ERS technical standard on bronchial challenge testing: pathophysiology and methodology of indirect airway challenge testing

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This international task force recently reported the general considerations for "direct" bronchoprovocation testing. In the present document, the task force provides updated recommendations on the pathophysiology and conduct of "indirect" challenge tests. http://ow.ly/FR1K30m99Ef

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ABSTRACT Recently, this international task force reported the general considerations for bronchial challenge testing and the performance of the methacholine challenge test, a "direct" airway challenge test. Here, the task force provides an updated description of the pathophysiology and the methods to conduct indirect challenge tests. Because indirect challenge tests trigger airway narrowing through the activation of endogenous pathways that are involved in asthma, indirect challenge tests tend to be specific for asthma and reveal much about the biology of asthma, but may be less sensitive than direct tests for the detection of airway hyperresponsiveness. We provide recommendations for the conduct and interpretation of hyperpnoea challenge tests such as dry air exercise challenge and eucapnic voluntary hyperpnoea that provide a single strong stimulus for airway narrowing. This technical standard expands the recommendations to additional indirect tests such as hypertonic saline, mannitol and adenosine challenge that are incremental tests, but still retain characteristics of other indirect challenges. Assessment of airway hyperresponsiveness, with direct and indirect tests, are valuable tools to understand and to monitor airway function and to characterise the underlying asthma phenotype to guide therapy. The tests should be interpreted within the context of the clinical features of asthma.

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Introduction

Bronchoprovocation tests measure the propensity to develop airflow obstruction when the airways are challenged with either a direct-acting stimulus such as methacholine, or indirectly by physical or pharmacological stimuli that induce airway narrowing through the activation of inflammatory or neuronal cells (figure 1). The measurement of such indirect airway hyperresponsiveness (AHR) is valuable as a diagnostic test for asthma, and can be used to understand the underlying pathophysiology of asthma and as a guide for therapy. In the first technical standard on bronchial challenge testing, we described the general considerations for bronchoprovocation testing and updated the method to perform a direct bronchoprovocation challenge test with aerosolised methacholine [1]. Here, we describe the basis for the indirect challenge tests and the most commonly used methods to conduct these tests.

In a broad sense, direct challenge tests such as methacholine are sensitive to detect asthma, but are not entirely specific for asthma, as subjects with other airway disorders may have direct AHR. A negative methacholine challenge test in a currently symptomatic patient is highly suggestive of a diagnosis other than asthma; however, there are rare instances where indirect AHR is present in the absence of direct AHR. In contrast, indirect challenge tests act through the generation of mediators from cells present in the airways, providing an assessment of the mechanisms that are involved in asthma pathophysiology. Because of this basis, indirect tests tend to be more specific for asthma, but generally less sensitive than direct tests to detect AHR and support diagnosis of asthma in the appropriate clinical context (figure 2) [2–4]. Thus, the indirect challenge tests are useful to understand the underlying pathobiology of asthma, and specific manifestations of asthma such as allergen or exercise-induced responses, while the direct challenge test with methacholine is a sensitive test that serves as a more general indicator of AHR that may not be specific for asthma. The prototypical clinical manifestation of indirect AHR is the development of bronchoconstriction in response to exercise challenge, a clinical disorder that is called exercise-induced bronchoconstriction (EIB) [5–7]. In this regard, there is substantial discordance between the severity of EIB and measures of direct AHR such as methacholine challenge. It is common that individuals with asthma do not have EIB but do have AHR to methacholine challenge, and in some cases there is enough discordance that subjects with a negative methacholine challenge have EIB.

Some of the indirect challenge tests have the potential to trigger severe bronchoconstriction, because the challenge test is conducted with a single strong stimulus for bronchoconstriction, in contrast to the methacholine challenge that is conducted in a graded manner from a low initial starting dose. In this technical standard, we review the pathophysiological basis for indirect challenge testing, and the methods to conduct several of the most common tests. The challenge tests that use a single strong hyperpnoea stimulus including exercise challenge and eucapnic voluntary hyperpnoea (EVH) are discussed initially, followed by the methodology for the graded challenge tests including hypertonic saline, mannitol and adenosine challenge. Specific allergen challenge, which is also a form of indirect AHR and is used predominantly as a research tool in specialised centres, is discussed briefly; a recent report that was reviewed by this task force provides a detailed description of specific allergen challenge [8].

Methods

This document is the second of two technical standards that were developed by a task force on bronchial challenge testing initially organised by the European Respiratory Society (ERS) and American Thoracic

Society (ATS) as outlined in the first technical standard [1]. Due to the extended timeline of the project, this document was completed under ERS-only sponsorship with the participation of the full international panel. The task force initially met and performed a structured literature review on bronchoprovocation testing from 1990 onward. The initial literature review was conducted through PubMed and Embase by University of Washington librarians. The specific search terms were detailed in appendix A of the first report [1], and included terms related to both direct and indirect challenge tests. The initial literature search identified 2235 potentially relevant citations that were reviewed by the chair of the task force, who selected papers that reported methodology, compared methods or reported the outcomes of testing in larger populations, eliminated references that were not relevant and grouped the references by topic. After the initial meeting of the task force, working groups were established who reviewed the literature related to each type of indirect challenge test, specifically those related to exercise, EVH and cold air challenge testing, osmotic challenge testing including hypertonic saline and mannitol challenge tests and adenosine challenge. Task force members initially reviewed the citations for potential inclusion in the document, and then subsequently updated that search in 2017 prior to the completion of each section.

370 documents were found to be relevant to the indirect challenge testing technical standard. This included 42 documents related to adenosine challenge, 32 related to hypertonic saline challenge, 66 related to mannitol challenge, 207 related to exercise challenge, 38 related to EVH, and 23 related to cold air challenge. A task force member with specific expertise in each area reviewed the documents for potential inclusion in the final technical standard and a subset of these references were included in the final document.

