 participants (70 asthmatic patients and 70 nonasthmatic subjects) was calculated to provide precision, which was defined as the width of the 95% exact binomial CI, between 0.12 and 0.18 for our estimates of sensitivity and specificity depending on the observed percentage (0.95–0.85). Comparisons between cohorts were made by using Kruskal-Wallis tests for continuous variables and χ^2 or Fisher exact tests for categorical variables. The sensitivity and specificity of the MCTs were computed with exact binomial 95% CIs, and comparisons between subgroups were made by using the Fisher exact test. *P* values (2-sided) of less than .05 were considered statistically significant.

RESULTS

Characteristics of the study participants

A summary of the clinical characteristics of the study participants (126 asthma and 93 healthy control participants) is shown in Table I. Participants with asthma were slightly older; had higher body mass index, reported eczema, sinusitis, rhinitis, food allergy, and gastroesophageal reflux disease more frequently; and had lower lung function and greater allergy skin test reactivity than nonasthmatic control participants. The majority of asthmatic patients (90%) were receiving an ICS alone or in combination with a long-acting β -agonist. Overall, their asthma was suboptimally controlled with an average Asthma Control Questionnaire score of 1.1 (<0.76 is defined as well controlled²⁰). Twenty-three percent reported receiving a prednisone burst and 29% reported having an urgent care visit for asthma in the preceding 12 months.

Sensitivity and specificity of the MCT

MCTs were completed in 125 asthmatic participants and 92 healthy control participants (1 in each group did not complete the testing). The overall sensitivity of the MCT for differentiating asthmatic participants from healthy control participants by using a PC₂₀ of 8 mg/mL or less to define a positive test result was 77%, and the specificity was 96% (Table II). The actual number of positive and negative test results in each group is provided in Table E1 in this article's Online Repository at www.jacionline.org. Increasing the threshold for a positive MCT result from 8 to 16 mg/mL did not noticeably improve the characteristics of the test (Fig 1 and see Fig E1 in this article's Online Repository at

TABLE I. Participants' characteristics

Characteristics	Healthy control participants (n = 93)	Asthmatic patients (n = 126)	P value*
General characteristics			
Male sex	37 (40)	42 (33)	.401
Race/ethnicity			
White	63 (68)	77 (61)	.062
African American	14 (15)	34 (27)	
Latino/Hispanic	10 (11)	13 (10)	
Others	6 (6)	2 (2)	
Age at enrollment (y), mean	32.9 ± 12.8	38.3 ± 15	.006
Former smoker	3 (3)	13 (10)	.084
BMI	25.9 ± 4.9	29.2 ± 7.6	.002
Other conditions			
Eczema	1 (1)	17 (13)	.002
Sinusitis	1 (1)	23 (18)	<.001
Hay fever/rhinitis	7 (8)	78 (62)	<.001
GERD	4 (4)	34 (27)	<.001
Food allergy	1 (1)	24 (19)	<.001
Family history of asthma	26 (28)	79 (63)	<.001
Atopy			
Median no. of positive allergy skin test results	0 (0-8)	3.5 (0-14)	<.001
≥1 Positive allergy skin test results	43 (46)	102 (81)	<.001
Lung function			
Percent predicted FEV ₁ (before BD)	95.1 ± 10.4	88.6 ± 11.9	<.001
Percent predicted FVC (before BD)	97.1 ± 10.7	96.7 ± 11.7	.720
Asthma characteristics			
Median age of onset (y)	NA	11.0 (1-53)	
Asthma treatment (%)			
ICS/LABA combination	NA	74 (59)	
ICS alone	NA	39 (31)	
No ICS	NA	13 (10)	
Antileukotriene alone or in combination	NA	30 (24)	
Asthma control			
Use of daily SABA	NA	27 (21)	
ACQ	NA	1.1 ± 0.7	
Urgent care visit (%) in 12 mo	NA	36 (29)	
Prednisone burst (%) in 12 mo	NA	29 (23)	

Data are presented as numbers (percentages), means ± SDs, or medians (ranges). Race/ethnicity was determined by self-identification to best descriptive category. ACQ, 7-item Asthma Control Questionnaire; BD, bronchodilator; BMI, body mass index; FVC, forced vital capacity; GERD, gastroesophageal reflux disease; LABA, long-acting β-agonist; NA, not applicable; SABA, short-acting β-agonist. *P value compares the phase I healthy control group (n = 93) versus the phase I asthmatic group (n = 126).

www.jacionline.org). An analysis of the sensitivity and specificity in a subset of age- and sex-matched subjects (80 participants from each group) produced similar results (data not shown).

Effect of participants' characteristics on the MCT

We investigated factors that might have influenced the diagnostic characteristics of the MCT (Table II and see Table E2 in this article's Online Repository at www.jacionline.org). The

sensitivity of the MCT in white subjects was less than that in African American subjects ($P = .015$). Sex did not have a significant effect on sensitivity ($P = .267$). Participants with atopy, which was defined as having 1 or more positive skin test results, had significantly higher MCT sensitivity compared with those without atopy ($P = .005$). The specificity of the MCT was high and similar among all subgroups. There was no significant difference in baseline demographics and asthma severity measurements by race (see Table E3). There was no significant difference in the percentage of atopic white and African American participants ($P = .624$).

