Aerosol Use in the Pulmonary Function Lab

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Aerosolized medications are frequently used in the pulmonary function laboratory. The 2 most common implementations are bronchodilators and bronchial challenge agents. Bronchodilator administration is not well standardized, largely because of the various methods of delivery available for clinical practice. Metered-dose inhalers used with spacer devices are the most common route for bronchodilator administration, but many laboratories use small-volume nebulizers. Interpretation of pre- and post-bronchodilator studies is confounded by the definitions of airway obstruction and bronchodilator responsiveness. Protocols for administering bronchial challenge aerosols (methacholine, mannitol, hypertonic saline) are well defined but are susceptible to some of the same problems that limit comparison of bronchodilator techniques. Bronchial challenges with inhaled aerosols are influenced not only by the delivery device but by the patient's breathing pattern, particularly in protocols that include deep inspiratory efforts. *Key words: aerosols; bronchodilators; bronchial challenge; methacholine; mannitol; FEV_1.* [Respir Care 2015;60(6):931–940. © 2015 Daedalus Enterprises]

Aerosols in the Pulmonary Function Testing Lab

Aerosolized agents are an essential component of pulmonary function testing (PFT).[1] Inhaled bronchodilators are widely used to assess reversibility of airway obstruction, particularly in patients suspected of having asthma.[2] Measurement of the FEV_1/FVC ratio after administration of a β agonist is the de facto test to establish the diagnosis of COPD.[3] Similarly, measurement of FEV_1 following bronchodilator administration is used to categorize the severity of airway obstruction. Improvement in air flow is the primary end point in the evaluation of new inhaled bronchodilators, and PFT provides the necessary tools for assessment. The other primary use of aerosols in the PFT lab involves inhaled bronchial challenge agents. As is the case for bronchodilators, bronchial provocation using an aerosolized agent is a key tool for making or excluding the diagnosis of asthma.[4] Inhalation of challenge aerosols also provides a means to quantify the degree of hyper-responsiveness.

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How Aerosols Are Administered in the PFT Lab

Bronchodilators

Bronchodilators can be administered by various methods for diagnostic purposes. Typically, a β agonist or an-

ticholinergic drug is delivered using the same method as might be employed by the patient. These methods include metered-dose inhalers (MDIs), powder inhalers, or small-volume nebulizers powered by compressed gas.[5] In some instances, the patient may be tested using the same device and dose prescribed for him/her.

Bronchial Challenge Agents

Bronchial challenge agents typically require a specific protocol depending on the agent.[6,7] Methacholine and histamine are usually administered via a small-volume nebulizer. To quantify the number of breaths of the challenge agent, a dosimeter may be used. Dosimeters can be triggered manually by the pulmonary function technologist or automatically by sensing the patient's inspiratory effort. Mannitol utilizes a proprietary powder inhaler to deliver the drug. Ultrasonic or thin-film nebulizers are used to administer hypertonic saline as an airway challenge agent.

Bronchodilator Testing

Despite the fact that inhaled bronchodilators are commonly used in conjunction with PFT, how they are administered is not universally standardized. The 2005 guidelines of the American Thoracic Society/European Respiratory Society (ATS/ERS) proposed some recommendations for bronchodilator administration.[1]

If the goal of measuring air flow following bronchodilator administration is to determine whether improvement is possible, patients typically do not need to withhold their medications. In fact, their treatment should be optimized and their condition stable. If the question to be answered is whether air flow limitation is reversible, then patients should withhold medications before baseline testing. Short-acting β agonists and anticholinergics such as ipratropium should be withheld at least 4 h before testing. Long-acting β agonists such as salmeterol, as well as slow-release preparations and oral aminophyllines, should be withheld for at least 12 h.

The ATS/ERS 2005 spirometry guidelines[1] suggest the following steps to standardize bronchodilator administration in the context of PFT: 1) use a valved holding chamber; 2) inhale 90 μg (90 μg in the United States equals 100 μg in Europe because of how the dose is measured) of short-acting β agonist (albuterol or similar) from below functional residual capacity to total lung capacity; 3) breath-hold for 5–10 s; 4) take 4 separate doses (total of 360 μg) at 30-s intervals, and 5) repeat spirometry after 10–15 min for short-acting β agonists and 30 min for short-acting anticholinergic agents.

