The usefulness of biomarkers of airway inflammation in managing asthma

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ABSTRACT

The goal of managing asthma is to maintain disease control. Current approaches to assessment of control do not include measurement of airway inflammation. This study was designed to assess the usefulness of biomarkers of airway inflammation in guiding asthma management decisions. A literature review was performed. Bronchial biopsy is a direct measure of airway inflammation but not practical for routine use. Enumeration of sputum eosinophils is very useful in guiding changes in controller medication to decrease asthma exacerbations, whereas measurement of exhaled nitric oxide has not proven to be useful in this regard. Serial measurement of airway hyperreactivity as a guide to asthma management yields inconclusive results. Use of indirect stimuli for bronchial challenge offers both practical and theoretical advantages in the assessment of airway hyperreactivity. Data on the analysis of exhaled breath condensate have not yet been studied adequately in guiding management decisions. Enumeration of sputum cell counts appears to be the most useful biomarker of airway inflammation in guiding asthma management decisions. Combined approaches using simple methods of measuring airway hyperreactivity and obtaining sputum samples hold promise for the future, particularly if rapid analysis of cellular products in sputum can be developed.

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Key words: Airway hyperresponsiveness, asthma, biomarker, exhaled breath condensate, exhaled nitric oxide, mannitol, sputum eosinophils

Asthma is a chronic inflammatory disease of the conducting airways characterized by symptoms of coughing, wheezing, chest tightness, and shortness of breath, which are frequently episodic in nature. At present, there is no evidence that asthma can be cured or prevented and the major goal of asthma management is to achieve "control" of the disease. The National Asthma Education and Prevention Program Expert Panel Report 3—Guidelines for the Diagnosis and Management of Asthma (EPR-3)[1] and other international asthma management guidelines[2,3] suggest that achieving control of asthma should result in a reduction both in current asthma-related functional impairment and in future asthma exacerbations or loss of lung function. Decisions about medication changes and other therapeutic interventions in asthma are predicated on whether a patient's disease is under control or not. Each of the aforementioned guidelines stresses the importance of routine monitoring of asthma control to continuously guide management decisions.

Current approaches to the assessment of asthma control include questioning of the patient (or caregiver) about current and recalled frequency of asthma symptoms, need for symptom-relieving medications, nocturnal awakenings due to asthma, functional limitations because of asthma, recent need for urgent care, and missed work or school, together with an objective assessment of lung function. Controlled asthma is characterized by minimal symptoms during the day or night, no limitation in routine daily activities or in exercise tolerance because of asthma symptoms, minimal need for reliever medications, and lung function at or near normal or personal best, combined with an absence of recent need for urgent care. This multifaceted approach to assessment of asthma control has been aided by the development of validated patient-centric questionnaires that can quickly provide a numeric score facilitating the assessment of asthma control. Three such questionnaires have been developed and validated in prospective studies and their routine use is also endorsed by the EPR-3.[1] These include the Asthma Control Test,[4] the Asthma Treatment Assessment Questionnaire,[5] and the Asthma Control Questionnaire.[6]

However, asthma is a disease of inflammation of the airways and it is believed that airway inflammation is largely responsible for asthma symptoms, exacerbations, and other aspects of disease activity. Indeed, treatments that reduce inflammation also have the most proven efficacy in management of asthma.[7] Well-described inflammatory changes in the asthmatic lung include infiltration of the airway by eosinophils, mast

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cells, and neutrophils together with histopathological abnormalities in the epithelium, the submucosa, and the muscle layers of the airway.[8–15] Currently recommended approaches to assessment of asthma control do not include measurement of airway inflammation and a focus of intense research in asthma is the identification and validation of direct or indirect means to assess airway inflammation. One objective of such studies is to identify a measure of inflammation that would be both useful and widely accessible in guidance of asthma management decisions to outcomes superior than observed with current approaches. Other objectives of measuring airway inflammation might include facilitating the diagnosis or differentiation of asthma from other mimicking conditions or the categorization of an individual asthma patient's phenotype based on the pattern of cellular inflammatory markers (*e.g.*, eosinophilic, neutrophilic, or paucicellular).[16] This brief review will critically evaluate the literature regarding the usefulness of several modalities of measuring airway inflammation including bronchial biopsy, enumeration of sputum eosinophils, measurement of nitric oxide in exhaled breath, measurements of airway hyperresponsiveness (AHR), and evaluation of physicochemical characteristics of the condensate from exhaled breath in assessing asthma control and guiding treatment decision making.