Comparison of participants with positive or negative MCT results

We compared the clinical characteristics of those asthmatic participants with positive (n = 96) and negative (n = 29) MCT results (Table III). In addition to African American race and atopy, lower percent predicted FEV₁ was associated with a positive MCT result ($P = .003$). Body mass index was similar in both groups. There were no significant differences in other asthma characteristics, including asthma control, triggers, diagnosis, or symptoms, between participants with a positive or negative MCT result (see Table E4 in this article's Online Repository at www.jacionline.org).

DISCUSSION

We have made important observations about the performance of the MCT in the diagnosis of asthma: (1) 23% of patients with physician-diagnosed asthma receiving active controller treatment had a negative test result, and (2) the sensitivity of the test varied with race and the presence of atopy.

The sensitivity and specificity of the MCT observed in previous studies have varied depending on the study population tested.^{5-10,12-14,21} In studies with large sample sizes, sensitivity ranged from 51%¹² to 100%,⁶ and specificity ranged from 49%¹⁴ to 100% (Table IV).^{5-10,12-14,21} However, the current ATS guidelines suggest that MCTs can be used to clinically exclude asthma if the PC₂₀ is greater than 16 mg/mL in the proper clinical setting.¹⁵ This conclusion was based mainly on studies before ICS use had become a mainstay treatment for asthma. Hence a negative MCT result has been generally interpreted in a clinical setting as indicating that it is unlikely that a patient with unexplained respiratory symptoms has asthma. Our study clearly demonstrates that clinicians should not trust that MCTs can conclusively exclude the presence of asthma. In 125 asthmatic patients receiving controller treatment and 92 nonasthmatic control subjects in our study, we observed a sensitivity of 77% and specificity of 96% with a threshold of 8 mg/mL. Increasing the threshold to 16 mg/mL did not substantially increase the sensitivity.

We also observed that race and the presence of atopy had a significant effect on sensitivity. The MCT was much less sensitive in white participants (69%) compared with African American participants (91%, $P = .015$). This is the first study of which we are aware showing a racial difference in MCT diagnostic sensitivity. Most of the previous studies did not report racial composition, the effect of race, or both. A study did evaluate the racial difference in PD₂₀ (provocative dose of methacholine causing a 20% fall in FEV₁) in postpartum African American women in comparison with white women and found that African American women

TABLE II. Overall sensitivity and specificity of the MCT to detect a physician's diagnosis of asthma by race, sex, and atopy

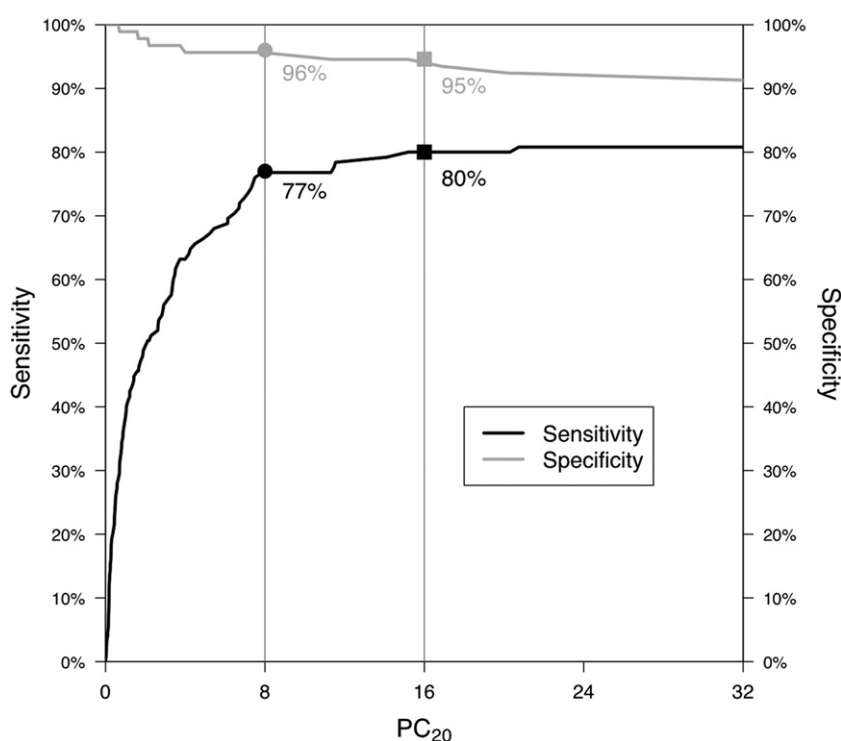
	No.	Sensitivity	Specificity	PPV	NPV
Overall	217*	77% (68% to 84%)	96% (89% to 99%)	96% (90% to 99%)	75% (66% to 83%)
Race					
White	139	69% (57% to 79%)	95% (86% to 99%)	95% (85% to 99%)	71% (60% to 81%)
African American	48	91% (76% to 99%)	93% (66% to 100%)	97% (83% to 100%)	81% (54% to 96%)
Sex					
Female	138	73% (62% to 83%)	98% (90% to 100%)	98% (91% to 100%)	71% (59% to 81%)
Male	79	83% (68% to 94%)	92% (78% to 99%)	92% (78% to 99%)	83% (67% to 93%)
Atopy†					
No	72	52% (30% to 74%)	94% (83% to 99%)	80% (51% to 96%)	81% (68% to 90%)
Yes	145	82% (73% to 90%)	98% (87% to 100%)	99% (93% to 100%)	70% (56% to 82%)

Values are presented as percentages (95% CIs). PC₂₀ of 8 mg/mL or less is defined as a positive test result.

NPV, Negative predictive value; PPV, positive predictive value.

*One participant in the asthmatic group and 1 participant in the nonasthmatic control group did not complete PC₂₀ measurement.

