

Airway Hyperresponsiveness in Asthma: Measurement and Clinical Relevance



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Airway hyperresponsiveness is a characteristic feature of asthma, and its measurement is an important tool in its diagnosis. With a few caveats, methacholine bronchial provocation by a 2-minute tidal breathing method is highly sensitive; a negative test result ($PC_{20} > 16$ mg/mL, $PD_{20} > 400$ µg) rules out current asthma with reasonable certainty. A PC_{20} value of less than 1 mg/mL/ PD_{20} value of less than 25 µg is highly specific (ie, diagnostic) but quite insensitive for asthma. For accurate interpretation of the test results, it is important to control and standardize technical factors that have an impact on nebulizer performance. In addition to its utility to relate symptoms such as cough, wheeze, and shortness of breath to variable airflow obstruction (ie, to diagnose current asthma), the test is useful to make a number of other clinical assessments. These include (1) evaluation of patients with occupational asthma, (2) evaluation of patients with exercise-induced respiratory symptoms, (3) evaluation of novel asthma medications, (4) evaluation of relative potency of inhaled bronchodilators, (5) as a biomarker to adjust anti-inflammatory therapy to improve clinical outcomes, and (6) in the evaluation of patients with severe asthma to rule out masqueraders such as laryngeal dysfunction. The actual mechanism of altered smooth muscle behavior in asthma that is assessed

by direct (eg, methacholine) or indirect (eg, allergen) bronchial provocation remains one of the most fundamental questions related to asthma that needs to be determined. The test is underutilized in clinical practice. © 2016 American Academy of Allergy, Asthma & Immunology (J Allergy Clin Immunol Pract 2017;5:649-59)

Key words: Airway hyperresponsiveness; Methacholine; Technical factors; Clinical practice; Sputum cell counts

INTRODUCTION (BY PAUL M. O'BYRNE AND PARAMESWARAN NAIR)

In 1945, Tiffeneau and Beauvallet¹ reported, for the first time, the use of inhaled acetylcholine to measure airway responsiveness in patients with asthma. Subsequently, Curry² demonstrated that patients with asthma developed bronchoconstriction after administration of intramuscular, intravenous, or nebulized histamine. This increased bronchoconstrictor response in patients with asthma was labeled "airway reactivity," which was described as nonspecific, because the increased responses occurred to all agonists that constricted airways smooth muscle, such as the

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The symposium was organized with an unrestricted educational grant from Methapharm Inc, Brantford, Ontario, Canada. P. Nair is supported by the Frederick E. Hargreave Teva Innovation Chair in Airway Diseases.

Conflicts of interest: P. Nair declares that the report was based on a symposium that was supported by an unrestricted grant from Methapharm Inc; has received consultancy fees from AstraZeneca, Roche, Sanofi, Knopp, and Teva; has received research support from AstraZeneca, Teva, Novartis, Roche, Sanofi, Boehringer-Ingelheim, and GlaxoSmithKline; and has received lecture fees from AstraZeneca, Novartis, and Teva. J. G. Martin has received travel support from Methapharm and has received lecture fees from AstraZeneca. D. C. Cockcroft has received consultancy fees and travel support from Methapharm; is on the Methapharm Canada advisory board; is employed by the University of Saskatchewan; and has received research support from AllerGen and AstraZeneca. M. Dolovich has received consultancy fees from Methapharm and Teva and has received research support from Boehringer-Ingelheim. C. Lemiere has received fees for participation in Metafarm, GlaxoSmithKline, Teva, and AstraZeneca advisory boards and is a member of the Canadian Thoracic Society Asthma Clinical Assembly. L.-P. Boulet is on the advisory boards for and has received

consultancy fees from AstraZeneca, Novartis, and Methapharm; has received research support from Altair, Amgen, Asmacure, AstraZeneca, Boehringer-Ingelheim, Boston Scientific, Genentech, GlaxoSmithKline, Novartis, Ono Pharma, Schering, and Wyeth; has received lecture fees from AstraZeneca, GlaxoSmithKline, Merck, and Novartis; received royalties from UpToDate; has received payment for the development of educational presentations from AstraZeneca, GlaxoSmithKline, Merck Frosst, Boehringer-Ingelheim, and Novartis; has received travel support from Novartis and Takeda; is a member of the Canadian Thoracic Society Respiratory Guidelines Committee; is Chair of GINA Guidelines Dissemination and Implementation Committee; and is Laval University Chair on Knowledge Transfer, Prevention and Education in Respiratory and Cardiovascular Health. P. M. O'Byrne is on the Joint Oversight Board for Long-Acting β_2 -Agonists Safety Study; has received consultancy fees from AstraZeneca, GlaxoSmithKline, Merck, and Boehringer-Ingelheim; and has received research support from AstraZeneca, Amgen, Genentech, and Axikin.

Report of the Frederick E. Hargreave Symposium on Airway Hyperresponsiveness, Hamilton, Ontario, Canada, August 6, 2016.

Received for publication October 19, 2016; accepted for publication November 1, 2016.

Available online February 2, 2017.

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2213-2198

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<http://dx.doi.org/10.1016/j.jaip.2016.11.030>

Abbreviations used

AHR- Airway hyperresponsiveness
 APSD- Aerosol particle size distribution
 EIB- Exercise-induced bronchoconstriction
 EVH- Eucapnic voluntary hyperpnea
 ICS- Inhaled corticosteroid
 OA- Occupational asthma
 TSLP- Thymic stromal lymphopoietin

cholinergic agonists carbacol³ and methacholine,⁴ prostaglandins D2⁵ and F2 α ,⁶ and cysteinyl leukotrienes.⁷

In the 1970s, there was a major research interest in identifying the best methods for measuring airway reactivity and expressing the results; the relationships between the degree of airway reactivity and various clinical manifestations of asthma; and its possible mechanisms. In 1979, a seminal meeting of researchers from around the world working in these areas was held at St Joseph's Hospital and McMaster University. The meeting was organized by Dr Freddy Hargreave and resulted in the publication of a book titled "Airway reactivity: Mechanisms and clinical relevance."⁸ The presentations and discussions that happened during the 3 days of meetings were hugely influential in determining future research directions in asthma.

