

Bronchoprovocation Testing in Asthma: An Update



Pasquale Comberiati, MD^{a,b}, Rohit K. Katial, MD^c,
Ronina A. Covar, MD^{c,*}

KEYWORDS

- Asthma • Airway inflammation • Airway remodeling
- Bronchoprovocation challenges • Bronchial hyperresponsiveness
- Bronchoprovocation testing

KEY POINTS

- Bronchial hyperresponsiveness (BHR) probably represents several inherent elements of the disease process, such as genetic predisposition, airway inflammation, and airway remodeling.
- Airway inflammation is thought to account for the transient and variable component of BHR, although treatments targeting airway inflammation do not normalize BHR.
- The permanent or persistent component of BHR, particularly to direct stimuli, correlates significantly with structural changes in the airway, such as basement membrane thickness and epithelial damage.
- Treatments significantly eliminating BHR are currently lacking.
- BHR, especially to indirect stimuli, could be a meaningful marker to monitor antiinflammatory treatment efficacy.

INTRODUCTION

Bronchial hyperresponsiveness (BHR) is defined as a heightened bronchoconstrictive response to airway stimuli. It complements the cardinal features in asthma, such as variable or reversible airflow limitation and airway inflammation. To what extent the mechanisms of airflow limitation, airway inflammation, and BHR overlap is still unclear, and how these 3 components come together probably accounts for the wide variability of asthma as a disease.^{1,2} Although BHR is considered a pathophysiologic

This article is an update of the previously published article on “Bronchoprovocation Testing in Asthma” in the August 2012 issue of the *Immunology and Allergy Clinics of North America*.

Conflict of Interests: None to declare.

^a Department of Clinical and Experimental Medicine, Section of Paediatrics, University of Pisa, 56126 Pisa, Italy; ^b Department of Clinical Immunology and Allergology, I.M. Sechenov First Moscow State Medical University, 119991 Moscow, Russia; ^c National Jewish Health, 1400 Jackson Street (J321), Denver, CO 80206, USA

* Corresponding author.

E-mail address: covarr@njhealth.org

Immunol Allergy Clin N Am 38 (2018) 545–571

<https://doi.org/10.1016/j.iac.2018.06.010>

0889-8561/18/© 2018 Elsevier Inc. All rights reserved.

immunology.theclinics.com

hallmark of asthma, it should be acknowledged that this property of the airway is dynamic, because its severity and even presence can vary over time, with disease activity, triggers or specific exposure, and with treatment.² In addition, it is important to recognize that there is a component that is not reflective of a specific disease entity. Indeed, BHR has been documented in asymptomatic subjects in the general population and in other diseases, such as chronic obstructive pulmonary diseases, congestive heart failure, cystic fibrosis, bronchitis, allergic rhinitis, and even in healthy subjects.^{3–5} Whether its presence in these conditions indicates a common inflammatory process is still poorly understood.

Bronchoprovocation challenges (BPCs), using pharmacologic or physical means to evaluate BHR, have been used to serve a variety of purposes. The most common indication of BPC is when a diagnosis of asthma in either a clinical or a research (clinical trials and epidemiologic) setting is in question (eg, in patients who have suggestive symptoms but no documented airflow limitation). In general, the reliability of the test is more useful in excluding a diagnosis than in confirming one, based on the higher negative predictive value compared with its positive predictive ability.^{6,7} This is more relevant in special situations, such as involvement in high-risk occupational tasks and environmental exposure (eg, military, scuba diving); however, because of the overlap in provocation doses causing a reduction in lung function at the time of a challenge between diseased and healthy subjects, setting an absolute level to define a diseased state is difficult.⁸

BPCs are also recommended in the evaluation of a patient suspected of having occupational asthma⁹ and exercise-induced respiratory symptoms.¹⁰ Assessment of BHR can also be considered for patients who are suspected of having poor symptom perception or for detecting peculiar physiologic features characteristic of certain asthmatics, such as presence of “excessive” airway closure (characterized by lack of plateau on the dose–response curve).¹¹ In addition to the utility in complementing the clinical diagnosis of asthma, which has the potential to reduce misdiagnosed or over-treated patients both in primary care settings^{12,13} and in patients with severe asthma, BPCs have also become an important research outcome measure to monitor intervention outcome and determine mechanisms underlying efficacy.¹ Finally, there is increasing evidence that BHR can serve as a biomarker to monitor asthma control and adjust antiinflammatory therapy with inhaled corticosteroids (ICSs).^{14,15}

BHR is related to various measures of asthma severity,^{3,16,17} and it has also been reported as a predictor of development of persistent asthma^{18–20} and airflow limitation in adulthood.^{21,22} These significant applications of BPCs make them a requirement and in many instances the gold standard in the evaluation of asthma. Hence, it is important to reflect on what these tests convey about asthma.

Central to the application and interpretation of BPCs is the understanding and standardizing of the numerous BPCs available. Recent evidence strongly suggests that various commonly used BPCs differ in their potential to serve as inflammatory biomarkers in relation to whether a direct (eg, methacholine and histamine) or indirect (eg, exercise, adenosine, hypertonic saline, and mannitol) stimulus is used to assess BHR. The response to a direct stimulus depends on the smooth muscle’s response to the chemical, whereas in indirect challenges, the reaction is caused by the smooth muscle’s responsiveness to the mediators induced by the stimulus. The information obtained from numerous studies with BPCs has provided valuable insights into the pathogenesis and pathophysiology of asthma and the relationships between airway inflammation and BHR.

This article covers the relationships between BHR and airway inflammation and highlights the gaps in our current knowledge relating to BHR in asthma.

WHAT FACTORS CONSTITUTE BRONCHIAL HYPERRESPONSIVENESS?

Genetic Component

Recent “genome-wide association studies” have revealed a substantial number of distinct genetic loci associated with susceptibility to asthma and with several key features of asthma, including lung function, BHR, and disease severity.²³ A genotype-specific difference in response to methacholine was recently noted in a trial assessing response to treatment with salmeterol in combination with ICS, where beta-2 adrenergic receptor genotype seemed to influence improvements in BHR to methacholine following treatment.²⁴ Furthermore, recent gene expression profiling of airway smooth muscle tissue revealed 8 novel genes that differentiated asthmatic from nonasthmatic patients, 4 of which were related to the severity of BHR.²⁵

There is also evidence of genetic susceptibility not only to asthma and atopy, but more specifically to BHR, from clustering of these conditions in families.²⁶ The narrow-sense heritability of BHR has been reported to be approximately 30%.²⁷ The association of asthma, atopy, and BHR was stronger between identical twins compared with dizygotic twins. The strong correlation in monozygotic twins suggests an overlap between these genetic factors involved in each of these traits.²⁸

Coinheritance and colocalization of a gene to a region of chromosome 5q31–q33 that determines BHR to both histamine and serum immunoglobulin E (IgE) levels have been reported.²⁹ In that study, elevated levels of serum IgE were coinherited in siblings with BHR, but BHR was not correlated in siblings concordant for elevated serum IgE. These findings suggest that different genetic and environmental factors influence serum IgE levels, not necessarily affecting BHR. There are also several airway inflammatory mediators that are linked to chromosome 5q31–q33, such as granulocyte macrophage colony stimulating factor (GMCSF); fibroblast growth factor-acidic; other colony-stimulating factors and receptors; the lymphocyte-specific glucocorticoid receptor 1; beta-2 adrenergic receptor; and interleukin (IL)-3, IL-4, IL-5, IL-9, and IL-13.²⁹ Colocalization of these genes in that region may account for the common pathophysiologic features of asthma involving IL-4 in isotype switch to IgE and IL-3, IL-5, and GMCSF for proliferation and activation of eosinophils.

There are other genetic studies that link atopy or serum IgE levels or asthma to BHR, located in other regions such as in chromosome 11³⁰ and in the IL-4 and IL-13 cytokine region of chromosome 5,³¹ more proximal to that found by Postma and colleagues.²⁹

These findings still await confirmation in other populations. Currently, the mechanisms linking genetic predisposition and BHR remain to be defined, and no definite gene has been identified to date to account for BHR. In addition, gene-environment interaction may be key in that exposure to early environmental factors (such as passive smoke or other factors related to tobacco smoke in early life, such as viral infections) may modulate the genetic susceptibility for asthma-related phenotype.^{32,33}

Transient and Permanent Components of Bronchial Hyperresponsiveness

In asthma, BHR can be variable both within and between individuals. The variability emanates from a different underlying cause in addition to genetic predisposition, which includes presence of airway structural changes that account for more permanent component and the degree or severity of airway inflammation that contributes to the transient or modifiable elements. O’Byrne and Inman³⁴ proposed the terms persistent and transient, respectively, to differentiate these elements. For example, recent exposures to allergen,^{35–38} ozone,³⁹ infection,^{40,41} or occupational triggers^{42,43}

contribute to the transient property, whereas structural changes affect the permanent or persistent component.

The role of airway smooth muscle in bronchoconstriction and BHR is well recognized, but whether there are unique inherent airway smooth muscle characteristics or whether airway smooth muscle is merely an effector of an abnormal inflammatory response is unclear.² Airway inflammation is critical in the pathogenesis of asthma. What specific component of the inflammatory cascade and to what extent inflammation affect BHR are disputed. Furthermore, the physiologic and structural changes characteristic of airway remodeling (ie, fibrosis, smooth muscle hypertrophy, extracellular matrix deposition, vascularity) or epithelial damage⁴⁴ probably have a greater impact over airway inflammation on the more permanent feature of BHR.⁴⁵ In a double-blind, randomized, placebo-controlled, parallel group study, the relationships between airflow limitation, airway inflammation, airway remodeling, and BHR before and after treatment with high-dose inhaled fluticasone propionate (FP 750 µg twice daily) were evaluated in a group of patients who had mild persistent asthma.⁴⁶ Multiple regression analysis indicated that the 40% of the variability in BHR consisted of 21% being related to reticular basement membrane thickness ($P = .009$), 11% to bronchoalveolar lavage epithelial cells ($P = .04$), and 8% to bronchoalveolar lavage eosinophils ($P = .046$), with spirometric indices and mast cells being insignificant predictors of BHR. The longitudinal data validated the cross-sectional model. Spirometric indices and cellular inflammation improved after 3 months of treatment, with no further improvement at 12 months, whereas BHR improved throughout the 1-year study, represented by 6 doubling dose changes. A reduction in reticular basement membrane thickness serving as an index of airway remodeling was evident much later, only after 12 months of treatment (mean change 1.9, 95% confidence interval [CI], 3–0.7 µm; $P = .01$ vs baseline, $P = .05$ vs placebo). Early changes in BHR (ie, 2 doubling dose magnitudes) could be accounted for by improvement in airway inflammation within 3 months and subsequent improvement by airway remodeling.⁴⁶ Hence, both airway inflammation and airway remodeling contribute to BHR, and this study provides cross-sectional and longitudinal support that a larger contribution comes from features of airway remodeling. Both features of airway inflammation and remodeling respond, albeit temporally different, to anti-inflammatory treatment with ICS, although BHR did not resolve completely.