This protocol assumes the use of an MDI delivers ~360 μg of drug to the airways. Other drugs may be used and at lower doses if clinically indicated. If an anticholinergic such as ipratropium is used, 4 puffs of 40 μg may be administered. The choice of whether to use a β agonist or an anticholinergic for reversibility testing is usually made based on the anticipated or prescribed therapy for an individual patient. For short-acting β agonists, spirometry is performed after waiting at least 10 min; for anticholinergics, a 30-min delay (or slightly longer) is recommended. If the bronchodilator is delivered via small-volume nebulizer, a standard dose (such as 2.5 mg of albuterol in 2.5 mL of solution) should be adopted, with waiting times similar to those used for an MDI after the solution has been completely nebulized. Small-volume nebulizers with aerosol outputs in the respirable range (1–5-μm mass median aerodynamic diameter) capable of delivering the charge dose in a reasonable interval (5–15 min) should be used.

The amount of drug delivered to the airways depends on the method of administration along with the particle size distribution, inspiratory flow, breath-hold time, and inspired volume.[8] Each laboratory should control the method used to standardize the definition of reversibility. The use of a valved holding chamber or similar device is recommended.[9] For both adult and pediatric patients, activation of the MDI by the technologist (rather than the patient) along with appropriate coaching of the inspiratory maneuver may achieve more consistent drug delivery. PFT labs should select appropriate devices (ie, MDIs, powder inhalers, small-volume nebulizers) based primarily on their particle deposition characteristics.[10] For pediatric patients, a well-fitting mask rather than a mouthpiece may be needed for optimal drug delivery.

Infection control of aerosol devices used in the PFT lab closely follows the same recommendations for devices used therapeutically.[11] Technologists should wear gloves while handling mouthpieces, masks, or other components that may come in direct physical contact with mucous membranes. Reusable nebulizers, mouthpieces, and other equipment must be cleaned, disinfected, rinsed with sterile water, and allowed to air-dry between uses. MDIs are designed for single-patient use; however, some laboratories have adopted a common canister protocol, which allows the MDI to be reused for multiple patients.[12] This protocol requires that a clean valved holding chamber be used for each patient and that the MDI nozzle be cleaned and disinfected between patients. The canister itself is wiped with alcohol between patient uses, and good handwashing technique is required. The common canister protocol may not be appropriate for testing high-risk populations (eg, patients with cystic fibrosis, immunocompromised patients, lung transplant recipients).

Protocols for aerosolized bronchodilator delivery in conjunction with PFT underlie even larger problems of diagnosing and categorizing airway obstruction. These problems include how obstruction and reversibility of air-flow limitation are defined.[13] The Global Initiative for Chronic