BRONCHIAL BIOPSY

Endoscopic bronchial biopsy and histopathological analysis is a direct and precise way of evaluating airway inflammation. Although it is extremely informative in research studies, because of its invasiveness, it is not a practical measure in routine clinical care as a means to assess airway inflammation or asthma control.[8,14,17,18]

COUNTING INFLAMMATORY CELLS IN SPUTUM SAMPLES

In the 1980s, Hargreaves and colleagues pioneered the notion that enumeration of eosinophils in sputum might serve as an indirect measure of airway inflammation in asthmatic patients.[19] Opinion now favors the use of "induced" sputum over "spontaneous" sputum and typically requires the nebulization of hypertonic saline into the airways. This semi-invasive but directly quantitative methodology of sputum analysis has been largely standardized, with proven reproducibility.[20,21] Recent studies suggest that other agents, including inhaled mannitol as a dry powder, might also be a useful and possibly simpler method of obtaining induced sputum.[22] Enumeration of the cellular content of sputum is relatively labor intensive, however, in that it requires laboratory analysis and the use of trained medical laboratory technologists. Regardless of the technical requirements, this approach shows great promise in guiding asthma management decisions.

A landmark study in 2002 by Green and colleagues[23] convincingly showed that when enumeration of sputum eosinophils was added to the standard guideline-recommended criteria for assessment of asthma control in making management decisions, specifically medication adjustments, asthma exacerbation rates were significantly reduced. In this single-center study, moderate-to-severe asthmatic patients were randomized to have medication adjustments made based on either standard British Thoracic Society criteria[3] or by those criteria together with measurement of sputum eosinophils repeatedly over a 12-month period. Thirty-four patients in each group completed the study and the outcome was a significantly lower number of asthma exacerbations in the latter group, who experienced 35 exacerbations compared with 109 in the former group. The average daily dose of inhaled corticosteroids (ICSs) or oral corticosteroids did not differ between the two groups.

In 2006, Jayaram and colleagues[24] further showed the benefit of counting sputum eosinophils in guiding treatment decisions in asthma. Over 2 years, in a randomized, parallel treatment group, multicenter study, both the total number of asthma exacerbations and the time to the first exacerbation were significantly reduced by using sputum eosinophil count–driven decisions about treatment, rather than using a clinical strategy based on symptoms and lung function. In that study, steroid requirements were not different between the two groups. Furthermore, an additional analysis suggested that sputum eosinophil enumeration is a cost-effective strategy.[25]

Other studies addressing this question have reported similar supportive findings (Table 1). A meta-analysis published in the Cochrane Review Database in 2007,[26] addressed the question of "Tailored Interventions Based on Sputum Eosinophils Versus Clinical Symptoms for Asthma in Children and Adults" and concluded that this strategy could result in ~50% reduction in the number of asthma exacerbations (odds ratio = 0.49) with a number needed to benefit of 6, as well as a reduction in the severity of exacerbations. Interestingly, the mean dose of ICSs per day was similar in both groups pooled in this meta-analysis. Sufficient data were not available to make any conclusion regarding this strategy in pediatric asthma.

The published literature, therefore, indicates that sputum eosinophil enumeration as a complementary tool in the clinical assessment of asthma control shows great benefit in guiding management decisions as it relates to decreasing the frequency and severity of asthma exacerbations. Significant technical support is required to conduct these measurements and further cost-effectiveness studies will be important to assess

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Table 1 **Summary of clinical trials of asthma management guided by sputum eosinophil enumeration**