Recent findings suggest that ventilation heterogeneity may be another mechanism that contributes to BHR, perhaps from both inflammatory and noninflammatory processes. A causal relationship has been hypothesized by a modeling study, which showed that an uneven distribution of ventilation and airway narrowing throughout the airways resulted in a greater overall increase in airway resistance following uniformly applied bronchoconstriction (ie, BHR).⁴⁷ Such results have been strengthened by subsequent studies, which showed that baseline ventilation heterogeneity (measured by the index S_{cond} derived from multiple breath nitrogen washout) is an independent determinant of BHR and strongly correlates with the severity of BHR in patients with asthma.^{48,49} Of interest, ventilation heterogeneity seems to be, at least in part, independent of steroid responsive inflammatory process, as a significant correlation with BHR persisted following 3 months of ICS treatment.⁴⁸ Whether this residual ventilation heterogeneity is caused by other inflammatory processes, structural changes, or airway remodeling requires further investigation.

What makes the issue more complex is that the actual method of bronchoprovocation testing may provide a diverse relationship between inflammation and BHR. Indirect challenges are presumed to be more reflective of airway inflammation, in contrast to direct stimuli, which are more related to the irreversible or persistent BHR. It is likely

that there are many variables contributing to BHR and in specific conditions, different mechanisms or a combination thereof are responsible.

THE LINK BETWEEN BRONCHIAL HYPERRESPONSIVENESS AND AIRWAY INFLAMMATION

Associations Between Bronchial Hyperresponsiveness and Other Inflammatory Biomarkers

Evidence in support of this relationship can be grouped according to associations between inflammatory and allergic markers and BHR, and modification of BHR and inflammatory markers using antiinflammatory medications.

Allergen challenges in animal and human studies using single or repeated doses induce airway reactivity that is associated with a variety of immunologic features, such as production of antigen-specific IgE levels, upregulation of T-helper cytokines, early and late-phase reactions, and eosinophilic inflammation.^{50–53} After allergen stimulation, the influx of inflammatory cells in the airways, notably eosinophils, precedes the development of late-phase response and correlates with an increase in BHR. The onslaught of inflammatory cells in the airways at the time of the allergen challenge suggests a causal relationship between BHR and airway inflammation. Whether the effect is caused by activation of eosinophil-releasing substances that can increase reactivity or by the epithelial damage itself, which enhances BHR, is not well established.

Associations between BHR and other inflammatory processes or markers that are not necessarily characteristic of purely eosinophilic process exist. Using an animal model for asthma, mice that had been sensitized with ovalbumin and then challenged repeatedly with ovalbumin aerosols were treated with monoclonal antibodies directed against IL-4, IL-5, interferon gamma (IFN γ), or tumor necrosis factor alpha (TNF α) during the challenge period.⁵⁴ The control antibody-treated mice showed hyperresponsiveness to methacholine and presence of eosinophils in bronchoalveolar lavage. Treatment with antibodies to IL-4 or IL-5 did not inhibit BHR, although IL-5 specifically inhibited bronchoalveolar lavage eosinophilia in ovalbumin-challenged mice. In contrast, IFN γ completely abolished, and TNF α partially but not significantly inhibited development of airway hyper-responsiveness in ovalbumin-challenged animals, with neither treatment having an effect on eosinophilia.⁵⁴

In another murine model, the ability to develop BHR also did not correlate with the degree of airway eosinophilia, inflammation, or total serum and ovalbumin-specific IgE levels in genetically different inbred mouse strains (BALB/c, B6D2F1, and C57BL/6).⁵⁵ Higher BHR was found in the BALB strain that was associated with lower levels of eosinophils and ovalbumin-specific IgE levels, compared with the B6D2F1 strain. Hence, there is an apparent dissociation between eosinophilia and antigen-specific IgE and BHR. In addition, the genetic background of the mice influenced the development of BHR.

A recent study in adults with allergic asthma evaluating the effects of repeated bronchoconstriction induced by either serial inhaled allergen (dust mite) or methacholine challenges on airway remodeling offered interesting observations. Both inhaled allergen and methacholine challenges promoted airway remodeling in terms of increases in subepithelial collagen band thickness and epithelial mucous glands. Notably, this effect seemed to be dissociated from eosinophilic inflammation, because a similar degree of airway reactivity and remodeling was seen following both challenges, but eosinophils into the airways increased only within the allergen-challenged group.⁵⁶

These observations, specifically that of inhibition of BHR using specific mediators independent of the effect on airway eosinophilia, and the dissociation between airway eosinophilia and BHR, suggest that other mechanisms are involved. T lymphocytes have been shown to play a role in intrinsic, nonatopic BHR and the development of antigen-induced BHR.^{57–59} Alveolar macrophages may play a role in the development of nonspecific BHR, because these were the only cell types significantly increased in rats receiving repeated antigen challenges.⁶⁰ This is supported by a study in people in whom nonspecific BHR to histamine was closely correlated with elevated eosinophils and macrophages and with the ratio of eosinophils to macrophages.⁶¹ Airway mast cells and eosinophils were found to correlate with BHR in asthmatics treated with corticosteroids.⁶² A study by Kelly and colleagues⁶³ also suggests a relation between bronchial responsiveness and both neutrophil numbers and macrophage activity.

Although still speculative, different immune profiles may be involved in the development of BHR based on atopy. Among nonatopics, elevated allergen-specific and polyclonal IL-10 production, TNF α , and IFN γ were associated with BHR, whereas BHR among atopic individuals was associated with eosinophilia, IL-5, and IgE.⁶⁴

There are numerous epidemiologic and clinical trial studies that present associations, albeit modest, between BHR and features of atopy or other biomarkers of eosinophilic inflammation in the airway (from sputum, bronchoalveolar lavage, and exhaled substances).^{61,65–73} The associations with various measures of atopy can be variable. For example, in a study by Jansen and colleagues,⁷⁴ eosinophilia (odds ratio [OR] = 2.06, 95% CI, 1.28–3.31) and skin test positivity (OR = 1.66, 95% CI, 1.02–2.71) were both significantly associated with BHR to histamine, but high serum total IgE levels were not (OR = 1.29, 95% CI, 0.81–2.03) associated with BHR in this middle-aged population. In addition, the significant association between BHR and positive skin test pertained to symptomatic subjects only (OR = 5.78, 95% CI, 1.63–20.51), independent of eosinophilia and high serum total IgE levels. In contrast, the relationship between peripheral blood eosinophilia and BHR was the same in asymptomatic subjects and symptomatic subjects.⁷⁴ These findings suggest that skin testing, serum total IgE level, and peripheral blood eosinophilia represent different features of the atopic phenotype, with eosinophilia having the most robust association with BHR.

The use of a noninvasive measure of airway inflammation such as exhaled nitric oxide concentrations has been applied clinically in the past decade, especially in children. Exhaled nitric oxide has been correlated with BHR both in corticosteroid naive and treated subjects.^{62,75,76} With significant associations reported between exhaled nitric oxide levels and BHR, the feasibility of using exhaled nitric oxide measurements makes it a more attractive surrogate biomarker over BHR; however, the association is still quite modest (with approximately 9%–16% of exhaled nitric oxide variability probably attributable to BHR). Therefore, different mechanisms underlie exhaled nitric oxide measurements and BHR.

Although numerous studies illustrate the link between BHR and various measures of airway inflammation, there are studies that show a lack of an association.^{44,77–82} Crimi and colleagues⁸² evaluated the relationships between BHR and the number and types of inflammatory cells in the sputum, bronchoalveolar lavage, and bronchial biopsy samples of patients who had mild-to-moderate asthma with sensitization to either house dust mite or animal dander. In this study, no association was found between BHR and the presence of inflammatory cells (eosinophils, neutrophils, lymphocytes, or macrophages) in the airways. It could also be possible that it is not just the presence or number of inflammatory cells per se, but the activation of these cells that would correlate with BHR. Perhaps the correlation between role for eosinophils in allergic

inflammation and BHR would be more apparent after an allergen challenge and not necessarily at steady state or baseline.^{56,83}

Modifying Bronchial Hyperresponsiveness Using Antiinflammatory, Allergen Avoidance, and Immunomodulatory Therapy

More than just associations of BHR with various markers of airway inflammation, convincing evidence supportive of the premise that BHR is indeed a biomarker of airway inflammation comes from numerous clinical trials demonstrating favorable effects of antiinflammatory medications such as ICSs and leukotriene-modifying agents on BHR and other inflammatory indices.^{46,84–87} Despite significantly improving symptoms and decreasing airway inflammation, ICSs produce at best a modest decrease in BHR as measured by histamine or methacholine challenges: a magnitude of 1.5 to 6 doubling dose reduction in BHR.^{15,42,86,88,89} This suggests that the effect of treatment on BHR is not absolute, despite normalization of lung function or amelioration of symptoms and inflammation. Leukotriene-modifying agents have been shown to reduce bronchial reactivity.^{90–93} A study in preschool children found bronchoprotection (measured by change in airway resistance after cold, dry air challenge) in those treated with montelukast (17%) compared with placebo-treated group (46%). In adolescents and adults, using exercise challenge after 12 weeks of treatment to measure the degree of protection against bronchoconstriction, montelukast therapy was associated with a 47.4% improvement in degree of inhibition using area under the curve, compared with placebo; maximal drop in percentage of forced expiratory volume in 1 s (FEV₁%) was 22% versus 32% in patients on montelukast therapy compared with placebo, respectively. There was no significant difference in doubling dose measurements of methacholine between the 2 groups after 12 weeks of treatment.

Environmental interventions to reduce aeroallergen exposure have also been demonstrated to modify bronchial reactivity.^{94,95} Children who lived in a house dust mite-free environment at high altitude for a month was found to have improvement in BPCs using adenosine monophosphate (2.15 doubling dose concentration) and exercise (decrease in FEV₁ of 27% at baseline and 21% after 1 month) but not with direct methacholine challenge.⁹⁴

In contrast to the interventions that target a broader influence on the inflammatory response, specific immunomodulation in human studies has afforded no significant effect on BHR, despite a substantial bearing on inflammatory markers. In a randomized, double-blind, parallel, placebo-controlled study in patients with allergic asthma (ie, sensitized to dust mite, grass pollen, or cat dander),⁹⁶ single infusion of anti-IL-5 (either 2.5 mg/kg or 10 mg/kg) failed to modify BHR to histamine before and after allergen challenge 1 week and 1 month after the dose, despite a decrease in peripheral blood and sputum eosinophils. Mepolizumab, a humanized monoclonal antibody against IL-5, has been found to reduce severe exacerbations and improve asthma symptoms and sputum and blood eosinophils in patients with refractory eosinophilic asthma, yet no effects on BHR to methacholine was seen after 1-year treatment.⁹⁷ These findings suggest that eosinophilia might not be the prime factor responsible for BHR in allergic asthma, because several inflammatory cells (eg, T cells, mast cells, and eosinophils) contribute to the late asthmatic response. In addition, anti-IgE (omalizumab) treatment for 16 weeks given to adults who have mild-to-moderate asthma afforded a marked reduction in free IgE; IgE positive cells in the airway mucosa; sputum eosinophil count; tissue eosinophils; cells positive for the high-affinity Fc receptor for IgE; CD3+, CD4+, and CD8+ T lymphocytes; B lymphocytes; and cells staining for IL-4, without an effect on BHR to methacholine responsiveness.⁹⁸ Similar findings were observed in a subsequent study, which showed that omalizumab reduced the BHR to indirect stimuli

(adenosine 5'-monophosphate) after 4 and 12 weeks of treatment, without affecting the response to methacholine.⁹⁹ Overall, these studies suggest that, at least in allergic asthma, specific therapy directed at eosinophil or IgE regulation does not obliterate bronchial responsiveness to methacholine.

