Specific Conductance Criteria for a Positive Methacholine Challenge Test: Are the American Thoracic Society Guidelines Rather Generous?

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BACKGROUND: American Thoracic Society (ATS) guidelines for methacholine challenge testing (MCT) discuss specific airways conductance (sG_{aw}) as a surrogate marker for forced expiratory volume in the first second (FEV_1) to diagnose airways obstruction. The guidelines suggest a cutoff value of 45% drop in sG_{aw} to diagnose a positive MCT. However, there is no available evidence that supports this cutoff value of 45%. We conducted this study to examine the relationship between FEV_1 and sG_{aw} during MCT. METHODS: One-hundred thirty-eight patients who had both sG_{aw} and FEV_1 measured during MCT between April 2003 and March 2004 were retrospectively evaluated. The tests were done according to the ATS guidelines. Data were first analyzed using linear regression modeling, comparing the change in FEV_1 to changes in sG_{aw}. Then the sensitivity and specificity were generated for different cut points, using receiver operating characteristic analysis. RESULTS: Thirty-eight patients had a positive MCT based on ATS FEV_1 criteria. A decrease of 20% in FEV_1 correlated with a drop of 56% in sG_{aw} (95% confidence interval 52% to 60%, r^2 0.35, $P < .001$). Using 20% decline from baseline in FEV_1 at different PC_{20} (provocational concentration that produced a ≥ 20% FEV_1 decrease) values (4 mg/mL, 8 mg/mL, and 16 mg/mL), we then analyzed the sensitivity, specificity, positive predictive value, and negative predictive value of the 45% decline in sG_{aw} and compared it with a 56% decline in sG_{aw}. Using receiver operating characteristic analysis, we were able to find that a cutoff of 51–52% performed better than either of the 2 values. CONCLUSIONS: Our study suggests that the ATS suggested cutoff value of 45% decline in sG_{aw} to diagnose a positive MCT may be rather generous, and a decline of 51% from baseline may provide a more accurate measure of airway hyper-responsiveness. Further studies using well defined subjects with and without asthma should be done to better assess the test characteristics of sG_{aw}. *Key words: asthma, methacholine challenge test, specific conductance, sG_{aw}.*
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Introduction

Methacholine challenge testing (MCT) is used to help in diagnosing asthma when other methods, including spirometry before and after administration of a bronchodilator, do not establish a diagnosis. The change in forced expiratory volume in the first second (FEV_1) is the primary outcome measure.[1] MCT is most accurate when the pre-test probability of asthma is 30–70%.[2] The test is usually considered positive when there is a ≥ 20% decline in FEV_1 from baseline, at a PC_{20} (provocational concentration that produced a ≥ 20% FEV_1 decrease) of < 8 mg/mL or < 16 mg/mL.[1] The best PC_{20} cut point to separate patients with asthma from those without asthma is in the range of 8–16 mg/mL.[1-3]

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The American Thoracic Society (ATS) guidelines for MCT discuss specific airways conductance (sG_{aw}) as an

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alternative marker for FEV_1 to diagnose airways obstruction.[1] They advise the use of specific conductance primarily in patients who cannot perform acceptable spirometry maneuvers.[1,4,5] However, it has been contested that, in the absence of substantial decline in the FEV_1, patients may have a false negative MCT if the change in specific conductance is not considered to make the diagnosis.[6] But there are no published data that allow us to confidently state the cutoff value of sG_{aw} that should be used to define a positive MCT.

Changes in specific conductance, with values ranging from 30% to 55%, have been used in numerous studies and in different populations, either to diagnose a positive MCT or to determine a significant bronchodilator response.[6-10] But in asthmatics most studies historically have used a 35% decline in sG_{aw} from baseline to define a positive MCT.[11-14] Such a drop alone, however, may not sufficiently discriminate asthmatics from groups that contain normal subjects and subjects with chronic obstructive pulmonary disease.[15] This is because sG_{aw} is more variable than FEV_1.[1]

Given this variability, the latest ATS guidelines suggest that a drop in sG_{aw} of 45% or more in an MCT is required for a positive MCT.[1] However, there are no published data that show the sensitivity or specificity of this cutoff for the diagnosis of asthma. Thus, we wanted to evaluate the test characteristics of sG_{aw} in a group of patients who had an MCT. In this study we had 2 goals. The first was to evaluate how changes in sG_{aw} compare to changes in FEV_1. The second was to assess the test characteristics of sG_{aw} using change in FEV_1 as the accepted standard.

