

Conflicting Methacholine Challenge Tests

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Introduction

An 11-year-old boy experienced almost daily wheezing, dyspnea on exertion, and sleep perturbation because of cough. In addition, nadir peak expiratory flow (PEF) was below 100 L/min, which was <30% of predicted (328 L/min),¹ compatible with severe persistent asthma.² After the initiation of therapy with 180 μ g of albuterol 3 times per day, the patient's domiciliary PEF rose to >200 L/min and his symptoms improved. The patient was subsequently prescribed fluticasone 220 μ g twice daily. After weeks of combined albuterol and fluticasone therapy, the patient's PEF was >300 L/min and his symptoms had regressed to a mild intermittent status.² Three weeks after the initiation of fluticasone therapy the patient presented to the hospital for a methacholine challenge test (test 1). The patient did not take his albuterol or fluticasone on the day of testing. The baseline spirometry was normal (Table 1). Methacholine challenge testing was performed with a modified 5-breath dosimeter technique, nebulizing Provocholine (Methapharm, Brantford, Ontario, Canada) with a DeVilbiss 646 nebulizer (DeVilbiss, Health Care, Somerset, Pennsylvania). The methacholine challenge test revealed a PC₂₀ (the provocative concentration of methacholine that resulted in a 20% decrease in forced expiratory volume in the first second [FEV₁]) of >20 mg/mL (Table 2). In response to this "negative" methacholine challenge, the patient was declared "nonasthmatic" by his pediatrician, and the albuterol and fluticasone therapy was discontinued. Shortly after the discontinuance of therapy, the patient's symptoms and variable PEF returned. Two weeks following the discontinuance of therapy, the patient returned for a repeat methacholine challenge test (test 2). The baseline spirometry was again normal (Table 3); however, this time

Table 1. Baseline Spirometry Results: Test 1

Test	Predicted	Measured	Percent of Predicted
FVC (L)	2.75	2.74	100
FEV ₁ (L)*	2.40	2.19	91
FEV ₁ /FVC (%)	88	82	91
FEF _{25-75%} (L/s)	2.75	2.16	79
FEF _{50%} (L/s)	3.12	2.53	81
FEF _{max} (L/s)	5.46	5.11	94
TET (s)	NA	6.03	NA

FVC = forced vital capacity

FEV₁ = forced expiratory volume in the first second

*FEV₁ variance from 3 FVC measurements = 6 mL

FEV₁/FVC = ratio of FEV₁ to FVC

FEF_{25-75%} = forced expiratory flow during the middle half of the FVC

FEF_{50%} = forced expiratory flow at 50% of the FVC

FEF_{max} = maximum forced expiratory flow

TET = total expiratory time

NA = not applicable

Table 2. Methacholine Challenge Test Results: Test 1

Protocol Step	Breaths	Cumulative Dose Units*	FEV ₁ (L)	Percent Change
Pre-spirometry	NA	NA	2.19	NA
2.5 mg/mL	3	7.5	2.15	-2
2.5 mg/mL	5	20	2.14	-3
5 mg/mL	5	45	2.08	-5
10 mg/mL	5	95	1.98	-10
			1.98	-10
20 mg/mL	5	195	1.95	-11

*Dose units calculated as breaths \times mg/mL

FEV₁ = forced expiratory volume in the first second

NA = not applicable

PD₂₀ (provocative dose of methacholine that results in a 20% fall in FEV₁) = 195 dose units

PC₂₀ (provocative concentration of methacholine that results in a 20% fall in FEV₁) = 20 mg/mL

the patient demonstrated substantial bronchoconstriction in response to methacholine inhalation, with a PC₂₀ of 8.85 mg/mL (Table 4). A PC₂₀ of 4–16 mg/mL is classified as borderline bronchial hyperresponsiveness;³ however, given the change in PC₂₀ and the accompanying changes in symptoms and PEF variability, methacholine challenge test 2 was interpreted as a positive test for asthma.