A proof of concept study targeting mast cell in patients with refractory asthma using KIT inhibition with imatinib demonstrated not only a reduction in mast cell activity (serum and BAL tryptase) but also a significant improvement in BHR using methacholine after 6 months in patients treated with imatinib (1.73 ± 0.60 doubling dose from baseline) compared with placebo (1.07 ± 0.60 doubling dose, $P = .048$).¹⁰⁰

Antiinflammatory compounds that have broader coverage might be more effective at targeting this outcome. A recent study in adults with mild, stable allergic asthma showed that treatment with the humanized monoclonal immunoglobulin G2 λ , AMG 157, which binds with thymic stromal lymphopoietin (TSLP) to inhibit receptor interaction, significantly reduced markers of persisting airway inflammation (ie, the fraction of exhaled nitric oxide; blood and sputum eosinophils) and attenuated not only allergen-induced airway responses, including the early and late asthmatic bronchoconstriction but also BHR to methacholine (1.36 vs 0.66 doubling dose in the AMG 157 vs placebo treated groups, respectively).¹⁰¹ TSLP is a protein released primarily from epithelial cells in response to irritating stimuli,¹⁰² which is thought to initiate signaling pathways leading to Th2 cell-driven inflammation resulting in the production of proinflammatory cytokines, including IL-5, IL-4, and IL-13. Although the biological mechanisms of AMG 157 have to be unraveled, it is likely that this anti-TSLP treatment acts by exerting a broad inhibition of regulatory networks. These findings suggest that TSLP has a key role not only in bronchoconstrictor responses evoked by the inhalation of allergens but also in maintenance of airway inflammation in patients with allergic asthma. However, at this stage it is unclear whether the reduction in eosinophils counts has a causal association with the inhibition of the allergen-induced responses or whether such changes follow another event responsible for the protective effect. This study also showed the utility of allergen-induced airway responses (including BHR to methacholine) as predictive model for the assessment of efficacy of new potential therapies for asthma.¹

A summary of potential new treatments for asthma and their effects on BHR is provided in [Table 1](#).^{103–114} Even if a general association exists between BHR and airway inflammation in asthma, such a relationship is not absolute and is therefore limited, because both measures can be influenced by different factors. It is likely that single measurements of BHR to pharmacologic stimuli cannot provide accurate information on airway inflammation. Depending on the BPC used, they could be suggestive of a different disease or immunologic pathway.

Correlations Between Direct and Indirect Challenges

The manner in which BPCs are conducted can be relevant in defining the association between BHR and airway inflammation; hence the distinction between direct and indirect stimuli is made. BPCs using direct stimuli such as methacholine and histamine provoke bronchoconstriction by acting primarily on airway smooth muscles and also on mucous glands and airway microvasculature. Methacholine (acetyl-b-methylcholine chloride), a synthetic derivative of acetylcholine, binds to specific muscarinic receptors, whereas histamine binds to H1 receptors on the airway smooth muscle. In contrast, indirect challenges use stimuli (eg, physical, osmotic, or pharmacologic) that affect inflammatory and neuronal cells and induce release of mediators or cytokines that cause bronchospasm. Examples of stimuli acting indirectly to assess BHR include exercise, adenosine monophosphate (AMP), dry powder mannitol, and

Table 1

Effect of treatment with potential new treatments for asthma, on allergen-induced early responses, late responses, methacholine airway hyperresponsiveness, and sputum eosinophilia

New Molecule	Dosing	Early Response	Late Response	AHR	Eosinophils
Inhaled heparin disaccharide ¹⁰³	Single	No	No	No	No
Inhaled PGE2 ¹⁰⁴	Single	Yes	Yes	Yes	Yes
Anti-CD11a ¹⁰⁵	8 wk	No	Yes	No	Yes
Antisense against IL-3, GM-CSF, and CCR3 ¹⁰⁶	4 d	Yes	No	No	Yes
CPG-Oligo ¹⁰⁷	4 wk	No	No	No	No
Anti-IL-13 ¹⁰⁸	2 wk	Yes	Yes	No	No
PDE4 inhibitor ¹⁰⁹	2 wk	No	Yes	Yes	Yes
Anti-Ox 40L ¹¹⁰	16 wk	No	No	No	No
$\alpha 7$ nicotinic agonist ¹¹¹	9 d	No	No	Yes	No
CysLT1/LT2 antagonist ¹¹²	8 d	Yes	Yes	Yes	Yes
Anti-M1 prime ¹¹³	12 wk	Yes	Yes	No	Yes
Nonsteroid GR agonist ¹¹⁴	1 wk	No	Yes	Yes	Yes
Anti-TSLP ¹⁰¹	12 wk	Yes	Yes	Yes	Yes

Abbreviations: AHR, airway hyperresponsiveness; GR, glucocorticoid receptor; No, no statistically significant effect; PGE2, prostaglandin E2; Yes, statistically significant attenuation.

From Nair P, Martin JG, Cockcroft DC, et al. Airway hyperresponsiveness in asthma: measurement and clinical relevance. *J Allergy Clin Immunol Pract* 2017;5(3):655; with permission.

eucapnic voluntary hyperpnoea (EVH). This section presents correlations between direct and indirect BPC and compares the effects of antiinflammatory therapy in regard to symptoms, markers of airway inflammation, and BHR using direct and indirect airway challenges.

The most common pharmacologic agents used for direct challenges are methacholine and histamine; their pharmacokinetic properties are known, and their safety has long been established. Bronchial responsiveness to methacholine and histamine correlates significantly ($r = 0.9$).¹¹⁵ BPCs using direct stimuli are considered to be highly sensitive in excluding a diagnosis of current asthma, particularly in patients who have asthma symptoms and normal lung function, but they have a relatively low specificity for asthma. Therefore, positive challenges are consistent with but not entirely diagnostic of asthma and must be interpreted with other features of asthma. A careful decision analysis taking into consideration pretest probability and posttest probability is recommended when interpreting the results, considering the continuous variable using PC₂₀ and the overlap in PC₂₀ results between healthy and diseased patients (Fig. 1).⁶

The pretest probability is an estimate of the likelihood of asthma before the procedure is obtained. The posttest probability is the likelihood of asthma taking into account the pretest probability and the methacholine provocation results, so that the actual PC₂₀ results represent the contribution of the BPC to the diagnosis. For example, in an epidemiologic survey of asthma with a randomly selected sample whose medical history is not as detailed, an estimate of prevalence or pretest probability would be 5%. Subjects who would have a PC₂₀ of 1 mg/mL could have an approximate 45% posttest likelihood of having asthma. When a patient presents with symptoms, the pretest probability is expectedly higher, so that if the estimate is between 30% and 70%, a PC₂₀ of 1 mg/mL could bring the posttest estimate to approximately 90%

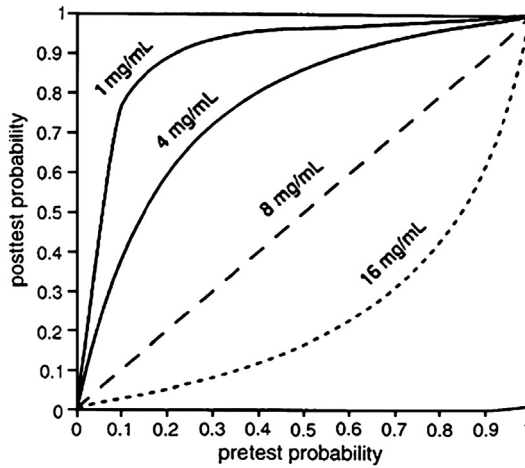


Fig. 1. Curves illustrating pretest and posttest probability of asthma after methacholine challenge test with 4 PC_{20} values. The curves represent a compilation of information from several sources. They are approximations presented to illustrate the relationships and principles of decision analysis. They are not intended to calculate precise posttest probabilities in patients. (From Crapo RO, Casaburi R, Coates AL, et al. Guidelines for methacholine and exercise challenge testing - 1999. *Am J Respir Crit Care Med* 2000;161(1):318; with permission. Copyright © 2000, American Thoracic Society.)

to 98%. The positive predictive value of methacholine challenge for asthma increases the lower PC_{20} (particularly for value <1 mg/mL in the moderate or greater range of BHR) and the higher the pretest probability. False-negative tests should be infrequent if BPC is performed at a time of current or recent symptoms, and potent functional (long-acting beta agonist in particular) or specific (anticholinergic) antagonist medications have been withheld for the appropriate duration prior testing.¹¹⁶

The reported sensitivity and specificity of the methacholine challenge test (MCT) has varied depending on the study population tested. In studies with large sample sizes, sensitivity ranged from 51%¹¹⁷ to 100%¹¹⁸ and specificity ranged from 49%¹¹⁹ to 100% (Table 2).^{117–131} Much of these data are based on older clinical trials. However, the current American Thoracic Society (ATS) guidelines suggest that MCTs can be used to exclude asthma if the PC_{20} is greater than 16 mg/mL in the proper clinical setting.⁶ This conclusion was based mainly on studies before ICS use had become a mainstay treatment for asthma. Hence a negative MCT result has been generally interpreted as indicating that it is unlikely that a patient with respiratory symptoms has asthma. A recent study conducted by the Asthma Clinical Research Consortium demonstrated that in 125 asthmatic subjects receiving controller treatment and 92 nonasthmatic control subjects an MCT, sensitivity of 77% and specificity of 96% were observed with a threshold of 8 mg/mL. Increasing the threshold to 16 mg/mL did not substantially increase the sensitivity.¹²⁷ In the latter trial, it was also observed that race and the presence of atopy had a significant effect on methacholine sensitivity. The MCT was much less sensitive in white participants (69%) compared with African Americans (91%, $P = .015$).¹²⁷

There are several issues in cross comparing the numerous studies evaluating the performance of the MCT. The study population may differ in several ways, racially, in the severity of asthma, degree of airflow limitation, presence or absence of asthma symptoms, presence of underlying atopy and obesity, and use of controller

Table 2
Selected studies evaluating the performance of methacholine challenge tests

Author (Reference), Year	Study Population	No.	Adults or Pediatrics	Sensitivity	Specificity	PPV	NPV	Definition of Positive PC ₂₀ (mg/mL)
Hopp et al, ¹²⁰ 1984	Asthma, healthy control	165	Pediatrics	98%	63%	57%	99%	800 BU ^a
Cockcroft et al, ¹¹⁸ 1992	College students	500	Adults	100%	93%	34%	100%	8
Nieminen, ¹²³ 1992	Outpatient pulmonary clinic referrals	791	Adults	89%	76%	71%	91%	2600 µg ^a
Perpina et al, ¹²⁴ 1993	Asthma, rhinitis, chronic bronchitis, healthy control	300	Adults	84%	86%	No information	No information	15
Goldstein et al, ¹²² 1994	Allergy clinic referrals	198	Adults, pediatrics	61%	100%	100%	74%	8
Goldstein et al, ¹²⁶ 2001	Suspected asthmatic patients with normal lung function in private clinic	121	Adults, pediatrics	86%	100%	100%	56%	8
Liem et al, ¹²¹ 2008	Manitoba birth cohort: asthma, healthy control	640	Pediatrics	66%	64%	No information	No information	4
Anderson et al, ¹¹⁷ 2009	Suspected asthmatic patients recruited from multiple study sites	375	Adults, pediatrics	51%	75%	78%	46%	16
Sverriid et al, ¹²⁵ 2010	Young adults randomly drawn from nationwide registration in Denmark	238	Adults, pediatrics (age 15–24 y)	69%	80%	49%	90%	8 µmol ^a
Carlsten et al, ¹¹⁹ 2011	Birth cohort with high risk for asthma	348	Pediatrics	80%	49%	27%	92%	3

Abbreviations: BU, breath units; NPV, negative predictive value; PPV, positive predictive value.