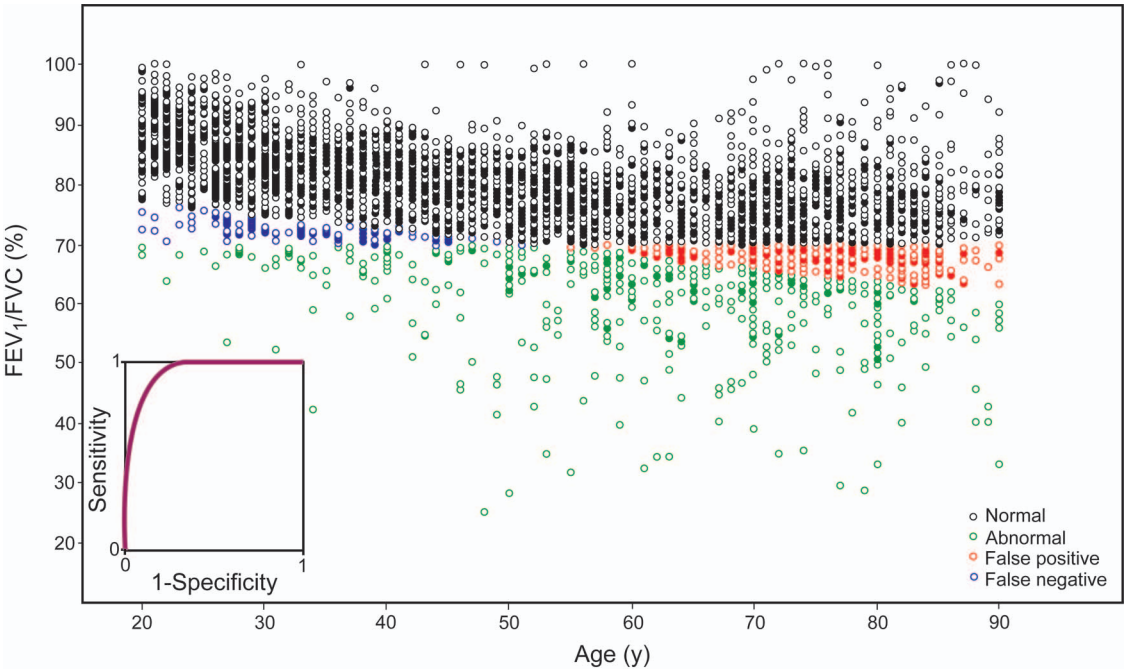

Fig. 1. FEV_1/FVC ratios for females in the National Health and Nutrition Examination Survey III study are plotted against their age. The graph compares the fixed cutoff of 70% to the lower limit of the normal range (defined as the lowest percentile) for making a diagnosis of airway obstruction. Black circles represent subjects with normal values (true negative) by either method, and green circles represent subjects with abnormal ratios (true positive) by either method. Red circles show false positives, that is, subjects whose FEV_1/FVC is < 0.70 but above the lowest 5th percentile. Blue circles represent younger subjects who are false negatives because their ratios are > 0.70 but below the lowest 5th percentile. Using the fixed cutoff introduces an age-related bias and may misclassify older subjects as having airway obstruction or younger patients as normal when their lung function is significantly below their lowest 5th percentile. The receiver operating characteristic curve is shown in the inset. Courtesy Philip H Quanjer.

Obstructive Lung Disease (GOLD) defines COPD as $FEV_1/FVC < 0.70$ after bronchodilator administration.[14] The GOLD criteria do not specify any particular methodology for bronchodilator administration. This definition, based on a fixed cutoff of 0.70, has been widely adopted for clinical and research purposes. Unfortunately, the construct of a fixed cutoff obscures the natural history of lung function. Both FEV_1 and FVC decrease with advancing age; FEV_1 decreases slightly more rapidly that FVC, and as a result, the ratio decreases with age.[15] In men, FEV_1/FVC reaches 0.70 usually in the fourth to fifth decade of life, and in women, slightly later (fifth decade). Using a fixed cutoff after bronchodilator administration misclassifies older adults as having airway obstruction and may miss real obstruction in young adults (Fig. 1). Numerous studies, as well as the ATS/ERS guidelines,[1] suggest using the lowest fifth percentile as the lower limit of the normal range to define airway obstruction.[16-18]

The second problem related to interpretation of post-bronchodilator pulmonary function is how reversibility is defined. Current ATS/ERS recommendations suggest that a post-bronchodilator increase in FEV_1 (or FVC) of $> 12\%$ *and* 0.2 L is the minimally significant change.[19] Some clinicians prefer an improvement in the percent predicted following bronchodilator administration; in this case, an increase of $> 9\%$ may be considered significant.[20] These definitions of reversibility are based on changes in FEV_1 that exceed those occurring in healthy subjects following bronchodilator administration. Healthy subjects typically have small improvements (3–10%).[19] However, requiring a 12% and 0.2-L increase may not be appropriate in older subjects with obstruction. The 12%/0.2-L criteria are not appropriate for many pediatric subjects.[21] Many patients with baseline airway obstruction show symptom relief even though they do not formally meet the ATS/ERS criteria. Subjects with COPD have shown quite variable responses to inhaled bronchodilators upon serial testing (Fig. 2).[22]

Another issue complicating the interpretation of post-bronchodilator pulmonary function is whether other tests besides spirometry should be used. Lung-volume measurements are used infrequently but may show significant changes, particularly in the presence of hyperinflation or air trapping.[23] Airway resistance and conductance, measured by body plethysmography, often improve significantly even though FEV_1 does not. Because of the variability in airway resistance and conductance in healthy subjects, improvements of 40–50% are necessary to conclude that reversibility is present.[24] Measurements of re-

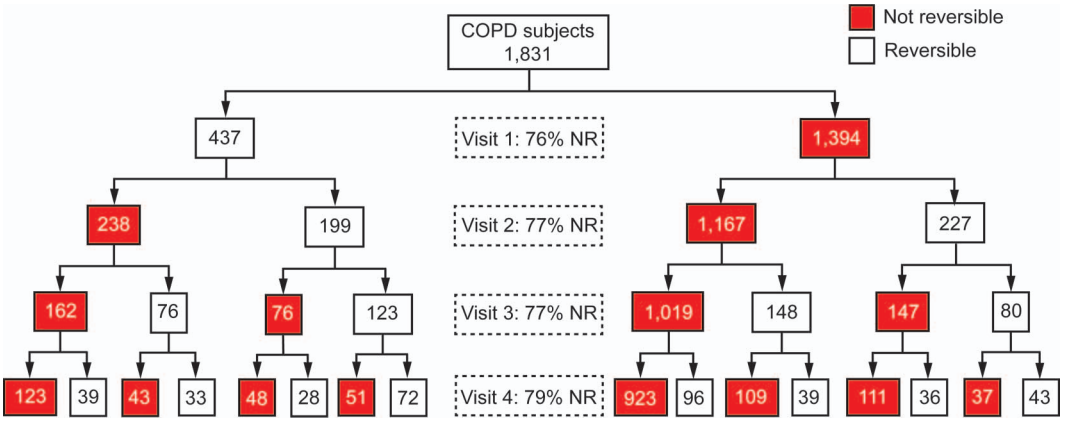

Fig. 2. Response to inhaled bronchodilator in 1,831 subjects with COPD on serial testing at 4 visits. Red boxes show the number of subjects who did not have a 12% and 0.2-L improvement following inhaled bronchodilator administration. White boxes show the number of subjects who met the criteria for a positive response at each visit. Although between 76% and 79% of the subjects were reversible at each of the 4 visits, only 50% (923/1,831) were not reversible (NR) at all visits, and only 4% (72/1,831) displayed consistent reversibility at all visits. Data are from Reference 22.