One important area that this meeting helped clarify is that airway responses to bronchoconstrictor agonists can be demonstrated in many subjects without asthma, if high enough concentrations of the agonist were inhaled. Four important differences have been shown to exist between responses in subjects with asthma and subjects without asthma (Figure 1). These are as follows:

1. Bronchoconstriction in those with asthma starts at a lower inhaled concentration of the agonist. In pharmacological terms, this is the threshold dose.
2. The slope of the concentration/response curve is steeper in those with asthma. This is airway reactivity.
3. The maximal bronchoconstrictor responses are greater in those with asthma.
4. Normal subjects achieve a "plateau response" to the bronchoconstrictor stimulus, whereas subjects with asthma generally do not.

Although the precise mechanisms that underlie these phenomenon remain to be elucidated,⁹ the recognition that "airway reactivity" was describing only 1 component of the responses measured resulted in a change in terminology to "airway hyperresponsiveness" (AHR). In August 2016, another meeting was held at St Joseph's Hospital and McMaster University, both to commemorate the original meeting and to discuss how far the field has advanced in almost 4 decades. This article is a summary of those presentations and discussions.

THE CONTRIBUTIONS OF AIRWAY SMOOTH MUSCLE TO AIRWAY RESPONSIVENESS (BY JAMES G. MARTIN)

Airway smooth muscle is the principal tissue mediating airway narrowing and not surprisingly has been a target of pharmacological therapy since the earliest times. Inhaled methacholine acts principally to cause airway narrowing by activation of muscarinic

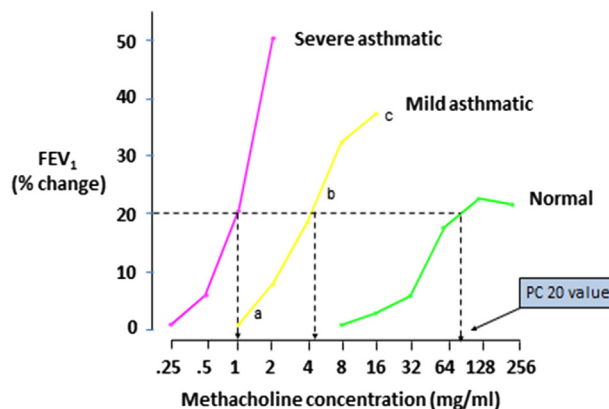


FIGURE 1. Airway responsiveness to inhaled methacholine measured in a normal subject, and subjects with mild and severe asthma. The response is measured by changes in FEV₁ and the results expressed as PC₂₀. Subjects with asthma have (A) reduced threshold responses to methacholine; (B) an increased slope of the methacholine dose-response curve; (C) an increase in the maximal bronchoconstrictor responses, when compared with subjects without asthma.

receptors on the smooth muscle of the airways. The degree of airway narrowing in response to stimulation of smooth muscle by methacholine and other bronchoconstrictive substances is a function of the force generated by the contracting muscle counterbalanced by a number of opposing forces.¹⁰ The lung elasticity transmitted through the parenchyma to the airway wall is an important limitation to airway narrowing. This bronchodilating influence is enhanced progressively as the airways narrow as a result of a phenomenon called airway-parenchymal interdependence. Breathing movements stretch the airway smooth muscle with every breath and in doing so exert a potent bronchodilating effect on the muscle.¹¹ The effect of lung elastic recoil on methacholine-induced increases in airway resistance is markedly enhanced by the simple effect of breathing below the usual functional residual capacity, converting the degree of airway narrowing from modest and limited constriction to severe narrowing comparable to an asthma attack.¹² Conversely, breathing above functional residual capacity attenuates the effect of methacholine. Similarly, healthy subjects without asthma who are not allowed to take large breaths during the methacholine challenge have an augmented response to inhaled methacholine.

So how is it that, in subjects with asthma, methacholine can induce such marked airway narrowing? One possible explanation that may account for the AHR of airways in subjects with asthma is the growth of airway smooth muscle, presumably stimulated by inflammatory mediators, growth factors, and perhaps by the act of bronchoconstriction itself.¹³ Numerous studies have documented the increased mass of muscle in the airways of subjects with asthma and several have related it to the severity of asthma.¹⁴ Muscle mass is increased in most instances by an increase in the number of cells, and in some cases by hypertrophy also.¹⁵ Evidence of cellular turnover can be found through immunostaining for cell proliferative markers such as proliferating cell nuclear antigen.¹⁶ The muscle that is wrapped in a spiral fashion around the airways is unchanged in orientation by the

remodeling process (G. Ijpma, unpublished data, 2016). This suggests that the processes driving growth are constrained, perhaps by mechanical stresses, to increase muscle such that it is added in an orderly manner to existing muscle bundles.

However, not all patients with asthma have an increase in airway smooth muscle so one is tempted to assume that the airway smooth muscle in those patients is intrinsically more contractile. It has been difficult to have evidence of enhanced contractility *ex vivo*. mRNA analyses of contractile proteins on tissue obtained by laser microdissection failed to show differences in those with mild asthma.¹⁷ In contrast, Western analysis has shown increases in some contractile apparatus proteins in those with moderate asthma.¹⁸ Detailed studies of mechanics on excised asthmatic tissues have not confirmed enhanced contractile responses.¹⁹ The most consistent finding has perhaps been an impaired capacity for relaxation. The tissue microenvironment therefore appears to be crucial in inducing the altered behavior of the airway smooth muscle. Exposure of airway smooth muscle *in vivo* and *ex vivo* to increased tone by administering methacholine or a thromboxane mimetic enhances the muscle's response to a subsequent administration of agonist.²⁰ However, the actual mechanism of altered smooth muscle behavior remains one of the most fundamental questions related to asthma that needs to be determined.