^a These studies used a provocative dose of methacholine to define a positive test result (PD₂₀).

From Sumino K, Sugar EA, Irvin CG, et al. Methacholine challenge test: diagnostic characteristics in asthmatic patients receiving controller medications. *J Allergy Clin Immunol* 2012;130(1):74; with permission.

medications. Finally, it is possible that the method by which the MCT is performed may influence the results. Previous studies and the ATS guidelines on MCTs have indicated that the 2 MCT methods, 5-breath dosimeter and 2-minute tidal breathing, are equivalent.^{128–130} However, 2 groups recently reported evidence of a lack of comparability between the 2 methods.^{128–132} These investigators found that patients with mild asthma ($PC_{20} > 2$ mg/mL) were more likely to have a positive MCT result with the tidal breathing method than with the 5-breath dosimeter method. The dosimeter method using repeated deep inhalations has been shown to cause bronchoprotective effect and false-negative results, especially in those with milder airway hyperresponsiveness,¹³³ therefore reducing the diagnostic sensitivity of the test. To allow comparable results from different devices or protocols, the latest European Respiratory Society (ERS) guidelines on methacholine challenge (endorsed by the ATS) recommend to avoid deep-breath methods and to base the test result on the delivered dose of methacholine causing a 20% decrease in FEV_1 (provocative dose [PD_{20}]), rather than using methacholine concentration (PC_{20}).¹³⁴ Indeed there is emerging evidence to show that the methacholine dose, expressed as PD_{20} , ideally eliminates the overestimation of dose when evaporation of the output is not considered and allows more consistent correlation than PC_{20} when comparing responses between different protocols.^{135,136} Estimated comparison for PC_{20} in mg/mL (when using the English Wright 2-min tidal protocol) and PD_{20} in μ g (calculated as described in ERS guidelines) is reported in Table 3.³

In 2010, the US Food and Drug Administration approved the dry powder mannitol test for determining BHR in patients 6 years of age or older. Mannitol dry powder is delivered in progressively increasing doses (0, 5, 10, 20, 40, 80, 160, 160, 160 mg), with forced expiratory volume in the first second of expiration (FEV_1) measured 1 minute after each dose. A positive response is a 15% reduction in FEV_1 at a total cumulative dose of 635 mg or a 10% reduction in FEV_1 from baseline between doses. The mannitol test has been suggested for confirming a diagnosis of asthma and to follow response to therapy.^{137–141} The advantage of the indirect tests over the direct such as methacholine is that they provide information on 2 key features of asthma, airway inflammation and BHR. In addition to a hyper-responsive smooth muscle, a positive response to mannitol or other indirect stimuli indicates both the presence of inflammatory cells and a sufficient concentration of mediators to cause bronchoconstriction. A negative test result indicates a missing element such as insufficient numbers of inflammatory cells or insufficient concentration of mediators or an unresponsive smooth muscle. Examples of negative tests include known asthmatic patients being treated

Table 3 Categorization of airway response to methacholine		
PD_{20} μmol (μg)	PC_{20} mgmL⁻¹	Interpretation
>2 (>400)	>16	Normal
0.5–2.0 (100–400)	4–16	Borderline AHR
0.13–0.5 (25–100)	1–4	Mid-AHR
0.03–0.13 (6–25)	0.25–1	Moderate AHR
<0.03 (<6)	<0.25	Marked AHR

Abbreviations: AHR, airway hyperresponsiveness; PC_{20} , provocative concentration causing a 20% fall in FEV_1 ; PD_{20} , provocative dose causing a 20% fall in FEV_1 .

From Coates AL, Wanger J, Cockcroft DW, et al. ERS technical standard on bronchial challenge testing: general considerations and performance of methacholine challenge tests. *Eur Respir J* 2017;49(5):1601526; with permission.

effectively with ICSs,^{142,143} people with eosinophilic bronchitis but no asthma,¹⁴⁴ or people with asymptomatic BHR to methacholine who may have airway injury such as elite athletes.^{121,145,146}

Sverrild and colleagues¹²⁵ evaluated methacholine and mannitol and reported a specificity of 80.2% (95% CI, 77.1%–82.9%) and 98.4% (95% CI, 96.2%–99.4%), respectively, with a positive predictive value of 48.6% versus 90.4%, whereas the sensitivity was 68.6% (95% CI, 57.1%–78.4%) and 58.8% (95% CI, 50.7%–62.6%), respectively. Other trials have reported a sensitivity range between 50% and 60%, with specificities in the high 90% range. Although the sensitivity does not seem to be very high, it is important to recognize that the benefit of current treatment on BHR, a previous diagnosis, or the period of current asthma has not always been taken into account when reporting the data. For example, the sensitivity of mannitol to identify a physician diagnosis of asthma increased from 59.8% to 89% when those taking inhaled steroids and negative to mannitol were excluded.¹⁴⁷ Overall, the distinct underlying mechanisms between methacholine and mannitol suggest that performing both tests may be useful in characterizing different phenotypes of BHR. Indeed, a recent study among community-managed adult asthmatics reported that 14% had BHR only to methacholine, whereas another 16% had responsiveness only to mannitol.¹³

Mannitol challenge seems to be more sensitive for exercise-induced bronchospasm than an exercise challenge but is not as sensitive as desired for diagnosing exercise-induced bronchospasm. However, because of its relatively high specificity, mannitol may serve as a confirmatory test in those with a positive MCT. A comprehensive discussion on this topic is beyond the scope of this article but 2 extensive reviews have been published discussing mannitol challenges.^{147,148}

Among asthmatics, in general, an investigation into the relationship between BHR to histamine and exercise illustrated that inhaled direct challenge would be more sensitive than exercise tests at demonstrating increased nonspecific airway reactivity.¹⁴⁹ In that study, 84% of asthmatics had a positive histamine challenge, whereas only half had evidence of a positive exercise test (ie, 10% drop in FEV₁). Despite the high estimate of asthma diagnosis using direct BPCs such as histamine or methacholine in symptomatic patients, this may compare poorly when indirect challenges are used for a specific purpose. For example, a study was undertaken to investigate the relationship between asthma symptoms and BHR to methacholine and EVH, among symptomatic elite summer-sport athletes because of a notable increase in the prevalence of asthma reports among athletes participating in the Olympic games.¹⁵⁰ In that study, athletes who reported asthma symptoms were twice as likely to have a positive response to EVH challenge (50%; mean reduction in FEV₁—of 25.4% ± 15%), compared with a positive methacholine challenge (18%; mean PD₂₀ of 1.69 mmol to 2.05 mmol). Although all subjects with positive methacholine challenge results had positive EVH challenge results, methacholine had a negative predictive value of only 61% and a sensitivity of 36% for identifying those responsive to EVH. These results suggest that EVH is a more sensitive test for the diagnosis of BHR in elite athletes, and the differences in response to the direct and indirect stimuli raise the possibility of a different pathogenesis involved.¹⁵⁰

Airway cooling associated with vigorous physical exercise has been thought of as a presumptive cause of exercise-induced asthma. The association of airway cooling with airway hyperreactivity is today folded into 2 potential theories: thermal and osmotic hypotheses. Thermal hypothesis refers to cooling of the airways after exercise that is followed by rapid airway warming, which causes vascular congestion, increased permeability, and edema. The osmotic hypothesis implicates evaporated water loss causing hyperosmolality; stimulation of mucosal drying leading to mast

cell degranulation; release of inflammatory mediators such as histamine, prostaglandin D₂, and the cysteinyl leukotrienes; and ultimately to edema and airway smooth muscle contraction.

Between indirect challenges specifically to hypertonic saline and exercise challenge, the responses to either stimulus seem to track better. From a community-based, cross-sectional survey, 382 children successfully completed a 4.5% hypertonic saline challenge with increasing inhalation periods, and 365 performed a 6-minute standardized, free running exercise challenge. The prevalence of BHR to hypertonic saline was 20.4%. The results showed similar low sensitivity and high specificity values to identify children who had current wheeze, using 4.5% hypertonic saline challenge (47% and 92%, respectively) and a standardized exercise challenge (46% and 88%, respectively). There was a moderate agreement of response to hypertonic saline and to exercise ($\kappa = 0.43$).¹⁵¹

There also seem to be differences between direct and indirect stimuli in the evaluation of response to intervention. Patients sensitized to house dust mites, who were transferred to a more favorable environment at high altitude, demonstrated no significant difference in BHR to methacholine challenge but developed a significant improvement in airway response to adenosine monophosphate, indicated by displacement of the dose-response curve to the right by 2 doubling concentrations ($P = .005$) and exercise ($P = .03$).⁹⁴ These results demonstrate the discordance in responses to direct and indirect challenges. The improvement in BHR to the indirect stimuli using AMP and exercise in contrast to the lack of response in BHR to methacholine suggests amelioration of airway inflammation following avoidance of house dust aeroallergens.⁹⁴

Indirect challenges compared with direct stimuli may also be more sensitive in detecting changes in airway reactivity with ICS treatment.^{84,85,152–155} BHR to methacholine and AMP was compared, as well as induced sputum analysis and exhaled nitric oxide measurements, before and after 2 weeks of treatment with oral or ICSs. Using multiple regression analysis, corticosteroid improvement in AMP PC₂₀ correlated negatively with changes in sputum eosinophils, bronchial epithelial cells, and exhaled nitric oxide and positively with changes in sputum lymphocytes. On the other hand, improvement in methacholine PC₂₀ correlated negatively with changes in the number of sputum eosinophils and positively with increase in sputum lymphocytes and FEV₁ percentage predicted. There was a stronger correlation between changes in sputum eosinophils and AMP PC₂₀ compared with methacholine PC₂₀ ($r = -0.43$, $P = .0001$ vs $r = -0.28$, $P = .004$, respectively). The total explained variance of the improvement in BHR was greater for changes in AMP compared with changes for methacholine (36% vs 22%, respectively). These suggest that improvement in BHR to adenosine may better detect changes in airway inflammation than improvement in methacholine PC₂₀. After inhalation of an aqueous solution administered as AMP, it is converted immediately to adenosine by the ubiquitous enzyme 5'-nucleotidase. Adenosine exerts its effects through interaction with specific adenosine (P1) receptors. The exact mechanism of action of AMP on airway wall is not well-defined, but the role of mast cells is crucial based on a variety of inflammatory mediators, such as histamine, prostaglandins, leukotrienes, and IL-8 release, from human mast cells enhanced by adenosine *in vitro*.^{156–159} Although there are studies that have shown differential BHR response to antiinflammatories such as ICSs, there are also those that show concordant effects of these medications on bronchial responsiveness to direct and indirect stimuli.^{143,160–163} Perhaps the variability in bronchial response

to direct and indirect stimuli from ICS therapy depends not only on the specific drug or stimulant but also on the duration of treatment, dosage, and delivery (see [Table 1](#)).

Bronchoprovocation Challenges in the Longitudinal Management of Asthma

Current international guidelines on asthma recommend the use of a stepwise management strategy for effective long-term control, with level and adjustment of antiinflammatory pharmacologic treatments based solely on symptoms, lung function, and reliever bronchodilator use.^{164–166} Once clinical control is achieved, the dose of ICS should be tapered to the minimal level required to maintain disease control, to minimize side effects associated with their prolonged use. However, this approach may not be adequate, because several studies have shown persisting BHR and airway inflammation in many asthmatics following ICS use despite good clinical control.^{46,101,167,168} The presence of ongoing airway inflammation is not only associated with an increased risk of airway remodeling and related long-term loss in lung function^{169,170} but it also predicts the risk of future exacerbations and loss of disease control when stepping-down treatment with ICSs.^{171,172} In addition, the well-documented flat dose–response curve to ICS for symptoms and lung function with a plateau for improvement above low doses¹⁷³ makes these 2 clinical markers insufficient to monitor the effects of antiinflammatory treatments.