Table 1. Bronchodilator Testing Problems

Test-related issues
MDI vs small-volume nebulizer vs other
Use of spacer or other device to enhance deposition
Dosage and delivery to the airways
Spirometry vs lung volumes, R_{aw}, sG_{aw}, FOT
Patient-related issues
12%/200 mL not appropriate for all subjects
Definition of obstruction, fixed cutoff vs lower limit of the normal range (elderly, pediatric)
Variability of responsiveness in COPD, asthma
Bronchial hyper-responsiveness not predicted by bronchodilator response
Unacceptable/non-repeatable spirometry

MDI = metered-dose inhaler
R_{aw} = resistance of the airways
sG_{aw} = specific conductance of the airways
FOT = forced oscillation technique

sistance by the forced oscillation technique may show similar improvements after bronchodilation, and this methodology is applicable to pediatric patients who may not be able to perform spirometry.[25]

Table 1 summarizes some of the problems surrounding pre/post-bronchodilator testing and interpretation of reversibility. Because of these, the consensus of most clinicians is that lack of response to a single measurement does not preclude a trial of bronchodilators. The consequences of false-positive and false-negative results must be considered. A false positive (apparent bronchodilator response when none is present) usually results in prescription of a bronchodilator, which may present risks, particularly in patients who have cardiovascular comorbidities.[26] A false negative (no apparent response in the presence of airway obstruction) can result in misclassification and misdiagnosis. A false-negative response to bronchodilator testing can be particularly insidious in cases in which an elderly patient is misclassified as having airway obstruction. Such individuals may be diagnosed as having COPD.[16] The following points should be considered in the performance and interpretation of bronchodilator studies: 1) reduce variability by using a consistent method of bronchodilator administration, 2) consider the rationale for testing (overall improvement vs reversibility), 3) recognize the variability of an individual patient's response (especially in COPD), 4) consider responses in addition to FEV_1 (residual volume, specific airway conductance, forced oscillation technique), and 5) a negative response does NOT preclude a clinical trial.

Inhaled Bronchial Challenge Agents

Bronchial challenges are designed to answer 2 basic questions: 1) Does the patient have asthma? 2) What is the degree of hyper-responsiveness? Inhalation challenge tests are widely used to assist in making or excluding the diagnosis of asthma.[4,27] Aerosols are used for both direct (methacholine, histamine) and indirect (mannitol, hypertonic saline) bronchial challenges. Table 2 lists the agents available for direct and indirect bronchial challenge. Direct challenge agents cause bronchoconstriction in the smooth muscle surrounding the airways, whereas indirect challenge agents provoke changes in the mucociliary lining of the airways, similar to those that accompany airway inflammation and narrowing. Direct challenge with methacholine has a high sensitivity and fair specificity if the challenge is performed close to the time when symptoms are present. Direct challenges are most useful to rule out

Table 2. Direct and Indirect Bronchial Challenge Agents

Direct
Cholinergic agonists
Methacholine*
Carbachol*
Acetylcholine*
Histamine*
Indirect
Physical stimuli
Exercise
Eucapnic voluntary hyperventilation
Hypertonic saline*
Mannitol*
Pharmacologic stimuli
Adenosine monophosphate*

* Aerosol challenge agents

asthma, that is, a negative direct challenge effectively eliminates current asthma. Indirect challenges such as exercise or mannitol have a high specificity in patients who have airway inflammation common to asthma. A positive indirect challenge confirms (rules in) the presence of asthma and is often associated with exercise-induced bronchospasm.[6] Unfortunately, inhalation challenges are subject to many of the same problems that plague bronchodilator studies, namely, the method of aerosol generation and delivery can greatly affect the outcome of the challenge.[28] Additional issues that arise during inhalation challenges include the fact that the patient may experience symptoms and physiologic changes that make it difficult to perform the required maneuvers (maximum forced exhalation).[29]

Neither direct nor indirect challenges are 100% sensitive and specific for asthma. Direct challenges (eg, methacholine) may result in a false positive in current smokers, patients with congestive heart failure or allergic rhinitis, and diseases that affect airway caliber (COPD, bronchitis, cystic fibrosis). Direct challenges can give a false negative if asthma-like symptoms are not present or if the patient has not withheld bronchodilators or other anti-asthma medications. Both direct and indirect challenges can result in a false positive if the patient has had a recent upper or lower respiratory tract infection.[6] Indirect challenges (such as exercise or mannitol) may give a false negative if the patient has recently exercised (ie, indirect challenges tend to cause a refractory period) or if medications are not withheld appropriately. Paroxysmal coughing sometimes occurs with indirect challenges, making it difficult to assess the degree of bronchial hyper-responsiveness.