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Table 3. Baseline Spirometry Results: Test 2

Test	Predicted	Measured	Percent of Predicted
FVC (L)	2.75	2.86	104
FEV ₁ (L)*	2.40	2.25	94
FEV ₁ /FVC (%)	88	79	89
FEF _{25-75%} (L/s)	2.75	2.10	77
FEF _{50%} (L/s)	3.12	2.55	82
FEF _{max} (L/s)	5.46	4.53	83
TET (s)	NA	5.11	NA

FVC = forced vital capacity
 FEV₁ = forced expiratory volume in the first second
 *FEV₁ variance from 3 FVC maneuvers = 4 mL
 FEV₁/FVC = ratio of FEV₁ to FVC
 FEF_{25-75%} = forced expiratory flow during the middle half of the FVC
 FEF_{50%} = forced expiratory flow at 50% of the FVC
 FEF_{max} = maximum forced expiratory flow
 TET = total expiratory time
 NA = not applicable

Table 4. Methacholine Challenge Test Results: Test 2

Protocol Step	Breaths	Cumulative Dose Units*	FEV ₁ (L)	Percent Change
Pre-spirometry	NA	NA	2.25	NA
2.5 mg/mL	3	7.5	2.14	-5
2.5 mg/mL	5	20	2.14	-5
			2.15	-4
5 mg/mL	5	45	2.02	-10
			2.01	-11
10 mg/mL	5	95	1.74	-23
			1.58	-30
			1.69	-25

*Dose units calculated as breaths × mg/mL
 FEV₁ = forced expiratory volume in the first second
 NA = not applicable
 PD₂₀ (provocative dose of methacholine that results in a 20% fall in FEV₁) = 83.46 dose units
 PC₂₀ (provocative concentration of methacholine that results in a 20% fall in FEV₁) = 8.85 mg/mL

Question

What are the possible causes for the different PC₂₀ values between test 1 and test 2?

Answers

- Poor spirometry technique and effort during methacholine challenge test 2.
- Spurious results due to technical or procedural factors.
- The patient had a respiratory infection when test 2 was conducted.
- The higher PC₂₀ on test 1 was an effect of fluticasone therapy.

Table 5. Strategies to Obtain Acceptable Spirometry Results From Children

Children >9 years of age
Avoid criticism
Use enthusiastic and positive language
Introduce an element of competition
Children 4–9 years of age
Avoid frightening the patient with loud coaching
Offer lots of praise
If the patient's attention span is short, take a break: color some pictures, go for a walk, get a drink
After Test Completion
Offer rewards (eg, stickers, small toys) regardless of the patient's performance, so he or she might look forward to the next visit

(Adapted from Reference 7.)

Discussion

Poor Patient Effort

Patient effort during spirometry testing may be poor or inconsistent due to inadequate instruction and coaching, physical fatigue, compromised cognitive function, or in-dolence. While obtaining quality spirometry tests from young children can be challenging, nearly all school-age children can produce acceptable spirometry measurements. Arets et al⁴ found that 98.4% of patients age 5–19 years old could produce multiple FEV₁ measurements with a variability <200 mL. In a study of 4,000 patients age 9–18 years old, Enright and colleagues⁵ found that American Thoracic Society spirometry acceptability criteria were met approximately 95% of the time.

Obtaining acceptable spirometry may be more difficult with young children; Crenesse et al⁶ found that only 55% of children ages 3–6 years old could reproduce forced vital capacity and FEV₁ values within 100 mL, and 21% of the patients could not exhale for longer than 1 second. Wanger⁷ offered good strategic advice for obtaining acceptable spirometry measurements from children (Table 5). It is doubtful that poor efforts were responsible for the conflicting results between methacholine challenge tests 1 and 2. The patient demonstrated consistent effort, with the FEV₁ varying only 6 mL and 4 mL in the baseline spirometry for test 1 and test 2, respectively. In addition, there was very little FEV₁ variability during both methacholine challenge tests when repeat measurements within a protocol step were made. The greatest variability was 160 mL between the 3 FEV₁ measurements that were >20% below baseline in methacholine challenge test 2 (10 mg/mL dose). I performed both of the patient's tests and, in my judgment, the patient's spirometry technique and effort were consistently good.