Study (yr)	Subjects (*n*)	Trial Type	Follow-Up Frequency and Time	Sputum Eosinophil Normal Cutoff	Primary Outcome	Secondary Outcomes
Green *et al.* (2002)[23]	Adults ($n = 74$)	Unicenter, randomized, single blind	Nine times over 12 mo	<3%	Severe asthma exacerbations were reduced ($p = 0.01$).	No change in the mean dose of ICS or oral prednisolone
Chlumsky *et al.* (2006)[70]	Adults with atopy ($n = 55$)	Unicenter, randomized, single blind, parallel group	Every 3 months for 18 mo	Graded: <3% led to step-down therapy, 4–8% led to no change, and 8% led to increase therapy	The rate of asthma exacerbations was reduced ($p = 0.013$) as was the time to first exacerbation ($p = 0.003$).	No change in ICS dose
Jayaram *et al.* (2006)[24]	Adults ($n = 117$)	Multicenter, randomized, single blind, parallel group	Every 3 months and for exacerbations for 2 yr	≤2%	Total number of exacerbations decreased and time to first exacerbation increased ($p = 0.04$ and $p = 0.06$ respectively).	No change in the mean cumulative ICS dose

ICS = inhaled corticosteroids.

this strategy, given substantial economic impact of asthma on overall health care.

Increases in sputum eosinophils have also shown additional usefulness as a prognostic indicator in identifying those patients who are at increased risk of losing asthma control after either inhaled steroid reduction[27–29] or total cessation.[30] At this time, the position adopted by the EPR-3[1] guidelines is that although minimally invasive biomarkers such as sputum eosinophil measurement might be useful, they still require further evaluation regarding their clinical usefulness before their routine use can be recommended.

MEASUREMENT OF EXHALED NITRIC OXIDE

An alternative strategy for evaluation of airway inflammation in asthma, which has the merits of being less invasive and significantly more convenient than bronchial biopsy or enumeration of eosinophils in sputum, is the automated measurement of nitric oxide in exhaled breath. Because inducible nitric oxide is produced by the conversion of alanine to citrulline by the enzymes of in the respiratory burst pathway, in theory, it can be generated in any tissue including those parts of the respiratory tract not associated with bronchial inflammation. Exclusion of a contribution to exhaled fraction of nitric oxide by inflammatory processes in the nose or sinuses is achieved by the placement of an occlusive nose clip. McFadden has reviewed the principles underlying the importance of airflow rate in the use of fractional exhaled nitric oxide (FeNO) in assessment of asthma.[31] An expiratory flow rate slow enough to allow equilibration of nitric oxide with the cells lining the bronchial wall is required to ensure that airway inflammation is being reflected in the measurement of FeNO. Movement of air at the high flow rate serves mainly to sample alveolar air rather than allowing for exchange across the bronchial wall. An analysis of studies comparing values of FeNO between asthmatic and nonasthmatic patients shows that differentiation is better seen at flow rates of 50 mL/s rather than 250 mL/s[32,33] and a cutoff of around 28–30 ppb seems to provide an optimal differentiation between asthmatic and nonasthmatic patients. Both the American Thoracic Society[34] and the European Respiratory Society[35] have recommended a flow rate of 50 mL/s for 6 seconds as the standard approach for measurement of FeNO. Precision in attaining ideal flow rates is improved by the use of an in-line resistance and patient feedback from a graphical user interface, and, typically, the procedure is repeated three times to ensure reproducibility on the resulting measurement. A small number of devices are now available on the commercial market to measure FeNO, with approval from the Food and Drug Administration for this purpose.[36]

The usefulness of FeNO measurement in guiding asthma management decisions remains uncertain. A

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2005 study by Smith *et al.*[37] comparing two groups of ICS-dependent asthmatic patients indicated a 45% (but statistically not significant) reduction in exacerbation rates over 12 months when FeNO measurement obtained every 2 months was used to guide treatment decisions. In this study, the amount of ICS used by the FeNO measurement-based management group was significantly less than that required in the control group. A subsequent study with similar design[38] resulted in an even smaller reduction in exacerbation rates but was associated with a somewhat higher use of ICS. This study included 118 patients (58 in the FeNO group and 60 in the control group), with evaluations at monthly intervals for the first 4 months and then every 2 months for the subsequent 8 months. The exacerbation rates were 0.33/patient per year in the FeNO group and 0.42/patient per year in the control group representing a 21% difference, which was not statistically significant. Furthermore, 11% more inhaled steroids were used by the group in whom therapeutic decision making was based on FeNO measurement. A subsequent multicenter, double-blind, parallel-group trial focused on a similar question[39] with evaluation of 546 children and young adults with asthma. Subjects were randomized to management based on the guidelines of the National Asthma Education and Prevention Program[1] or by measurement of FeNO. There was no significant difference in the number of days with asthma symptoms, asthma exacerbations, or pulmonary function between the two groups, even though the group treated using FeNO measurements received higher ICS doses. An additional small number of studies in children and adolescents on the utility of FeNO in therapeutic decision making[40–42] have been published. These studies are summarized in Tables 2 and 3, and a 2009 meta-analysis published in the Cochrane Review Database of studies[43] evaluating whether tailored intervention based on exhaled nitric oxide versus clinical symptoms resulted in better outcomes in adults or children, concluded that this approach resulted in no significant difference between groups for the primary outcome of asthma exacerbations or for other outcomes (clinical symptoms, FeNO level, and spirometry) and mixed results with respect ICS doses. The conclusion therefore at this time is that FeNO monitoring in asthma cannot achieve outcomes better than those resulting from the close clinical monitoring and patient support provided in clinical trials. Thus, despite the appeal of its simplicity relative to the somewhat more invasive approach of sputum induction, measurement of FeNO does not yield the improved asthma management outcomes seen with eosinophil enumeration.