Hypertonic saline (and mannitol) may be used to promote airway clearance as well as for bronchial challenge. Hypertonic saline (3.0–7.0%) has been used to assist with clearance of secretions in cystic fibrosis as well as in infants with bronchiolitis. A hypertonic saline challenge may

Methacholine* Dosing Schemes

Doubling dose		Quadrupling dose	
0.031	mg/mL	0.0625	mg/mL
0.0625	mg/mL	0.250	mg/mL
0.125	mg/mL	1.0	mg/mL
0.250	mg/mL	4.0	mg/mL
0.50	mg/mL	16.0	mg/mL
1.0	mg/mL		
2.0	mg/mL		
4.0	mg/mL		
8.0	mg/mL		
16.0	mg/mL		

* Also used for histamine challenge

Fig. 3. Two methacholine dosing schemes. Methacholine may be administered in 9 doubling doses from 0.031 mg/mL up to 16 mg/mL or using a quadrupling scheme with 5 doses ranging from 0.625 mg/mL up to 16 mg/mL. A similar scheme can be used for histamine challenge. Not shown is an optional diluent step; normal saline or similar is administered before the first dose of methacholine and used to establish the target FEV_1 for calculating a 20% decrease. Data are from Reference 30.

be used to assess the safety of the agent for therapeutic purposes. In the standard challenge procedure, a high-output ultrasonic nebulizer or jet nebulizer is used to administer hypertonic saline (4.5%) over progressively increasing intervals (eg, 30 s and 1, 2, 4, and 8 min) with FEV_1 measured after each dose. A decrease in FEV_1 of $> 15\%$ is considered consistent with increased bronchial hyperreactivity. To evaluate patients with cystic fibrosis, a β agonist is administered before the saline challenge. A decrease of $> 20\%$ or intolerable symptoms would cause the patient to be ineligible for hypertonic saline therapy.

In 2000, the ATS published guidelines for performance of direct challenges using methacholine.[30] The recommendations suggest 2 protocols that can be used with the assumption that either will produce similar results when applied in a clinical setting evaluating subjects with suspected asthma.[30] The suggested protocols are the 2-min tidal breathing and 5-breath dosimeter methods. Although these protocols differ significantly in their methodologies (see below), both can be interpreted by expressing their results in terms of the provocative concentration of methacholine that produces a 20% decrease in FEV_1 (PC_{20}).

For each of the protocols, 2 methacholine dosing schemes are suggested (Fig. 3). Each protocol starts with a very low concentration of methacholine (0.031 or 0.0625 mg/mL) and increases with either doubling or quadrupling doses up to a maximum of 16 mg/mL. Because the 9-dose doubling scheme takes significantly longer to administer, the 5-dose quadrupling scheme is used more frequently. It should be noted that other dosing schemes are sometimes used; in particular, the dosing scheme supplied with the commercially available brand of methacholine (Provocholine, Methapharm, Brantford, Ontario, Canada) uses 5 dilutions

Table 3. 5-Breath Dosimeter Protocol

Perform baseline spirometry
Patient inspires slowly (5 s) to TLC
Dosimeter triggers for 0.6 s during inspiration
Output of 0.009 mL/actuation recommended
Patient breath holds at TLC (5 s)
Repeat 5 times (2-min total time)
FEV_1 measured at 30 and 90 s (3–4 efforts)
Record highest FEV_1
If FEV_1 falls $< 20\%$, repeat with next dose
If FEV_1 falls $> 20\%$ or last dose given, administer bronchodilator

TLC = total lung capacity

Table 4. 2-Minute Tidal Breathing Protocol

Perform baseline spirometry
Adjust nebulizer output to calibration level
Output of 0.13 mL/min is recommended
Patient breathes tidally for 2 min (timer)
FEV_1 measured at 30 and 90 s (3–4 efforts)
Record highest FEV_1
If FEV_1 falls $< 20\%$, repeat with next dose
If FEV_1 falls $> 20\%$ or last dose given, administer bronchodilator

but increases up to a maximum of 25 mg/mL.[31] A diluent step or dose is optional before the first dose of methacholine. The diluent is typically the solution used to prepare the methacholine (saline). If a diluent step is included, the control value for FEV_1 is taken from it; otherwise, the baseline FEV_1 is used to calculate a target 20% decrease in FEV_1.