Pathophysiological basis and rationale

Ventilation with large amounts of air during exercise and other related stimuli will trigger EIB in ~30–60% of patients with asthma [9–13]. Cross-sectional screening studies have found rates of EIB between 10% and 18% in the general population, suggesting that EIB may be more common than previously recognised in some populations and that certain individuals who have not been diagnosed with asthma may have this disorder [14, 15]. Atopy is a recognised risk factor for EIB, suggesting that subjects with allergic rhinitis or atopic dermatitis with exercise-related symptoms may have EIB [16–19]. There is evidence that EIB is a manifestation of airway inflammation since patients with EIB, relative to asthmatics without this aspect of asthma, have higher levels of cysteinyl leukotrienes (CysLTs) [20, 21], and 8-isoprostanes [22] in their airways; furthermore, the level of exhaled nitric oxide is also correlated with the severity of EIB [23–28]. A recent quantitative immunopathology study revealed that the density of intra-epithelial mast cells is increased in subjects with asthma who have EIB as compared with subjects with asthma who do not have EIB and nonasthmatic controls [29]. In addition, there is a relationship between the percentage of sputum eosinophils and the severity of EIB [30]. Some studies indicate that EIB and other features of asthma occur frequently in high-level athletes, such as cross-country skiers without a prior history of asthma, after exposure to training environments in which they inspire large volumes of cold dry air that may result in injury to the airways [31–36]. Identification of this disorder is particularly important, since asthma triggered on the playing field was the most common cause of nontraumatic death during sports in a community-based survey [37].

Although the susceptibility to EIB varies markedly among subjects, the severity of bronchoconstriction induced on any one occasion is strongly related to the amount of ventilation achieved, up to a maximal plateau [38], as well as the water content and temperature of the inspired air. During periods of high ventilation, a large volume of air is equilibrated to the humidified conditions of the lower airways over a short period of time, leading to the transfer of both water and heat to the inspired air with resulting

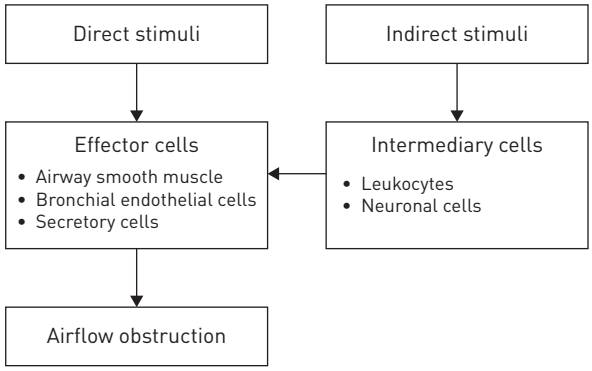

FIGURE 1 Conceptual framework for the difference between direct and indirect challenge tests. Indirect challenge tests act indirectly through the activation of leukocytes and/or neuronal cells that lead to the subsequent development of airflow obstruction. Reproduced and modified from [3] with permission from the publisher.

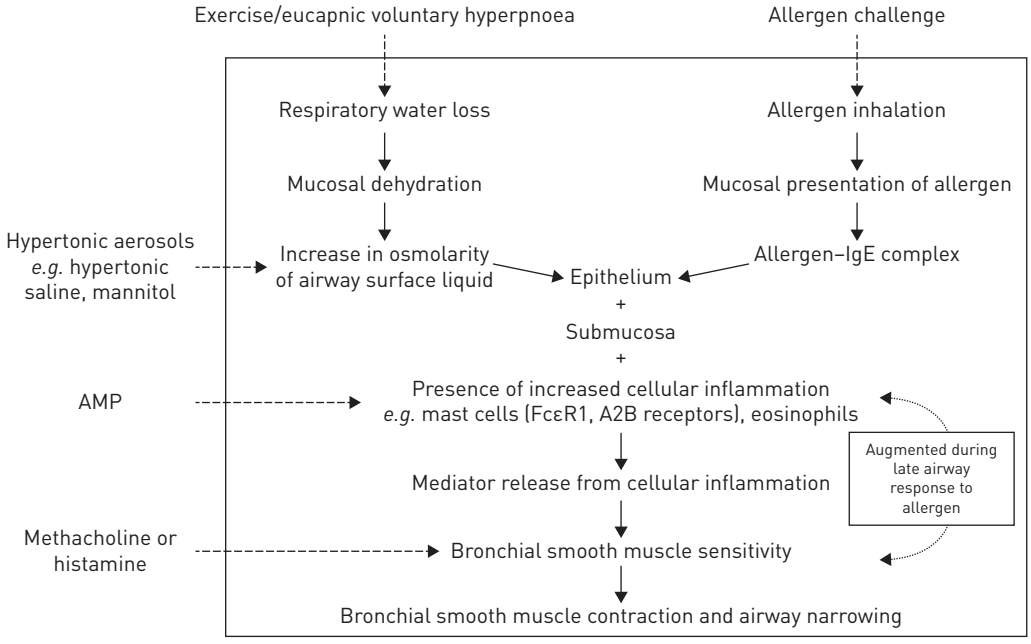

FIGURE 2 Mechanisms of action of indirect challenge tests used in the clinical and research setting. In contrast to the indirect mechanisms of indirect airway hyperresponsiveness, methacholine and histamine are examples of direct challenge tests that cause airway narrowing directly through airway smooth muscle contraction. Reproduced and modified from [4] with permission from the publisher.

osmotic stress as well as cooling of the airways that results from this vaporisation of water [5]. The role of cooling in isolation remains controversial, since very cold temperatures are required to accentuate airway narrowing, such cold air is not required for bronchoconstriction and EIB can occur when the inspired air is above body temperature [5]. Although the precise nature of the initial stimulus remains incompletely understood, there is strong evidence that mediators including CysLTs and prostaglandin D_2 are released into the airways in response to exercise challenge [39–44] and that the mediators that are released are derived from cells residing in the airways, including mast cells and eosinophils [40, 41, 45]. Sensory nerve involvement has been clearly demonstrated following hyperpnoea-induced bronchoconstriction in animal models [46, 47] and studies in humans are supportive of sensory nerve involvement [48]. Despite these inflammatory events triggered by exercise, there is no clear evidence of a cellular influx into the airways or an increase in direct AHR following exercise challenge [49].