Over the past 20 years, there have been many attempts to manage asthma using strategies based on measurements of BHR. In a parallel group study involving 75 adults with mild-to-moderate asthma, Sont and colleagues¹⁷⁴ compared titration of treatment with ICS based either on standard clinical guidelines (symptoms and lung function alone) or additionally on the degree of BHR to methacholine (BHR strategy) over a 2-year period. Participants whose treatment was adjusted based on their BHR had less mild exacerbation (0.23 vs 0.43 exacerbation/year/patient in the control group) and greater improvement in lung function, but required higher doses of ICSs compared with subjects whose treatment was titrated according to recommended clinical guidelines. Of interest, bronchial biopsy findings (available from 49 subjects) showed greater reduction in thickness of the subepithelial reticular layer in the BHR strategy group than in the reference strategy group. The changes in BHR in both strategy groups were correlated with eosinophil counts in the biopsies ($r = -0.48$, $P = .003$). These results provide more indirect but strong evidence of BHR being a surrogate marker of inflammation in asthma. BHR documented as a mean PC_{20} to methacholine of 0.47 mg/mL before treatment was only marginally reduced using the BHR strategy (increase in PC_{20} 1.1 doubling concentrations, not statistically significant).¹⁷⁴ This confirmed previous studies showing that long-term treatment with ICSs does not necessarily normalize BHR to methacholine, emphasizing the limited effects of current pharmacologic therapies on the permanent component of BHR.¹⁷⁰

A similar study in 206 children (aged 6–16 years) with moderate persistent asthma did not confirm the full clinical benefit of adjusting ICSs dose based on BHR to methacholine. Over a 2-year period, Nuijsink and colleagues¹⁷⁵ found no difference in the number of symptom-free days using the BHR strategy, although a better prebronchodilator FEV_1 was observed in a subgroup of children with allergic asthma. This improvement was also achieved with a higher dose of ICSs in the BHR strategy. This beneficial effect on lung function was lost at follow-up after 3 to 7 years of usual care, when the mean dose of ICSs had diminished from 550 mg/d at the end of the study to 235 mg/d.¹⁷⁶ This suggests that a BHR-guided treatment strategy may need to be sustained in order to preserve lung function. Moreover, as with the study

by Sont and colleagues, BHR persisted at the end of the study and did not differ between the treatment groups.

Koenig and colleagues¹⁷⁷ found similar results in adults with mild-to-moderate asthma, although over a shorter duration of treatment (40 weeks), with suboptimal retention rate (70%). BHR strategy with dose adjustment every 8 weeks (instead of every 12 weeks as the Sont and colleagues¹⁷⁴ study) led to use of higher doses of ICSs (either with or without the combination of a long-acting B2-agonist) with no difference in symptom-free days, lung function, albuterol use, and airway responsiveness compared with reference strategy groups.

BHR to indirect stimuli correlates better with airway inflammation and may represent a more clinically relevant marker to guide adjustment of antiinflammatory treatment and monitor asthma control. It is well documented that the regular use of ICSs attenuates or even abolishes the airway reactivity to indirect stimuli.^{143,178} On the other hand, increase in BHR to mannitol and AMP after stepping down of ICSs in clinically controlled asthmatics has been shown to predict the risk of a subsequent exacerbation and failure of dose reduction.^{142,179} Moreover, BHR to mannitol has shown advantages over other current biomarkers of airway inflammation, because it can also be present in asthmatics with noneosinophilic inflammatory phenotypes and normal FeNO level.¹⁸⁰ Given these observations, a recent randomized controlled trial investigated whether tracking BHR to mannitol (using the lower PD10 threshold) could perform better in the management of adults with mild-to-moderate asthma in primary care setting receiving inhaled ciclesonide, compared with twice monthly clinical or spirometric assessments (standard care).¹⁸¹ Over a period of 12 months, patients managed on the basis of mannitol BHR experienced fewer mild (but not severe) exacerbations and significant improvement not only in symptoms and reliever bronchodilator use but also in BHR to mannitol and methacholine and airway inflammation measured by FeNO levels. However, in keeping with previous findings, the BHR strategy resulted in exposure to a two-fold higher dose of ciclesonide, albeit no significant adrenal suppression (measured by urinary cortisol) was seen compared with the lower doses in the reference strategy group.¹⁸¹

Overall, higher ICS doses are needed to affect BHR than that required to achieve clinical improvement. This was recently confirmed in post hoc pooled analysis of data from 4 randomized clinical trials, which demonstrated a dose-response for improvement in both inflammatory outcomes (ie, FeNO, serum eosinophilic cationic protein, and blood eosinophil) and BHR, when using ICSs above low doses (up to 800 mg/d, beclomethasone equivalent), but not for symptoms and lung function.¹⁷³ Further prospective studies are warranted to define whether asthma can be controlled better, safely, and more economically using treatment strategies aimed at reducing BHR.

SUMMARY

BHR is an important feature of asthma, and it is considered a useful tool in the diagnosis, monitoring, managing, and prognostication of a complex condition. With its many valuable uses, it probably represents several inherent elements of the disease process, such as genetic predisposition, airway inflammation, structural changes, and airway remodeling. Airway inflammation seems to account for the transient and variable component of BHR. Associations between BHR to direct and indirect stimuli and inflammatory markers are present. In addition, antiinflammatory treatment can reduce airway inflammation and BHR but not close to eliminating it completely. The exact mechanisms that explain the association between BHR and airway inflammation are unknown. The permanent or persistent component of BHR, particularly to direct

stimuli, correlates significantly with structural changes in the airway, such as basement membrane thickness and epithelial damage. It might be this component that is resistant or refractory to the effects of available interventions. A few trials of specific immunomodulatory therapy have shown considerable improvements in markers of airway inflammation, without significantly modifying airway reactivity. On the other hand, the inflammatory component may explain the variable component that modulates over time and with antiinflammatory therapy and thus is better reflected by the BHR measured with indirect challenges such as mannitol or exercise or EVH. There is certainly a need to develop interventions that will affect the more permanent feature of BHR. There is also a need to include biomarker measurements in the routine management and surveillance of asthma to improve long-term control. BHR, particularly to indirect stimuli, may provide a reliable marker to best manage asthma in addition to currently recommended clinical monitoring, although further prospective studies are needed to clearly define its role.

REFERENCES

1. Nair P, Martin JG, Cockcroft DC, et al. Airway hyperresponsiveness in asthma: measurement and clinical relevance. *J Allergy Clin Immunol Pract* 2017;5(3):649–59.
2. Chapman DG, Irvin CG. Mechanisms of airway hyper-responsiveness in asthma: the past, present and yet to come. *Clin Exp Allergy* 2015;45(4):706–19.
3. Woolcock AJ, Peat JK, Salome CM, et al. Prevalence of bronchial hyperresponsiveness and asthma in a rural adult population. *Thorax* 1987;42(5):361–8.
4. Peat JK, Toelle BG, Marks GB, et al. Continuing the debate about measuring asthma in population studies. *Thorax* 2001;56:406–11.
5. Hoppers JJ, Postma DS, Rijcken B, et al. Histamine airway hyper-responsiveness and mortality from chronic obstructive pulmonary disease: a cohort study. *Lancet* 2000;356:1313–7.
6. Crapo RO, Casaburi R, Coates AL, et al. Guidelines for methacholine and exercise challenge testing-1999. This official statement of the American Thoracic Society was adopted by the ATS Board of Directors, July 1999. *Am J Respir Crit Care Med* 2000;161(1):309–29.
7. Rijcken B, Schouten JP, Weiss ST, et al. Long term variability of bronchial responsiveness to histamine in a random population sample of adults. *Am Rev Respir Dis* 1993;148:944–94.
8. Cockcroft DW, Berscheid BA, Murdock KY. Unimodal distribution of bronchial responsiveness to inhaled histamine in a random human population. *Chest* 1983;83(5):751–4.
9. Vandenplas O, Suojalehto H, Aasen TB, et al. Specific inhalation challenge in the diagnosis of occupational asthma: consensus statement. *Eur Respir J* 2014;43(6):1573–87.
10. Weiler JM, Brannan JD, Randolph CC, et al. Exercise-induced bronchoconstriction update-2016. *J Allergy Clin Immunol* 2016;138(5):1292–5.
11. Busse WW, Wanner A, Adams K, et al. Investigative bronchoprovocation and bronchoscopy in airway diseases. *Am J Respir Crit Care Med* 2005;172(7):807–16.
12. Aaron SD, Vandemheen KL, FitzGerald JM, et al. Reevaluation of diagnosis in adults with physician-diagnosed asthma. *JAMA* 2017;317(3):269–79.
13. Manoharan A, Lipworth BJ, Craig E, et al. The potential role of direct and indirect bronchial challenge testing to identify overtreatment of community managed asthma. *Clin Exp Allergy* 2014;44(10):1240–5.

14. Galera R, Casitas R, Martínez-Cerón E, et al. Does airway hyperresponsiveness monitoring lead to improved asthma control? *Clin Exp Allergy* 2015;45(9): 1396–405.
15. Brannan JD. Bronchial hyperresponsiveness in the assessment of asthma control: Airway hyperresponsiveness in asthma: its measurement and clinical significance. *Chest* 2010;138(2 Suppl):11S–7S.
16. Josephs LK, Gregg I, Mullee MA, et al. Nonspecific bronchial reactivity and its relationship to the clinical expression of asthma. A longitudinal study. *Am Rev Respir Dis* 1989;140:350–7.
17. Weiss ST, Van Natta ML, Zeiger RS. Relationship between increased airway responsiveness and asthma severity in the childhood asthma management program. *Am J Respir Crit Care Med* 2000;162:50–6.
18. Westerhof GA, Coumou H, de Nijs SB, et al. Clinical predictors of remission and persistence of adult-onset asthma. *J Allergy Clin Immunol* 2018 Jan;141(1): 104–9.
19. Sears MR, Greene JM, Willan AR, et al. A longitudinal, population based, cohort study of childhood asthma followed to adulthood. *N Engl J Med* 2003;349:1414–22.
20. Jansen DF, Schouten JP, Vonk JM, et al. Smoking and airway hyperresponsiveness especially in the presence of blood eosinophilia increase the risk to develop respiratory symptoms: a 25-year follow-up study in the general adult population. *Am J Respir Crit Care Med* 1999;160:259–64.
21. Brutsche MH, Downs SH, Schindler C, et al. Bronchial hyperresponsiveness and the development of asthma and COPD in asymptomatic individuals: SAPALDIA cohort study. *Thorax* 2006;61:671–7.
22. Rasmussen F, Taylor DR, Flannery EM, et al. Risk factors for airway remodeling in asthma manifested by a low postbronchodilator FEV1/vital capacity ratio: a longitudinal population study from childhood to adulthood. *Am J Respir Crit Care Med* 2002;165(11):1480–8.
23. Portelli MA, Hodge E, Sayers I. Genetic risk factors for the development of allergic disease identified by genome-wide association. *Clin Exp Allergy* 2015;45:21–31.
24. Wechsler ME, Kunselman SJ, Chinchilli VM, et al. Effect of beta2-adrenergic receptor polymorphism on response to longacting beta2 agonist in asthma (LARGE trial): a genotype stratified, randomised, placebo-controlled, crossover trial. *Lancet* 2009;374:1754–64.
25. Yick CY, Zwinderman AH, Kunst PW, et al. Gene expression profiling of laser microdissected airway smooth muscle tissue in asthma and atopy. *Allergy* 2014; 69:1233–40.
26. Hopp RJ, Bewtra AK, Biven R, et al. Bronchial reactivity pattern in nonasthmatic parents of asthmatics. *Ann Allergy* 1988;61(3):184–6.
27. Palmer LJ, Burton PR, James AL, et al. Familial aggregation and heritability of asthma-associated quantitative traits in a population-based sample of nuclear families. *Eur J Hum Genet* 2000;8:853–60.
28. Clarke JR, Jenkins MA, Hopper JL, et al. Evidence for genetic associations between asthma, atopy, and bronchial hyperresponsiveness: a study of 8- to 18-year-old twins. *Am J Respir Crit Care Med* 2000;162(6):2188–93.
29. Postma DS, Bleeker ER, Amelung PJ, et al. Genetic susceptibility to asthmad-bronchial hyperresponsiveness coinherited with a major gene for atopy. *N Engl J Med* 1995;333(14):894–900.
30. Ruffilli A, Bonini S. Susceptibility genes for allergy and asthma. *Allergy* 1997; 52(3):256–73.