Methods

Subjects

All adult patients with clinical suspicion of asthma, who were referred to our pulmonary function laboratory for an MCT between April 2003 and March 2004, were retrospectively evaluated. The laboratory conducted MCT with simultaneous measurements of spirometric variables and sG_{aw} during that time frame. Patients who completed the testing, regardless of their results, were enrolled. Patients who could not complete the testing were excluded. Also, patients who were sent for MCT for reasons other than suspicion of asthma, such as occupational testing, were excluded. The study design was approved by our local institutional review board.

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Pulmonary Function Testing

Spirometry was conducted using standard techniques[16] (VM Autobox 6200, SensorMedics, Yorba Linda, California). Forced expiratory maneuvers were performed according to the ATS guidelines, and the best effort was analyzed. Airways resistance and sG_{aw} were determined by variable-pressure body plethysmography (VM Autobox 6200, SensorMedics, Yorba Linda, California). Body plethysmography measurements were gathered by taking the average of the best 3 adequate and acceptable attempts.[17] The test was not deemed adequate until the open-shutter panting maneuver showed a relatively closed loop and the panting frequency was approximately 1.5 Hz. The entire testing was performed with the patient in a seated position. The pulmonary function test data were expressed as a percentage of predicted normal values.[18,19]

Methacholine Challenge Protocol

Serial dilutions of methacholine chloride (Provocholine, Methapharm, Branford, Ontario, Canada) were prepared in normal saline solution containing 0.4% phenol (pH 7.0) and passed through bacterial-retentive filters with 0.2-μm porosity. Methacholine aerosol was delivered using a Respirgard II (Vital Signs, Totowa, New Jersey) filtered medication nebulizer. Following a control inhalation of diluent, each patient took 5 slow inhalations from functional residual capacity to total lung capacity from the starting concentration of 0.25 mg/mL. Plethysmographic studies were performed, followed by the FVC maneuvers. Studies were performed after 90 seconds and within 5 min of exposure to the methacholine inhalation. If the reduction in FEV_1 was $< 20\%$ from baseline, 5 inhalations of increasing concentrations of methacholine, at 0.25 mg/mL, 1 mg/mL, 4 mg/mL, and 16 mg/mL, were administered. We modified our protocol from the ATS guidelines that suggest using 0.0625 as the starting concentration. This was based on departmental consensus of the senior pulmonary staff of a large teaching hospital, reviewing thousands of patients tested over several decades with no adverse events occurring at or below 0.25 mg/mL methacholine concentration. Testing was continued until a 20% drop in FEV_1 was obtained or the highest concentration was given. Post-bronchodilator study was done after administration of ipratropium and/or albuterol. Testing terminated when post-test FEV_1 returned to baseline or to a value of less than 10% from baseline.

Data Analysis

Results from the test using the highest concentration of methacholine that the subject was administered were used for analysis. Data were analyzed using linear regression modeling (SAS 9.1, SAS Institute, Cary, North Carolina),

comparing the change in FEV_1 to changes in sG_{aw}. Correlations were reported with 95% confidence intervals. For the analyses, an alpha level of .05 was used to assess statistical significance.

The test characteristics of the value derived through this analysis were then compared with the ATS suggested value of 45% using receiver operating characteristic curve analysis. The patients were defined as positive or negative for asthma based on their FEV_1 $PC_{20.}$ Given the controversy as to the "best" discriminating value for PC_{20}, we did 3 separate analyses, using 4, 8, or 16 mg/mL as the dose of methacholine that defined a positive test. For each cutoff we performed logistic regression to assess the relationship between sG_{aw} and the diagnosis of asthma. We then constructed 2×2 tables to determine the sensitivity, specificity, positive predictive value, and negative predictive value of the potential diagnostic cutoffs for sG_{aw}. Finally, we used statistics software (StatsDirect, StatsDirect, Cheshire, United Kingdom) to find the best cutoff for sG_{aw}. For these analyses we also asked the software to maximize the Youden index (sensitivity + specificity – 1), as this index is felt to be the one of the better methods for choosing an optimal cutoff.[20,21] After determining the sensitivity, specificity, positive predictive value, and negative predictive value for each of these cutoffs, we calculated 95% confidence intervals for these measures according to the efficient-score method described by Newcombe.[22]

Results

One-hundred forty-seven patients who were suspected of having asthma underwent an MCT during the study period. Nine patients were excluded due to either lack of completion of the test or incomplete data. Thus, our study population consisted of 138 patients. All had a normal spirometry preceding the MCT. Their demographics are shown in Table 1. No criteria were set for baseline sG_{aw}, due to lack of commonly accepted validated normal predicted values for this measurement.

Table 1. Patient Demographics

Total number of patients with the methacholine challenge test	147
Patients excluded	9
Total number of patients included in the study	138
Sex	
Female	98
Male	40
Smoking History	
Past smokers	19
Active smokers	1
Non-smokers	118
Age (mean ± SD y)	48 ± 7
Race	
Caucasian	46
African American	88
Other	4

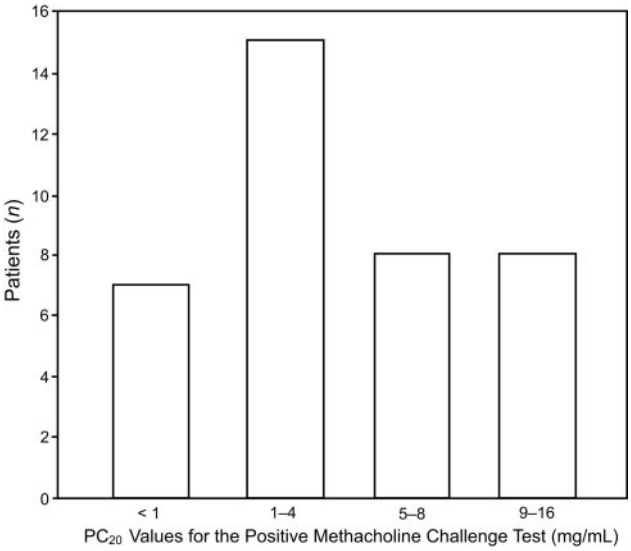

Fig. 1. Distribution of patients with a positive methacholine challenge test, according to their PC_{20} (provocational concentration that produced a ≥ 20% decrease in forced expiratory volume in the first second) values.

During the testing, patients had both spirometric and specific conductance analysis in response to methacholine. Thirty-eight patients had a positive test according to the ATS FEV_1 criteria. Of these 38 patients, 8 patients (21%) had a borderline positive test with a PC_{20} between 8 mg/mL and 16 mg/mL, 8 patients (21%) had a PC_{20} between 4 mg/mL and 8 mg/mL, and 22 patients (58%) had a PC_{20} less than or equal to 4 mg/mL. (Fig. 1) One-hundred patients had a negative MCT (< 20% drop in FEV_1) after 16 mg of methacholine inhalation. None of the patients with a positive test had an occupational exposure to explain their responsiveness.

Values for FEV_1 and sG_{aw} were retrieved. Using a linear regression model, we found that a decrease of 20% in FEV_1 correlated with a drop of 56% in sG_{aw} (95% confidence interval 52–60%, r^2 0.35, $P < .001$) (Fig. 2). In 7 patients who had zero change in FEV_1 there was a decline in sG_{aw} of 31% (95% confidence interval 27–37%, r^2 0.32, $P < .001$).

We then assessed the test characteristics of the ATS recommended 45% decline in sG_{aw} as well as the 56% decline in sG_{aw} found above using the receiver operating characteristic curve. As there is some controversy as to how best to define a positive MCT, we performed 3 separate analyses using 3 different PC_{20} values. The values for PC_{20} that we evaluated were 4 mg/mL, 8 mg/mL, and 16 mg/mL. As shown in Figure 3, the optimal cutoff was