Indications

Exercise challenge is the most straightforward method to determine whether exercise-related respiratory symptoms represent EIB rather than dysfunctional breathing, airflow obstruction related to chronic asthma or other cardiovascular pathology. In addition, patients with EIB generally demonstrate AHR to methacholine; however, there is significant discordance between AHR to methacholine and the severity of EIB [29, 50, 51]. Although supportive of the potential diagnosis of EIB, a positive methacholine challenge test does not rule in EIB, and a negative methacholine challenge does not entirely exclude EIB [50, 52, 53], as some athletes with a positive hyperpnoea challenge test have a negative methacholine challenge [51, 54]. In general, AHR to methacholine is a test that is sensitive for asthma but is not very specific, while EIB is less sensitive and more specific for asthma [13, 53, 55–59]. The sensitivity of indirect challenge tests varies with the population that is being studied, as one multicentre trial found that in subjects with relatively normal lung function, mild symptoms and mild AHR, direct and indirect tests had similar sensitivity for a clinical diagnosis of asthma and for detection of EIB [52].

The knowledge that an individual subject has EIB can be used to guide therapy targeted at prevention of symptoms and signs related to this disorder. Previously, testing for EIB or asthma was required by some governing bodies to obtain approval for the use of certain asthma medications during athletic competition. In subjects engaged in demanding or lifesaving work that may require intense exercise (*e.g.* military, police or firefighting work), a test for EIB may be indicated [60]. In children, identification and treatment of EIB may be particularly relevant because of the important effects of physical activity for physical and psychological development [61]. As a model stimulus of indirect AHR, exercise challenge can serve as a means to study the efficacy and optimal dose of preventative therapy for asthma [62].

Safety considerations and contraindications

Unlike progressive challenge tests such as the methacholine or mannitol challenge tests with an incremental increase in the dose of the stimulus, exercise, EVH and cold air challenge tests build rapidly to a strong stimulus for bronchoconstriction, making it imperative that the laboratory has appropriate equipment available to manage severe bronchoconstriction. A physician should be present or immediately available during the study, and cardiopulmonary resuscitation equipment should be immediately available. During exercise, arterial oxygen saturation should be estimated by pulse oximetry, and blood pressure and ECG should be monitored. The patient should be observed for undue stress (*e.g.* severe wheezing or shortness of breath, chest pain, lack of coordination) or adverse signs (*e.g.* ECG abnormalities, falling blood pressure, decrease in oxygen saturation) during the test. Because of the risk of severe bronchoconstriction, it is recommended that the forced expiratory volume in 1 s (FEV_1) before challenge should be $\geqslant$75% predicted and pulse oximetry saturation should be >94% [63]. Additional contraindications include significant cardiovascular disease, such as inducible cardiac ischaemia, uncontrolled hypertension, aortic aneurysm or life-threatening arrhythmias (see table 1 in Coates *et al.* [1]). A 12-lead ECG should be obtained in subjects with cardiovascular risk factors, while a three-lead ECG may be acceptable in individuals with a low pretest probability of heart disease and in children. Pregnancy is a contraindication to testing due to the potential risk to the fetus. Safety recommendations are based on physiological considerations and on substantial experience reported in the literature [60, 63].

Patient preparation

The patient should report to the laboratory in comfortable clothes and running or gym shoes, having consumed no more than a light meal. Both short- and long-term medications should be withheld before testing to prevent the possibility of a false negative test [7] (table 1), unless the goal is to assess the efficacy of therapy to prevent EIB [64]. It should be noted that regular use of β_2-agonists increases the severity of EIB [65, 66]. Some studies have demonstrated a preventative effect of a single high dose of an inhaled corticosteroid prior to EVH challenge [67] or prior to exercise challenge [68], but most studies have found that lower standard doses of inhaled corticosteroids used daily require a longer period of use for optimal efficacy [69]. Hence, maintenance therapy with inhaled corticosteroids can suppress EIB [62, 69]. Vigorous

TABLE 1 Withholding times prior to indirect challenge testing

	Withholding time	Maximum duration of protection[#]
SABA (albuterol, terbutaline)	8 h	<6 h
LABA (salmeterol, eformoterol)	36 h	12 h
LABA in combination with an ICS (salmeterol/fluticasone, formoterol/budesonide)	36 h	NA
Ultra-LABAs (indacaterol, olodaterol, vilanterol)	48 h	NA
ICS (budesonide, fluticasone propionate, beclomethasone)	6 h	NA
Long-acting ICS (fluticasone furoate, ciclesonide)	24 h	NA
Leukotriene receptor antagonists (montelukast, zafirlukast)	4 days	24 h
Leukotriene synthesis inhibitors (zileuton/slow-release zileuton)	12 h/16 h	4 h
Antihistamines (loratadine, cetirzine, fexofenadine)	72 h	<2 h
Short-acting muscarinic acetylcholine antagonist (ipratropium bromide)	12 h	<0.5 h
Long-acting muscarinic acetylcholine antagonist (tiotropium bromide, aclidinium bromide, glycopyrronium)	72 h	NA
Cromones (sodium cromoglycate, nedocromil sodium)	4 h	2 h
Xanthines (theophylline)	24 h	NA
Caffeine	24 h	NA
Vigorous exercise	4 h	<4 h

Examples of specific medications within the class are provided in parenthesis. The withholding times recommended for short- and long-acting β_2-agonists differs from the recommendation in the original reference, reflecting the recommendations of this task force and the uncertainty in the precise duration of the inhibitory effect of these medications on indirect airway hyperresponsiveness. SABA: short-acting β_2-agonist; LABA: long-acting β_2-agonist; ICS: inhaled corticosteroid; NA: not available. [#]: refers to the potential effects of a single dose and may not apply to chronic dosing. Reproduced and modified from [7] with permission from the publisher.

exercise should be avoided for ⩾4 h before testing, because a "warm up" exercise may cause a period where the subject is refractory to additional exercise challenge [60, 70, 71]. The time of day should be consistent in repeated studies, as the severity of EIB is greater in the afternoon compared to the morning, indicating an influence of diurnal variability [72]. In addition, dietary factors including a low salt diet [41] and supplemental omega-3 fatty acids [43, 44], as well as antioxidants [73], may influence EIB severity.