Various brands of small-volume nebulizers may be used to deliver methacholine. Both reusable and disposable devices can be employed, but each should have consistent aerosol output with a mass median aerodynamic diameter in the range of 1.0–3.6 μm. For the 5-breath dosimeter method (Table 3), an output of 0.009 mL/actuation is recommended.[30] Each breath is triggered (either manually or by patient inspiratory effort) causing the dosimeter to activate the nebulizer for 0.6 s. Actuation occurs while the patient is inspiring maximally to total lung capacity, followed by a breath-hold. For the 2-min tidal breathing method (Table 4), the recommended nebulizer output is 0.13 mL/min.[30] The patient breathes tidally for a 2-min period.

For each of these protocols, the nebulizer may be evaluated by measuring its output.[32] This can be done using a measured amount of water and a laboratory balance. For the dosimeter method, the nebulizer with solution is weighed before and after a specific number of actuations, and the output is computed from the difference in weight divided by the actuations. For the tidal breathing method, the nebulizer with solution is weighed before and after a fixed number of minutes, with the output being equal to the difference in weight divided by the number of minutes. The actual nebulizer output for the tidal breathing method can usually be adjusted by altering the driving pressure/flow to the device. For the dosimeter method, the nebulizer output can be adjusted by changing the actuation time or, in some devices, by altering the driving pressure. If separate nebulizers are used for each dose of methacholine, they should be labeled after the optimal output has been calculated and/or confirmed. Each device may require a slightly different driving pressure/flow and should be marked appropriately. If disposable nebulizers are used, a representative sample should be evaluated for output; repeated measurements may be required for different lots of the same device.[30] If a single nebulizer is used for the challenge, any residual methacholine should be discarded by emptying and shaking the cup before proceeding to the next higher dose.

Table 5. Interpretation of Methacholine Challenge

PC_{20}	Interpretation*
> 16 mg/mL	Normal bronchial responsiveness
4.0–16 mg/mL	Borderline bronchial hyper-responsiveness
1.0–4.0 mg/mL	Mild bronchial hyper-responsiveness (positive test)
< 1 mg/mL	Moderate-to-severe bronchial hyper-responsiveness

* Good quality spirometry (no obstruction before test) and improvement in FEV_1 following bronchodilator after the challenge
PC_{20} = provocative concentration of methacholine that produces a 20% decrease in FEV_1

Either a mouthpiece or aerosol mask may be used to administer the methacholine. To optimize deposition in the airways, a nose clip is recommended, even when an aerosol mask is used. To minimize the amount of aerosol exhaled by the patient, a disposable bacterial filter may be connected to the exhalation port of the nebulizer. Performing the test in a negative-pressure room is ideal, but may not be practical for all labs. Technologists or other personnel who are sensitive to methacholine should not be present in the immediate area when testing is performed.[33]

Table 5 lists the PC_{20} values recommended by the ATS for interpretation of hyper-responsiveness following methacholine challenge. PC_{20} may be calculated as:

$$PC_{20} = \text{antilog}\left[\text{logC1} + \frac{(\text{logC2} - \text{logC1})(20 - \text{R1})}{\text{R2} - \text{R1}}\right]$$

where C1 is the second to last methacholine concentration, C2 is the final methacholine concentration (causing $\geq 20\%$ decrease in FEV_1), R1 is the percent decrease in FEV_1 after C1, and R2 is the percent decrease in FEV_1 after C2. Note that PC_{20} uses an exponential model to interpolate the exact concentration of drug that causes a 20% decrease in FEV_1. This is different from the provocative dose, which

Fig. 4. Mannitol challenge kit, marketed as Aridol. Each kit contains numbered blister packs with capsules of mannitol powder along with a disposable proprietary powder inhaler. Courtesy Pharmaxis.

is simply the dose of drug corresponding to the $\geq 20\%$ decrease in FEV_1. If the patient has a 20% decrease in FEV_1 following the first dose of methacholine (or after the diluent dose), PC_{20} cannot be calculated and should be reported as < 1 mg/mL. If the patient does not have a 20% decrease after the highest dose has been administered, PC_{20} should be reported as > 16 mg/mL. In addition to FEV_1, tests such as specific airway conductance or forced oscillation may be useful in confirming an asthma diagnosis.[34]

Mannitol (Aridol in the United States) is a recent addition to the armamentarium of inhaled bronchial challenge agents. Mannitol is an indirect agent designed for rapid assessment of patients with asthma-like symptoms, particularly those related to exercise.[35] Mannitol is a sugar alcohol and is supplied as a powder with a proprietary inhaler (Fig. 4). As the powder is inhaled, an osmotic gradient is established in the airway mucosa, stimulating release of the same mediators responsible for exercise-induced bronchospasm. Table 6 lists the doses used in the standard kit.