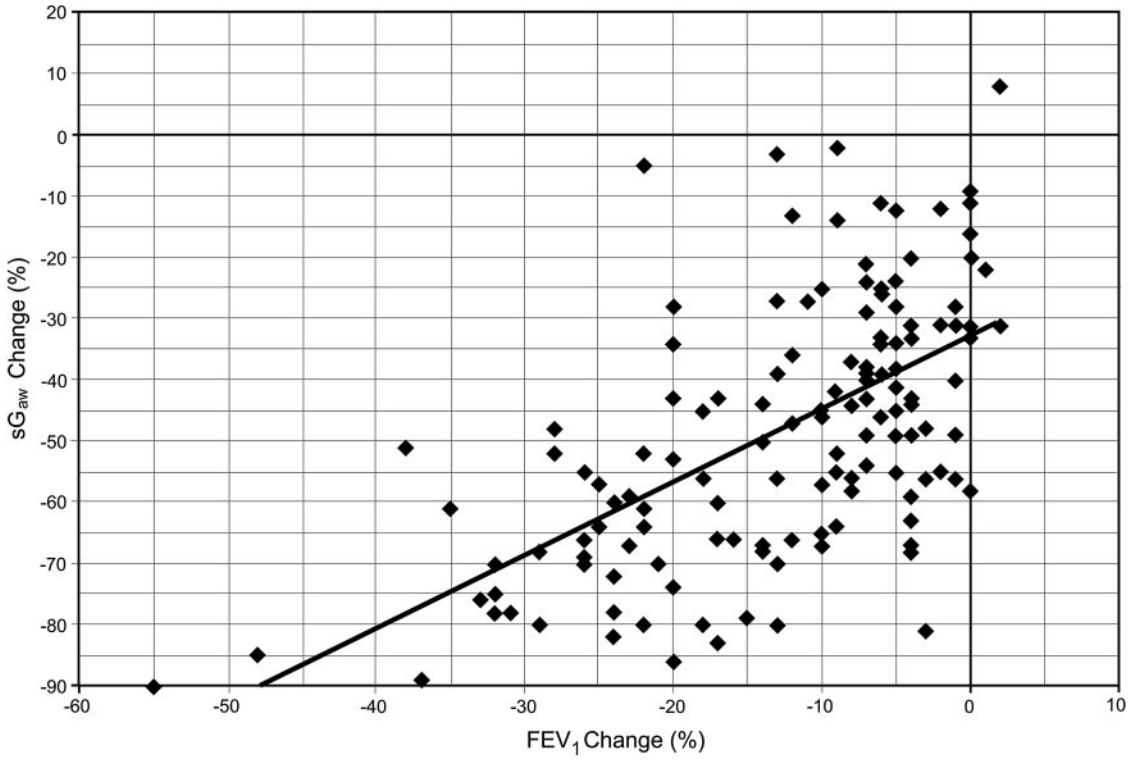

Fig. 2. Correlation of percentage drops in specific conductance of the airways (sG_{aw}) and change in forced expiratory volume in the first second (FEV_1) in the methacholine challenge tests. A drop in FEV_1 of 20% correlates to a drop in sG_{aw} of 56%. Data are shown with fitted linear regression line.

a drop in sG_{aw} of 52%, 51%, and 51% when the diagnostic cutoff was a PC_{20} of 4 mg/mL, 8 mg/mL, and 16 mg/mL, respectively. As can be seen in Table 2, Table 3, and Table 4, the test characteristics of the cutoffs of 45%, 56%, and 51% at the 3 different PC_{20} are relatively similar, but the best cutoff (as measured by the Youden index) is in the range of 51–52%.

Discussion

Our study shows that a 20% drop in FEV_1 correlates with a 56% drop in sG_{aw}. However, neither a 56% drop in sG_{aw} nor the ATS-recommended drop of 45% had the most ideal test characteristics to define a group of patients with a positive MCT when a 20% decline in FEV_1 is used as the diagnostic criterion. Instead, an intermediate cutoff value of 51–52% performs best to diagnose asthma in a group of patients with a clinical suspicion of asthma and a 20% decline in FEV_1.

The measurement of sG_{aw} to diagnose a positive MCT has been used rather infrequently in the United States. In one survey, 78% of the investigators who used bronchoprovocation testing used only the FEV_1 criterion, and only 12% used sG_{aw} measurements routinely.[23] Another survey of laboratories showed that sG_{aw} assessment was done in $<$ 20% of cases.[6] Though there are no new studies that estimate the current usage of sG_{aw}, the lack of equipment required to measure sG_{aw} and unfamiliarity with methods to measure it may explain the relatively uncommon use.[23] Nonetheless, it has been argued that measurement of sG_{aw} complements spirometric analysis and may be more sensitive in diagnosing an MCT and change in airway caliber than spirometry alone.[6,10,24,25] Such studies encourage more frequent use of sG_{aw} in reactive airways disease, but due to lack of validated diagnostic cutoff points, they use empirical values for a diagnostic sG_{aw} drop.