Prior to any of the challenge tests, it is critical to obtain reliable baseline spirometry since the response to exercise, EVH or cold air challenge is based on the change in FEV_1 from baseline. Accordingly, baseline spirometry should be obtained following ATS/ERS guidelines immediately prior to beginning of the challenge test [74]. It is ideal to obtain two separate baseline spirometry tests separated by 10–20 min before the challenge test to confirm the stability of spirometry.

Exercise challenge testing

Exercise testing can be accomplished using a motorised treadmill or a cycle ergometer. The rapid increase in ventilation during treadmill running makes it the preferable test; however, a cycle ergometer can be used effectively, providing that the work rate is increased rapidly to reach the target ventilation or heart rate. Although peak oxygen uptake averages ~10% less on a cycle ergometer than on treadmill, the lung function response is comparable as long as similar exercise intensity and ventilation are achieved [60, 75]. An outdoor free-run asthma screening test has been used in the community setting to screen for EIB [76–80], although environmental conditions are hard to standardise and safety measures are more difficult to provide in this setting. Sports-specific tests have also been applied in competitive athletes [81, 82].

Regardless of the mode of exercise test, the protocol should be designed to reach the target heart rate or minute ventilation over a short period of time, in the order of 2–3 min (table 2). The rapid rise in work rate is needed because a warm-up period or prolonged lower-level exercise may decrease the severity of EIB [70, 71, 83]. For this reason, the incremental work rate profile used in standard cardiopulmonary exercise testing may be less sensitive for the detection of EIB [60]. Heart rate and rhythm should be monitored with a continuous ECG, and an initial rise in blood pressure with exercise should be confirmed.

Following the rapid increase in work, the target level should be maintained for 6 min [60, 63]. If the equipment is available, it is preferable to achieve a ventilation target rather than a heart rate target to monitor the intensity of the challenge. Ventilation should reach 60% of the predicted maximum voluntary ventilation (MVV, estimated as $FEV_1 \times 40$) [63, 84]. Monitoring ventilation requires the patient to breathe through a mouthpiece or mask, which adds some burden to conducting the test. An acceptable alternative is a target heart rate of >85% of the predicted maximum (calculated as 220 − age in years) [84, 85]; however, the approach based on heart rate may not always achieve the target ventilation [86], and higher heart rate targets can be associated with more bronchoconstriction [83]. Measurement of pulmonary gas exchange during exercise makes it possible to quantify the intensity of exercise as a fraction of predicted peak oxygen uptake, confirming the adequacy of exercise challenge. However, adding pulmonary gas exchange measurements is difficult when using a dry air setup and is usually not necessary.

The inspired air should be relatively dry and <25°C. This can be accomplished by conducting the study in an air-conditioned room (with ambient temperature at 20–25°C) with low relative humidity (⩽50%). The temperature and relative humidity should be recorded. An ideal system delivers dry air through a mouthpiece and a two-way valve from a talc-free reservoir filled with medical-grade compressed air [60]. The use of compressed air is preferred because it is completely dry and will cause greater water loss from

TABLE 2 Characteristics of hyperpnoea challenge tests

Vigorous exercise, usually on a treadmill
Rapid increase in ventilation over the first 2–3 min to reach the target
Maintain target ventilation for ⩾4 min, preferably 6 min
Target ventilation is 60% of maximum (MVV or $FEV_1 \times 40$)
Heart rate of >85% of maximum can serve as a surrogate for ventilation target
Inspired air should be dry, and ambient temperature <25°C
Nose clips should be used during the exercise challenge
Serial assessments of spirometry for 30 min after exercise
Two spirometry manoeuvres are acceptable at each time point
Use the best technically valid FEV_1 at each time point
Frequent deep inspiration following exercise challenge may affect result

MVV: maximum voluntary ventilation; FEV_1: forced expiratory volume in 1 s.

the airways, thus generally increasing the sensitivity of the test [38]. This may be particularly important for the detection of mild EIB, and during tests that are of shorter than the ideal 6-min plateau phase. During exercise, the patient should wear a nose clip, as nasal breathing decreases water loss from the airways [60].

Treadmill protocol

On the treadmill, speed and grade are progressively advanced during the first 2–3 min of exercise until the target level is obtained. The degree of physical fitness and body weight will strongly influence the grade and speed necessary to obtain the desired ventilation or heart rate. A reasonable procedure is to quickly advance to a rapid, but comfortable speed at a treadmill incline of 5.5% (3°) then, raise the slope until the desired heart rate or ventilation is obtained, up to an incline of 10%. Nomograms have been proposed to predict speed and grade that will elicit the desired heart rate, but they have not been validated extensively [60]. Another approach is to use a nomogram relating oxygen consumption per kilogram to speed and slope of the treadmill; however, the relationship between oxygen consumption and the percentage of maximum ventilation is variable [60]. The test ends when the patient has exercised at the target ventilation or heart rate for 6 min. The treadmill challenge protocol has a high degree of repeatability [87–89].

Bicycle ergometer protocol

For cycle ergometer exercise, work rate is rapidly increased using the electromagnetic braking system to achieve the target ventilation. Direct measurement of ventilation is easier with the stable position on an ergometer and is the preferred target. The target heart rate or ventilation should be reached within 2–3 min. A valid test requires the target exercise intensity to be sustained for 6 min, although sustained exercise of $\geqslant$4 min may be acceptable if the subject becomes fatigued. Although the repeatability of the bicycle protocol has not received extensive study, the repeatability in a limited number of individuals was excellent [60, 90].

Eucapnic voluntary hyperpnoea

EVH is an alternative to exercise challenge that was standardised in the early 1980s [91, 92] and does not require exercise. The study utilises medical dry air from a reservoir with an admixture of 4.9% carbon dioxide, which enables the study subject to breathe at high ventilation without the adverse consequences of hypocapnia. The subject is instructed to perform voluntary hyperpnoea for 6 min aiming at a target ventilation of 85% of MVV and with a minimum ventilation threshold of 60% of MVV [93, 94].

Like other indirect challenge tests, EVH has a lower sensitivity for asthma compared to direct tests such as methacholine challenge, but is fairly specific for asthma [6]. Like exercise challenge, the volume and water content of the inspired air are important determinants of the severity of bronchoconstriction following EVH [94, 95], and the use of certain asthma medications before the challenge can alter the sensitivity and specificity of the test (table 1) [67]. When standardised properly, the EVH test has a high degree of repeatability [95]. This method has been used to identify the origin of exercise-related symptoms in subjects with respiratory disorders [96], in elite athletes [97], in school-aged athletes [98] and in people with a health club membership [99]. In addition, EVH has been used to assess the efficacy of inhaled corticosteroids in infants and young children [100]. When compared to exercise challenge, a consistent finding is a higher sensitivity of EVH than exercise challenge for the detection of bronchoconstriction [81, 97, 101, 102].

Cold air challenge

Cold air has a low water-carrying capacity, resulting in greater heat and water transfer necessary to condition the inspired air at any minute ventilation. Since this greater heat and water flux is thought to be instrumental in the pathogenesis of EIB, performing exercise or EVH with cold air inhalation might be expected to increase the stimulus for bronchoconstriction. Cold air generators that produce dry air at below-freezing temperatures are commercially available and are in use in some laboratories; however, the additive effects of cold air depend on the specific protocol that is used. In adults, some studies demonstrate an enhancing effect of cold compared to ambient air [103], while others found no additive effect of cold air [104, 105]. An increase in minute ventilation elicited by the use of cold air explains some of the effects of cold air [106]. In children, cold air inhalation enhances the response to EHV in those with asthma [55, 107]. The addition of cold air appears to shorten the stimulus time necessary during challenge testing from 6 to 4 min [84]. In patients with symptoms specifically associated with exercise in the cold, exercise challenge while breathing cold dry air may be useful to enhance the sensitivity and specificity of the response [82, 97]. Part of the response to exercising in cold temperatures may be due to exposure of the face and body to cold temperatures, and not just the airways [108, 109]. Using cold air as part of an

exercise or EVH challenge elicits responses that are discordant from direct challenge tests such as methacholine or histamine [110, 111].

An exercise or EVH challenge using cold air is conducted as previously described, but with a source of frigid air for inspiration. Typically, subjects breathe through a heat exchanger or similar device that generates cold, dry air or exercise under ambient conditions of cold air, such as might be found outdoors during the winter or inside an ice skating arena [97]. When using a cold air generator, the device is either held by the patient or is supported in such a way as to deliver the air immediately before inspiration. The target range for inspired air temperature is −10–−20°C, and should be recorded by the technologist during the challenge.

Selection of the hyperpnoea challenge protocol

Although all the methods are supported in the literature as valid challenge tests for EIB, we recommend the use of the treadmill protocol monitoring heart rate as a surrogate for ventilation because of the wide availability of this method and general familiarity to both the physician and the patient with running on a treadmill. The other methods are also acceptable but require the use of specialised equipment (*e.g.* bicycle ergometer in bike challenge, feedback mechanism to maintain target ventilation in EVH, cold air generator) or protocols.

Post-challenge spirometry protocol

Serial measurements of lung function by FEV_1 over the first 30 min after exercise or EVH are used to determine whether the test is positive and quantify the severity of bronchoconstriction. Many laboratories conduct the first spirometry measurements immediately after challenge, and then 3, 6, 10, 15 and 30 min after challenge. An acceptable alternative is to start the assessment at 5 min after challenge; however, the earlier time intervals may be useful to detect the onset of severe bronchoconstriction if present at the cessation of the challenge test [60, 84]. Note that when performing spirometry measurements immediately after stopping exercise, the result might be artificially low due to reduced effort during spirometry in the setting of post-exercise hyperpnoea and dyspnoea, particularly in children. There may be some slight differences in the results when different numbers of spirometry manoeuvres are used, since deep inspiration during spirometry testing may inhibit bronchoconstriction [112]. It should be noted that the bronchoprotective effects of a deep inspiration is reduced in asthma and has been related to inflammation, suggesting that deep inspiration could add to the discriminant value of the tests [113–115]; however, we feel that the number of deep inspirations for the spirometry manoeuvre should be kept as constant as possible to minimise the variability of the test. At least two acceptable tests within 0.15 L should be obtained at each testing interval, unless FEV_1 or forced vital capacity are <1 L, in which case two acceptable tests should be within 0.10 L. The best FEV_1 at each interval is reported. Because of the challenge in carrying out repeated spirometry efforts following exercise, it is adequate to accept a duration of expiration of 2–3 s for the forced manoeuvre to measure FEV_1; however, it is important to vigorously coach the patient to inhale fully even in the presence of chest tightness.

In most cases, the nadir in FEV_1 occurs within 5–10 min of cessation of exercise, but can occasionally occur as late as 30 min post-exercise [116]. We recommend serial measurements for the entire 30 min since it is important to determine the nadir in FEV_1 to fully assess the severity of EIB.

Positive test threshold and interpretation of hyperpnoea challenge tests

The presence of EIB is defined by plotting FEV_1 as a percentage decline from the pre-exercise baseline FEV_1 at each post-exercise interval. A decrease of ⩾10% from the baseline FEV_1 is accepted as an abnormal response relative to population normal values [63, 116–118], but the specificity is higher with a criterion of 15% from baseline. The basis of these recommendations are studies in normal children that demonstrate an upper 95% confidence limit of the FEV_1 fall as 8.2% [118] and 10% [119]. A method to quantify the overall severity of EIB is to measure the area under the curve for post-exercise time multiplied by the percentage fall in FEV_1 at each time point over the 30 min after exercise [85]; this quantitative assessment is used predominantly in the research setting as a specific threshold for a positive test has not been established. The threshold for a positive response based on the maximum fall in FEV_1 also depends on the indication for the test, such that a more sensitive test (*i.e.* 10%) might be useful to understand the origin of symptoms in athletes, while a more specific test (*i.e.* 15–20%) may be needed for research studies [7].