The patient inhales from the powder inhaler, with nose clip in place, from functional residual capacity to total lung capacity with a 5-s breath-hold. The first capsule contains no active drug and serves as the control. Duplicate measurements of FEV_1 are made 60 s after the inhalation. The target FEV_1 is a 15% decrease from the control value or a 10% decrease between doses. If neither target is reached, the patient continues to the next higher dose. For the last 4 doses, multiple capsules are used (see Table 6). The technologist administering the test needs to make sure each dose is administered rapidly to maintain the osmotic gradient created in the airway.

Table 6. Mannitol Dosing Regimen

Dose No.	Dose (mg)	Cumulative Dose (mg)	Capsules/Dose
1	0	0	1
2	5	5	1
3	10	15	1
4	20	35	1
5	40	75	1
6	80	155	2 × 40 mg
7	160	315	4 × 40 mg
8	160	475	4 × 40 mg
9	160	635	4 × 40 mg

Table 7. Interpretation of Mannitol Challenge

PD_{15}	Interpretation
> 635 mg/mL	Normal
> 155 mg/mL	Mild bronchial hyper-responsiveness
≤ 155 mg/mL	Moderate bronchial hyper-responsiveness
≤ 35 mg/mL	Severe bronchial hyper-responsiveness

PD_{15} = provocative dose of mannitol required to produce a 15% decrease in FEV_1

If the patient has a 15% decrease in FEV_1 or a 10% decrease between doses, the test is considered positive, and the cumulative dose is reported as the provocative dose. If the patient inhales all 9 doses (cumulative dose of 635 mg) without a 15% decrease in FEV_1, the test is considered negative. The powder sometimes causes excessive coughing (with or without a significant decrease in FEV_1), which also suggests airway hyper-responsiveness. Table 7 categorizes the response to inhaled mannitol by cumulative dose.

Mannitol and methacholine have similar sensitivity and specificity when used to evaluate subjects with symptoms of exercise-induced bronchospasm.[36] However, there is significant variability in how individual patients respond to either drug, particularly if gauged against exercise response. Because mannitol is a physical indirect agent, it is recognized by the International Olympic Committee (along with exercise, eucapnic hyperventilation, and hypertonic saline) as a preferred means to determine airway hyper-responsiveness in elite athletes.[37]

Safety During Bronchoprovocation

Both methacholine and mannitol have similar safety profiles when used according to recommended protocols. Methacholine is considered safe and effective for patients 5 y of age and older. It is contraindicated in women who are pregnant, who may become pregnant, or who are lactating. Methacholine produces asthma-like symptoms in susceptible patients.[31] It is not recommended when the patient has a reduced FEV_1, but has been shown to be safe even if FEV_1 is low.[38] Safe use of methacholine aerosol requires careful attention to the patient's symptoms as well as terminating the test when FEV_1 decreases below a fixed threshold (ie, 20%).[30]

Mannitol is safe and effective and may be used in patients 6 y of age and older. It is not recommended for women who are pregnant or lactating; its use in these patients must be carefully considered, weighing the benefit of measuring airway hyper-responsiveness against the risk.[39] Like methacholine, it is not recommended in patients whose FEV_1 is already reduced ($< 1.0–1.5$ L or $< 70\%$ of predicted). Mannitol may cause significant cough and/or throat irritation in some individuals. Testing should be terminated when FEV_1 decreases below a fixed threshold (ie, 15% or 10% between doses).

Aerosolized methacholine and mannitol are both designed to induce bronchospasm as a diagnostic test. Because there is a possibility of severe bronchospasm, these tests should be conducted by technologists who are familiar with the actions and side effects of the drugs and only under the supervision of a physician.[30] Medications and equipment to treat severe bronchospasm must be immediately available in the testing area. Some patients may have comorbidities for which severe coughing or induced bronchospasm may be harmful. These include, but are not limited to, recent myocardial infarction, severe angina, uncontrolled hypertension, and upper or lower respiratory tract infections.