Those who suggest that sG_{aw} may be of use in MCT argue that it may be positive in those patients with asthma who have a negative methacholine test (as measured by a PC_{20} of 16 mg/mL or less). Thus, to ideally assess the utility of sG_{aw}, one would evaluate its test characteristics in a group of patients who have a negative MCT but who are diagnosed with asthma through some other means. However, trying to define such a population in a validated manner would be quite difficult. Thus, we chose to study the test characteristics of sG_{aw} in a group of patients who clearly had asthma given the clinical suspicion and a positive MCT. Our study suggests that, compared to the accepted 45% cutoff for change in sG_{aw}, a cut point of 51–52% may be more accurate.

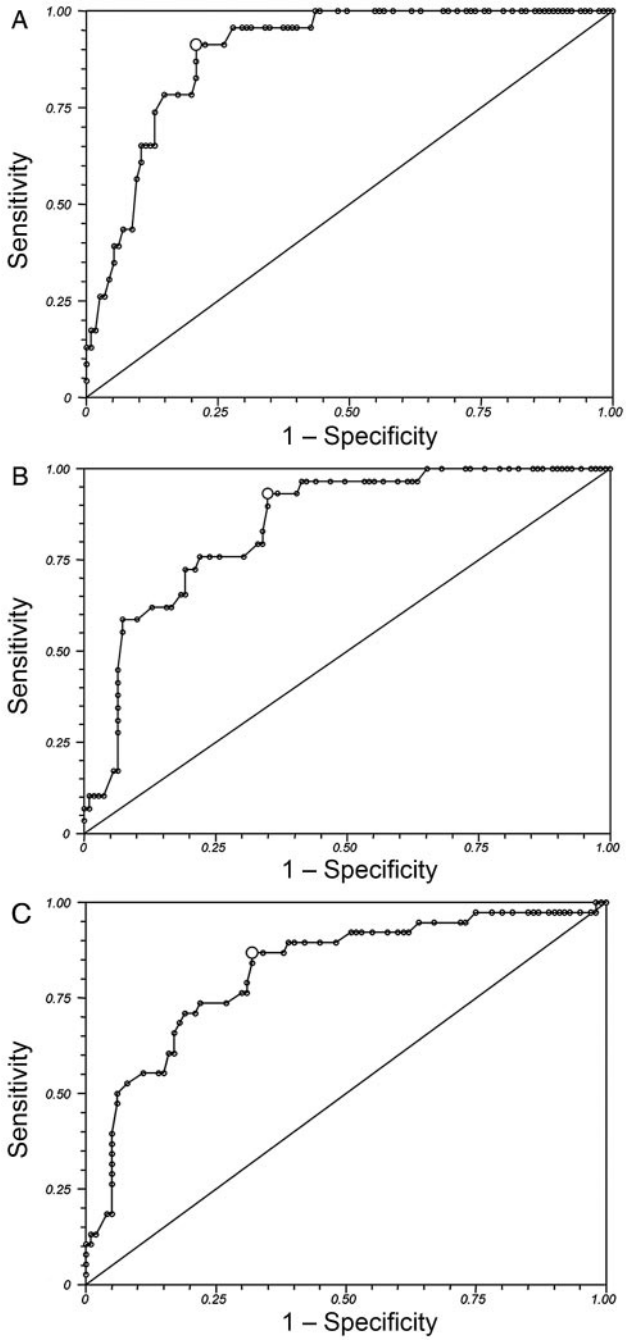

Fig. 3. Receiver operating characteristic analysis at the 3 different methacholine PC_{20} (provocational concentration that produced a ≥ 20% decrease in forced expiratory volume in the first second) cutoffs. A: 4 mg/mL. B: 8 mg/mL. C: 16 mg/mL. In each panel, the large hollow dot represents the "ideal" cut point, as generated by maximizing the Youden index.