For EVH, the threshold for a positive test is usually set at a decrease in FEV_1 of ⩾10% below baseline based on the characteristics of the EVH test in normal subjects [96]. Exercise or EVH challenge tests that include the addition of cold air should be interpreted in the same manner as the test conducted without cold air.

A β_2-agonist bronchodilator may be administered at any time to reverse bronchoconstriction if the patient experiences symptoms that are too severe, if there is severe bronchoconstriction that could progress further or if FEV_1 has not recovered to within 10% of baseline when the patient is ready to leave the laboratory. The clinician should be alert to other possible causes of dyspnoea including cardiovascular disease and upper airway abnormalities including fixed upper airway obstruction (*i.e.* subglottic stenosis) and inducible laryngeal obstruction including paradoxical vocal cord dysfunction and paradoxical arytenoid motion [120]. Upper airway abnormalities may be apparent if full flow–volume loops with inspiratory flow are obtained during spirometry; however, other techniques, such as direct laryngoscopy immediately after or during exercise may be needed to clarify the diagnosis [120].

Incremental indirect airway challenge tests

In addition to the hyperpnoea challenge tests that use a single strong stimulus for bronchoconstriction, several incremental challenge tests have been developed to measure indirect AHR. As water transfer during a period of hyperpnoea results in osmotic stress to the lower airways, the inhalation of aerosols with different osmotic properties than the airway surface liquid has been developed to model this stimulus in an incremental manner [121]. The response to either hypertonic or hypotonic aerosols is associated with the response to exercise [6, 122, 123] and EHV [124] challenge tests, and the response to hypertonic aerosols is also associated with mast cell infiltration of the airways and sputum eosinophils [125, 126]. Challenge with hypertonic saline causes mediator release following challenge that is similar to that described for exercise challenge [127] and can be inhibited by specific mediator antagonists [128]. In model systems, hypertonic saline leads to the release of neuropeptides that are involved in the development of bronchoconstriction [129]. Similarly, the use of dry powdered mannitol has been developed as an incremental challenge test that challenges the lower airways with increasing levels of osmotic stress [130]. A positive response to mannitol challenge is generally associated with a positive test for EIB [131, 132], but mannitol challenge is neither entirely sensitive or specific for the detection of EIB [52, 133]. Additionally, a recent study demonstrated that AHR to mannitol is associated with mast cell and eosinophil infiltration of the airways [134], consistent with prior evidence of the release of mast cell mediators following mannitol challenge [39], and a reduction in AHR to mannitol with a mast cell stabiliser [135]. Another incremental challenge test to define indirect AHR is the dose response to inhaled adenosine. Although not extensively studied, the response to adenosine challenge is generally correlated with the response to exercise challenge [136]. Furthermore, the response to adenosine challenge is associated with markers of airway inflammation, including the exhaled nitric oxide fraction [137, 138]. The mechanism of adenosine-induced AHR may be through the priming of mast cells [139], adding further evidence of a strong relationship between mast cell infiltration of the airways and indirect AHR.

Hypertonic saline challenge

Although the inhalation of either hypertonic or hypotonic aerosols will induce bronchoconstriction in susceptible individuals, the use of hypotonic aerosols is uncommon, due in part to a fatal episode of asthma triggered during distilled water challenge [140]. Hypertonic saline challenge is an incremental challenge in which 4.5% sterile saline is delivered by an ultrasonic nebuliser for increasing periods of time [124, 141]. The test should be conducted with an ultrasonic nebuliser with a flow of $\geqslant$1.2 $mL \cdot min^{-1}$ and the capacity to hold $\geqslant$100 mL hypertonic saline solution. The commonly used ultrasonic nebulisers provide a flow of 1.5–3.0 $mL \cdot min^{-1}$ [142, 143]. Because the "dose" or volume of hypertonic saline required to induce a 15% fall in FEV_1 is the primary outcome measurement, the precise output from the nebuliser should be established gravimetrically by the laboratory conducting the test. Ideally, the hypertonic saline should be delivered though a two-way nonrebreathing valve, and the inner walls of the tubing should be smooth and of constant length from test to test, as the deposition in the tubing has the potential to alter the characteristics of the test. A saliva collection reservoir should be used, as the test tends to cause ongoing salivation. During the test, the sterile 4.5% hypertonic saline is given for increasing durations of time until the longest duration is administered, or there is a >15% fall in FEV_1 from the pretest baseline. If FEV_1 falls between 10% and 15% compared with prechallenge FEV_1, the same dose step is repeated. Spirometry is used to assess the FEV_1 30 and 90 s after the end of each round of inhaled hypertonic saline. Hypertonic saline is initially administered for 30 s, then 1, 2, 4 and 8 min in subsequent cycles. When the test is positive, or symptoms are present, a short-acting inhaled β_2-agonist should be administered, and the patient monitored until fully recovered to within 10% of baseline FEV_1.

The contraindications for the test are generally the same as for other indirect challenge tests, with a baseline FEV_1 >70% pred considered safe for this type of test. The test is considered positive if there is a >15% fall in FEV_1 from the prechallenge FEV_1 during the test [123, 124]. The provocative dose causing a 20% fall in FEV_1 (PD_{15}) of hypertonic saline is established by linear interpolation of a plot of the cumulative dose (mL or g) of hypertonic saline administered during the test and the FEV_1 at the end of

each nebulisation cycle. The severity of AHR to hypertonic saline can be classified as mild (PD_{15} >6 mL), moderate (PD_{15} 2.1–6.0 mL) and severe (PD_{15} <2.0 mL), although precise cut-offs are uncertain, as large population studies have not been conducted [144–146]. Another outcome measure described for this test is the dose response slope (DRS), which is the percentage fall in FEV_1 divided by the amount of hypertonic saline inhaled. In a large study of children, the DRS had greater separation for current wheezing and for asthma than the PD_{15} hypertonic saline [144].