†No atopy is defined as a negative allergy skin test result, and the presence of atopy is defined as 1 or more positive allergy skin test results.

**FIG 1.** Relationship of various PC₂₀ thresholds and sensitivity/specificity of MCTs. Increasing the threshold for a positive MCT result from 8 to 16 mg/mL did not meaningfully change the sensitivity and specificity of the test.

had a lower PD₂₀, but the difference was no longer significant after adjusting for FEV₁ and IgE.²² In our study all asthma severity and control measures assessed were similar between African American and white participants (see Table E3). Thus the racial differences in methacholine reactivity observed were not based on asthma severity. Possible explanations for the lower MCT sensitivity in white participants might include disparities in the diagnosis of asthma and the threshold for treatment between races, leading to a higher likelihood of overdiagnosis among white participants. However, such a disparity was not supported by our measures of asthma severity, control measures, and treatment regimens. Second, there might be fundamental differences in the pathobiology of asthma by race. Further investigation is necessary to answer these questions.

We also found that asthmatic participants without atopy had a much lower sensitivity (53%) compared with that seen in atopic participants (83%, $P = .005$). This result confirms the previous reports of high rates of AHR in patients with atopy.^{7,23} A recent study that investigated the effect of atopy and sex on the performance of the MCT in 640 children (228 asthmatic patients and 223 healthy control participants) reported that the sensitivity was much lower in nonatopic compared with atopic subjects regardless of the PC₂₀ threshold. The sensitivity was lowest in nonatopic boys, and the authors concluded that the MCT was not helpful in diagnosing asthma in nonatopic boys.⁷ We did not observe a difference by sex among adults. The low sensitivity of MCTs in nonatopic participants in our study and in others indicates that MCTs should not be used as a diagnostic tool for asthma in this population.

TABLE III. Comparison of the characteristics of asthmatic participants with positive and negative MCT results

	Positive MCT result (n = 96)	Negative MCT result (n = 29)	P value
Characteristics			
Male sex	35 (36)	7 (24)	.314
Race/ethnicity			
White	53 (55)	24 (83)	.056
Black	31 (32)	3 (10)	
Others	12 (12)	2 (7)	
Age at enrollment (y), mean	39.1 ± 15.0	35.3 ± 15.1	.225
Former smoker	11 (11)	2 (7)	.72
BMI	29.2 ± 7.3	29.4 ± 8.8	.822
Atopy			
Median total no. of positive allergy skin test results	4 (0-14)	3 (0-14)	.113
≥1 Positive allergy skin test results	84 (88)	18 (62)	.005
Lung function			
Percent predicted FEV ₁	87.1 ± 11.9	93.8 ± 10.8	.003
Percent predicted FVC	96.4 ± 12.2	98.2 ± 10.3	.486
Peak flow (L/min)	418.6 ± 79.7	394.1 ± 98.8	.234
Asthma characteristics			
Family history of asthma	61 (64)	18 (62)	>.999
Median age of onset (y)	11.5 (1-53)	10.0 (1-52)	.495
Asthma treatment (%)			
ICS/LABA combination	54 (56)	19 (65)	.311
ICS alone	33 (34)	6 (21)	
No ICS	9 (10)	4 (14)	
Antileukotriene alone or in combination	19 (20)	11 (38)	.079
Asthma control			
Use of daily SABA	23 (24)	4 (14)	.364
Mean ACQ	1.1 ± 0.7	1.0 ± 0.6	.268
Urgent care visit in 12 mo	29 (30)	7 (24)	.69
Prednisone burst in 12 mo	22 (23)	6 (21)	.998

PC₂₀ of 8 mg/mL or less is defined as a positive test result. One participant in the asthmatic group and 1 participant in the nonasthmatic control group did not complete PC₂₀ measurements. Values are presented as numbers (percentages), means ± SDs, or medians (ranges).

ACQ, Asthma Control Questionnaire; BMI, body mass index; FVC, forced vital capacity; LABA, long-acting β-agonist; SABA, short-acting β-agonist.

It is possible that the method by which the MCT was performed might have influenced the results of the MCT sensitivity. Some previous studies and the ATS guidelines on MCTs have indicated that the 2 MCT methods, 5-breath dosimeter method and 2-minute tidal breathing method, are equivalent.²⁴⁻²⁶ However, Cockcroft and Davis²⁷ recently reported evidence of a lack of comparability between the 2 methods. Cockcroft and Davis found that patients with mild asthma (PC₂₀ >2 mg/mL) were more likely to have a positive MCT result with the tidal breathing method than with the 5-breath dosimeter method. They also reported that the submaximal inhalation dosimeter method, in which deep inhalation is restricted, resulted in a significantly lower PC₂₀ compared with the standard dosimeter method with deep breathing (4.1 vs 1.8 mg/mL, $P < .0001$).²⁸ The authors speculated that the inhalation to total lung capacity required for the 5-breath dosimeter method might provide bronchoprotection, as shown in nonasthmatic control subjects.²⁹ Accordingly, we cannot extend our results to other methods of MCT that do not require deep breaths.

The specificity of MCTs observed in our study was high, and none of the participants' characteristics had an effect on the

specificity. This is likely because our study evaluated the ability of MCTs to differentiate asthmatic participants from nonasthmatic healthy control participants and did not include participants with lung diseases that are known to cause false-positive MCT results (ie, sarcoidosis, chronic obstructive pulmonary disease, bronchiectasis, and smokers). We recruited our control participants from the general community with the requirement of good health without a clinical diagnosis of allergies. In comparison with our asthma participants our healthy control group was slightly younger, weighed less, and had a higher prevalence of atopy (46%) than previously reported (approximately 30%).^{30,31} However, this higher prevalence of atopy in the control group did not affect the specificity of MCTs when evaluated by atopic status.