MEASUREMENT OF AHR (BY DONALD W. COCKCROFT)

Airway hyperresponsiveness (AHR), an objective measure of variable airflow obstruction, is measured using provocation tests with chemical (histamine, methacholine, adenosine monophosphate [AMP], mannitol, etc) or physical (exercise or its surrogate eucapnic voluntary hyperpnea [EVH]) stimuli.²¹ Bronchoprovocation is recommended as a diagnostic investigation in subjects with symptoms (possibly asthmatic) and normal flow rates. Stimuli used to measure AHR have been categorized as direct or indirect.^{21,22} Direct stimuli (histamine, methacholine) act on smooth muscle receptors, whereas indirect stimuli (exercise, EVH, AMP, mannitol) act through an intermediate pathway, often involving inflammatory cell mediator release. In contrast to indirect stimuli, direct stimuli (eg, methacholine) are more dependent on muscle function (including airway caliber), less dependent on airway inflammation, require much (~3 orders of magnitude) lower dose, exhibit no refractoriness, are not inhibited by cromones, and have high sensitivity and relatively low specificity for asthma (Table I). The 2 types of test are, therefore, complementary. AHR is most widely measured with methacholine, and methacholine is the only inhalation challenge agent currently approved by regulatory agencies; this review will focus on methacholine challenge methods.

Methacholine is inhaled in increasing amounts targeting a reduction in FEV₁. Results are expressed as the provocation concentration of methacholine causing a 20% fall in FEV₁ or PC₂₀.²³⁻²⁵ There are many noncomparable methods, 2 of which were outlined in 2000 by the American Thoracic Society.²³ These were the 2-minute tidal breathing method developed by Freddy Hargreave²⁴ and the 5-breath American Academy of Allergy, Asthma & Immunology (AAAAI) method modified by Freddy.²⁵ Definitions were as follows: PC₂₀ more than 16 mg/mL, normal; 4 to 16 mg/mL, borderline; 1 to 4 mg/mL, mild AHR; 0.25 to 1 mg/mL, moderate AHR; and less than 0.25 mg/mL, marked AHR.²³ One study suggested that these 2 methods were

TABLE I. Comparison of direct and indirect bronchial provocation tests

Parameter	Direct (eg, methacholine)	Indirect (eg, mannitol)
Muscle function	+++++	++
Airway caliber	+++++	±
Inflammation	Nil to ++	+++++
Dose needed	Low	High
Refractory period	±	+++++
Cromolyn inhibition	Nil	+++++
Sensitivity	High	Low
Specificity	Lowish	High
Diagnostic use	Rule out	Rule in

equivalent²⁵; however, this is not the case.^{26,27} The repeated deep inhalations from the dosimeter cause marked bronchoprotection especially in those with mild AHR.^{28,29} This is the region where most clinical positive challenges will occur. The dosimeter method as outlined in 2000, therefore, markedly reduces the diagnostic sensitivity, which, in our opinion, is the major clinical value of the test.

Traditional jet nebulizers (Wright, de Vilbiss) used for bronchoprovocation are inefficient and becoming difficult to obtain. Much of the weight loss (up to 75% or more) during use, the conventional manner in which we have determined output, is evaporation rather than nebulization.³⁰ Because the response to methacholine is dose rather than concentration dependent,^{31,32} it is important to standardize methods to ensure that they deliver the same dose at a given PC₂₀. One way to achieve between-method comparisons would be to express the result as a provocative dose (PD₂₀), ideally eliminating the (up to 75% or more) overestimate of dose when evaporation is not considered. We estimate that a tidal breathing PC₂₀ of 16 mg/mL equates to a (solute output) PD₂₀ of 400 µg.³² Methacholine, inhaled in doubling doses at 5-minute intervals, has a partial (~50%) cumulative effect²⁴; this will change with differing time intervals. To keep this effect consistent, the time interval requires standardization; we recommend 5 minutes. New methacholine guidelines are in preparation based on the above principals.

Important caveats regarding interpretation of methacholine challenge tests include the following. Perhaps the most important is the need for the symptoms under consideration to be clinically current (ie, within the past few days). AHR may not be present in nonexposed atopic individuals³³ and can resolve rapidly in those with occupational asthma.³⁴ This is a common reason for poor performance of challenges in epidemiologic studies³⁵ where current is defined as within the past year. Expiratory flows should be normal because airflow obstruction causes (nonasthmatic) responsiveness^{36,37} due to geometric factors. AHR testing may be of limited use in guiding treatment of cough because a positive test result does not predict response³⁸ and a negative test result does not predict nonresponse³⁹ to asthma therapy. Careful attention needs to be paid to medications and their withhold time; inadvertent use of inhibiting drugs, especially antimuscarinic agents, can cause false negatives. A few elite athletes with exercise-induced bronchoconstriction (EIB) have negative methacholine test results.⁴⁰ AHR severity does not equate to asthma severity; most individuals with mild AHR do not have asthma, whereas most subjects with moderate AHR have only mild asthma. Subjects with more severe asthma will not require AHR testing.

In summary, methacholine challenge provides an objective test to measure AHR. Standardization is important to achieve a consistent and reproducible dose both within and between methods. With a few caveats, the test is highly sensitive; a negative test result ($PC_{20} > 16$ mg/mL, $PD_{20} > 400$ μ g) rules out current asthma with reasonable certainty. A PC_{20} value of less than 1 mg/mL/ PD_{20} value of less than 25 μ g is highly specific (ie, diagnostic) but quite insensitive for asthma.³³

TECHNICAL FACTORS IN THE ASSESSMENT OF AHR (BY MYRNA DOLOVICH)

The assessment of AHR involves the inhalation of a challenge aerosol produced via liquid nebulization with a specific breathing pattern to administer the drug.⁴¹ These components are influenced by a number of technical factors that can alter the generation and delivery of aerosol to the lung. Although liquid aerosols of histamine and methacholine have been the choice pharmaceutical, recent developments have produced and successfully tested a dry powder formulation of methacholine, inhaled via a dry powder inhaler.⁴²

There are a number of marketed nebulizers that are used to produce challenge aerosols for AHR testing. The Wright jet nebulizer, originally designed and produced in the United Kingdom,⁴³ was the most widely used delivery system for AHR tests, providing aerosols of increasing concentrations of histamine or methacholine to subjects breathing tidally. In 1975, the Rosenthal-French Dosimeter coupled to a DeVilbiss jet nebulizer⁴⁴ was shown to give equivalent outcomes to the Wright nebulizer but using breath-actuation with high inspiratory airflow rates providing unit doses of the challenge aerosol.²⁵ Recent developments in nebulizer design have yielded portable, high efficiency devices, providing low-flow, small-particle aerosols,^{45,46} promoting increased delivery with uniform distribution of drug to the whole lung. Using these nebulizers will result in marked differences in PC_{20} or PD_{20} , necessitating the modification of procedures to align with current AHR test methods and results.