An advantage of hypertonic saline as a test of indirect AHR is the ability to conduct an induced sputum test at the same time, to characterise features of airway inflammation [126, 147]. Although hypertonic saline challenge has not been used extensively in population based studies, there is a strong scientific basis that the response to hypertonic saline is similar to other indirect tests such as exercise challenge [123] and EVH [148]. Population studies, predominantly in children, support the ability of hypertonic saline challenge to discriminate between subjects with and without asthma and related features of asthma [149–151]. Further support for the connection between AHR to hypertonic saline and airway inflammation are studies that reveal that anti-inflammatory therapies including inhaled steroids [126, 145, 147, 152] and cromones [153] alter the severity of AHR to hypertonic saline.

Mannitol challenge testing

The mannitol challenge test has undergone considerable evaluation and has received regulatory approval in the United States and Europe. The test is conducted using a capsule-based dry powdered inhaler device that is used to deliver increasing doses of mannitol to the lower airways. The dry powder is prepared by the manufacturer by spray-drying mannitol into particles that are of respirable size. Inhalation of dry powdered mannitol rapidly increases the osmolarity of the airway surface liquid [130, 131]. After baseline spirometry is obtained, a capsule without any mannitol is inhaled through the delivery system. The capsule is placed in the inhaler and pierced by simultaneously depressing the two buttons on each side of the dry powder inhaler. The individual is instructed to exhale fully away from the inhaler, tilt the head back slightly and place the inhaler in their mouth tilted upward at a 45° upright angle with lips sealed around the mouthpiece, and then take a slow deep inhalation. The subject should be instructed to hold their breath for 5 s after inhalation. Nose clips may be used if desired. During inhalation, a "rattling" sound should be heard as the capsule spins within the inhaler. When 5 s has passed, the patient is instructed to exhale through the mouth (away from the inhaler), to remove the nose clip, and to breathe normally. 1 min after the start of inhalation, two acceptable spirometry manoeuvres should be obtained, with the highest FEV_1 retained as the baseline FEV_1. Following spirometry, increasing doses of mannitol (5, 10, 20, 40, 2×40, 4×40, 4×40 and 4×40 mg) are administered using the same cycle of the inhaled dose from the dry powder inhaler, followed by two acceptable spirometry manoeuvres conducted 1 min after administration of the dose. When multiple capsules are required for the dose, the timer is started at the start of inhalation of the final dose. Because there is a cumulative dose effect, the next dose should be administered as soon as possible following spirometry, and delays between doses should be avoided. The test is stopped when there is a 15% decrease from baseline FEV_1, a 10% decrease in FEV_1 between two consecutive doses or the cumulative dose reaches the total of 635 mg.

The contraindications for mannitol challenge testing are like those for other indirect challenge tests, with a few additional considerations. First, known hypersensitivity to mannitol or to the gelatine used to make the capsules is an absolute contraindication. Second, moderate airflow limitation (FEV_1 <70% pred or <1.5 L) is considered a contraindication to testing. As cough is frequently induced by inhaled mannitol, the test should be avoided in patients with medical conditions that would be exacerbated by frequent coughing. Finally, as mannitol is a pregnancy category C drug and is a drug with potential excretion in breastmilk, administration of mannitol challenge should be avoided in pregnant women and in nursing mothers. Following mannitol challenge with a positive test or significant respiratory symptoms (*e.g.* wheezing, dyspnoea, cough), a short-acting inhaled β_2-agonist should be administered, and the patient monitored until fully recovered to within 10% of baseline FEV_1. In the case of a negative result, if the patient has significant respiratory symptoms, a short-acting inhaled β_2-agonist may be considered.

A mannitol challenge test is considered positive if there is a 15% decrease from baseline FEV_1 in response to the cumulative total dose, or a 10% decrease in FEV_1 between two consecutive mannitol doses. The test is considered negative when a cumulative dose of 635 mg of mannitol has been administered and the FEV_1 has not fallen by ⩾15% from baseline. Sensitivity to mannitol is expressed as the PD_{15} derived from the dose-response curve (PD_{15} mannitol). The PD_{15} mannitol can be used to classify the severity of indirect AHR as mild (>155 mg), moderate (>35 and ⩽155 mg) or severe (⩽35 mg) (figure 3) [84, 154, 155]. Reactivity to mannitol can also be expressed as the response dose ratio (RDR), which is the percentage decrease in FEV_1 at the end of challenge divided by the cumulative dose of mannitol administered to induce that decrease in FEV_1 [130, 143]. The PD_{15} is generally used in clinical practice to "rule in" or

establish a diagnosis of asthma, and for providing an estimate of the severity of AHR. The RDR makes it possible to include subjects who do not reach the cut-off in the analysis, providing a continuous measurement of indirect AHR without censored data, which may make it particularly useful for epidemiological studies as has been shown for similar methods of assessment of direct AHR [156, 157]. Additionally, the mannitol challenge test has utility as a screening test for asthma [158, 159], to monitor the effectiveness of long-term controller therapy for asthma [160–163], and to determine the optimal dose of inhaled corticosteroid therapy [164–166]. In cross-sectional studies in a clinical setting, the sensitivity of the mannitol bronchial challenge test to identify asthma ranges from ~40% to 59%, while the specificity ranges from ~78% to nearly 100% [52, 159, 167].

Adenosine challenge testing

The adenosine bronchial challenge test is used primarily in the research setting, because specific thresholds for the interpretation of the test have not been clearly established. The adenosine challenge test is usually performed with an inhaled solution of AMP because AMP is more soluble in sterile normal saline than adenosine, and is rapidly metabolised to adenosine in the lungs [168]. The sodium salt of AMP is available as a dry crystalline powder from chemical suppliers; however, pharmaceutical grade AMP is not currently available for this test, and should only be used with appropriate regulatory approval. The use of a 2-min tidal breathing as well as a five-breath dosimeter protocol with doubling concentrations of AMP ranging from 3.125 to 400 mg·mL^{-1} were described in the 2003 ERS task force on indirect airway challenges [2]. Because of the higher output of modern nebulisers, a lower starting concentration of AMP should be considered [169]. The test is stopped once a 20% fall in FEV_1 occurs and is generally reported as the PC_{20} AMP. Safety considerations are like those for other indirect challenge tests.