31. Marsh DG, Neely JD, Breazeale DR, et al. Linkage analysis of IL4 and other chromosome 5q31.1 markers and total serum immunoglobulin E concentration. *Science* 1994;264(5162):1152–6.
32. Meyers DA, Postma DS, Stine OC, et al. Genome screen for asthma and bronchial hyper-responsiveness: interactions with passive smoke exposure. *J Allergy Clin Immunol* 2005;115(6):1169–75.
33. Colilla S, Nicolae D, Pluzhnikov A, et al. Collaborative Study for the Genetics of Asthma. Evidence for gene–environment interactions in a linkage study of asthma and smoking exposure. *J Allergy Clin Immunol* 2003;111(4):840–6.
34. O'byrne PM, Inman MD. Airway hyperresponsiveness. *Chest* 2003;123:411S–6S.
35. Cartier A, Thomson NC, Frith PA, et al. Allergen-induced increase in bronchial responsiveness to histamine: relationship to the late asthmatic response and change in airway caliber. *J Allergy Clin Immunol* 1982;70(3):170–7.
36. De Monchy JG, Kauman HF, Venge P, et al. Bronchoalveolar eosinophilia during allergen-induced late asthmatic reactions. *Am Rev Respir Dis* 1985;131(3):373–6.
37. Pin I, Freitag AP, O'Byrne PM, et al. Changes in the cellular profile of induced sputum after allergen-induced asthmatic responses. *Am Rev Respir Dis* 1992;145(6):1265–9.
38. Metzger WJ, Richerson HB, Worden K, et al. Bronchoalveolar lavage of allergic asthmatic patients following allergen bronchoprovocation. *Chest* 1986;89(4):477–83.
39. O'Byrne PM, Walters EH, Gold BD, et al. Neutrophil depletion inhibits airway hyperresponsiveness induced by ozone exposure. *Am Rev Respir Dis* 1984;130(2):214–9.
40. Empey DW, Laitinen LA, Jacobs L, et al. Mechanisms of bronchial hyperreactivity in normal subjects after upper respiratory tract infection. *Am Rev Respir Dis* 1976;113(2):131–9.
41. Laitinen LA, Elkin RB, Empey DW, et al. Bronchial hyperresponsiveness in normal subjects during attenuated influenza virus infection. *Am Rev Respir Dis* 1991;143(2):358–61.
42. Mapp C, Boschetto P, dal Vecchio L, et al. Protective effect of antiasthma drugs on late asthmatic reactions and increased airway responsiveness induced by toluene diisocyanate in sensitized subjects. *Am Rev Respir Dis* 1987;136(6):1403–7.
43. Fabbri LM, Boschetto P, Zocca E, et al. Bronchoalveolar neutrophilia during late asthmatic reactions induced by toluene diisocyanate. *Am Rev Respir Dis* 1987;136(1):36–42.
44. Jeffery PK, Wardlaw AJ, Nelson FC, et al. Bronchial biopsies in asthma. An ultra-structural, quantitative study and correlation with hyperreactivity. *Am Rev Respir Dis* 1989;140(6):1745–53.
45. Boulet LP, Laviolette M, Turcotte H, et al. Bronchial subepithelial fibrosis correlates with airway responsiveness to methacholine. *Chest* 1997;112(1):45–52.
46. Ward C, Pais M, Bish R, et al. Airway inflammation, basement membrane thickening and bronchial hyperresponsiveness in asthma. *Thorax* 2002;57(4):309–16. XA.
47. Venegas JG, Winkler T, Musch G, et al. Self-organized patchiness in asthma as a prelude to catastrophic shifts. *Nature* 2005;434:777–82.
48. Downie SR, Salome CM, Verbanck S, et al. Ventilation heterogeneity is a major determinant of airway hyperresponsiveness in asthma, independent of airway inflammation. *Thorax* 2007;62:684–9.

49. Kaminsky DA, Daud A, Chapman DG. Relationship between the baseline alveolar volume-to-total lung capacity ratio and airway responsiveness. *Respirology* 2014;19:1046–51.
50. Elwood W, Barnes PJ, Chung KF. Airway hyperresponsiveness is associated with inflammatory cell infiltration in allergic brown Norway rats. *Int Arch Allergy Immunol* 1992;99(1):91–7.
51. Elwood W, Lötvall JO, Barnes PJ, et al. Characterization of allergen-induced bronchial hyperresponsiveness and airway inflammation in actively sensitized brown-Norway rats. *J Allergy Clin Immunol* 1991;88(6):951–60.
52. Boulet LP, Cartier A, Thomson NC, et al. Asthma and increases in nonallergic bronchial responsiveness from seasonal pollen exposure. *J Allergy Clin Immunol* 1983;71(4):399–406.
53. Palmqvist M, Pettersson K, Sjostrand M, et al. Mild experimental exacerbation of asthma induced by individualised low-dose repeated allergen exposure. A double-blind evaluation. *Respir Med* 1998;92(10):1223–30.
54. Hessel EM, Van Oosterhout AJ, Van Ark I, et al. Development of airway hyperresponsiveness is dependent on interferon- γ and independent of eosinophil infiltration. *Am J Respir Cell Mol Biol* 1997;16(3):325–34.
55. Wilder JA, Collie DD, Wilson BS, et al. Dissociation of airway hyperresponsiveness from immunoglobulin E and airway eosinophilia in a murine model of allergic asthma. *Am J Respir Cell Mol Biol* 1999;20(6):1326–34.
56. Grainge CL, Lau LC, Ward JA, et al. Effect of bronchoconstriction on airway remodeling in asthma. *N Engl J Med* 2011;364(21):2006–15.
57. De Sanctis GT, Itoh A, Green FH, et al. T-lymphocytes regulate genetically determined airway hyperresponsiveness in mice. *Nat Med* 1997;3(4):460–2.
58. Gavett SH, Chen X, Finkelman F, et al. Depletion of murine CD4⁺T lymphocytes prevents antigen-induced airway hyperreactivity and pulmonary eosinophilia. *Am J Respir Cell Mol Biol* 1994;10(6):587–93.
59. Krinzman SJ, De Sanctis GT, Cernadas M, et al. Inhibition of T cell costimulation abrogates airway hyperresponsiveness in a murine model. *J Clin Invest* 1996;98(12):2693–9.
60. Kamachi A, Nasuhara Y, Nishimura M, et al. Dissociation between airway responsiveness to methacholine and responsiveness to antigen. *Eur Respir J* 2002;19(1):76–83.
61. Ferguson AC, Wong FW. Bronchial hyperresponsiveness in asthmatic children. Correlation with macrophages and eosinophils in bronchoalveolar fluid. *Chest* 1989;96:988–91.
62. Gibson PG, Saltos N, Borgas T. Airway mast cells and eosinophils correlate with clinical severity and airway hyperresponsiveness in corticosteroid-treated asthma. *J Allergy Clin Immunol* 2000;105(4):752–9.
63. Kelly C, Ward C, Stenton CS, et al. Number and activity of inflammatory cells in bronchoalveolar lavage fluid in asthma and their relation to airway responsiveness. *Thorax* 1988;43:684–92.
64. Heaton T, Rowe J, Turner S, et al. An immunoepidemiological approach to asthma: identification of in-vitro T-cell response patterns associated with different wheezing phenotypes in children. *Lancet* 2005;365(9454):142–9.
65. Kirby JG, Hargreave FE, Gleich GJ, et al. Bronchoalveolar cell profiles of asthmatic and nonasthmatic subjects. *Am Rev Respir Dis* 1987;136(2):379–83.
66. Wardlaw AJ, Dunnette S, Gleich GJ, et al. Eosinophils and mast cells in bronchoalveolar lavage in subjects with mild asthma. Relationship to bronchial hyperreactivity. *Am Rev Respir Dis* 1988;137(1):62–9.

67. Pliss LB, Ingenito EP, Ingram RH Jr. Responsiveness, inflammation, and effects of deep breaths on obstruction in mild asthma. *J Appl Physiol* (1985) 1989; 66(5):2298–304.
68. Woolley KL, Adelroth E, Woolley MJ, et al. Granulocyte-macrophage colony-stimulating factor, eosinophils and eosinophil cationic protein in subjects with and without mild, stable, atopic asthma. *Eur Respir J* 1994;7(9):1576–84.
69. Bradley BL, Azzawi M, Jacobson M, et al. Eosinophils, T-lymphocytes, mast cells, neutrophils, and macrophages in bronchial biopsy specimens from atopic subjects with asthma: comparison with biopsy specimens from atopic subjects without asthma and normal control subjects and relationship to bronchial hyper-responsiveness. *J Allergy Clin Immunol* 1991;88(4):661–74.
70. Chetta A, Foresi A, Del Donno M, et al. Bronchial responsiveness to distilled water and methacholine and its relationship to inflammation and remodeling of the airways in asthma. *Am J Respir Crit Care Med* 1996;153:910–7.
71. Pin I, Radford S, Kolendowicz R, et al. Airway inflammation in symptomatic and asymptomatic children with methacholine hyperresponsiveness. *Eur Respir J* 1993;6(9):1249–56.
72. Pizzichini E, Pizzichini MM, Efthimiadis A, et al. Indices of airway inflammation in induced sputum: reproducibility and validity of cell and fluid-phase measurements. *Am J Respir Crit Care Med* 1996;154(2 Pt 1):308–17.
73. Foresi A, Leone C, Pelucchi A, et al. Eosinophils, mast cells, and basophils in induced sputum from patients with seasonal allergic rhinitis and perennial asthma: relationship to methacholine responsiveness. *J Allergy Clin Immunol* 1997;100(1):58–64.
74. Jansen DF, Rijcken B, Schouten JP, et al. The relationship of skin test positivity, high serum total IgE levels, and peripheral blood eosinophilia to symptomatic and asymptomatic airway hyperresponsiveness. *Am J Respir Crit Care Med* 1999;159(3):924–31.
75. Jatakanon A, Lim S, Kharitonov SA, et al. Correlation between exhaled nitric oxide, sputum eosinophils, and methacholine responsiveness in patients with mild asthma. *Thorax* 1998;53(2):91–5.
76. Reid DW, Johns DP, Feltis B, et al. Exhaled nitric oxide continues to reflect airway hyper-responsiveness and disease activity in inhaled corticosteroid-treated adult asthmatic patients. *Respirology* 2003;8(4):479–86.
77. Chan-Yeung M, Leriche J, Maclean L, et al. Comparison of cellular and protein changes in bronchial lavage fluid of symptomatic and asymptomatic patients with red cedar asthma on follow-up examination. *Clin Allergy* 1988; 18(4):359–65.
78. Djukanović R, Wilson JW, Britten KM, et al. Quantitation of mast cells and eosinophils in the bronchial mucosa of symptomatic atopic asthmatics and healthy control subjects using immunohistochemistry. *Am Rev Respir Dis* 1990;142(4): 863–71.
79. Adelroth E, Rosenhall L, Johansson SA, et al. Inflammatory cells and eosinophilic activity in asthmatics investigated by bronchoalveolar lavage. The effects of antiasthmatic treatment with budesonide or terbutaline. *Am Rev Respir Dis* 1990;142(1):91–9.
80. Ollerenshaw SL, Woolcock AJ. Characteristics of the inflammation in biopsies from large airways of subjects with asthma and subjects with chronic airflow limitation. *Am Rev Respir Dis* 1992;145(4 Pt 1):922–7.
81. Iredale MJ, Wanklyn SA, Phillips IP, et al. Non-invasive assessment of bronchial inflammation in asthma: no correlation between eosinophilia of induced sputum

- and bronchial responsiveness to inhaled hypertonic saline. *Clin Exp Allergy* 1994;24(10):940–5.
82. Crimi E, Spanevello A, Neri M, et al. Dissociation between airway inflammation and airway hyperresponsiveness in allergic asthma. *Am J Respir Crit Care Med* 1998;157(1):4–9.
 83. Oddera S, Silvestri M, Penna R, et al. Airway eosinophilic inflammation and bronchial hyperresponsiveness after allergen inhalation challenge in asthma. *Lung* 1998;176(4):237–47.
 84. Meijer RJ, Kerstjens HA, Arends LR, et al. Effects of inhaled fluticasone and oral prednisolone on clinical and inflammatory parameters in patients with asthma. *Thorax* 1999;54(10):894–9.
 85. Nielsen KG, Bisgaard H. The effect of inhaled budesonide on symptoms, lung function, and cold air and methacholine responsiveness in 2-to 5-year-old asthmatic children. *Am J Respir Crit Care Med* 2000;162(4 Pt 1):1500–6.
 86. van den Berge M, Kerstjens HA, Meijer RJ, et al. Corticosteroid-induced improvement in the PC20 of adenosine monophosphate is more closely associated with reduction in airway inflammation than improvement in the PC20 of methacholine. *Am J Respir Crit Care Med* 2001;164(7):1127–32.
 87. The Childhood Asthma Management Program Research Group. Long-term effects of budesonide or nedocromil in children with asthma. *N Engl J Med* 2000;343(15):1054–63.
 88. Juniper EF, Kline PA, Vanzieleghem MA, et al. Effect of long-term treatment with an inhaled corticosteroid (budesonide) on airway hyperresponsiveness and clinical asthma in nonsteroid-dependent asthmatics. *Am Rev Respir Dis* 1990;142:832–6.
 89. Kerrebijn KF, van Essen-Zandvliet EE, Neijens HJ. Effect of long-term treatment with inhaled corticosteroids and beta-agonists on the bronchial responsiveness in children with asthma. *J Allergy Clin Immunol* 1987;79:653–9.
 90. Bisgaard H, Nielsen KG. Bronchoprotection with a leukotriene receptor antagonist in asthmatic preschool children. *Am J Respir Crit Care Med* 2000;162(1):187–90.
 91. Le JA, Busse WW, Pearlman D, et al. Montelukast, a leukotriene-receptor antagonist, for the treatment of mild asthma and exercise-induced bronchoconstriction. *N Engl J Med* 1998;339(3):147–52.
 92. Kononowa N, Michel S, Miedinger D, et al. Effects of add-on montelukast on airway hyperresponsiveness in patients with well controlled asthma: a pilot study. *J Drug Assess* 2013;2:49–57.
 93. Fischer AR, McFadden CA, Frantz R, et al. Effect of chronic 5-lipoxygenase inhibition on airway hyperresponsiveness in asthmatic subjects. *Am J Respir Crit Care Med* 1995;152:1203–7.
 94. Benckhuijsen J, van den Bos JW, van Velzen E, et al. Differences in the effect of allergen avoidance on bronchial hyperresponsiveness as measured by methacholine, adenosine 5'monophosphate, and exercise in asthmatic children. *Pediatr Pulmonol* 1996;22(3):147–53.
 95. van Velzen E, van den Bos JW, Benckhuijsen JA, et al. Effect of allergen avoidance at high altitude on direct and indirect bronchial hyperresponsiveness and markers of inflammation in children with allergic asthma. *Thorax* 1996;51(6):582–4.
 96. Leckie MJ, ten Brinke A, Khan J, et al. Effects of an interleukin-5 blocking monoclonal antibody on eosinophils, airway hyperresponsiveness, and the late asthmatic response. *Lancet* 2000;356(9248):2144–8.

97. Haldar P, Brightling CE, Hargadon B, et al. Mepolizumab and exacerbations of refractory eosinophilic asthma. *N Engl J Med* 2009;360(10):973.
98. Djukanović R, Wilson SJ, Kraft M, et al. Effects of treatment with anti-immunoglobulin E antibody omalizumab on airway inflammation in allergic asthma. *Am J Respir Crit Care Med* 2004;170(6):583–93.
99. Prieto L, Gutiérrez V, Colás C, et al. Effect of omalizumab on adenosine 5'-monophosphate responsiveness in subjects with allergic asthma. *Int Arch Allergy Immunol* 2006;139(2):122–31.
100. Cahill KN, Katz HR, Cui J, et al. KIT inhibition by imatinib in patients with severe refractory asthma. *N Engl J Med* 2017;376(20):1911–20.
101. Gauvreau GM, O'Byrne PM, Boulet L-P, et al. Effects of an anti-TSLP antibody on allergen-induced asthmatic responses. *N Engl J Med* 2014;370:2102–10.
102. Allakhverdi Z, Comeau MR, Jessup HK, et al. Thymic stromal lymphopietin is released by human epithelial cells in response to microbes, trauma, or inflammation and potently activates mast cells. *J Exp Med* 2007;204:253–8.
103. Duong M, Cockcroft D, Boulet LP, et al. The effect of IXV-0142, a heparin-derived hypersulfated disaccharide, on the allergic airway responses in asthma. *Allergy* 2008;63:1195–201.
104. Gauvreau GM, Watson RM, O'Byrne PM. Protective effects of inhaled PGE2 on allergen-induced airway responses and airway inflammation. *Am J Respir Crit Care Med* 1999;159:31–6.
105. Gauvreau GM, Becker AB, Boulet LP, et al. The effects of an anti-CD11a mAb, efalizumab, on allergen-induced airway responses and airway inflammation in subjects with atopic asthma. *J Allergy Clin Immunol* 2003;112:331–8.
106. Gauvreau GM, Boulet LP, Cockcroft DW, et al. Antisense therapy against CCR3 and the common beta chain attenuates allergen-induced eosinophilic responses. *Am J Respir Crit Care Med* 2008;177:952–8.
107. Gauvreau GM, Hessel EM, Boulet LP, et al. Immunostimulatory sequences regulate interferon-inducible genes but not allergic airway responses. *Am J Respir Crit Care Med* 2006;174:15–20.
108. Gauvreau GM, Boulet LP, Cockcroft DW, et al. Effects of interleukin-13 blockade on allergen-induced airway responses in mild atopic asthma. *Am J Respir Crit Care Med* 2011;183:1007–14.
109. Gauvreau GM, Boulet LP, Schmid-Wirlitsch C, et al. Roflumilast attenuates allergen-induced inflammation in mild asthmatic subjects. *Respir Res* 2011;12:140.
110. Gauvreau GM, Boulet LP, Cockcroft DW, et al. OX40L blockade and allergen-induced airway responses in subjects with mild asthma. *Clin Exp Allergy* 2014;44:29–37.
111. Boulet LP, Gauvreau GM, Cockcroft DW, et al. Effects of ASM-024, a modulator of acetylcholine receptor function, on airway responsiveness and allergen-induced responses in patients with mild asthma. *Can Respir J* 2015;22:230–4.
112. Gauvreau GM, Boulet LP, FitzGerald JM, et al. A dual CysLT1/2 antagonist attenuates allergen-induced airway responses in subjects with mild allergic asthma. *Allergy* 2016;71:1721–7.
113. Gauvreau GM, Harris JM, Boulet LP, et al. Targeting membrane-expressed IgE B cell receptor with an antibody to the M1 prime epitope reduces IgE production. *Sci Transl Med* 6, 243ra85. *Ann Neurosci* 2014;21(4):150.
114. Gauvreau GM, Boulet LP, Leigh R, et al. A nonsteroidal glucocorticoid receptor agonist inhibits allergen-induced late asthmatic responses. *Am J Respir Crit Care Med* 2015;191:161–7.

115. Juniper EF, Frith PA, Dunnett C, et al. Reproducibility and comparison of responses to inhaled histamine and methacholine. *Thorax* 1978;33:705–10.
116. Cockcroft DW. Direct challenge tests. *Chest* 2010;138:18S–24S.
117. Anderson SD, Charlton B, Weiler JM, et al. Comparison of mannitol and methacholine to predict exercise-induced bronchoconstriction and a clinical diagnosis of asthma. *Respir Res* 2009;10:4.
118. Cockcroft DW, Murdock KY, Berscheid BA, et al. Sensitivity and specificity of histamine PC20 determination in a random selection of young college students. *J Allergy Clin Immunol* 1992;89:23–30.
119. Carlsten C, Dimich-Ward H, Ferguson A, et al. Airway hyperresponsiveness to methacholine in 7-year-old children: sensitivity and specificity for pediatric allergist-diagnosed asthma. *Pediatr Pulmonol* 2011;46:175–8.
120. Hopp RJ, Bewtra AK, Nair NM, et al. Specificity and sensitivity of methacholine inhalation challenge in normal and asthmatic children. *J Allergy Clin Immunol* 1984;74:154–8.
121. Liem JJ, Kozyrskyj AL, Cockcroft DW, et al. Diagnosing asthma in children: what is the role for methacholine bronchoprovocation testing? *Pediatr Pulmonol* 2008;43:481–9.
122. Goldstein MF, Pacana SM, Dvorin DJ, et al. Retrospective analyses of methacholine inhalation challenges. *Chest* 1994;105:1082–8.
123. Nieminen MM. Unimodal distribution of bronchial hyperresponsiveness to methacholine in asthmatic patients. *Chest* 1992;102:1537–43.
124. Perpina M, Pellicer C, de Diego A, et al. Diagnostic value of the bronchial provocation test with methacholine in asthma. A Bayesian analysis approach. *Chest* 1993;104:149–54.
125. Sverrild A, Porsbjerg C, Thomsen SF, et al. Airway hyperresponsiveness to mannitol and methacholine and exhaled nitric oxide: a random-sample population study. *J Allergy Clin Immunol* 2010;126:952–8.
126. Goldstein MF, Veza BA, Dunskey EH, et al. Comparisons of peak diurnal expiratory flow variation, postbronchodilator FEV(1) responses, and methacholine inhalation challenges in the evaluation of suspected asthma. *Chest* 2001;119:1001–10.
127. Sumino K, Sugar EA, Irvin CG, et al, American Lung Association Asthma Clinical Research Centers. Methacholine challenge test: diagnostic characteristics in asthmatic patients receiving controller medications. *J Allergy Clin Immunol* 2012;130:69–75.
128. Prieto L, Ferrer A, Domenech J, et al. Effect of challenge method on sensitivity, reactivity, and maximal response to methacholine. *Ann Allergy Asthma Immunol* 2006;97:175–81.
129. Wubbel C, Asmus MJ, Stevens G, et al. Methacholine challenge testing: comparison of the two American Thoracic Society-recommended methods. *Chest* 2004;125:453–8.
130. Siersted HC, Walker CM, O'Shaughnessy AD, et al. Comparison of two standardized methods of methacholine inhalation challenge in young adults. *Eur Respir J* 2000;15:181–4.
131. Cockcroft DW, Davis BE, Todd DC, et al. Methacholine challenge: comparison of two methods. *Chest* 2005;127(3):839–44.
132. Cockcroft DW. Methacholine challenge methods. *Chest* 2008;134:678–80.
133. Cockcroft DW, Davis BE. The bronchoprotective effect of inhaling methacholine by using total lung capacity inspirations has a marked influence on the interpretation of the test result. *J Allergy Clin Immunol* 2006;117:1244–8.

134. Coates AL, Wanger J, Cockcroft DW, et al. ERS technical standard on bronchial challenge testing: general considerations and performance of methacholine challenge tests. *Eur Respir J* 2017;49(5) [pii:1601526].
135. Dell SD, Bola SS, Foty R, et al. Provocative dose of methacholine causing a 20% drop in FEV1 should be used to interpret methacholine challenge tests with modern nebulizers. *Ann Am Thorac Soc* 2015;12:357–63.
136. Schulze J, Roswich M, Riemer C, et al. Methacholine challenge – comparison of an ATS protocol to a new rapid single concentration technique. *Respir Med* 2009;103:1898–903.
137. Cockcroft D, Davis B. Direct and indirect challenges in the clinical assessment of asthma. *Ann Allergy Asthma Immunol* 2009;103:363–70.
138. Porsbjerg C, Brannan JD, Anderson SD, et al. Relationship between airway responsiveness to mannitol and to methacholine and markers of airway inflammation, peak flow variability and quality of life in asthma patients. *Clin Exp Allergy* 2008;38:43–50.
139. Porsbjerg C, Backer V, Joos G, et al. Current and future use of the mannitol bronchial challenge in everyday clinical practice. *Clin Respir J* 2009;3:189–97.
140. Brannan JD, Koskela H, Anderson SD. Monitoring asthma therapy using indirect bronchial provocation tests. *Clin Respir J* 2007;1:3–15.
141. Kim MH, Song WJ, Kim TW, et al. Diagnostic properties of the methacholine and mannitol bronchial challenge tests: a comparison study. *Respirology* 2014;19: 852–6.
142. Leuppi JD, Salome CM, Jenkins CR, et al. Predictive markers of asthma exacerbations during stepwise dose reduction of inhaled corticosteroids. *Am J Respir Crit Care Med* 2001;163:406–12.
143. Koskela HO, Hyvarinen L, Brannan JD, et al. Sensitivity and validity of three bronchial provocation tests to demonstrate the effect of inhaled corticosteroids in asthma. *Chest* 2003;124:1341–9.
144. Singapuri A, McKenna S, Brightling CE, et al. Mannitol and AMP do not induce bronchoconstriction in eosinophilic bronchitis: further evidence for disassociation between airway inflammation and bronchial hyperresponsiveness. *Respirology* 2010;15:510–5.
145. Sue-Chu M, Brannan JD, Anderson SD, et al. Airway hyperresponsiveness to methacholine, adenosine5-monophosphate, mannitol, eucapnic voluntary hyperpnoea and field exercise challenge in elite cross country skiers. *Br J Sports Med* 2010;44:827–32.
146. Porsbjerg C, Rasmussen L, Thomsen SF, et al. Response to mannitol in asymptomatic subjects with airway hyper-responsiveness to methacholine. *Clin Exp Allergy* 2007;37:22–8.
147. Anderson SD, Brannan JD. Bronchial provocation testing: the future. *Curr Opin Allergy Clin Immunol* 2011;11(1):46–52.
148. Anderson SD. Indirect challenge tests: airway hyperresponsiveness in asthma: its measurement and clinical significance. *Chest* 2010;138(Suppl 2):25S–30S.
149. Anderton RC, Cu MT, Frith PA, et al. Bronchial responsiveness to inhaled histamine and exercise. *J Allergy Clin Immunol* 1979;63:315–20.
150. Holzer K, Anderson SD, Douglass J. Exercise in elite summer athletes: challenges for diagnosis. *J Allergy Clin Immunol* 2002;110:374–80.
151. Riedler J, Reade T, Dalton M, et al. Hypertonic saline challenge in an epidemiologic survey of asthma in children. *Am J Respir Crit Care Med* 1994;150: 1632–9.

152. O'Connor BJ, Ridge SM, Barnes PJ, et al. Greater effect of inhaled budesonide on adenosine 5'-monophosphate-induced than on sodium-metabisulfite-induced bronchoconstriction in asthma. *Am Rev Respir Dis* 1992;146:560–4.
153. Doull IJ, Sandall D, Smith S, et al. Differential inhibitory effect of regular inhaled corticosteroid on airway responsiveness to adenosine 5' monophosphate, methacholine, and bradykinin in symptomatic children with recurrent wheeze. *Pediatr Pulmonol* 1997;23:404–11.
154. Weersink EJ, Douma RR, Postma DS, et al. Fluticasone propionate, salmeterol xinafoate, and their combination in the treatment of nocturnal asthma. *Am J Respir Crit Care Med* 1997;155:1241–6.
155. Reynolds CJ, Togias A, Proud D. Airways hyperresponsiveness to bradykinin and methacholine: effects of inhaled fluticasone. *Clin Exp Allergy* 2002;32:1174–9.
156. Church MK, Holgate ST, Hughes PJ. Adenosine inhibits and potentiates IgE-dependent histamine release from human basophils by an A2-receptor mediated mechanism. *Br J Pharmacol* 1983;80:719–26.
157. Hughes PJ, Holgate ST, Church MK. Adenosine inhibits and potentiates IgE-dependent histamine release from human lung mast cells by an A2- purinoceptor mediated mechanism. *Biochem Pharmacol* 1984;33:3847–52.
158. Peachell PT, Columbo M, Kagey-Sobotka A, et al. Adenosine potentiates mediator release from human lung mast cells. *Am Rev Respir Dis* 1988;138:1143–51.
159. Forsythe P, McGarvey LP, Heaney LG, et al. Adenosine induces histamine release from human bronchoalveolar lavage mast cells. *Clin Sci* 1999;96:349–55.
160. Fuller RW, Choudry NB, Eriksson G. Action of budesonide on asthmatic bronchial hyper-responsiveness: effects on directly and indirectly acting bronchoconstrictors. *Chest* 1991;100:670–4.
161. Vathenen AS, Knox AJ, Wisniewski A, et al. Effect of inhaled budesonide on bronchial reactivity to histamine, exercise, and eucapnic dry air in patients with asthma. *Thorax* 1991;46:811–6.
162. Rodwell LT, Anderson SD, Seale JP. Inhaled steroids modify bronchial responses to hyper-osmolar saline. *Eur Respir J* 1992;5:953–62.
163. du Toit JI, Anderson SD, Jenkins CR, et al. Airway responsiveness in asthma: bronchial challenge with histamine and 4.5% sodium chloride before and after budesonide. *Allergy Asthma Proc* 1997;18:7–14.
164. Global Initiative for Asthma (GINA). Global strategy for asthma management and prevention. 2018. Available at: <https://ginasthma.org/2018-gina-report-global-strategy-for-asthma-management-and-prevention/>.
165. National Asthma Education and Prevention Program. Expert panel report 3 (EPR-3): guidelines for the diagnosis and management of asthma, full report 2007. Bethesda (MD): US Department of Health and Human Services, National Institutes of Health, National Heart, Lung, and Blood Institute; 2007.
166. British Thoracic Society, Scottish Intercollegiate Guidelines Network. British guideline on the management of asthma. *Thorax* 2014;69(suppl 1):1–192.
167. van den Toorn LM, Overbeek SE, de Jongste JC, et al. Airway inflammation is present during clinical remission of atopic asthma. *Am J Respir Crit Care Med* 2001;164:2107–13.
168. Hanxiang N, Jiong Y, Yanwei C, et al. Persistent airway inflammation and bronchial hyperresponsiveness in patients with totally controlled asthma. *Int J Clin Pract* 2008;62(4):599–605.
169. Laprise C, Lavolette M, Boutet M, et al. Asymptomatic airway hyperresponsiveness: relationships with airway inflammation and remodelling. *Eur Respir J* 1999; 14(1):63–73.

170. Saglani S, Lloyd CM. Novel concepts in airway inflammation and remodelling in asthma. *Eur Respir J* 2015;46(6):1796–804.
171. Deykin A, Lazarus SC, Fahy JV, et al. Sputum eosinophil counts predict asthma control after discontinuation of inhaled corticosteroids. *J Allergy Clin Immunol* 2005;115:720–7.
172. Dweik RA, Sorkness RL, Wenzel S, et al. Use of exhaled nitric oxide measurement to identify a reactive, at-risk phenotype among patients with asthma. *Am J Respir Crit Care Med* 2010;181:1033–41.
173. Anderson WJ, Short PM, Jabbal S, et al. Inhaled corticosteroid dose response in asthma: Should we measure inflammation? *Ann Allergy Asthma Immunol* 2017;118(2):179–85.
174. Sont JK, Willems LN, Bel EH, et al. Clinical control and histopathologic outcome of asthma when using airway hyperresponsiveness as an additional guide to long-term treatment. *Am J Respir Crit Care Med* 1999;159(4 Pt 1):1043–51.
175. Nuijsink M, Hop WC, Sterk PJ, et al. Long-term asthma treatment guided by airway hyperresponsiveness in children: a randomised controlled trial. *Eur Respir J* 2007;30:457e66.
176. Nuijsink M, Vaessen-Verberne AA, Hop WC, et al. Long-term follow-up after two years of asthma treatment guided by airway responsiveness in children. *Respir Med* 2013;107(7):981–6.
177. Koenig SM, Murray JJ, Wolfe J, et al. Does measuring BHR add to guideline derived clinical measures in determining treatment for patients with persistent asthma? *Respir Med* 2008;102(5):665–73.
178. Brannan JD, Perry CP, Anderson SD. Mannitol test results in asthmatic adults receiving inhaled corticosteroids. *J Allergy Clin Immunol* 2013;131(3):906–7.
179. Prieto L, Ferrer A, Domenech J, et al. Predictive value of the response to inhaled adenosine 5'-monophosphate for reducing the dose of inhaled corticosteroids in asthma. *J Investig Allergol Clin Immunol* 2013;23(5):351–8.
180. Porsbjerg C, Lund TK, Pedersen L, et al. Inflammatory subtypes in asthma are related to airway hyperresponsiveness to mannitol and exhaled NO. *J Asthma* 2009;46(6):606–12.
181. Lipworth BJ, Short PM, Williamson PA, et al. A randomized primary care trial of steroid titration against mannitol in persistent asthma: STAMINA trial. *Chest* 2012;141(3):607–15.