The efficacy of both methacholine and mannitol can be affected by the concomitant use of bronchodilators or other anti-asthma preparations. Patients who use inhaled β agonists on a daily basis may have a reduced response to those drugs when used to reverse bronchospasm induced during bronchial challenge.[40] The effects of β blockers on bronchial hyper-reactivity is not well defined, but these agents may diminish the ability of β agonists to reverse induced bronchospasm.[41]

The method of aerosol administration can also influence the measurement of bronchial hyper-responsiveness. Both the 5-breath dosimeter method (methacholine) and the standard mannitol protocol require the patient to inspire maximally from functional residual capacity to total lung capacity and perform a breath-hold. The effect of this type of deep inspiration is well documented.[42] In normal patients, deep inspiration causes mild bronchodilation, and this may also be seen in patients with mild asthma. In some patients who have marked airway hyper-responsiveness, deep inspiration may have little or no effect, instead of having a bronchoprotective effect. The result of deep inspiration during the administration of the bronchial challenge aerosol is to attenuate the resulting bronchoconstriction. For methacholine challenge, there may be as much as a 2-fold difference in PC_{20}, depending on whether a deep inspiration or tidal breathing technique is used.[43] The effect of deep inspiration must be accounted for when selecting a bronchial challenge protocol and when interpreting the results.

Summary

Aerosolized bronchodilators are widely used to assess reversibility of airway obstruction and, in many instances, to establish a diagnosis of either COPD or asthma. In spite of their ubiquitous use, neither the equipment nor protocols are widely standardized. This lack of standardization is largely caused by the number of different inhaled bronchodilator medications and their varied delivery mecha-

nisms. Even when standardized within an individual PFT lab, the response to inhaled bronchodilators is markedly variable, especially in patients with COPD.

Aerosolized bronchial challenge agents are similarly widely used. Protocols for methacholine challenge and, more recently, mannitol challenge are standardized. However, bronchial challenges using inhaled aerosols are subject to many of the same variables that are problematic in bronchodilator testing (method of nebulization, patient performance). Bronchial challenge with inhaled agents poses little risk to the patient when the prescribed protocols are followed because testing can be terminated at a predetermined threshold (unlike exercise-induced asthma or eucapnic voluntary hyperventilation tests). The method of aerosol administration can significantly affect the measured hyper-responsiveness, especially in protocols than involve deep inspiration.

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Discussion

Berlinski: Thank you for that nice presentation. Based on what you said, it would be fair to say that the tidal breathing method would be the most appropriate method for our patients. In addition, you have flow-dependent dosing with DPI [dry powder inhaler]. If you do a deep inhalation, you have a bronchoprotective effect. The 2-min tidal breathing method could be used with younger children to adults.

Ruppel: Yes, I think you're going to see less use of the dosimeter because even the modified dosimeter method is going to be somewhat problematic. But there are a lot of dosimeters around. I didn't mention it, but the folks here below 2 mg/mL, they're pretty consistent, so if there's significant airway hyper-responsiveness, the method doesn't make quite as much difference. But because the dosimeter delivers about half as much total drug by certain estimations, that probably does play a role somewhere in the middle here. These folks here would probably be protected by deep inspiration, but the good news is that if you can get the drug down in the airways and we continue using PC_{20} [provocative concentration that produces a 20% decrease in FEV_1], not the cumulative breath units or some of the other things we used in the past, you're probably going to be able to detect and answer the first question: does the patient have asthma? Whether you'll be able to classify the responsiveness of the patient is another story.

‡ **Suggett:** I've got a doubt about the DPI use of mannitol. You touched on flow dependence, and it was also mentioned this morning in terms of adherence and using the right methodology. For this type of device (DPI), in which you have to disperse the powder to get a good fine-particle fraction, but you also have the potential of flow dependence influencing the degree of powder dispersion, are there any data to show consistency of dosing across different patients in reality?

Ruppel: I believe there is some literature from Sandy Anderson's group,[1] who looked at that in deposition studies. They saw that it did work and was well controlled, but there was pretty good deposition in the lower airways as well as in the larger airways, with some variability in the penetration index that was not flow-dependent. The biggest issue we've seen from a practical view point is if the patient inhales too rapidly, the little capsule spins too fast, static charge builds up within the device, which makes it hard to get the capsule out, some of the powder adheres to the filter in the device, and a lot of the drug impacts in the upper airway, and the patient begins to cough. The trick is to get the patient to inhale rapidly enough to cause the spinning action but not so fast that all those other things happen; it's really kind of an art that the technologist who's using it has to carefully teach the patient. The Aridol folks give you a little practice device to use, but you know how patients are: they're going to do what they're going to do, and when you tell them to take a big breath, they'll suck in really hard, and you're off to the cough.

‡ **Suggett:** It looks like it is a conventional capsule-based DPI, and I am therefore pretty sure there will be variability with that.

Ruppel: Yes. We were more concerned with the static charge that was building up in there and getting the capsule out, so we actually tried having technologists put their hands on an antistatic mat that you'd use if you were working on sensitive electronics, and it didn't help at all. The best thing is to wear gloves and have tweezers to get the capsule out, but it's very technique-dependent.

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