As in the case of methacholine challenge [1], there are several important considerations that affect the standardisation and interpretation of the adenosine challenge test. First, the well-described bronchoprotective effect of deep inspiration may alter the characteristics of the tests when the dosimeter method is used. Second, modern nebulisers have the potential to deliver much higher doses of the drug to the lower airways, such that the dose rather than the concentration should be considered to better standardise the test based on the delivery characteristics of the nebuliser. Consistent with these considerations, one study demonstrated a lower PC_{20} AMP using the tidal breathing method over the dosimeter method [170]. Another consideration is that the relatively high concentrations of AMP in this dosing scheme have been shown to alter nebuliser delivery during the tidal breathing cycle [171]. A novel delivery system was recently described using two different dry powdered formulations of adenosine; preliminary testing in a modest number of subjects with and without asthma has been reported [172, 173].

Additional studies are needed to fully standardise the delivery of adenosine for the adenosine bronchial challenge test, and to establish well defined thresholds and predictive values for a positive and negative test. The initial studies of AMP challenge indicate that it more closely reflects active airway inflammation in patients with asthma than direct challenge tests [137, 174, 175], and may have a stronger relationship

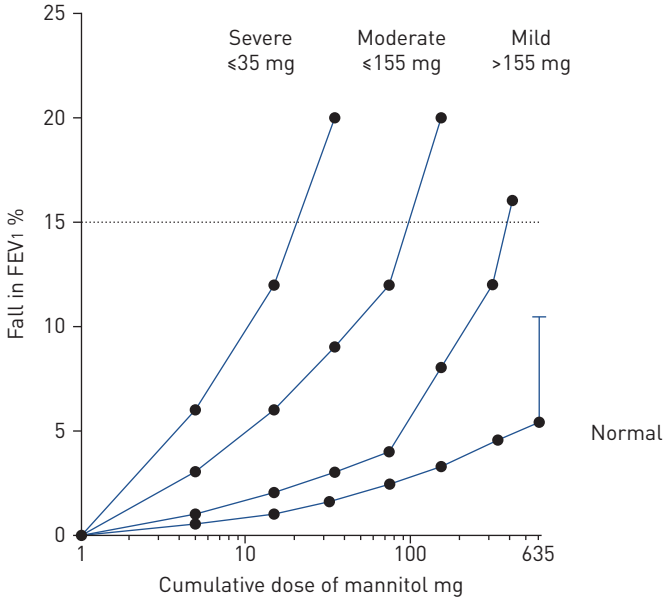

FIGURE 3 Classification of the severity of airway hyperresponsiveness according to the response to dry powdered mannitol challenge. The cumulative dose of mannitol required to provoke a 15% reduction in forced expiratory volume in 1 s (FEV_1) from baseline is calculated based on the final two cumulative doses of mannitol and the reduction in FEV_1 at each dose. The mean maximum response (+1 SD) in FEV_1 after a cumulative dose of 635 mg is shown in the error bar for normal subjects. Reproduced and modified from reference [84] with permission from the publisher, including data from the normal control group in reference [155].

with the bronchodilator response [176]. Like other indirect challenge tests, the adenosine challenge test may be particularly useful to ascertain the effects of anti-inflammatory therapies such as inhaled steroids in asthma [177–181].

Inhaled allergen challenge

Inhaled allergen challenge is a specific indirect stimulus in allergen-sensitised asthmatics and is used primarily as a model of antigen-specific T-helper type-2 cell-driven asthma, allowing investigation of the links between allergen-induced inflammatory events and subsequent changes in airway physiology [182, 183]. More recently, allergen challenge has been used to define aspects of the innate immune response to allergen [184, 185]. Although sometimes used clinically to demonstrate the relationship between relevant stimuli in symptomatic subjects, the allergen challenge is primarily a research tool, often used to assess the efficacy of novel potential asthma-controlling medications [8, 186]. For the sake of safety and reproducibility, allergen challenges should only be performed in specialised research centres with ample expertise and experience with the methodology of allergen challenge and asthma management. To increase safety, incremental inhalations of an allergen based on the gradual decline in FEV_1 during the early airway response to allergen is recommended. Irrespective of the inhalation method, allergen should only be administered in asthmatics where prechallenge clinical stability is confirmed by strict spirometry and AHR criteria [8].

Summary

The assessment of AHR is exceedingly valuable as a specific diagnostic test for asthma, to understand the basis for asthma symptoms and to recognise the underlying asthma phenotype and thereby to guide and monitor therapy. Direct challenge tests including methacholine challenge have the advantage of a high level of sensitivity, and are therefore most useful to exclude the diagnosis of asthma in the presence of asthma-like symptoms [1]; however, direct AHR is not specific for asthma and can be present in other airway disorders. Depending upon the patient population being examined, indirect tests can have similar sensitivity to methacholine challenge for the detection of asthma [52]. The major strength of indirect challenge tests is that they are more specific for asthma, and can reveal important aspects of the pathophysiology of asthma, and the response to a specific stimulus such as exercise or allergen challenge.

The most commonly used indirect challenge tests, such as exercise challenge and EHV, have a single strong stimulus for bronchoconstriction and need to be used with caution in individuals with symptoms suggestive of poorly controlled asthma or with reduced lung function. Incremental indirect challenge tests, such as hypertonic saline and mannitol challenge, have been developed that can be used to assess indirect AHR across a larger spectrum of individuals with asthma. The assessment of indirect AHR can be useful in several different ways, including diagnostic testing for asthma, epidemiological studies of asthma, and the selection of asthma therapy. As in the case of direct AHR testing, it is crucial that the results of the test are interpreted in the appropriate clinical context and in relation to the features of asthma.

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