Measurement of exhaled nitric oxide has also been studied for its potential application to other aspects of asthma care, including in establishing a diagnosis of asthma. A 2004 study by Smith *et al.*[44] described the performance of FeNO in correctly identifying asthmatic patients among patients referred with a diagnosis of possible asthma to a tertiary referral center in New Zealand. Based on clinical history and either bronchodilator reversibility of 12% or bronchial hyperresponsiveness to methacholine, 17 of 47 referred patients were determined to have asthma. With a cutoff of 20 ppb, the diagnostic performance of FeNO measurement included a sensitivity of 88% and a specificity of 79% with a negative predictive value of 92%. Gelb *et al.* in 2006[45] were able to show the added predictive value of FeNO measurement over and above forced expiratory volume in 1 second (FEV_1) measurement, in terms of predicting asthma exacerbations over the subsequent year. Also, Silkoff *et al.*[46] showed a potential role for FeNO measurement in assessing compliance with use of ICS and asthmatic patients by documenting elevations in FeNO on discontinuation of inhaled steroids.

AIRWAY HYPERRESPONSIVENESS

AHR is a diagnostic feature of asthma and is closely associated with airway inflammation. In clinical practice, its measurement is used mainly for its diagnostic purposes in mild asthma but in research studies it is being evaluated also for its prognostic usefulness. The most widely used test for measurement of AHR is the methacholine bronchial provocation test or challenge. Methacholine is delivered as a wet aerosol and, at a high dose, most individuals will experience bronchoconstriction to inhaled methacholine. AHR is typically diagnosed when a 20% fall in FEV_1 is observed at inhaled concentrations of methacholine of $\leq$16 mg/mL. In a given asthmatic patient, changes over time in the concentration of methacholine necessary to elicit a 20% fall in FEV_1 might be reflective of changes in underlying airway inflammation.

Sont and colleagues[47] investigated the usefulness of serial measurements of methacholine responsiveness in guiding asthma management. They studied 75 mild-to-moderate asthma in patients assigned to management strategies focusing on clinical parameters with or without the addition of methacholine challenges, assessed at 3 monthly intervals over 2 years. Changes in bronchial responsiveness were used to make the decision to intensify or decrease therapy with ICS. Patients treated according to the AHR strategy had a 1.8-fold significantly lower rate of mild exacerbations than did patients in the reference strategy group (0.23 and 0.43 exacerbation/year per patient, respectively; $p = 0.03$). FEV_1 also improved to a significantly greater extent in the AHR strategy group ($p < 0.05$). At all time points throughout the study, greater amounts of steroids were required by the group managed by the AHR

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Table 2 **Summary of clinical trials of asthma management guided by fractional exhaled nitric oxide (FeNO) measurement**

Study (yr)	Subjects (*n*)	Trial Type	Follow-Up Time	FeNO Cutoff	Primary Outcome	Secondary Outcomes
Pijnenburg, *et al.* (2005)[42]	Children or adolescents ($n = 40$)	Unicenter, double-blind, parallel groups	Two, 4, 12, and 24 wk and 12 mo	<30 ppb	Higher FeNO levels in patients with relapse after ICS withdrawal ($p = 0.01$).	None
Smith *et al.* (2005)[37]	Adolescents and adults ($n = 97$)	Unicenter, single blind, placebo controlled	Every 2 mo for 12 mo in phase II	<15 ppb at 250 mL/s or 35 ppb 50 mL/s	Lower frequency of asthma exacerbations in the FeNO-managed group ($p = 0.27$).	Decreased mean daily ICS dose in the FeNO-managed group ($p = 0.008$).
Fritsch *et al.* (2006)[41]	Children or adolescents ($n = 47$)	Unicenter, single-blind, parallel groups	Every 6 wk for 6 mo	<20 ppb	No change in the FEV_1 with addition of FeNO measurement to treatment algorithm ($p > 0.05$).	No change in number of asthma exacerbations, symptoms, and β-agonist use with addition of FeNO measurement to treatment algorithm ($p > 0.05$).
Shaw *et al.* (2007)[38]	Adults ($n = 118$)	Unicenter, single blind	Every 4 wk for 4 mo, and then every 2 mo for 8 mo	<26 ppb twice in a row (or 16 ppb)	Small but not significant decrease in the number of severe asthma exacerbations in FeNO-based management ($p = 0.43$).	Decrease in final daily ICS dose in FeNO ($p = 0.028$) with an 11% higher total ICS ($p = 0.40$).
Szefler *et al.* (2008)[39]	Children or adolescents ($n = 546$)	Multicenter, double-blind, parallel groups	Every 2 wk for 46 wk	<20 ppb	No difference in asthma symptom days with FeNO-based management ($p = 0.78$).	No difference in number of days of acute asthma exacerbations with FeNO-based management ($p = 0.11$).
De Jongste *et al.* (2009)[40]	Atopic children or adolescents ($n = 113$)	Multicenter single-blind, parallel groups	Every 3 wk for 30 wk	<20 ppb for ages 6–10 yr old or 25 ppb if >10 yr old	No change in the number of symptom-free days in the last 12 wk with FeNO-based management ($p = 0.95$)	No decrease in number of exacerbations with FeNO-based management ($p > 0.05$).

FEV_1 = forced expiratory volume 1 s; ICS = inhaled corticosteroids.

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Table 3 **Summary of the observational studies on the use of mannitol to measure airway hyperreactivity**

Study (yr)	Subjects (*n*)	Trial Type	Measured Markers	Inhaled Mannitol Criteria	Clinical Diagnosis Of Asthma	Primary Outcomes
Porsbjerg *et al.* (2008)[50]	Adults ($n = 53$)	Unicenter	Methacholine, sputum eosinophils, FeNO, peak flow variability	PD_{15}	GINA guidelines (2006)	Higher levels of sputum eosinophils ($p < 0.001$) and FeNO ($p < 0.05$) in patients with airway hyperreactivity to mannitol and methacholine.
Porsbjerg *et al.* (2009)[69]	Adults without ICS therapy ($n = 62$)	Unicenter	FeNO, spirometry, sputum eosinophils	PD_{15}	GINA guidelines (2006)	Lower PD_{15} to inhaled mannitol ($p = 0.02$) and higher FeNO ($p = 0.001$) in eosinophilic asthma.
Sverrlid *et al.* (2009)[57]	Children and adults aged 14–24 yr ($n = 238$)	Unicenter	Hypertonic saline challenge, spirometry	PD_{15}	GINA guidelines (2007)	Increased frequency of mannitol airway hyperreactivity in asthmatic young adults ($p < 0.001$).
Wood *et al.* (2010)[62]	Adults ($n = 55$)	Unicenter	Hypertonic saline challenge, spirometry	PD_{15}	Physician's diagnosis	Higher total sputum cell counts with hypertonic saline in comparison with mannitol provocation testing ($p = 0.003$) but no difference in the number of eosinophils and neutrophils ($p = 0.446$, $p = 0.507$).

FeNO = fractional exhaled nitric oxide; GINA = Global Initiative for Asthma; PD_{15} = provocation dose causing a 15% decline in FEV_1 from baseline; ICS = inhaled corticosteroids; FEV_1 = forced expiratory volume in 1 s.

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strategy. In contrast, a more recent study in children did not find a change in number of symptom-free days when AHR, assessed by methacholine, and symptom strategy were used for guiding dose of ICS compared with symptom strategy alone.[48] Thus, the published data are inconclusive about the usefulness of this strategy in guiding asthma management decisions. The practical efficacy of methacholine challenge as a routine tool for guiding asthma therapy has also been questioned.[49]

Methacholine is a muscarinic analog of histamine, which causes airway smooth muscle contraction, without the associated systemic symptoms of flushing, syncope, and cough, associated with use of histamine. Methacholine acts directly on airway smooth muscle receptors to induce bronchoconstriction, whereas indirect bronchoconstrictors act by causing mediator release from inflammatory cells, sensory nerve stimulation, and, perhaps, other mechanisms. Indirect stimuli used to measure AHR have included exercise, adenosine monophosphate (also delivered by wet nebulization), and mannitol (delivered by dry-powder inhaler). Among the theoretical advantages of using indirect stimuli to diagnose AHR is that all of the common "natural" stimuli that provoke asthma attacks, such as allergens, cold air, exercise, and sulfur dioxide, also act indirectly and it is felt that airway responsiveness to indirect stimuli appears to provide a better reflection of changes in inflammation[49–53] than does responsiveness to methacholine. The AHR measured by indirect challenges improves to a greater extent than AHR measured by methacholine with anti-inflammatory therapy including both ICS[51,54] and allergen avoidance.[55,56] However, although measurement of AHR by indirect stimuli might be more reflective of inflammation, it is less sensitive than methacholine challenge in mild and/or well-controlled asthma.[49,57,58]

For the reasons outlined previously, AHR measured by indirectly acting stimuli might be considered the better choice for serial determination in evaluating and monitoring asthma control. Mannitol is approved by regulatory authorities in Europe and Australia and is available in a convenient and standardized kit with prefilled dry-powder capsules and a single-use dry-powder inhaler device.[22,59] There is a standard protocol for dose delivery resulting in ease of administration to patients, including children, making it simpler to use than either methacholine or adenosine monophosphate. To date, inhaled mannitol testing has not been specifically studied as a guide for therapeutic decision making, either in assessing therapeutic control or treatment of exacerbations.

COMPARISON OF BIOMARKERS

Correlations among various biomarkers in their clinical efficacies or their usefulness when used in combination with other biomarkers in asthma management have been reported. A number of studies suggest that FeNO should correlate well with eosinophilic-type inflammation in the airways, and, accordingly, sputum eosinophil counts because of the increased production of nitric oxide by inflammatory processes. One early study noting this association was reported by Obata and colleagues, where a direct correlation between FeNO and sputum eosinophils was observed in red oak pollen-allergic asthmatic patients pre- and postallergen challenge.[60] The study by Green *et al.* on sputum eosinophil-guided management, previously described, also showed that patients with improvement in asthma control achieved by suppressing eosinophil sputum counts had equally significant improvements in other biomarkers including FeNO and methacholine AHR ($p = 0.01$ and $p = 0.03$, respectively).[23] The Shaw study also correlated FeNO with sputum eosinophil counts throughout the study ($r^2 = 0.455$; $p = 0.001$)[38] as did the study by Smith *et al.*[37] Furthermore, patients in clinical remission of asthma also showed correlation between their tissue eosinophil counts with sputum eosinophils and FeNO.[10]

The correlation between measures of airway inflammation and bronchial hyperreactivity has more conflicting data. Leuppi *et al.* in 2001 did not find a correlation between mannitol AHR and FeNO levels,[61] whereas the aforementioned study by Van den Toorn[10] did relate adenosine but not methacholine AHR to both sputum esosinophils and FeNO. In addition, a study by Van den Berge *et al.* noted that the correlation between mannitol AHR is more closely associated with sputum eosinophil counts rather than with AHR measured with methacholine in adult asthmatic patients.[51] There is speculation that ICS may obscure such relationships in some studies, because corticosteroids likely have unequal influence on the different parameters.[50] One study indicated that AHR measured by the indirect stimulus mannitol might be more sensitive to improvement with inhaled budesonide than was AHR to methacholine.[54] Other studies have shown correlations between indirect measures of AHR such as hypertonic saline or mannitol and sputum eosinophils[62] including a study in children by Frisch *et al.*, where FeNO levels were found to correlate significantly with bronchial hyperreactivity as measured by hypertonic saline.[41]

Although generally supporting concordance between the various studied biomarkers there are some conflicting data that likely reflect the phenotypical variety of asthma and the differential effects of corticosteroid or other anti-inflammatory therapies. Additional research is required to more fully elucidate the interconnections and the potential applicability of using several simultaneous biomarker strategies to measure inflammation.

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BIOMARKERS IN EXHALED BREATH CONDENSATE (EBC)

An alternative noninvasive approach to assessing airway inflammation is the analysis of the condensate of exhaled breath. Exhaled breath condensate (EBC) is the liquid phase collected by cooling exhaled breath and its best studied characteristic in asthma is a physicochemical one, pH. In 2000, Hunt and colleagues showed that the pH of EBC was significantly lower in patients with acute asthma when compared with well-controlled asthmatic patients, and that the EBC pH normalized with improvement.[63] A subsequent larger study of children confirmed that pH in EBC was significantly lower in patients with acute asthma than in patients with either stable asthma, allergic rhinitis, eczema, or normal controls (7.25 versus 7.32, $p < 0.05$; 7.25 versus 7.48, $p < 0.02$; 7.25 versus 7.78, $p < 0.0001$; 7.25 versus 7.78, $p < 0.0001$, respectively) and that pH normalized with resolution of asthma symptoms.[64] Although there is a lack of diagnostic specificity of the pH of EBC (because it can be reduced in chronic obstructive pulmonary disease and cystic fibrosis, smoking, and infection), these studies do suggest some potential efficacy in monitoring disease activity longitudinally.

EBC contains an array of compounds that might also have efficacy in the diagnosis, assessment of severity, and monitoring of airway diseases. These include the products of cellular inflammation (leukotrienes, prostaglandins, cytokines, and hydrogen ions [pH]), products of oxidative stress (hydrogen peroxide and B-isoprostane), and products of nitrosative stress (nitrates and nitrites). A variety of analytical methods have been used in the identification of these compounds including enzyme immunoassays, gas chromatography, mass spectrometry, HPLC, and others. This topic is well reviewed by Hoffmeyer (2009) and Kharitonov (2006).[65,66] A more recent approach to the analysis of EBC in asthma includes the detection of small molecules, including the development of an "electronic nose." There are studies on its use in diagnosing asthma[67,68] but there are no prospective trials on the use of EBC analysates for asthma management, and this continues to be an area requiring additional investigation.

CONCLUSIONS

Progress on the translation of the inflammatory pathology of asthma into clinically useful tools for tailoring asthma therapy continues to develop, with a previous focus on measurement of forced exhaled nitric oxide, sputum eosinophils, and airway hyperreactivity. Enumeration of sputum eosinophils has proven very useful but somewhat impractical because of technical limitations. Serial measurements of AHR with methacholine have also been helpful in this regard, albeit to a lesser extent, but are also limited by practical considerations. In contrast, measurement of FeNO, appealing because of its relative simplicity, does not seem to be nearly as useful in guiding therapy. Analysis of EBC merits further consideration because, to date, it has not been studied to guide therapy.

Looking forward, inhaled mannitol bronchoprovocation has significant potential because, in addition to being a relatively easy method to measure AHR, it represents an indirect stimulus well correlated with airway inflammation and it also has the advantage of providing a reliable method of obtaining induced sputum.[57] Furthermore, analysis of such induced sputum might prove useful in permitting discrimination between eosinophilic and noneosinophilic asthma, or other phenotypes, with implications for treatment decisions. A recent study by Porsbjerg and colleagues showed correlation between AHR determined by inhaled mannitol and asthma phenotypes classified by inflammatory cells seen in induced sputum: eosinophilic asthma, neutrophilic asthma, mixed granulocytic asthma, and paucigranulocytic asthma.[69] One might also foresee the day when alternative strategies to assess neutrophil- and eosinophil-derived products in expectorated sputum would be more useful than cell counting, particularly if developed for rapid analysis in an office setting. In the near future, therefore, assessment of asthma control might include inhalation of mannitol from a standardized dry-powder inhaler with pre- and postspirometry to determine the degree of AHR, and, if sputum is produced, rapid automated analysis of that sputum in the office setting for evidence of eosinophil products or products of other cells to determine the degree and nature of airway inflammation.

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