We attempted to characterize the participants with negative MCT results by comparing their demographics and asthma characteristics with those with positive MCT results. Other than differences in atopy and race, which were discussed earlier, only FEV₁ was significantly different. Patients with lower airway caliber tend to have more AHR,^{32,33} but the small difference we observed in baseline FEV₁ is unlikely to completely explain the large difference in PC₂₀. Several previous studies have indicated that forced expiratory flow between 25% and 75% of vital capacity might correlate better with PC₂₀.^{33,34} Unfortunately, expiratory flow between 25% and 75% of vital capacity values were not obtained during our study. Moreover, the difference in PC₂₀ cannot be attributed to a difference in the use of ICSs because similar asthma treatment regimen patterns were observed in both groups. A longitudinal study evaluating MCT performance after withdrawal of controller medications and testing for AHR by using alternative methods can provide additional information.

There are several limitations to our study. Our participants had mostly mild-to-moderate persistent asthma and were receiving chronic treatment, and therefore our results might not be generalizable to patients with severe asthma or those not taking a controller medicine. However, the MCT is used most often in patients with normal lung function whose diagnosis is in question, and therefore we believe our study results are relevant to the most common clinical setting.

Second, we defined participants as having asthma if they had a medical history of physician-diagnosed asthma and were treated chronically with asthma medications as the gold standard. Thus it is possible that our study included subjects who might have received a misdiagnosis or overdiagnosis from their primary physician, which could have decreased the sensitivity of MCTs. Luks et al³⁵ evaluated asthma overdiagnosis in a Canadian community sample with physician-diagnosed asthma and found that up to 30% were overdiagnosed. Similarly, a study in the Netherlands found up to 26% of patients who were given ICSs by primary care physicians had no proper indication.³⁶ However, we were not able to clinically distinguish those participants who had positive or negative MCT results based on respiratory symptoms or control measurements (Table III and see Table E4) indicating lack of clinical difference by MCT positivity. Regardless, the gold standard for defining asthma is controversial, and the selection of participants with asthma for this study is consistent with most clinical definitions.

In summary, in patients with physician-diagnosed asthma receiving asthma controller therapy, the overall sensitivity of the MCT was 77% and the specificity was 96% for the diagnosis of asthma, and this sensitivity was markedly influenced by race and atopy. Given the reduced sensitivity of the MCT, clinicians

TABLE IV. Selected studies evaluating the performance of MCTs

Author (reference)	Year	Study population	No.	Adults or pediatrics	Sensitivity	Specificity	PPV	NPV	Definition of positive PC ₂₀ (mg/mL)
Hopp et al ⁵	1984	Asthma, healthy control	165	Pediatrics	98%	63%	57%	99%	800 BU*
Cockcroft et al ⁶	1992	College students	500	Adults	100%	93%	34%	100%	8
Nieminen ⁹	1992	Outpatient pulmonary clinic referrals	791	Adults	89%	76%	71%	91%	2600 μ g*
Perpina et al ¹⁰	1993	Asthma, rhinitis, chronic bronchitis, healthy control	300	Adults	84%	86%	No information	No information	15
Goldstein et al ⁸	1994	Allergy clinic referrals	198	Adults, pediatrics	61%	100%	100%	74%	8
Goldstein et al ²¹	2001	Suspected asthmatic patients with normal lung function in private clinic	121	Adults, pediatrics	86%	100%	100%	56%	8
Liem et al ⁷	2008	Manitoba birth cohort: asthma, healthy control	640	Pediatrics	66%	64%	No information	No information	4
Anderson et al ¹²	2009	Suspected asthmatic patients recruited from multiple study sites	375	Adults, pediatrics	51%	75%	78%	46%	16
Sverrild et al ¹³	2010	Young adults randomly drawn from nationwide registration in Denmark	238	Adults, pediatrics (age 15-24 y)	69%	80%	49%	90%	8 μ mol*
Carlsten et al ¹⁴	2011	Birth cohort with high risk for asthma	348	Pediatrics	80%	49%	27%	92%	3

BU, Breath units; NPV, negative predictive value; PPV, positive predictive value.

*These studies used a provocative dose of methacholine to define a positive test result (PD₂₀).

should not use MCTs as the sole method to exclude the diagnosis of asthma, especially in white and nonatopic individuals. Furthermore, clinical researchers should be aware of the limitations of using PC₂₀ as an inclusion criterion or as an outcome measure for a clinical trial.

Clinical implications: The MCT should be not be used as the sole method for excluding the diagnosis of asthma, especially in white and nonatopic subjects.

REFERENCES

- Sutherland ER, King TS, Icitovic N, Ameredes BT, Bleecker E, Boushey HA, et al. A trial of clarithromycin for the treatment of suboptimally controlled asthma. *J Allergy Clin Immunol* 2010;126:747-53.
- Weiss ST, Van Natta ML, Zeiger RS. Relationship between increased airway responsiveness and asthma severity in the childhood asthma management program. *Am J Respir Crit Care Med* 2000;162:50-6.
- Wechsler ME, Kunselman SJ, Chinchilli VM, Bleecker E, Boushey HA, Calhoun WJ, et al. Effect of beta2-adrenergic receptor polymorphism on response to long-acting beta2 agonist in asthma (LARGE trial): a genotype-stratified, randomised, placebo-controlled, crossover trial. *Lancet* 2009;374:1754-64.
- Mastrorade JG, Anthonisen NR, Castro M, Holbrook JT, Leone FT, Teague WG, et al. Efficacy of esomeprazole for treatment of poorly controlled asthma. *N Engl J Med* 2009;360:1487-99.
- Hopp RJ, Bewtra AK, Nair NM, Townley RG. Specificity and sensitivity of methacholine inhalation challenge in normal and asthmatic children. *J Allergy Clin Immunol* 1984;74:154-8.
- Cockcroft DW, Murdock KY, Berscheid BA, Gore BP. Sensitivity and specificity of histamine PC₂₀ determination in a random selection of young college students. *J Allergy Clin Immunol* 1992;89:23-30.
- Liem JJ, Kozyrskyj AL, Cockcroft DW, Becker AB. Diagnosing asthma in children: what is the role for methacholine bronchoprovocation testing? *Pediatr Pulmonol* 2008;43:481-9.
- Goldstein MF, Pacana SM, Dvorin DJ, Dunskey EH. Retrospective analyses of methacholine inhalation challenges. *Chest* 1994;105:1082-8.
- Nieminen MM. Unimodal distribution of bronchial hyperresponsiveness to methacholine in asthmatic patients. *Chest* 1992;102:1537-43.
- 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;104:149-54.
- Pekkanen J, Pearce N. Defining asthma in epidemiological studies. *Eur Respir J* 1999;14:951-7.
- Anderson SD, Charlton B, Weiler JM, Nichols S, Spector SL, Pearlman DS. Comparison of mannitol and methacholine to predict exercise-induced bronchoconstriction and a clinical diagnosis of asthma. *Respir Res* 2009;10:4.
- Sverrild A, Porsbjerg C, Thomsen SF, Backer V. Airway hyperresponsiveness to mannitol and methacholine and exhaled nitric oxide: a random-sample population study. *J Allergy Clin Immunol* 2010;126:952-8.
- Carlsten C, Dimich-Ward H, Ferguson A, Becker A, Dybuncio A, Chan-Yeung M. Airway hyperresponsiveness to methacholine in 7-year-old children: sensitivity and specificity for pediatric allergist-diagnosed asthma. *Pediatr Pulmonol* 2011;46:175-8.
- 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;161:309-29.
- National Institutes of Health, National Heart, Lung and Blood Institute, National Asthma Education and Prevention Program. Expert Panel Report 2: Guidelines for the Diagnosis and Management of Asthma (EPR-2 1997). Bethesda: National Institutes of Health; 1997. Report no. 07-4051.
- Brannan JD. Bronchial hyperresponsiveness in the assessment of asthma control: airway hyperresponsiveness in asthma: its measurement and clinical significance. *Chest* 2010;138:11S-7S.
- Forezi A, Mastropasqua B, Chetta A, D'Ippolito R, Testi R, Olivieri D, et al. Step-down compared to fixed-dose treatment with inhaled fluticasone propionate in asthma. *Chest* 2005;127:117-24.
- Juniper EF, O'Byrne PM, Guyatt GH, Ferrie PJ, King DR. Development and validation of a questionnaire to measure asthma control. *Eur Respir J* 1999;14:902-7.
- National Institutes of Health, National Heart, Lung and Blood Institute, National Asthma Education and Prevention Program. Expert Panel Report-3: Guidelines for the Diagnosis and Management of Asthma (EPR-3, 2007). Bethesda, Md: National Institutes of Health; 1997. Report No. 07-4051.
- Goldstein MF, Veza BA, Dunskey EH, Dvorin DJ, Belecanech GA, Haralabatos IC. Comparisons of peak diurnal expiratory flow variation, postbronchodilator FEV₁(1)

- responses, and methacholine inhalation challenges in the evaluation of suspected asthma. *Chest* 2001;119:1001-10.
22. Sherman CB, Tollerud DJ, Heffner LJ, Speizer FE, Weiss ST. Airway responsiveness in young black and white women. *Am Rev Respir Dis* 1993;148:98-102.
 23. Hewitt DJ. Interpretation of the "positive" methacholine challenge. *Am J Ind Med* 2008;51:769-81.
 24. Prieto L, Ferrer A, Domenech J, Perez-Frances C. Effect of challenge method on sensitivity, reactivity, and maximal response to methacholine. *Ann Allergy Asthma Immunol* 2006;97:175-81.
 25. Wubbel C, Asmus MJ, Stevens G, Chesrown SE, Hendeles L. Methacholine challenge testing: comparison of the two American Thoracic Society-recommended methods. *Chest* 2004;125:453-8.
 26. Siersted HC, Walker CM, O'Shaughnessy AD, Willan AR, Wiecek EM, Sears MR. Comparison of two standardized methods of methacholine inhalation challenge in young adults. *Eur Respir J* 2000;15:181-4.
 27. 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;117:1244-8.
 28. Todd DC, Davis BE, Hurst TS, Cockcroft DW. Dosimeter methacholine challenge: comparison of maximal versus submaximal inhalations. *J Allergy Clin Immunol* 2004;114:517-9.
 29. Skloot G, Togias A. Bronchodilation and bronchoprotection by deep inspiration and their relationship to bronchial hyperresponsiveness. *Clin Rev Allergy Immunol* 2003;24:55-72.
 30. Kerkhof M, Dubois AE, Postma DS, Schouten JP, de Monchy JG. Role and interpretation of total serum IgE measurements in the diagnosis of allergic airway disease in adults. *Allergy* 2003;58:905-11.
 31. Gergen PJ, Turkeltaub PC, Kovar MG. The prevalence of allergic skin test reactivity to eight common aeroallergens in the U.S. population: results from the second National Health and Nutrition Examination Survey. *J Allergy Clin Immunol* 1987;80:669-79.
 32. De Meer G, Heederik D, Postma DS. Bronchial responsiveness to adenosine 5'-monophosphate (AMP) and methacholine differ in their relationship with airway allergy and baseline FEV(1). *Am J Respir Crit Care Med* 2002;165:327-31.
 33. Simon MR, Chinchilli VM, Phillips BR, Sorkness CA, Lemanske RF Jr, Szefer SJ, et al. Forced expiratory flow between 25% and 75% of vital capacity and FEV1/forced vital capacity ratio in relation to clinical and physiological parameters in asthmatic children with normal FEV1 values. *J Allergy Clin Immunol* 2010;126:527-34, e1-8.
 34. Litonjua AA, Sparrow D, Weiss ST. The FEF25-75/FVC ratio is associated with methacholine airway responsiveness. The normative aging study. *Am J Respir Crit Care Med* 1999;159:1574-9.
 35. Luks VP, Vandemheen KL, Aaron SD. Confirmation of asthma in an era of over-diagnosis. *Eur Respir J* 2010;36:255-60.
 36. Lucas AE, Smeenk FW, Smeele IJ, van Schayck CP. Overtreatment with inhaled corticosteroids and diagnostic problems in primary care patients, an exploratory study. *Fam Pract* 2008;25:86-91.

APPENDIX E1

MEMBERS OF THE RESEARCH GROUP FOR THE MeCIS TRIAL

Baylor College of Medicine, Houston, Tex: N. A. Hanania (principal investigator); M. Sockrider (co-principal investigator); L. Bertrand, RN, RPFT (principal clinic coordinator); M. Atik, MD (coordinator).

Duke University Medical Center, Durham, NC: L. Williams (principal investigator); J. Sundy (co-principal investigator); G. Dudek (principal clinic coordinator); A. Dugdale; C. Foss; D. Jaggers, R. Newton (coordinators)

Illinois Consortium, Chicago, Ill: L. Smith (principal investigator); J. Moy; E. Naureckas;

(co-principal investigators); J. Hixon (principal clinic coordinator); A. Brees; M. Morley; V. Zagaja (coordinators)

Indiana University, Asthma Clinical Research Center, Indianapolis, Ind: M. Busk (principal investigator); P. Puntenney (principal clinic coordinator); N. Busk; J. Hutchins (coordinators)

Louisiana State University Health Sciences Center, Ernest N. Morial Asthma, Allergy, and Respiratory Disease Center, New Orleans, La: W. R. Summer (principal investigator); G. Meyaski (principal clinic coordinator); A. Antoine (coordinator)

National Jewish Health, Denver, Colo: R. Katial (principal investigator); H. Currier (principal clinic coordinator)

Nemours Children's Clinic—University of Florida Consortium, Jacksonville, Fla: J. Lima (principal investigator); K. Blake; J. Lang (co-principal investigators); M. McRae (principal clinic coordinator)

New York University—Columbia University Consortium, New York, NY: J. Reibman (principal investigator); E. DiMango; L. Rogers (co-principal investigators); K. Carapetyan (principal clinic coordinator, at New York University), E. Simpson; N. Surinder (clinic coordinators at Columbia University)

North Shore—Long Island Jewish Health System, New Hyde Park, NY: R. Cohen (principal investigator); R. Ramdeo (principal clinic coordinator)

University of Vermont, Burlington, Vt: C. G. Irvin (principal investigator); A. E. Dixon; D. A. Kaminsky (co-principal investigators); S. M. Burns (principal clinic coordinator); L. V. Griffes; J. E. Lippmann (coordinators)

Ohio State University Medical Center—Nationwide Children's Hospital, Columbus, Ohio: J. Mastronarde (principal investiga-

tor); J. Parsons (co-principal investigator); K. McCoy; C. Benninger (co-investigator); J. Drake (principal clinic coordinator); R. Compton; D. Cosmar (coordinators)

University of Miami at Miami—University of South Florida, Tampa, Fla: A. Wanner (principal investigator, Miami); R. Lockey (principal investigator, Tampa); E. Mendes (principal clinic coordinator for University of Miami); S. McCullough (principal clinic coordinator for University of South Florida); D. Miller; M. Grandstaff; P. Rebolledo (coordinators)

University of Missouri—Kansas City School of Medicine, Kansas City, Mo: G. Salzman (principal investigator); D. Pyszczyński (co-principal investigator); P. Haney (principal clinic coordinator)

St Louis Asthma Clinical Research Center of Washington University, St Louis, Mo: M. Castro (principal investigator); L. Bacharier; K. Sumino (co-investigators); J. Tarsi (principal clinic coordinator); S. De Martino (coordinator)

University of California, San Diego, San Diego, Calif: S. Wasserman (principal investigator);

J. Ramsdell (co-principal investigator); T. Greene (principal clinic coordinator); K. Kinninger (clinic coordinator)

University of Alabama, Birmingham, Ala: W. Bailey (research group chair)

Data Coordinating Center, Johns Hopkins University Center for Clinical Trials, Baltimore, Md: R. Wise (center director); J. Holbrook (deputy director); E. Brown (principal coordinator); A. Adler; D. Amend-Libercci; M. Daniel; A. Lears; G. Leatherman; C. Levine; D. Nowakowski; N. Prusakowski; S. Roettger; D. Shade; E. Sugar; C. Wei

Steering Committee: W. Bailey (chair); M. Busk; M. Castro; R. Cohen; A. Dozor; N. Edelman; N. Hanania; J. Holbrook; C. Irvin; R. Katial; E. Lancet; J. Lima; J. Mastronarde; J. Reibman; G. Salzman; L. Smith; W. Summer; J. Sundy; A. Wanner; S. Wasserman; R. Wise

American Lung Association Data and Safety Monitoring Board: V. Chinchilli (chair); P. Lanken; C. R. Rinaldo; D. Tashkin

Project Office, American Lung Association, New York, NY: N. Edelman (scientific consultant); E. Lancet; S. Rappaport

American Lung Association Scientific Advisory Committee: N. Schachter (chair); L. A. Baggott; W. C. Bailey; A. L. Brannen; M. Castro; B. Christman; A. Chung; R. M. Donaldson; C. Holloway; T. A. Mahr; J. A. Neubauer; J. Samet; E. Swenson; D. Upson; D. Weiss; R. Wise

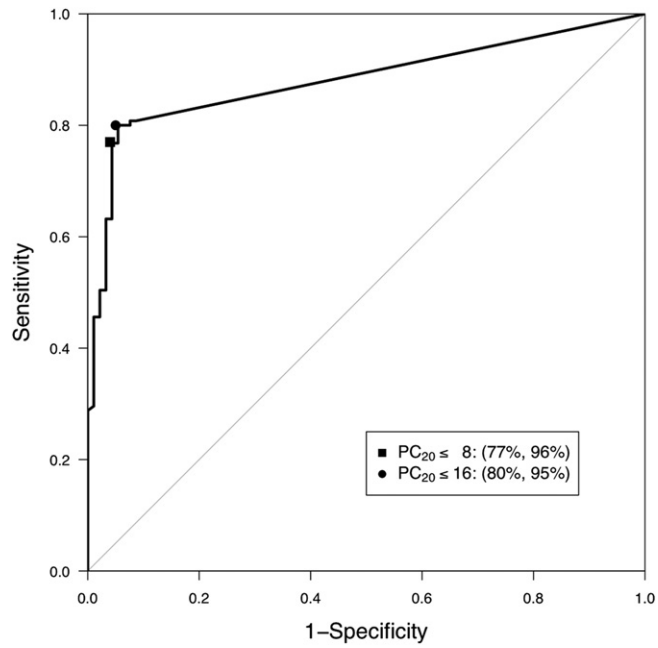


FIG E1. Receiver operating curve of sensitivity and specificity of various PC_{20} thresholds. Increasing the threshold from 8 to 16 mg/mL does not increase the area under the curve.

TABLE E1. Numbers of participants with positive and negative MCT results with a PC₂₀ threshold from 1 to 16 mg/mL

PC ₂₀ ≤1 mg/mL	Asthmatic patients	Healthy control subjects
Positive	48	1
Negative	77	91
	Sensitivity	Specificity
	38%	99%
PC ₂₀ ≤2 mg/mL	Asthmatic patients	Healthy control subjects
Positive	62	2
Negative	63	9
	Sensitivity	Specificity
	50%	98%
PC ₂₀ ≤4 mg/mL	Asthmatic patients	Healthy control subjects
Positive	79	4
Negative	46	88
	Sensitivity	Specificity
	63%	96%
PC ₂₀ ≤8 mg/mL	Asthmatic patients	Healthy control subjects
Positive	96	4
Negative	29	88
	Sensitivity	Specificity
	77%	96%
PC ₂₀ ≤16 mg/mL	Asthmatic patients	Healthy control subjects
Positive	100	5
Negative	25	87
	Sensitivity	Specificity
	80%	95%

One participant in the asthmatic group and 1 participant in the nonasthmatic control group did not complete PC₂₀ measurement.

TABLE E2. Numbers of participants with positive and negative MCT results by race, with a positive result defined as PC₂₀ less than 8 mg/mL

White race	Asthmatic patients	Healthy control subjects
Positive	53	3
Negative	23	59

African American race	Asthmatic patients	Healthy control subjects
Positive	31	1
Negative	3	13

One participant in the asthmatic group and 1 participant in the nonasthmatic control group did not complete PC₂₀ measurement.

TABLE E3. Participant characteristics of asthmatic patients stratified by race

Characteristics	White race (n = 77)	African American race (n = 34)	P value
Male sex	27 (35)	9 (26)	.502
Age at enrollment (y), mean	37.1 ± 15.4	40.5 ± 14.5	.235
Former smoker	11 (14)	2 (6)	.343
BMI	28.6 ± 7.7	31.7 ± 7.7	.051
Other conditions			
Eczema	10 (13)	6 (18)	.725
Sinusitis	14 (18)	8 (24)	.694
Hay fever/rhinitis	49 (64)	19 (56)	.574
GERD	22 (29)	6 (18)	.325
Food allergy	17 (22)	6 (18)	.782
Family history of asthma	44 (57)	26 (76)	.058
Atopy			
Median no. of positive skin test results	3 (0-14)	5 (0-14)	.108
≥1 Positive skin test results	61 (79)	29 (85)	.624
Lung function			
Percent predicted FEV ₁ (before BD)	88.9 ± 11.9	90.5 ± 12.6	.48
Percent predicted FVC (before BD)	96.8 ± 12.1	98.1 ± 11.4	.318
Asthma characteristics			
Median age of onset (y)	10 (1-52)	13 (1-53)	.467
Asthma treatment			
ICS/LABA combination	47 (61)	22 (65)	.186
ICS alone	19 (25)	11 (32)	
No ICS	11 (14)	1 (3)	
Antileukotriene alone or in combination	17 (22)	13 (38)	.125
Asthma control			
Use of daily SABA	14 (18)	8 (24)	.694
Mean ACQ score	1.0 ± 0.6	1.2 ± 0.8	.49
Urgent care visit (%) in 12 mo	20 (26)	13 (38)	.281
Prednisone burst (%) in 12 mo	15 (19)	11 (32)	.218

Data are presented as numbers (percentages), means ± SDs, or medians (ranges).