Table 2. Test Characteristics of the Cutoffs of 45%, 56%, and 52% at the PC_{20} of 4 mg/mL of Methacholine, Along With the Respective Youdin Index

	45%	56%	52%
Sensitivity (%, 95% CI)	96 (76–100)	78 (56–92)	91 (70–98)
Specificity (%, 95% CI)	70 (60–78)	80 (71–87)	79 (70–86)
Positive predictive value (%, 95% CI)	39 (26–52)	44 (29–60)	47 (32–62)
Negative predictive value (%, 95% CI)	99 (92–100)	95 (88–98)	98 (92–100)
Youden index	0.66	0.58	0.70

PC_{20} = provocational concentration that produced a ≥ 20% decrease in forced expiratory volume in the first second
CI = confidence interval

Table 3. Test Characteristics of the Cutoffs of 45%, 56%, and 52% at the PC_{20} of 8 mg/mL of Methacholine, Along With the Respective Youdin Index

	45%	56%	52%
Sensitivity (%, 95% CI)	97 (80–100)	76 (56–89)	93 (76–99)
Specificity (%, 95% CI)	53 (43–63)	70 (60–78)	65 (55–74)
Positive predictive value (%, 95% CI)	35 (25–47)	40 (27–54)	42 (30–54)
Negative predictive value (%, 95% CI)	98 (90–100)	92 (83–96)	97 (90–100)
Youden index	0.50	0.46	0.58

PC_{20} = provocational concentration that produced a ≥ 20% decrease in forced expiratory volume in the first second
CI = confidence interval

Table 4. Test Characteristics of the Cutoffs of 45%, 56%, and 52% at the PC_{20} of 16 mg/mL of Methacholine, Along With the Respective Youdin Index

	45%	56%	52%
Sensitivity (%, 95% CI)	89 (74–97)	74 (57–86)	87 (71–95)
Specificity (%, 95% CI)	55 (45–65)	73 (63–81)	68 (58–77)
Positive predictive value (%, 95% CI)	43 (32–55)	51 (37–64)	51 (38–63)
Negative predictive value (%, 95% CI)	93 (83–98)	88 (79–94)	93 (84–97)
Youden index	0.44	0.47	0.55

PC_{20} = provocational concentration that produced a ≥ 20% decrease in forced expiratory volume in the first second
CI = confidence interval

We used the 5-breath dosimeter protocol for methacholine administration. In healthy persons and patients with mild asthma, the deep inhalation that precedes an FVC maneuver causes transient bronchodilation that may last for up to 6 min.[26,27] This response is blunted or absent in patients with severe asthma, and may actually cause bronchoconstriction.[1,28] In patients being clinically tested for a diagnosis of asthma, this effect of deep inhalations on airways may contribute to a disproportionate response between the FEV_1 and sG_{aw}.[1] This variability has the poten-

tial to alter our results. Because most MCTs are performed in individuals who are either non-asthmatic or mildly asthmatic, there is likely to be a bronchodilator response, and this variability would be less significant.[1] This holds true for our study patients also, as none of the patients had abnormal spirometry at baseline.

Recent data also show that inhibition of deep inhalation for 10 min or more during methacholine inhalation increases airway narrowing in normal people, while inhibiting it for 15 min increases airway narrowing in asthmatics.[28] This is a limitation to our study, as we did not inhibit the deep inhalation effect, and thus may have missed "borderline asthmatics." Deep inhalation may not affect all segments of the airway similarly, but past studies have shown that the changes in sG_{aw} usually follow the decline in FEV_1 in patients who undergo MCT.[15,24,29]

In our study, individuals who had zero change in FEV_1 at the highest concentration of methacholine (16 mg/mL) showed an average decline of 31% in sG_{aw}, with wide variability. This conforms to prior studies, which have shown considerable decline in sG_{aw} after methacholine administration in the absence of history or symptoms of asthma.[30,31] This further suggests that the current ATS recommendations for using a cutoff of 45% drop in sG_{aw} to define a positive MCT may be rather generous. The above results, however, are from a clinically derived population and may not hold true for a "healthy normal" population. This correlation needs to be confirmed in "healthy normals" in future studies.