Technical factors influencing aerosol production and quality are presented in Table II. Critical factors are aerosol particle size distribution (APSD), and the inhalation technique, in particular, the inhalation flow rate. The standardized methods to obtain APSD data have been published in a number of regulatory guidances and pharmacopeias. The statistics from the APSD, namely, mass median aerodynamic diameter and fine particle fraction, relate to the overall quality of the aerosol and allow projections of lung deposition and distribution to be made. The emitted dose provides the amount of test substance that could be inhaled into the mouth.⁴⁷ Both APSD and emitted dose require measuring absolute amounts of challenge substance. Formulation factors also need to be considered and controlled. And the airway disease status of the subject markedly influences the dose inhaled and the distribution within the lung of that dose.^{48,49}

PC_{20} or PD_{20} is predicated on knowing the concentration of challenge substance delivered to the subject. Currently, the nominal concentration of the solution that causes the 20% decrease in FEV_1 is used. However, drug concentration in the inhaled liquid aerosol and reservoir fluid increases with nebulization, with this change occurring throughout nebulization. To allow comparisons between subjects and for subsequent tests in the same subject, the concentration of drug in the reservoir should be measured as well as the volume or weight changes of

TABLE II. Technical factors influencing aerosol production and quality

Aerosol generation
– Nebulizer output
• Jet flow rate
• Fill volume
• Solvent evaporation/concentration changes
• Drug delivery rate
• Side vent/auxiliary air
• Baffle orientation
– Aerosol particle (MMAD)/droplet (VMD) size, shape, density
– Continuous vs intermittent generation
– Subject interface
– Residual liquid or dead volume
– Ambient temperature, humidity
– Environmental contamination
– Reproducibility and reliability of output
Aerosol inhalation
– Aerosol particle/droplet size distribution (FPF)
– Inspiratory flow rate
– Volume inhaled
– Inspiratory time
– Breath-hold
– Spontaneous vs controlled breathing
– Nose vs mouth breathing
– Airway caliber
– Emitted dose/delivered dose/respirable dose
– Lung deposition efficiency
Drug: Challenge substance
– Fill volume
– Temperature of solution
– Diluent
– pH
– Surface tension
– Vapor pressure at air-liquid interface
– Initial, final concentration of solution
– Assay methodology

FPF, Fine particle fraction; MMAD, mass median aerodynamic diameter; VMD, volume median diameter.

the reservoir contents to accurately determine the drug output⁵⁰ (see Table E1 in this article's Online Repository at www.jaci-inpractice.org). Drug output estimated from weight change converted to milligrams of drug using the nominal drug concentration can overestimate output by approximately 25% compared with absolute measurements of drug output.⁴⁹

For ongoing calibration purposes and assurances that the operating characteristics of the jet nebulizer have not changed, one can operate the nebulizer for a fixed time and with a specific volume of fluid in the reservoir, weighing it before and after this specific time. This approach will provide a quick check on the reproducibility of the nebulizer output, but will not measure the extent of concentration changes in the challenge aerosol.

The dose of challenge aerosol inhaled at a specific inspiratory flow rate affects the response to the aerosol. Less methacholine was deposited in the lung with a more central deposition pattern when delivered as fast versus slow vital capacity breaths from the R-F Dosimeter. The PC_{20} response increased with the decreasing dose of deposited aerosol 8. Figure E1 in this article's Online