Data are shown for white and African American subjects. Other racial/ethnic categories were excluded.

ACQ, Asthma Control Questionnaire; BD, bronchodilator; BMI, body mass index; FVC, forced vital capacity; GERD, gastroesophageal reflux disease; LABA, long-acting β-agonist; NA, not applicable; SABA, short-acting β-agonist.

TABLE E4. Comparison of detailed clinical asthma history in participants with positive and negative MCT results

Asthmatic patients (n = 126)	Positive MCT results (n = 83/96 [86%] included)	Negative MCT results (n = 25/29 [86%] included)	P value
Original dx made by:			
Primary care doctor	62 (77)	14 (64)	.23
Lung doctor	11 (14)	3 (14)	
Allergy doctor	8 (10)	5 (23)	
Current f/u			
Primary care doctor	50 (60)	11 (50)	.45
Lung doctor	15 (18)	7 (32)	
Allergy doctor	18 (22)	4 (18)	
Symptoms in past 3 mo			
Cough			
Never	37 (64)	12 (67)	.15
1-2 times/wk	20 (34)	4 (22)	
Daily	1 (2)	2 (11)	
Sputum			
Never	44 (75)	15 (83)	.78
1-2 times/wk	11 (19)	3 (17)	
Daily	4 (7)	0 (0)	
Chest tightness			
Never	30 (53)	10 (59)	.91
1-2 times/wk	23 (40)	6 (35)	
Daily	4 (7)	1 (6)	
Wheezing			
Never	26 (59)	11 (65)	>.99
1-2 times/wk	14 (32)	5 (29)	
Daily	4 (9)	1 (6)	
Shortness of breath			
Never	23 (49)	9 (56)	.78
1-2 times/wk	17 (36)	6 (38)	
Daily	7 (15)	1 (6)	
Nocturnal symptoms			
Never	43 (74)	9 (50)	.12
1-2 times/wk	11 (19)	7 (39)	
Daily	4 (7)	2 (11)	
Triggers			
Respiratory tract infection	63 (85)	21 (95)	.29
Irritants	68 (86)	20 (83)	.75
Emotions	30 (38)	7 (32)	.57
Drugs	7 (11)	0 (0)	.19
Food additives	11 (14)	1 (5)	.28
Weather changes	74 (89)	19 (79)	.30
Exercises	60 (74)	16 (70)	.67
Cleaning supplies	50 (65)	12 (55)	.37
Exposure to animals			
Cat	50 (60)	10 (40)	.07
Dog	27 (33)	8 (32)	.96
Rodent	11 (13)	3 (12)	>.99
Season			
Winter	42 (51)	13 (52)	.9
Spring	35 (42)	7 (28)	.20
Summer	16 (19)	7 (28)	.35
Fall	33 (40)	8 (32)	.48
Family history			
Father	13 (16)	2 (8)	.51
Mother	18 (22)	5 (20)	.86
Siblings	28 (34)	7 (28)	.59
Children	15 (18)	2 (8)	.35
None	34 (41)	12 (48)	.53

(Continued)

TABLE E4. (Continued)

Asthmatic patients (n = 126)	Positive MCT results (n = 83/96 [86%] included)	Negative MCT results (n = 25/29 [86%] included)	P value
Asthma control			
Last seen by doctor for breathing problem			
Never	4 (5)	0	.46
Within the last year	44 (53)	11 (44)	
>1 y	35 (42)	14 (56)	
Last visit in ED or urgent care for breathing problem			
Never	23 (28)	12 (48)	.11
Within last year	11 (13)	4 (16)	
>1 y	49 (59)	9 (36)	
Last overnight stay in hospital because of breathing problem			
Never	45 (54)	15 (60)	.12
Within last year	1 (1)	2 (8)	
>1 y	37 (45)	8 (32)	
Last ICU admission because of asthma attack			
Never	64 (77)	20 (80)	.49
Within last year	1 (1)	1 (4)	
>1 y	18 (22)	4 (16)	
Asthma treatment			
ICS (noncombination)	26 (31)	6 (24)	.48
ICS/LABA combination	47 (57)	14 (56)	.96
Oral steroids in past 12 mo	11 (13)	4 (16)	.75
Injectable corticosteroids	2 (4)	0	>.99
Immunotherapy	4 (5)	1 (4)	>.99

Additional information on asthma history was obtained from a subset of participants (positive MCT result, 83/96 [86%]; negative MCT result, 25/29 [86%]). A PC₂₀ of 8 mg/mL or less is defined as a positive test result. One participant in the asthmatic group and 1 participant in the nonasthmatic control group did not complete PC₂₀ measurement.

dx, Diagnosis; f/u, follow-up; LABA, long-acting β -agonist.