Another potential limitation of our study is the possibility of spectrum bias. Spectrum bias refers to the understanding that a test may have characteristics when applied to different subgroups.[32] For the purposes of our study, the implication would be that the reported characteristics of the sG_{aw} as a predictive test for asthma may vary if used in a group of patients who are different than those reported in our study. As can be shown in Table 1, most were non-smokers, and we had a reasonable representation of both sexes and both white and African American patients. However, the most likely factor that would be important for the issues of spectrum bias would be the pre-test probability of asthma. There is no objective and reproducible way for us to measure that. In our institution we tend to send patients for MCT in whom we have an intermediate pre-test probability of asthma. So patients who are quite likely to have asthma are typically treated empirically. This means that patients who have some symptoms suggestive of asthma (cough, unexplained episodic dyspnea) along with a normal spirometry are the most likely group to undergo MCT. Other practitioners at other institutions may have slightly different criteria for ordering an MCT and thus may not find exactly similar test characteristics in their populations.

While our study does suggest a strong correlation that a 56% decline in sG_{aw} corresponds better to a 20% fall in FEV_1, it does not suggest that these 2 variables are somehow interchangeable. This is better depicted by the receiver operating characteristic curve analysis that shows that 51–52% decline in sG_{aw} performs better than both the 45% and 56% cutoff values. One should be cautious while interpreting large declines in sG_{aw} in the absence of a 20% decline in FEV_1 in an MCT. In our study, 27 subjects had a significant fall in sG_{aw} ($\geq$ 52%), but did not drop their FEV_1 by 20%. Do these patients have underlying bronchial hyper-responsiveness and does this drop have any clinical importance will be questions that can be asked in future studies.

In the interim, if one chooses to use sG_{aw} in the absence of FEV_1 as a diagnostic criterion, a decline of 51% from baseline may provide a more accurate measure of airway hyper-responsiveness until additional prospective studies further address this issue. Contrary to some previous reports, we would caution against more frequent use of sG_{aw} alone in the MCT. More studies are needed to determine the clinical importance of changes in sG_{aw} in an MCT and how these changes relate to a decline in FEV_1. Prospective studies involving well-defined subjects with and without asthma should be conducted to better assess these test characteristics of sG_{aw} in MCT.

Conclusions

Our study suggests that in people who are tested for clinical suspicion of asthma, the ATS suggested cutoff value of 45% decline in sG_{aw} to diagnose a positive MCT may be rather generous. A decline of 51% in sG_{aw} from baseline may provide a more accurate measure of airway hyper-responsiveness. Further studies using well-defined subjects with and without asthma should be done to better assess the test characteristics of sG_{aw}.

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REFERENCES

1. Crapo RO, Casaburi R, Coates AL, Enright PL, Hankinson JL, Irvin CG, et al. Guidelines for methacholine and exercise challenge testing-1999. Am J Respir Crit Care Med 2000;161(1):309-329.
2. Perpina M, Pellicer C, de Diego A, Compte L, Macian V. Diagnostic value of the bronchial provocation test with methacholine in asthma: Bayesian analysis approach. Chest 1993;104(1):149-154.
3. Rijcken B, Schouten JP, Weiss ST, Rosner B, De Vries K, Van der Lende R. Long term variability of bronchial responsiveness to histamine in a random population sample of adults. Am Rev Respir Dis 1993;148(4 Pt 1):944-949.

4. Cockroft DW, Berscheid BA. Measurement of responsiveness to inhaled histamine: Comparison of FEV and sGaw. Ann Allergy 1983; 51(3):374-377.
5. Habib MP, Pare PD, Engel LA. Variability of airways response to inhaled histamine in normal subjects. J Appl Physiol 1979;47(1):51-58.
6. Parker AL, McCool FD. Pulmonary function characteristics in patients with different patterns of methacholine airway hyperresponsiveness. Chest 2002;121(6):1818-1823.
7. Morice AH, Waterhouse JC, Peers EM, Parry-Billings M. Use of whole body plethysmography to compare bronchodilator inhaler efficacy. Respiration 1998;65(2):120-124.
8. Hellinckx J, De Boeck K, Bande-Knops J, van der Poel M, Demedts M. Bronchodilator response in 3-6.5 years old healthy and stable asthmatic children. Eur Respir J 1998;12(2):438-443.
9. Van Noord JA, Smeets J, Clément J, Van de Woestijne KP, Demedts M. Assessment of reversibility of airflow obstruction. Am J Respir Crit Care Med 1994;150(2):551-554.
10. Bussamra MH, Cukier A, Stelmach R, Rodrigues JC. Evaluation of the magnitude of the bronchodilator response in children and adolescents with asthma. Chest 2005;127(2):530-535.
11. Ishii M, Hida W, Suzuki S, Ichinose M, Sasaki H, Takishima T. Comparison of intermittent and continuous inhalation provocation tests. Ann Allergy 1989;62(3):223-228.
12. Donna E, Danta I, Kim CS, Wanner A. Relationship between deposition of and responsiveness to inhaled methacholine in normal and asymptomatic subjects. J Allergy Clin Immunol 1989;83(2 Pt 1): 456-461.
13. Clini E, Vitacca M, Scalvini S, Quadri A, Foglio K. Methacholine inhaled challenge: study of correlation among different indices expressing the result. Monaldi Arch Chest Dis 1996;51(3):194-198.
14. Munakata M, Ohe M, Homma Y, Kawakami Y. Pulmonary dysanapsis, methacholine airway responsiveness and sensitization to airborne antigen. Respirology 1997;2(2):113-118.
15. Greenspon LW, Gracely E. A discriminant analysis applied to methacholine bronchoprovocation testing improves classification of patients as normal, asthma, or COPD. Chest 1992;102(5):1419-1425.
16. American Thoracic Society. Standardization of spirometry, 1994 update. Am J Respir Crit Care Med 1995;152(3):1107-1136.
17. Crapo RO, Morris AH, Gardner RM. Reference spirometric values using techniques and equipment that meet ATS recommendations. Am Rev Respir Dis 1981;123(6):659-664.
18. American Thoracic Society. Lung function testing: selection of reference values and interpretative strategies. Am Rev Respir Dis 1991; 144(5):1202-1218.
19. Scott GC, Braun SR. A survey of the current use and methods of analysis of bronchoprovocational challenges. Chest 1991;100(2):322-328.
20. Schisterman EF, Perkins NJ, Liu A, Bondell H. Optimal cut-point and its corresponding Youden Index to discriminate individuals using pooled blood samples. Epidemiology 2005;16(1):73-81.
21. Perkins NJ, Schisterman EF. The inconsistency of "optimal" cutpoints obtained using two criteria based on the receiver operating characteristic curve. Am J Epidemiol 2006;163(7):670-675.
22. Newcombe RG. Two-sided confidence intervals for the single proportion: comparison of seven methods. Stat Med 1998;17(8):857-872.
23. Cropp GJA, Bernstein IL, Boushey HA. Guidelines for bronchial inhalation challenges with pharmacologic and antigenic agents. ATS News 1980;6:11-19.
24. Goldstein MF, Pacana SM, Dvorin DJ, Dunsky EH. Retrospective analyses of methacholine inhalation challenges. Chest 1994;105(4): 1082-1088.
25. Houghton CM, Woodcock AA, Singh D. A comparison of plethysmography, spirometry and oscillometry for assessing the pulmonary effects of inhaled ipratropium bromide in healthy subjects and patients with asthma. Br J Clin Pharmacol 2005;59(2):152-159.
26. Moore BJ, Verburgt LM, King GG, Paré PD. The effect of deep inspiration on methacholine dose-response curves in normal subjects. Am J Respir Crit Care Med 1997;156(4 Pt 1):1278-1281.
27. Pellegrino R, Sterk PJ, Sont JK, Brusasco V. Assessing the effect of deep inhalation on airway calibre: a novel approach to lung function in bronchial asthma and COPD. Eur Respir J 1998;12(5):1219-1227.
28. King GG, Moore BJ, Seow CY, Paré PD. Airway narrowing associated with inhibition of deep inspiration during methacholine inhalation in asthmatics. Am J Respir Crit Care Med 2001;164(2):216-218.
29. Michoud MC, Ghezzo H, Amyot R. A comparison of pulmonary function tests used for bronchial challenges. Bull Eur Physiopathol Respir 1982;18(4):609-621.
30. Fish JE, Rosenthal RR, Batra G, Menkes H, Summer W, Permutt S, Norman P. Airway response to methacholine in allergic and nonallergic subjects. Am Rev Respir Dis 1976;113(5):579-586.
31. Corrao WM, Braman SS, Irwin RS. Chronic cough as the sole presenting manifestation of bronchial asthma. N Eng J Med 1979; 300(12):633-637.
32. Willis BH. Spectrum bias-why clinicians need to be cautious when applying diagnostic test studies. Fam Pract 2008;25(5):390-396.