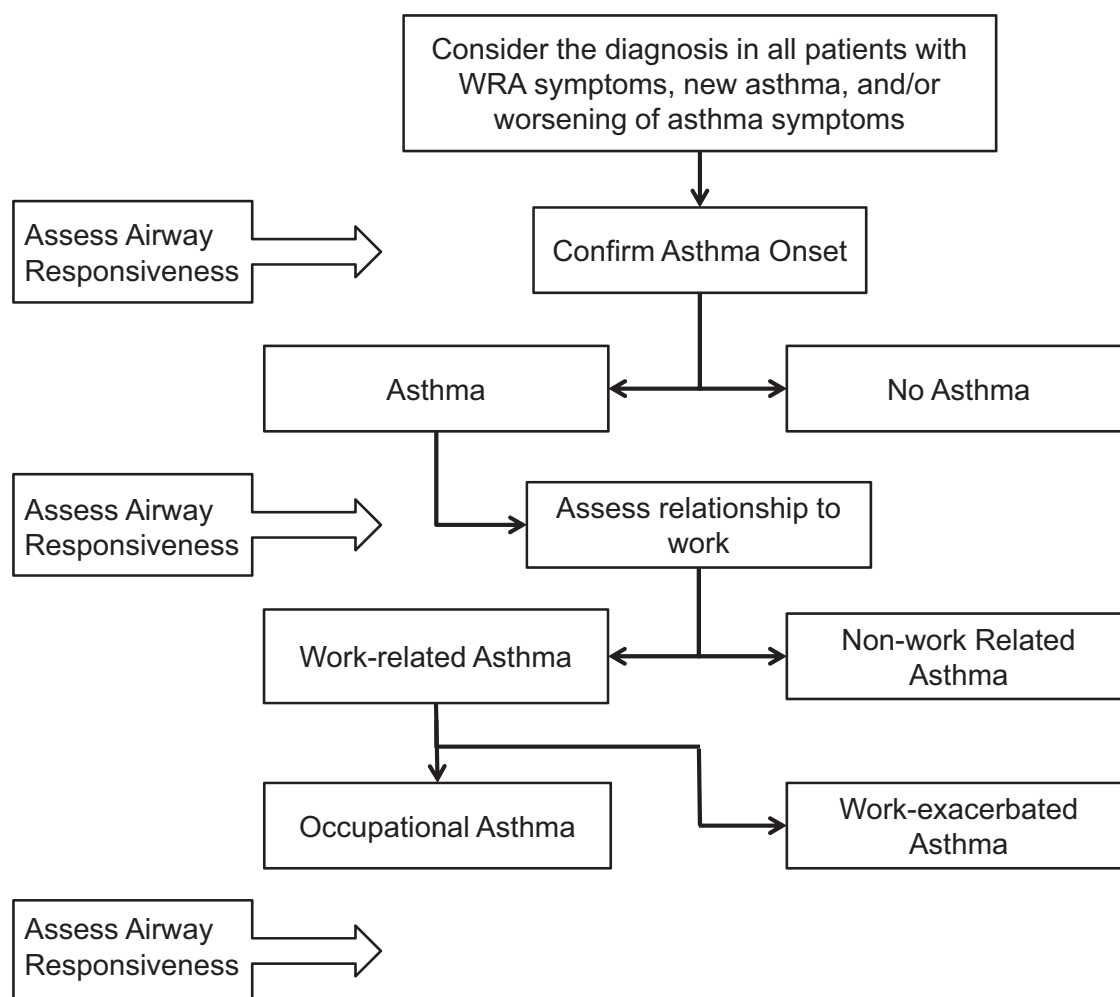


FIGURE 2. Flow chart of investigation of WRA.⁵² WRA, Work-related asthma.

Repository at www.jaci-inpractice.org illustrates the distribution of ventilation before and after methacholine challenge.⁵¹

In summary, controlling technical factors that have an impact on nebulizer performance is important, as well as standardizing the inhalation breathing pattern. To accurately validate the concentration and/or dose of drug inhaled, premeasurements and postmeasurements of concentration and fluid volume changes should be performed.

ASSESSMENT OF AIRWAY RESPONSIVENESS TO MANAGE OCCUPATIONAL ASTHMA (BY CATHERINE LEMIERE)

The diagnosis of occupational asthma (OA) requires a comprehensive investigation involving the performance of different and complementary tests. The assessment of airway responsiveness is crucial in the evaluation of a patient suspected of having OA. It should be performed at different stages of the investigation of OA: at the beginning of the investigation to confirm the diagnosis of asthma; during the investigation to confirm the relationship between the onset of asthma and the exposure to a specific agent at the workplace; after the diagnosis has been established to assess the sequels of OA after removal from exposure (Figure 2).⁵²

The diagnosis of asthma needs to be objectively confirmed in every patient suspected of having OA. Beach et al⁵³ performed a systematic review of 24 studies that assessed the sensitivity and specificity of methacholine challenge in subjects suspected to have OA. They found respective sensitivities of 79.3% and 68% for high- and low-molecular-weight agents and respective specificities of 51% and 64% for high- and low-molecular-weight agents for diagnosing OA. However, only 2 of these 24 studies recruited more than 100 patients (102 and 209). The sample size of the remaining studies varied from 4 to 75 subjects. Furthermore, the work status of the workers was not taken into account in the systematic review. We recently assessed 1012 patients suspected of having OA. When performed in a worker still at work, a methacholine challenge test had a 97% negative predictive value for diagnosing OA.⁵⁴ However, although a negative methacholine challenge makes the diagnosis of OA very unlikely in a symptomatic patient still working, a few cases of OA have been reported in subjects with a negative methacholine challenge. These cases had a prominent eosinophilic airway inflammation when exposed to the offending agent.^{55,56}

The next step of the investigation of OA is to demonstrate a relationship between asthma onset and work exposure. The same systematic review⁵³ analyzed the sensitivity and specificity of serial measures of airway responsiveness at and away from work.

Only 3 studies including mixed agents and 2 studies including low-molecular-weight agents were available. This limited number of studies with small sample sizes prevents establishing a firm conclusion regarding the usefulness of serial airway responsiveness for the diagnosis of OA. A change in airway responsiveness induced by exposure to the offending agent is likely to be associated with OA. Vandenplas et al⁵⁷ showed that changes in airway responsiveness precede changes in airway caliber in subjects with OA.

In some Canadian provinces, airway responsiveness is assessed 2 years after removal from exposure in addition to airflow obstruction and the need of medication in order to evaluate the sequels of the workers.⁵⁸ Although airway responsiveness can improve even 5 years after removal from exposure,⁵⁹ the maximum improvement of this test occurs during the first 2 years after removal from exposure.⁶⁰

In conclusion, the assessment of airway responsiveness is useful at different stages of the investigation and management of OA. Combining the assessment of airway responsiveness and airway inflammation is likely to improve further the diagnosis of OA.

ASSESSMENT OF AHR TO EVALUATE EXERCISE-INDUCED RESPIRATORY SYMPTOMS (BY LOUIS-PHILIPPE BOULET)

Most subjects with asthma may experience a fall in expiratory flows after exercise, usually associated with respiratory symptoms.⁶¹ This may occur more frequently when asthma is not optimally controlled, or after exposure to sensitizing agents or respiratory infections. EIB results from smooth muscle contraction, associated with the release of mediators such as histamine, prostaglandins, and cys-leukotrienes and possibly neural (eg, cholinergic) influences.^{62,63} The main trigger of EIB is considered to be airways dehydration from exercise-induced increased ventilatory requirements, resulting in a rise in airway fluid osmolarity.⁶² A correlation has been reported between the intensity of eosinophilic airway inflammation, as assessed by induced sputum analysis, and the magnitude of EIB as well as with response to inhaled mannitol, a surrogate of exercise.^{64,65}

Contrary to other conditions that may mimic asthma such as exercise-induced laryngeal obstruction,⁶⁶ which occurs mostly during exercise, EIB develops typically after exercise, with airway caliber increasing and airway responsiveness decreasing during the effort.⁶⁷ However, contrary to allergen exposure in sensitized subjects, it does not induce significant airway inflammation nor increases airway responsiveness in subjects with asthma.⁶⁸

Compared with nonathletes, high-level athletes show an increased prevalence of various respiratory ailments such as asthma, EIB, allergic or nonallergic rhinitis, chronic cough, vocal cord dysfunction/exercise-induced laryngeal obstruction, and recurrent respiratory infections.⁶⁹ Asthma has been reported in up to one-fourth of summer sports athletes and up to one-half of winter sports athletes, but AHR is even higher, its prevalence depending on the sport practiced and the type of bronchoprovocation test, from 25% to 80% in athletes performing endurance sports, particularly swimmers and winter athletes, in opposition to less than 20% in power and speed sports athletes.^{70,71}