Spurious Results Due to Technical or Procedural Factors

There are numerous technical and procedural factors that can affect methacholine challenge results.³ First, the methacholine powder must be mixed correctly with normal saline, with or without 0.4% phenol (preservative), under sterile conditions. Methacholine should be stored at about 4°C, but should be allowed to warm to room temperature before administration. Provocholine can be stored for up to 5 months without loss of potency. There is no evidence to suspect that the results of methacholine challenge test 1 were affected by diminished methacholine potency. We follow the stated recommendations for handling and storage, and label methacholine vials with the expiration date.

Another important factor in methacholine administration is nebulizer performance. It is suggested that the nebulizer output should be 0.009 mL \pm 10% from a 0.6-s dosimeter-controlled nebulization time.³ Factors that affect the performance of the DeVilbiss 646 nebulizer include the gas flow, the opening or closing of the nebulizer air vent (open position increases output), the position of the impinger arm, and the distance between the capillary tube and the jet orifice. Enright has suggested the use of a spark-plug-gapper tool to standardize the gap between the capillary tube and the jet orifice.³ I use the same nebulizer for each methacholine dose to avoid output variability between nebulizers. While I do not believe that nebulizer performance was responsible for the conflicting methacholine challenge results, I cannot state this affirmatively, because I did not measure nebulizer output of the 2 nebulizers used for methacholine tests 1 and 2. Finally, the patient's inspiratory flow, volume, and breath-hold time can also affect methacholine deposition. These variables can be difficult to control and should be practiced with the patient before methacholine is delivered. While holding the nebulizer level, wearing nose clips, and ensuring that the mouthpiece is not blocked by the tongue or teeth, the patient should be instructed to inhale slowly from the functional residual capacity to the total lung capacity (inspiratory time approximately 5 s), followed by a 5-s breath-hold.³ It is recommended that spirometry is repeated 30 s and 90 s after methacholine inhalation and that methacholine doses be delivered 5 min apart.^{3,8} Pulmonary function technologists should make every effort to follow their laboratory's methacholine protocol with as much precision as possible to minimize the effect of procedural variation on test results.

The Effect of Respiratory Infections on Airway Responsiveness

Respiratory infections can increase airway responsiveness for weeks, in both asthmatic and nonasthmatic indi-

viduals.^{3,9,10} The presence of atopy and a lower baseline FEV₁ are predisposing factors for infection-related increases in airway responsiveness.¹¹ In addition, the type of respiratory infection may influence the state of airway responsiveness.¹² Methacholine challenge testing should be postponed in patients with recent respiratory infections to avoid false-positive results. The patient in this case did not have signs or symptoms of a respiratory infection.