This increased prevalence of AHR in elite athletes seems to be due to environmental exposures, such as cold air in winter athletes or chlorine derivatives in swimmers, but the mechanical stress to airways caused by marked ventilatory efforts and

dehydration may be involved.^{72,73} Exercise-induced epithelial damage, increased airway permeability, and plasma exudation possibly play a role in airway responses.⁶² In elite swimmers and cross-country skiers, AHR has been associated with marked airway remodeling, including collagen deposition underneath basement membrane.^{74,75} Curiously, AHR in athletes is often associated with minimal or no airway inflammation, mostly neutrophilic.⁷⁶ Furthermore, airway responsiveness may normalize after cessation of training and competition, sometimes after only a few weeks.^{77,78}

In athletes, exercise-related respiratory symptoms do not correlate well with lung function or airway responsiveness.⁷⁹ When expiratory flows are normal—they may even be supra-normal in swimmers—bronchoprovocation tests are needed to confirm EIB/AHR.^{69,80} Although there is a relatively good correlation between indirect bronchoprovocation tests such as EVH, exercise, or mannitol challenges and direct tests such as methacholine challenge, athletes may have a positive response to one type of test but not to the other.^{80,81} To ensure optimal sports' performance, EIB should be minimized. To do so, in addition to usual preventative measures, optimal asthma control should be ensured and medications inhibiting EIB such as fast-acting beta-2 agonists may be used before exercise.⁸⁰ There is however a tolerance to the bronchoprotective effects of inhaled β_2 -agonists after regular use.⁸² Inhaled β_2 -agonists should therefore be used infrequently, their need being reduced by taking regular anti-inflammatory agents such as inhaled corticosteroids (ICSs). More research is required on the effects of combined environment factors and exercise on long-term airway responses and on how to reduce the risk of developing asthma and AHR in athletes.

ASSESSMENT OF AHR TO EVALUATE NEW MEDICATIONS (BY PAUL M. O'BYRNE)

Measurements of methacholine AHR have been extensively used to evaluate the efficacy and duration of effect of functional antagonists, such as inhaled β_2 -agonists. Early studies compared functional antagonists with different mechanisms of action, such as theophylline and inhaled β_2 -agonists,⁸³ different formulations of short-acting inhaled β_2 -agonists,⁸⁴ and more recently short-acting β_2 -agonists compared with long-acting β_2 -agonists.⁸⁵

Another important contribution of measuring methacholine AHR was in demonstrating the onset of action and efficacy of ICSs. This was initially done in milder patients, not being treated with ICS and in whom AHR improved and, in some patients, normalized.⁸⁶ These results helped support a place for ICS as asthma treatment, even in milder patients. The use of methacholine AHR to optimize doses of ICS has also been studied and demonstrated that this approach significantly improved FEV₁, as well as reduced mild asthma exacerbations⁸⁷; however, higher doses of ICS were needed to achieve this clinical benefit, and this approach is not widely used to manage patients.

The most recent use of methacholine AHR in drug development is evaluating the efficacy of new asthma treatments after inhaled allergen challenge. The allergen-induced changes in methacholine AHR was described by Cockcroft et al⁸⁸ to occur in allergic asthmatic subjects who develop allergen-induced airway responses. The magnitude of the effect was greater in individuals who developed both allergen-induced early and late asthmatic responses,⁸⁹ and lasted up to 1 week in most subjects.⁹⁰ Allergen-induced AHR is attenuated by treatment with

TABLE III. Effect of treatment with potential new treatments for asthma, on allergen-induced early responses, late responses, methacholine AHR, and sputum eosinophilia

New molecule	Dosing	Early response	Late response	AHR	Eosinophils
Inhaled heparin disaccharide ⁹⁶	Single	No	No	No	No
Inhaled PGE2 ⁹⁷	Single	Yes	Yes	Yes	Yes
Anti-CD11a ⁹⁸	8 wk	No	Yes	No	Yes
Antisense against IL-3, GM-CSF, and CCR3 ⁹⁹	4 d	Yes	No	No	Yes
CPG-Oligo ¹⁰⁰	4 wk	No	No	No	No
Anti-IL-13 ¹⁰¹	2 wk	Yes	Yes	No	No
PDE4 inhibitor ¹⁰²	2 wk	No	Yes	Yes	Yes
Anti-Ox 40L ¹⁰³	16 wk	No	No	No	No
$\alpha 7$ nicotinic agonist ¹⁰⁴	9 d	No	No	Yes	No
CysLT1/LT2 antagonist ¹⁰⁵	8 d	Yes	Yes	Yes	Yes
Anti-M1prime ¹⁰⁶	12 wk	Yes	Yes	No	Yes
Nonsteroid GR agonist ¹⁰⁷	1 wk	No	Yes	Yes	Yes
Anti-TSLP ¹⁰⁸	12 wk	Yes	Yes	Yes	Yes

GR, Glucocorticoid receptor; No, no statistically significant effect; PGE2, prostaglandin E2; Yes, statistically significant attenuation.

ICS administered before allergen challenge,⁹¹ but the effect is transient, lasting less than 24 hours.⁹² Other treatments that were shown to attenuate allergen-induced AHR included leukotriene receptor antagonists⁹³ and cromones.⁹⁴

Allergen-induced airway responses, including methacholine AHR, are now used as a useful tool for the evaluation of potential new therapies for asthma⁹⁵ (Table III). A recent study of a potential asthma treatment evaluated in this way is a human mAb that binds human thymic stromal lymphopoietin (TSLP) (AMG 157) and prevents interaction with its receptor. Treatment with AMG 157 had 2 important effects.¹⁰⁸ First, it markedly reduced baseline markers of persisting airway inflammation, normalizing the increased levels of fractional exhaled nitric oxide and both peripheral blood and sputum eosinophilia in the mild allergic asthmatic subjects. Second, it significantly reduced allergen-induced airway responses, including methacholine AHR (see Figure E2 in this article's Online Repository at www.jaci-inpractice.org), and all indexes of airway inflammation after allergen challenge. These results have important implications, in that they not only indicate that the constitutive release of TSLP is critical for the maintenance of airway inflammation in allergic asthma but also that allergen-induced TSLP release is involved in the allergen-induced changes in airway eosinophilia and bronchoconstrictor responses. The results of this study support further development of AMG 157 in asthma, and also the utility of allergen-induced responses in evaluating potential new treatments.

ASSESSMENT OF AHR IN A SEVERE ASTHMA CLINICAL PRACTICE (BY PARAMESWARAN NAIR)

AHR is a characteristic feature of asthma, and its measurement is an important tool in its diagnosis. As already discussed in this symposium, in addition to its utility to relate symptoms such as cough, wheeze, and shortness of breath to variable airflow obstruction (ie, to diagnose current asthma), the test is useful to make a number of other clinical assessments. These include (1) evaluation of patients with OA, (2) evaluation of patients with exercise-induced respiratory symptoms, (3) evaluation of novel asthma medications, (4) evaluation of the relative potency of inhaled bronchodilators,¹⁰⁹ and (5) and as a biomarker to adjust anti-inflammatory therapy to improve clinical outcomes.⁸⁷ This

section will focus specifically on my experience in applying this test to evaluate and manage patients with severe asthma as defined by the European Respiratory Society/American Thoracic Society recommendation.¹¹⁰

The pulmonary function laboratory of the Firestone Clinic accepts referrals for methacholine provocation test from general practitioners and from specialists. Since 2006, all the clinical information is captured on a database that is approved by the Research Ethics Board of St Joseph's Healthcare. Of the 11,110 requests for methacholine tests, 721 (6.5%) were specifically for patients to be assessed in the severe asthma clinic. We did not perform the test in 135 patients because their baseline FEV₁ was less than 65% of the predicted values (25 of them demonstrated >15% reversibility to salbutamol). The first observation to make is that the test, by the 2-minute tidal breathing method, can be safely performed by following the recommended guidelines. Of the 586 patients who had the test done, only 6 patients (1%) experienced chest tightness lasting for 30 minutes more than 2 points on a 10-point Borg scale.

The second observation is the confirmation of the previously reported phenomenon of overdiagnosis of asthma.¹¹¹ A total of 318 patients (54.3%) had a PC₂₀ methacholine value of more than 16 mg/mL at the time of assessment, indicating that their current symptoms were not due to variable airflow limitation. Of the 152 patients who were referred to as severe asthma (who had been evaluated previously, on average, by 2 specialists within the past 2 years), less than half (n = 74) were confirmed to have severe asthma. The chances of being correctly diagnosed increased with the severity of AHR (see Table E2 in this article's Online Repository at www.jaci-inpractice.org).

The third observation is the relationship between AHR and bronchitis assessed by sputum cytometry.¹¹² The distinct entity of eosinophilic bronchitis without AHR (n = 26) contributed to some of the patients being mislabeled as suffering from asthma (Table E2). Of the patients confirmed to have severe asthma (n = 74), majority (n = 45) had a T_H2-type inflammation characterized by persistent sputum eosinophilia (>3% with or without degranulation), while a smaller proportion had persistent neutrophilic bronchitis (total cell count >15 million cells/g and >65% neutrophils) or pleotropic bronchitis. A total of 14 patients had persistently normal sputum cell counts. A combination of

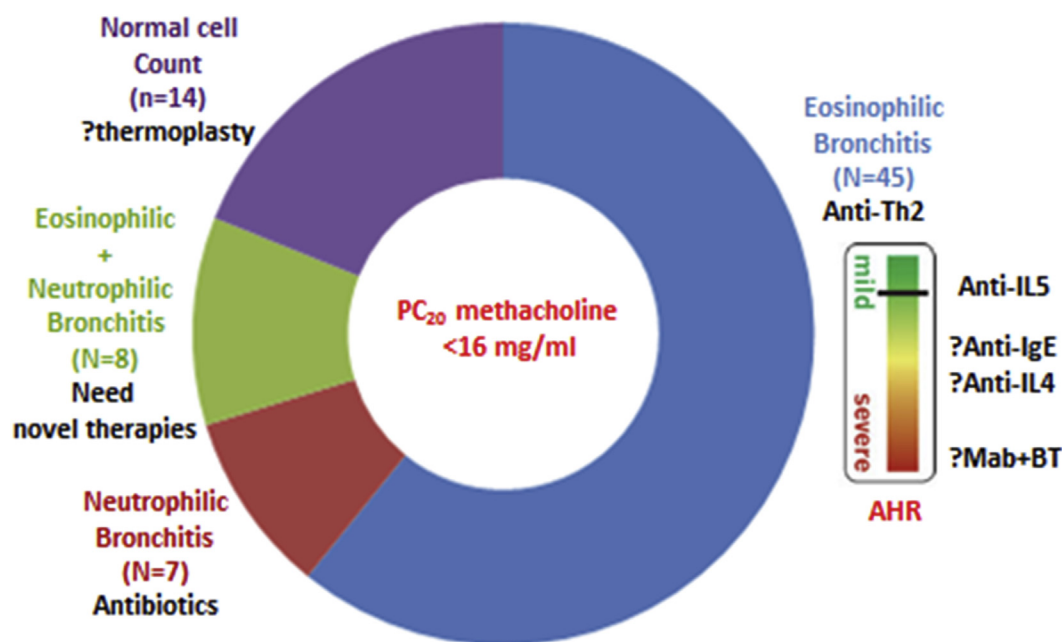


FIGURE 3. Heterogeneity of bronchitis in a severe asthma population (PC_{20} methacholine < 16 mg/mL) and the choice of therapies guided by the endotype.

assessment of AHR and sputum cell counts is used to select the appropriate therapy for these patients with severe asthma (Figure 3).^{113,114} It is important to consider measurements of AHR as an outcome measure to evaluate new antiasthma interventions such as anti-IL4/IL-13 mAbs and thermoplasty.

The fourth observation is the economic impact of this overdiagnosis, specifically, of severe asthma. Excluding the 26 patients with normal airway responsiveness who required inhaled or ingested steroids for their eosinophilic bronchitis, the 37 patients (16 of whom were getting recurrent neutrophilic bronchitis that was not recognized by their physicians) who did not have asthma, let alone severe asthma, had on average over the past year 3.5 emergency room visits, 1.2 hospitalizations (on average for 2.5 days), and 2.6 inappropriate courses of prednisone. Applying unit costs estimated from the British Columbia administrative databases¹¹⁵ of \$325 for an emergency room visit, \$2450 for a hospital admission for asthma, \$2000 for 1 year of asthma medications, a crude estimate of the total direct cost, inappropriately incurred by the health care system over 1 year, is \$218,855. This could have been potentially prevented by a methacholine test and a sputum cytometry that would cost approximately \$11,000 (unit costs of \$300 $\times$ 37 patients).¹¹⁶ It is therefore extremely important that a thorough economic analysis of the utility of methacholine provocation test be performed.

In summary, assessment of airway hyperresponsiveness is a safe and effective method to evaluate patients with severe asthma, prevent overdiagnosis, and thereby limit costs of inappropriate hospital visits and medications, and to select patients with targeted therapy with new biologics and other technologies. It appears that the test is generally underutilized because of a combination of irrational concern over its safety and a lack of appreciation of the need to understand the components of an airway disease (ie, AHR and airway inflammation)¹¹⁷ that contribute to symptoms and severity in an individual patient.

We gratefully acknowledge Sarah Svenningsen, PhD (McMaster University), and Jacque Bramley (University of Saskatchewan) for their assistance with the preparation of this manuscript.

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APPENDIX

TABLE E1. Estimation of methacholine concentration in the nebulizer reservoir⁴⁹

1. Change in weight (W) and change in drug concentration ($[\]$) in nebulizer fluid over time: Drug output at time t determined by: Output _{t} = $W_{\text{initial}} \times [\]_{\text{initial}} - W_t \times [\]_t$
2. For quick calibration: Gravimetric (Estimated) output: Change in weight $\times$ Nominal concentration
3. Absolute drug content in aerosol over time

TABLE E2. Distribution of AHR measurements and relation to type of bronchitis and severe asthma diagnosis.

PC ₂₀	n	Confirmed as severe asthma	EB	NB	EB + NB	Normal
>16	63	None	26	16	0	21
4-16	16	10	7	0	0	3
2-4	11	10	8	1	0	1
0.25-2	36	30	18	3	4	5
<0.25	26	24	12	3	4	5
Total	152	74	71	23	8	35

EB, Eosinophilic bronchitis; NB, neutrophilic bronchitis.

CHANGE IN LUNG DISTRIBUTION OF INHALED ^{18}F [FDG] AEROSOL 1.5 μm VMD, 3D PET

Asthmatic subject
pre and post MIT; @ 8mg/ml, ΔFEV_1 23.6%

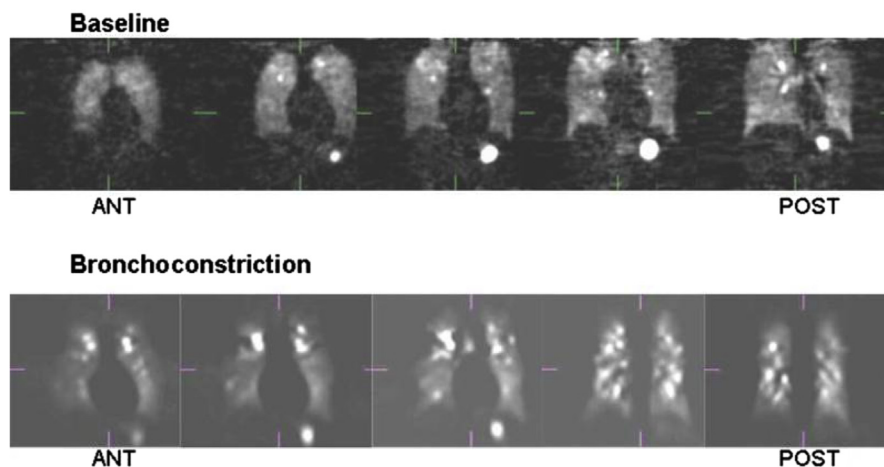


FIGURE E1. Example of the distribution of aerosol from a subject with asthma obtained before and after a methacholine inhalation challenge test (MIT). These 5 coronal slices from a positron emission tomography scan represent Anterior (ANT) to Posterior (POST) areas of the lung and demonstrate the nonuniformity of the distribution of bronchoconstriction of the aerosol used to perform the MIT. There is also a decrease in volume of each lung slice compared with the baseline scan. Reprinted with permission of the American Thoracic Society. Copyright © 2016 American Thoracic Society. Dolovich M, Labiris R. Imaging drug delivery and drug responses in the lung. *Proc Am Thorac Soc* 2004;1:329-37.⁵¹ *The American Journal of Respiratory and Critical Care Medicine* is an official journal of the American Thoracic Society.

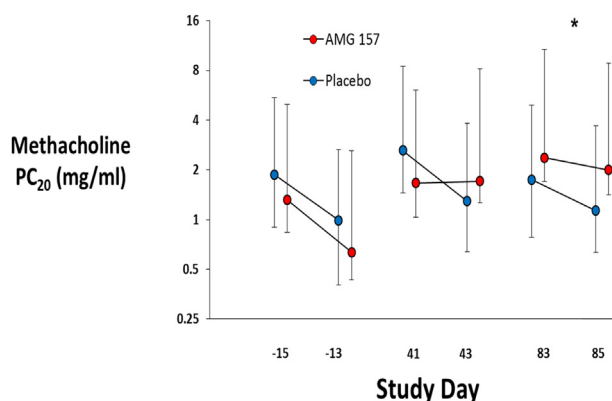


FIGURE E2. Allergen-induced methacholine AHR measured before (day -15) and after allergen (day -13) at screening and again after 6 weeks (days 41 and 43) and 12 weeks (days 83 and 85) of treatment with the anti-TSLP human mAb, AMG 157 (red circles), or placebo (blue circles). Allergen inhalation significantly decreased methacholine PC_{20} during the screening challenges in both study groups and after placebo treatment at 6 and 12 weeks. AMG 157 significantly attenuated the allergen-induced changes in methacholine PC_{20} by week 12 (* $P < .05$).¹⁰⁸

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