The Effect of Corticosteroids on Airway Responsiveness

Airway inflammation and bronchial hyperreactivity are key features of asthma. Though bronchial hyperreactivity is probably not caused exclusively by airway inflammation, corticosteroids have been shown to increase PC₂₀¹³⁻¹⁷ and improve the bronchodilator effect of deep inspiration.¹³ Corticosteroid use at the time of methacholine challenge test is not uncommon; Haynes et al¹⁸ found that 16.8% of patients had taken inhaled corticosteroids within 1 week of their methacholine challenge test. There are currently no recommendations for withholding corticosteroids prior to methacholine tests. The effect of corticosteroids on bronchial responsiveness may be prolonged, albeit probably not longer than a couple of weeks. Vathenen et al¹⁴ studied the effects of inhaled budesonide on bronchial responsiveness to histamine in 40 asthmatic patients. They found an increase in the PD₂₀ (the provocative dose of histamine that caused a 20% decrease in FEV₁) after the very first dose of budesonide, which continued to improve over the subsequent 6-week study period. Bronchial responsiveness to histamine returned to baseline 1 week after the discontinuance of budesonide treatment. van Rensen et al¹⁵ studied the effects of fluticasone 500 μ g twice daily on inflammatory markers in patients with asthma. After 2 weeks and 4 weeks of treatment, histamine PC₂₀, sputum eosinophilia, and exhaled nitric oxide were significantly improved over baseline and compared to placebo; however, after a 2-week washout period these physiologic gains were lost. Convery and colleagues¹⁶ also found that increases in methacholine PC₂₀ following 6 weeks of fluticasone treatment were lost after 2 weeks of treatment cessation. Marabini et al¹⁷ studied the effects of beclomethasone withdrawal in asthmatics who had been treated for greater than 3 months. After 3 weeks of beclomethasone discontinuance, methacholine responsiveness was significantly increased in both patients who had a normalized PC₂₀ due to corticosteroid treatment and those who had persistent hyperreactivity. It is notable that an acute increase in methacholine responsiveness was seen even in 3 patients who had taken beclomethasone for 3 years.

It is quite likely that the higher PC₂₀ in methacholine challenge test 1 (>20 mg/mL) was due to fluticasone ther-

apy. At the time of methacholine challenge test 1, the patient had been taking fluticasone for 20 days and had taken a dose the evening before the testing day. Methacholine challenge test 2, which showed a PC₂₀ of 8.85 mg/mL, was performed 14 days after fluticasone therapy had been discontinued. The fact that increased symptoms and PEF variability accompanied the reduction in PC₂₀ favors fluticasone discontinuance as the source of the PC₂₀ disparity.

Case Scrutiny

Methacholine challenge tests are administered to assess bronchial reactivity when some possibility of asthma exists. In other words, there is a clinical suspicion of asthma or there is a possibility of asthma that needs to be ruled out. By definition, suspicion includes some element of doubt. When one reviews this case, it is difficult to understand why there was much doubt about an asthma diagnosis. The patient's classic asthma symptoms and variable PEF values, which both improved in a stepwise fashion with β agonist, then corticosteroid therapy, made the pre-test probability of asthma very high. Based on these findings it could be easily argued that a methacholine challenge wasn't necessary. In addition, there was clearly a lack of appreciation for the effect of corticosteroid therapy on PC₂₀, which led to the false conclusion that the patient did not have asthma and the ill-advised decision to discontinue clearly beneficial therapy. It is important for the interpreting physician to be aware of any pertinent medications that the patient is taking at the time of methacholine challenge testing. We provide space for documentation of such information on our methacholine-challenge worksheet (Appendix) for the interpreting physician to review. Armed with that information, the interpreting physician can communicate any possible pharmacologic influences on PC₂₀ to the referring physician.

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Appendix

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PULMONARY MEDICINE LABORATORY

INPATIENT OUTPATIENT

DATE OF STUDY:

PHYSICIAN:

CIRCLE MEDICATIONS/LAST DOSE

Prednisone/ _____ Ipratropium/ _____
 Inhaled Steroids/ _____ Antihistamines/ _____
 Nasal Steroids/ _____ Leukotriene Modifiers/ _____
 Salmeterol/ _____ Theophylline/ _____
 Albuterol/ _____ Cromolyn/ _____

Smoking : Y / N PPD:

Last cigarette:

DOSAGE	FREQ	DOSE UNITS	FEV1	%CHANGE
Baseline Spirometry	---	0		
2.50 mg/ml	3	7.50		
2.50 mg/ml	5	20.00		
5.00 mg/ml	5	45.00		
10.00 mg/ml	5	95.00		
20.00 mg/ml	5	195.00		
Post Bronchodilator				

INTERPRETATION: