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Clinical Usefulness of Inflammatory Markers in Asthma

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Abstract

Asthma is a significant and increasing health problem. Airway inflammation and hyperresponsiveness are key pathophysiological mechanisms underlying asthma. Currently, effective treatments target these two processes and can lead to clinically important improvements in disease control. At present, decisions to initiate or modify therapy are based on symptoms and measures of airway caliber, with no direct assessment of airway inflammation or hyperresponsiveness. It is now possible to measure airway inflammation using noninvasive markers such as exhaled gases, induced sputum and serum measurements. Exhaled nitric oxide (eNO) and induced sputum eosinophils show the greatest promise as clinically useful markers of airway inflammation in asthma. Induced sputum can now be applied to the diagnosis of airway diseases, based on its ability to detect eosinophilic bronchitis in cough, and to differentiate between eosinophilic and non-eosinophilic asthma. The place of induced sputum and eNO in the ongoing monitoring of patients with asthma are now being investigated in controlled trials.

Asthma has been defined as a chronic inflammatory disease of the airways characterized by variable airflow obstruction and bronchial hyperresponsiveness. Airway inflammation is recognized as central to the development and progression of asthma, and a considerable amount of research has defined the mechanisms associated with inflammation in asthma.[1] Anti-inflammatory treatments, particularly inhaled corticosteroids, have targeted airway inflammation resulting in substantial improvements in asthma control.[2] Despite this, the direct measurement of inflammatory markers is not part of the routine clinical assessment in the management of asthma. If measures of airway inflammation are to become widely adopted for use in clinical practice, a number of requirements need to be fulfilled. The investigation needs to be clinically beneficial either in the diagnosis or management of asthma; it needs to be validated and reproducible other than in centers with a particular research interest and finally, it needs to be acceptable in terms of cost and safety.

This article explores the techniques of sputum induction, exhaled gas measures and blood or serum measures of eosinophilic inflammation as noninvasive measures of airway inflammation in terms of their potential clinical usefulness in asthma.

1. Induced Sputum

Sputum induction aims to directly sample inflammatory cells from the lower respiratory tract using an inhalation of hypertonic saline. In asthma, hypertonic saline indirectly causes bronchoconstriction, through the degranulation of mast cells.[3] In addition to this, it enhances sputum expectoration, probably by reducing the viscosity of tracheobronchial mucus, increasing mucociliary clearance and/or increasing the volume of airway secretions.[4,5]

Sputum is thus a mixture of saliva and lower airway secretions, allowing examination of the inflammatory cells and mediators present in the airway lumen. This is confirmed by the good correlation of sputum cell counts with those obtained by bronchial lavage.[6,7] The prevalence of inflammatory cells differs between differing airway compartments. In the airway lumen, the dominant cells (in decreasing frequency) are macrophages, neutrophils, lymphocytes and bronchial epithelial cells. Eosinophils are seldom present in normal sputum (table I). The population of inflammatory cells in the airway lumen is different from that in the airway epithelium and lamina propria, and consequently there is little correlation between sputum cell counts and bronchial biopsy.[7,8]

Table I. Normal ranges for induced sputum cell counts and eosinophil cationic protein (ECP) [data from Cai et al.[9]]

Variable	Mean	Standard deviation	Median	Interquartile range
Total cell count ($\times 10^6$/ml)	2.90	3.30	2.15	1.19–2.95
Viability (%)	66.40	20.66	69.85	53.44–81.21
Neutrophils (%)	26.26	13.66	24.86	16.88–35.15
Eosinophils (%)	0.81	2.93	0	0.00–2.75
Macrophages (%)	67.88	13.17	69.47	61.91–74.52
Lymphocytes (%)	1.12	2.15	0.75	0.29–1.31
Columnar epithelial cells (%)	3.77	5.19	2.15	0.60–4.41
Squamous (%)	17.30	20.44	8.75	3.44–25.50
ECP (μg/L)	708	2130	188	56–403

1.1 Induction Method and Sputum Examination

Sputum induction has mostly been carried out using varying concentrations of saline delivered by an ultrasonic nebulizer. The concentration of saline used has varied in studies from 0.9–7%, with some investigators changing concentrations during the procedure.[10] Saline concentration and nebulizer output are likely to affect success and safety. In stable asthma, hypertonic saline is more effective at inducing sputum than 0.9% saline,[11] but this may not be the case in acute asthma.[12] The tonicity of saline has been shown not to influence cell counts,[13] but its effect on fluid phase markers is not known. The duration of inhalation is important, with longer inhalation times sampling more of the distal airways, resulting in changes to sputum cell counts and fluid phase markers.[14,15] Ultrasonic nebulizers are the most effective way of delivering saline.[11] Lower output devices are probably as effective and potentially safer.[16]

Induction may be preceded by pretreatment with a β_2-adrenoceptor agonist (β_2-agonist) to reduce the risk of bronchospasm. Pretreatment with β_2-agonists does not influence cell counts.[11] Alternatively, sputum induction may be combined with bronchial provocation challenge as a single test.[17,18] There are advantages and disadvantages with each approach. The combined procedure is an efficient way to assess both airway inflammation and airway hyperresponsivenes. It may be more uncomfortable for the individual, since a greater degree of bronchoconstriction occurs with the combined procedure than with sputum induction alone. Both approaches have a high success rate for obtaining a satisfactory sputum sample. Regardless of the technique used, lung function needs to be monitored regularly throughout the procedure, and short-acting β_2-agonists must be available for rescue. In addition, oxygen saturation may need to be monitored in some circumstances and infection control procedures maintained at all times for the protection of staff and patients. An adequate sputum specimen can be obtained in 60–100% of adults and children older than 6 years.[10,19] Sputum induction has been performed in individuals with severe corticosteroid-dedependent asthma[16] and during acute exacerbations of asthma, using isotonic saline in the latter case.[12,20]

The main adverse effect of sputum induction is airflow obstruction caused by inhalation of hypertonic saline. In most circumstances, airflow obstruction can be managed; however, there are certain situations in which severe airflow obstruction can develop. This occurs in patients with unstable asthma, acute asthma, severe asthma, chronic airflow obstruction and severe airway hyperresponsiveness. These effects must be anticipated and carefully managed. This can be done by assessing asthma control and severity before the sputum induction begins, monitoring lung function periodically during the induction, and having skilled staff who can detect and effectively treat bronchoconstriction when it occurs. Pretreatment with a β_2-agonist delays the onset of bronchoconstriction and reduces its severity. However, it does not obviate the need to monitor lung function. Sputum examination can be performed either on samples selected from saliva[10] or by examining the whole expectorate.[6,21] Both methods have been validated, although the selection method leads to less squamous contamination and a higher concentration of cells and eosinophil cationic protein (ECP).[22] To ensure good cell viability, sputum should be processed within 2 hours of collection. Cells are then dispersed from surrounding mucus with dithiothreitol, to give a single cell suspension that is suitable for preparing cytospins for differential cell counts, using cytochemistry and immunohistochemistry. The cell-free supernatant can be stored and mediators of inflammation measured.

Both total and differential cell count measurements in induced sputum are reproducible in the same individual.[21,23,24] Sputum induction has been carried out in healthy volunteers, defining normal ranges for cell indices in adults[25] and children;[9] this is essential if sputum is to be used to assess clinical disease. In healthy normal volunteers, macrophages and neutrophils pre-

dominate, while eosinophils represent less than 2.75% of cells (table I). Sputum eosinophils are the best-validated measure of disease in induced sputum.

1.2 Relationship between Inflammation Markers in Induced Sputum and Clinical Disease

Sputum eosinophilia has long been associated with asthma. Eosinophils release pro-inflammatory cytokines and mediators which are thought to play a crucial role in the pathogenesis of asthma. Sputum eosinophilia has been noted in up to 80% of corticosteroid-naive asthmatics[24] and sputum eosinophils, ECP, albumin and fibrinogen are elevated in patients with asthma compared with controls.[9,10,14,17,23,24] The intensity of sputum eosinophilia increases with disease activity in asthma. This has been demonstrated following allergen challenge where increases in the number of eosinophils correlate with the magnitude of the late response and airway hyperresponsiveness.[26,27] In trials of corticosteroid withdrawal, increases in sputum eosinophils preceded worsening symptoms and changes in lung function and bronchial responsiveness.[28] Increases in sputum eosinophils have also been associated with natural exacerbations of asthma[29] and occupational asthma.[30] Prognostically, children who have increased methacholine responsiveness but who do not have elevated sputum eosinophils do not demonstrate symptoms of current or past asthma.[31] Allergen avoidance has been shown to reduce sputum eosinophil numbers in children.[32]

However, there is at best a weak relationship between sputum eosinophils or ECP and asthma when assessed by lung function, symptoms or bronchial responsiveness.[10,18,33-35] This observation reflects the heterogeneity and complexity of the pathophysiological mechanisms in asthma and may make the direct measurement of airway inflammation in asthma more important as an additional measure of disease activity. There are now two clinical situations where the heterogeneity of airway inflammation appears to be important; acute severe asthma and non-eosinophilic asthma. An increase in the number of sputum neutrophils have been observed in patients with acute severe exacerbations of asthma;[36] the neutrophil influx and elastase release in acute asthma is associated with infection of the respiratory tract with virus responsible for exacerbation of asthma.[37] In the latter setting, there is also extensive cell lysis which is reflected by elevated levels of sputum lactate dehydrogenase. This appears to be clinically important, since individuals with more intense inflammation and cell lysis have a longer duration of hospitalization and more severe airflow obstruction.[37]

In chronic corticosteroid-dependent asthma, bronchial biopsies show that individuals can be divided into those with elevated sputum eosinophils and those without; those with elevated sputum eosinophils had thicker basement membranes and more episodes of endotracheal intubation.[38] A similar differentiation is possible with sputum analysis, where the group with non-eosinophilic asthma respond poorly to inhaled corticosteroids.[39] In chronic persistent asthma, induced sputum can therefore identify subgroups of patients with differing responses to corticosteroid therapy.

1.3 Assessment of Treatment Response

Characterizing airway inflammation in patients with asthma may be most relevant in assessing treatment response to corticosteroids. Pavord et al.[39] studied 23 corticosteroid-naive asthmatics and treated them with inhaled budesonide. Participants were divided into those with and without elevated sputum eosinophils (eosinophils >3%). Treatment with budesonide led to a significant fall in eosinophil numbers in patients with elevated sputum eosinophils and a greater increase in bronchial reactivity and symptom score compared with patients with non-eosinophilic inflammation. This has also been seen in chronic obstructive pulmonary disease, where individuals with sputum eosinophilia are more likely to respond to corticosteroids.[40] This suggests that sputum eosinophilia may predict short-term responsiveness to corticosteroids in clinical asthma.

A more difficult question is to determine what is the desired response of sputum eosinophils to inhaled corticosteroids. Treatment with varying doses of inhaled corticosteroids led to a fall in sputum eosinophil numbers that varied from 69–85% from baseline.[41-44] However, the dose-response effect on sputum eosinophils appears to be flat[41] and does not correlate with changes in bronchial responsiveness.[43,44] What remains to be determined is what constitutes a clinically important effect, whether this can be used to measure the effectiveness of anti-inflammatory treatment in both the short and long term, and how this will complement our current methods of assessing disease control in asthma.

In addition, direct measurement of sputum eosinophilia has led to the identification of the entity of eosinophilic bronchitis without asthma as an important cause of chronic cough;[45] this condition may affect 15–20% of individuals with chronic cough presenting to a tertiary referral center[46] and predicts responsiveness of cough to corticosteroids.[47] The absence of sputum eosinophils indicates a lack of response of cough to corticosteroids.[48]

A further application of noninvasive markers is the prediction of success of dose reduction in patients with asthma that is well controlled. When asthma is controlled, therapy should be reduced to the minimum required to maintain such control.[49] The ability to predict which patient will lose asthma control

would be a significant advance over the current clinical approach of trial and error. Sputum eosinophil numbers have a high positive value for diagnosing and predicting loss of control of asthma.[50,51]

2. Exhaled Nitric Oxide

The use of exhaled gases to measure airway inflammation is a recent concept for a noninvasive marker. A number of gases have been measured, including carbon monoxide, hydrogen peroxide and reactive oxygen species. However, the best characterized of these gases in the context of asthma is nitric oxide (NO). NO has numerous effects in the lung as a bronchodilator, vasodilator and neurotransmitter.[52] NO is formed from the amino acid L-arginine, through the action of nitric oxide synthase (NOS). A calcium calmodulin-dependent isoform of NOS is expressed normally in epithelial cells, endothelial cells and macrophages, leading to low basal levels of NO production. Inflammatory stimuli (tumor necrosis factor-α, interferon [IFN]-γ, interleukin-1β and endotoxin) can dramatically increase NO production through the induction of NOS in bronchial epithelial cells, airway smooth muscle cells, fibroblasts, macrophages, neutrophils and eosinophils.[52,53] NO may also promote helper T cell type 2 responses by reducing levels of IFNγ; NO has been shown in animal studies to increase the migration of eosinophils,[54] a fact that is particularly relevant to airway inflammation in asthma.

2.1 Methodology for the Measurement of Exhaled Nitric Oxide

The techniques to measure exhaled nitric oxide (eNO) have been validated in adults, the elderly, children (including infants)[55-57] and intubated or tracheotomized patients,[58] Standardized methodology has been published.[59,60] Generally, eNO is measured via chemiluminescence, using a reaction with ozone to generate light which is measured photometrically. Commercially available analyzers can now detect NO at levels of less than 1 part per billion (ppb) in less than 3 seconds; normal values are thought to be 8–14 ppb. Exhaled air is sampled directly or by using an impermeable reservoir bag.

eNO levels may be affected by a number of technical factors. Oral/nasal contamination, a low exhalation rate and high atmospheric levels of NO increase eNO while a high exhalation rate, plastic tubing, leakage from the reservoir bag, smoking (including passive smoking), recent alcohol intake and caffeine intake decrease eNO.[61] Patients should breathe air free of NO or use a NO scrubber and mouthwash to reduce oral contamination. Nasal contamination is reduced by inhaling through the mouth and then exhaling against an expiratory resistance to close the nasopharynx. A display can be used to illustrate expiratory flow rate so the patient can maintain a flow within the desired range, as a low flow rate may reduce the amount of NO exhaled. At least 6 seconds of exhalation is needed to record a plateau value from which the reading is taken, with three consistent readings performed. The ability to measure eNO online has considerable advantages for clinical practice, and the ability to apply this to children too young to perform lung function tests is an advantage over sputum induction.

2.2 Relationship of Exhaled Nitric Oxide and Clinical Disease

It is well established that levels of eNO are higher in corticosteroid-naive asthma patients compared with healthy individuals.[61] eNO levels increase during acute exacerbations[62] and after allergen challenge[63] and correlates with bronchial hyper- responsiveness and sputum eosinophils.[41] Treatment with corticosteroids leads to a rapid and sustained reduction in eNO;[64,65] however, as in sputum induction there appears to be a plateau effect at doses of budesonide 400 μg/day.[41] In patients with asthma who have been treated with inhaled corticosteroids, the relationship between clinical asthma, lung function and eNO (as well as sputum eosinophils) is less clear.[66,67] Berlyne et al.[68] assessed the value of both eNO and sputum eosinophils in diagnosing asthma (table II). They found the two tests to be of comparable value in diagnosing asthma, but there was a decrease in the sensitivity and specificity of the tests with the use of inhaled corticosteroids. Of particular interest was the observation that in patients with asthma, treatment with inhaled corticosteroid led to an improvement in bronchial responsiveness and a decrease in sputum eosinophils and eNO levels; however, there were no significant correlations between changes in these three markers suggesting that they monitored different aspects of the asthma phenotype.[43]

3. Blood and Serum Measures of Airway Inflammation

While the asthma phenotype is expressed as a disorder of the airways, the recruitment of inflammatory cells to the airways requires general activation of the immune system. The latter effect may be assessed by measurements on blood, serum or even urine samples. Blood and serum measures of airway inflammation have particular relevance in children, in whom there are greater difficulties compared with adults in performing lung function tests, and physicians and parents are reluctant to commit children to long-term inhaled corticosteroid use. Blood and serum markers that have been used to assess disease activity in asthma have again focused on eosinophilic inflammation and in-

clude blood eosinophil numbers or activation markers such as ECP.[69-71] Peripheral blood eosinophil counts are available in all pathology laboratories, are well validated and results can be obtained potentially within a few hours. Serum measures of eosinophil activation have included ECP and eosinophil peroxidase (EPO),[70,71] which can be assayed using commercially available immunoassays. One limitation of these techniques, particularly in the pediatric population, is the requirement of venipuncture. Urinary measures of ECP are also available and may be more widely acceptable for routine disease monitoring.

Peripheral blood eosinophils have been shown to increase in acute exacerbations of asthma,[69] and serum ECP and EPO reflect eosinophil activation.[70] Serum ECP has been found to be higher in symptomatic asthma patients than in asymptomatic patients and individuals without asthma.[71] Elevated levels of both serum ECP and peripheral blood eosinophils predict eosinophilic airway inflammation.[72] Serum ECP has been shown to correlate statistically with pulmonary function and bronchial responsiveness; however, the relationships are not strong and the clinical importance is uncertain.[66,73,74] In addition, Matsumoto et al.[75] demonstrated that serum ECP levels were lower in asthma patients with a longer duration of illness, and who were older, potentially limiting the usefulness of serum ECP levels in assessing chronic asthma in adults. In children with stable asthma in whom ICS has been withdrawn, 71% had a deterioration in symptoms and there was a significant increase in peripheral blood eosinophils, serum ECP and serum EPO, but this did not correlate with FEV_1 or bronchial responsiveness.[76] Lower baseline peripheral blood eosinophil count, urinary eosinophil-derived protein X and eosinophil-derived neurotoxin, serum ECP and EPO were associated with a lower risk of exacerbation in the withdrawal arm and the odds ratio increased with increasing baseline levels. None of these markers of inflammation showed superiority over the others. This suggests that these measures may be useful in research settings, but their place in clinical practice remains to be established. When compared with sputum measures, serum ECP and blood eosinophils were less accurate than sputum eosinophils.[77]

Table II. Sensitivity and specificity (%) of exhaled nitric oxide (eNO) and induced sputum in the diagnosis of asthma[68]

	Not using ICS	Using ICS
eNO		
Sensitivity	81	51
Specificity	90	81
Sputum eosinophilia		
Sensitivity	78	85
Specificity	79	65

ICS = inhaled corticosteroid.

4. Clinical Application of Noninvasive Measures of Airway Inflammation

The key pathophysiological features of asthma are airway hyperresponsiveness and airway inflammation. Currently available treatments target these processes; long-acting β_2-agonists provide effective control of airway hyperresponsiveness while inhaled corticosteroids decrease eosinophilic airway inflammation. Treatment applications in asthma are based upon disease severity.[49] Since asthma is a chronic disease whose severity may change over time, it is important to adjust treatment to appropriately match treatment requirements with disease severity. Failure to do this leads to undertreatment with the associated risk of impaired quality of life and severe exacerbations. Alternatively, overtreatment may occur, with the risk of excessive adverse effects. At present, in patients with asthma, symptoms and lung function are used to monitor severity and adjust treatment. These measures are imperfectly correlated with the underlying pathophysiological processes in asthma, namely airway hyperresponsiveness and airway inflammation. Using objective measures of airway hyperresponsiveness and airway inflammation may lead to better management of asthma. This hypothesis has been partly addressed for measures of airway hyperresponsiveness, and studies are now needed to confirm the benefit of measures of airway inflammation in asthma. The value of objective tests in asthma management was demonstrated by Sont et al.,[78] who randomized patients to inhaled corticosteroid dose titration based on current guidelines (symptoms, lung function) combined with measurement of airway hyperresponsiveness by regular methacholine challenge testing. There were larger improvements in lung function, reduced airway variability, a lower exacerbation rate and, of particular note, a decline in thickness of the lamina reticularis, when treatment was based on measures of airway responsiveness. Both measures of airway inflammation and airway hyperresponsiveness are useful during down-titration of corticosteroids.[50,51,79]

A number of centers have adopted the use of induced sputum in the clinical assessment of asthma[80] and chronic cough.[81] Induced sputum can be used to identify individuals with eosinophilic bronchitis[81] and to assess gastroesophageal and oropharyngeal reflux contributing to respiratory symptoms by detecting the presence of lipid-laden macrophages.[82] In our center we assessed the role of induced sputum eosinophils in the diagnosis and assessment of adult patients referred to a tertiary center for the management of asthma or the diagnosis of episodic respiratory symptoms, such as chronic cough, breathlessness or chest tightness (figure 1, table III).[83] We found that sputum eosinophilia could not be predicted from clinical parameters such as a prior diagnosis of asthma, the pattern of symptoms, lung function

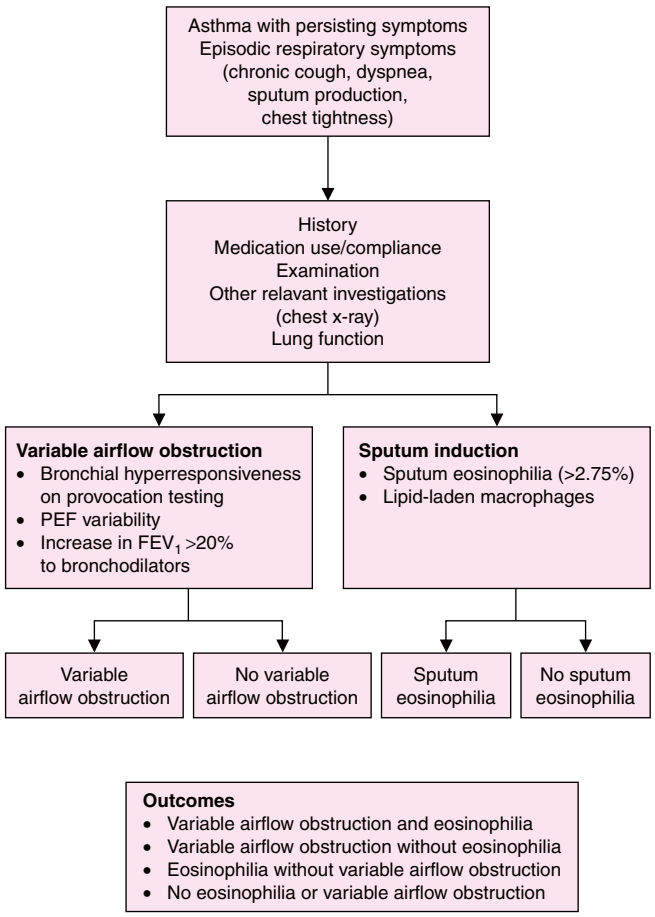

Fig. 1. The use of induced sputum in assessing airway inflammation in patients with persisting episodic respiratory symptoms.

at presentation, nor the presence of variable airflow obstruction (VAO). VAO was defined as bronchial hyperresponsiveness, increased diurnal variability of peak expiratory flow, or a post-bronchodilator FEV_1 response greater than 20%. After a standard clinical assessment and spirometry, patients were subjected to bronchial hyperresponsiveness and induced sputum tests using the combined protocol.[17,18] This evaluation identified four groups of patients, based on the presence or absence of airway inflammation and airway hyperresponsiveness. Patients with both VAO and sputum eosinophilia (asthma with eosinophilic bronchitis) were treated with inhaled corticosteroids. If they were found to be already receiving these medications, compliance was checked along with inhaler technique. If these were found to be adequate, additional anti-inflammatory treatment was offered, such as increasing the dose of the inhaled corticosteroid, a leukotriene antagonist or a trial of oral corticosteroids.

The second group of patients had VAO in the absence of sputum eosinophilia. This finding of non-eosinophilic asthma was more often found in patients with asthma already receiving treatment with inhaled corticosteroids. In these patients it is assumed that eosinophilic inflammation is suppressed by inhaled corticosteroids, and that symptoms occur with persistence of airway hyperresponsiveness. These patients may benefit from additional treatment that controls airway hyperresponsiveness, such as the addition of a long-acting β-agonist. The addition of a long-acting β-agonist to inhaled corticosteroid therapy has been shown to reduce symptoms and improve lung function. Using induced sputum identifies this group in whom increasing intranasal corticosteroid use is less likely to be of benefit and thus avoids increasing the dose of corticosteroid unnecessarily. In fact, in this group of patients it may be possible to decrease the dose of inhaled corticosteroid and use long-acting β-agonists to control symptoms of asthma.

The third outcome was sputum eosinophilia without VAO. These patients have eosinophilic bronchitis without asthma.[45-47] In symptomatic patients, particularly those with chronic cough and sputum eosinophilia, inhaled corticosteroids are effective in controlling symptoms and normalizing sputum eosinophils. It may also be worthwhile investigating the presence of allergic rhinitis and sinusitis in these patients, since these conditions are associated with eosinophilic bronchitis. The long-term outcomes for these patients are not known, but may include the development of asthma.

Finally, patients may be found to have no eosinophilia and no evidence of VAO. These individuals have neither current asthma nor eosinophilic bronchitis, and another cause for their persisting symptoms should be considered. In the case of cough, gastroesophageal reflux is a potential cause and induced sputum may be helpful if lipid-laden macrophages are identified.[82] Other common contributors are chronic sinusitis, postviral cough, upper airway dysfunction or hyperventilation. In the case of ex-smokers, chronic bronchitis should be considered. Sputum neutrophilia may suggest smokers' bronchitis, an occupational irritant, or the presence of ongoing infection. In patients already taking inhaled corticosteroids, such a finding should alert the physician to reviewing carefully the original diagnosis of asthma and perhaps reassessing the patient's condition after a steroid-free interval. It should be emphasized that the clinical and therapeutic implications of these different phenotypes require validation by further research, particularly with respect to the efficacy of treatment options based on inflammatory cell counts.

Alternatively, eNO could be used in place of induced sputum as a surrogate measure of eosinophilic airway inflammation; this may particularly appeal to clinicians involved in pediatric practice, where sputum induction is more difficult and measurement of eNO provides an immediate result. The disadvantage with

Table III. Asthma phenotypes defined by noninvasive testing

	Eosinophilic asthma	Non-eosinophilic asthma	Eosinophilic bronchitis without asthma	Undefined respiratory symptoms
Characteristics				
Symptoms	+	+	+	+
Variable airflow obstruction[a]	+	+	–	–
Eosinophilic bronchitis	+	–	+	–
Possible causes				
	Asthma	Asthma	Rhinitis	Gastroesophageal reflux
	Noncompliance to medication	Smoking	Drug reaction	Rhinitis
	Allergen exposure	Occupational dust exposures		Vocal cord dysfunction
	Corticosteroid-resistant asthma			Smoking
				Hyperventilation
Treatment options				
	ICS	Long-acting β_2-agonist	ICS	According to identified cause
	OCS	Low-dose ICS	OCS	
	Leukotriene receptor antagonist			

a A >20% response of forced expiratory flow in 1 second to bronchodilator therapy, bronchial hyperresponsiveness on provocation testing or increased diurnal variability of peak expiratory flow.

ICS = inhaled corticosteroid; **OCS** = oral corticosteroid; **+** = positive finding; **–** = negative finding.

eNO is its sensitivity particularly to inhaled corticosteroids. In addition, without a measure of airway hyperresponsivenss, eNO alone does not differentiate patients with eosinophilic bronchitis from those with eosinophilia and VAO. To date no reviews have assessed the use of eNO in the diagnosis and management of chronic cough.

5. Conclusion

In recent years several methods that are noninvasive measures of airway inflammation have been developed and validated in research settings. These methods include induced sputum, eNO and serum ECP. Induced sputum eosinophils have a promising place in the assessment of airway inflammation in asthma. The next stage in the development of these techniques is to determine their place in routine clinical practice. By studying airway inflammation in clinical asthma we have gained valuable insight into the heterogeneity of the disorder, particularly given that the relationship between airway inflammation, bronchial reactivity and clinical symptoms of asthma is complex. The response of these measures to treatment suggests that they each measure a specific component of the asthma phenotype. If we are to apply this information clinically, the next stage is to see how best to assess the natural history of asthma and airways disease in the context of both airway inflammation and bronchial responsiveness and to systematically examine the impact of treatment on airway inflammation, bronchial responsiveness and symptom control, both in the short and long term. Such investigation is required to see whether monitoring airway inflammation will be of use in clinical practice, but more importantly it may offer significant benefits for asthma management in the form of tailoring treatment and controlling symptoms of asthma more succinctly thereby limiting potential adverse events as well as maximizing treatment benefit.

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References

1. Chung KF. Chronic inflammation in asthma. Eur Respir Rev 1998; 8: 999-1006
2. Barnes PJ, Pedersen S. Efficacy and safety of inhaled corticosteroids in asthma. Am Rev Respir Dis 1993; 148: S1-26
3. Finney MJB, Anderson SD, Black JL. Terfenadine modifies airway narrowing induced by the inhalation of non-isotonic aerosols in subjects with asthma. Am Rev Respir Dis 1990; 141: 1151-7
4. Umeno E, McDonald DM, Nadel JA. Hypertonic saline increases vascular permeability in the rat trachea by producing neurogenic inflammation. J Clin Invest 1990; 85: 1905-8
5. Davviskas E, Anderson SD, Gonda I, et al. Inhalation of hypertonic saline aerosol enhances mucociliary clearance in asthmatic and healthy subjects. Eur Respir J 1996; 9: 725-32
6. Fahy J, Wong H, Lui J, et al. Comparison of samples collected by sputum induction and bronchoscopy from asthmatic and healthy subjects. Am J Respir Crit Care Med 1995; 152: 53-8
7. Keatings VM, Evans DJ, O'Connor BJ, et al. Cellular profiles in asthmatic airways: a comparison of induced sputum, bronchial washings and bronchoalveolar lavage fluid. Thorax 1997; 52: 372-4

8. Gibson PG, Saltos N, Borgas T. Airway mast cells and eosinophils correlate with clinical severity in corticosteroid-treated asthma. J Allergy Clin Immunol 2000; 105: 752-9
9. Cai Y, Carty K, Henry RL, et al. Persistence of sputum eosinophilia in children with controlled asthma compared with healthy children. Eur Respir J 1998; 11: 848-53
10. Pin I, Gibson PG, Kolendowicz R, et al. Use of induced sputum cell counts to investigate airway inflammation in asthma. Thorax 1992; 47: 25-9
11. Popov TA, Pizzichinini MMM, Pizzichini E, et al. Some technical factors influencing the induction of sputum for cell analysis. Eur Respir J 1995; 8: 559-65
12. Wark PAB, Simpson JL, Hensley MJ, et al. Safety of sputum induction with isotonic saline in adults with acute severe asthma. Clin Exp Allergy 2001; 31: 1-10
13. Bacci E, Cianchetti S, Paggiaro PL, et al. Comparison between hypertonic and isotonic saline induced sputum in the evaluation of airway inflammation in subjects with moderate asthma. Clin Exp Allergy 1996; 26: 395-400
14. Holz O, Jorres RA, Koschyk S, et al. Changes in sputum composition during sputum induction in healthy and asthmatic subjects. Clin Exp Allergy 1998; 28: 284-92
15. Gerschman NH, Liu H, Wong HH, et al. Fractional analysis of sequential induced sputum samples during sputum induction: evidence that different lung compartments are sampled at different time points. J Allergy Clin Immunol 1999; 104: 322-8
16. Pizzichini MMM, Pizzichini E, Clelland L, et al. Sputum in severe exacerbations of asthma. Kinetics of inflammatory indices after prednisone treatment. Am J Respir Crit Care Med 1997; 155: 1501-8
17. Gibson PG, Wlodarczyk J, Hensley MJ, et al. Epidemiological association of airway inflammation with asthma symptoms and airway hyperresponsiveness in childhood. Am J Respir Crit Care Med 1998; 158: 136-45
18. Iredale MJ, Wanklyn SA, Phillips IP, et al. Non-invasive assessment of bronchial inflammation in asthma: no correlation between eosinophilia and induced sputum and bronchial responsiveness to inhaled hypertonic saline. Clin Exp Allergy 1994; 24: 940-5
19. Gibson PG, Henry RL, Thomas P. Non-invasive assessment of airway inflammation in children: induced sputum, exhaled nitric oxide, and breath condensate. Eur Respir J 2000; 16: 1008-15
20. Norzila MZ, Fakes K, Henry RL, et al. Interleukin-8 secretion and neutrophil recruitment accompanies induced sputum eosinophil activation in children with acute asthma. Am J Respir Crit Care Med 2000; 161: 769-74
21. in-'t-Veen JC, de Gouw HW, Smits HH, et al. Repeatability of cellular and soluble markers of inflammation in induced sputum from patients with asthma. Eur Respir J 1996; 9: 2441-7
22. Spanevello A, Beghe B, Bianchi A, et al. Comparison of two methods of processing induced sputum: selected versus entire sputum. Am J Respir Crit Care Med 1998; 157: 665-8
23. Gibson PG, Girgis-Gabardo A, Morris MM, et al. Cellular characteristics of sputum from patients with asthma and chronic bronchitis. Thorax 1989; 44: 693-9
24. Pizzichini E, Pizzichini MMM, 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: 308-17
25. Belda J, Leigh R, Parameswaran K, et al. Induced sputum cell counts in healthy adults. Am J Respir Crit Care Med 2000; 161: 475-8
26. Pin I, Freitag AP, O'Byrne PM, et al. Changes in the cellular profile of induced sputum after allergen-induced asthmatic responses. Am J Respir Crit Care Med 1992; 145: 1265-9
27. Fahy JV, Liu J, Wong H, et al. Analysis of cellular and biochemical constituents of induced sputum after allergen challenge: a method for studying allergic airway inflammation. J Allergy Clin Immunol 1994; 93: 1031-9
28. Gibson PG, Wong BJ, Hepperle MJ, et al. A research method to induce and examine a mild exacerbation of asthma by withdrawal of inhaled corticosteroid. Clin Exp Allergy 1992; 225: 525-32
29. Twaddell SH, Gibson PG, Carty K, et al. Assessment of airway inflammation in children with acute asthma using induced sputum. Eur Respir J 1996; 9: 2104-8
30. Lemiere C, Pizzichini MM, Balkissoon R, et al. Diagnosing occupational asthma: use of induced sputum. Eur Respir J 1999 Mar; 13(3): 482-8
31. Pin I, Radford S, Kolendowicz R. Airway inflammation in symptomatic and asymptomatic children with methacholine hyperresponsiveness. Eur Respir J 1993; 6: 1249-56
32. Piacentini GL, Martinati L, Mingoni S, et al. Influence of allergen avoidance on eosinophilic phase of airway inflammation in children with allergic asthma. J Allergy Clin Immunol 1996; 97: 1079-84
33. Ronchi MC, Piragino C, Rosi E, et al. Do sputum eosinophils and ECP relate to the severity of asthma? Eur Respir J 1997; 10: 1809-13
34. Virchow JG, Holscher U, Virchow C. Sputum ECP levels correlate with parameters of airflow obstruction. Am Rev Respir Dis 1992; 146: 604-6
35. Louis RL. Lau C, Bron AO, et al. The relationship between airways inflammation and asthma severity. Am J Respir Crit Care Med 2000; 161: 9-16
36. Fahy JV, Kim KW, Liu J, et al. Prominent neutrophilic inflammation in sputum from subjects with asthma exacerbation. J Allergy Clin Immunol 1995; 95: 843-52
37. Wark PAB, Johnston SL, Moric I, et al. Neutrophil degranulation and cell lysis is associated with clinical severity in virus-induced asthma. Eur Respir J. In press
38. Wenzel SE, Schwartz LB, Langmack EL, et al. Evidence that severe asthma can be divided pathologically into two inflammatory subtypes with distinct physiologic and clinical characteristics. Am J Respir Crit Care Med 1999; 160: 1001-8
39. Pavord ID, Brightling CE, Woltmann G, et al. Non-eosinophilic corticosteroid unresponsive asthma. Lancet 1999; 353: 2213-4
40. Pizzichini E, Pizzichini MM, Gibson PG, et al. Sputum eosinophilia predicts benefit from prednisone in smokers with chronic obstructive bronchitis. Am J Respir Crit Care Med 1998; 158: 1511-7
41. Jatakonen A, Kharitonov S, Lim S, et al. Effect of differing doses of inhaled budesonide on markers of airway inflammation in patients with mild asthma. Thorax 1999; 54: 108-14
42. Lim S, Jatakonen A, Gilbey JM, et al. Effect of inhaled budesonide on lung function and airway inflammation: assessment by various inflammatory markers in mild asthma. Am J Respir Crit Care Med 1999; 159: 22-30
43. van Renson EL, Straathof KC, Veselic-Charvat MA, et al. Effect of inhaled steroids on airway hyperresponsiveness, sputum eosinophils, and exhaled nitric oxide levels in patients with asthma. Thorax 1999; 54: 403-8
44. Gershman NH, Wong HH, Liu JT, et al. Low and high dose fluticasone propionate in asthma; effects during and after treatment. Eur Respir J 2000; 15: 11-8
45. Gibson PG, Dolovich J, Denburg JA, et al. Chronic cough, eosinophilic bronchitis without asthma. Lancet 1989; I: 1346-8
46. Carney IK, Gibson PG, Muree-Allen K, et al. A systematic evaluation of mechanisms in chronic cough. Am J Respir Crit Care Med 1997; 156: 211-6
47. Gibson PG, Hargreave FE, Girgis-Garbardo A, et al. Chronic cough with eosinophilic bronchitis: examination for variable airflow obstruction and response to corticosteroid. Clin Exp Allergy 1995; 25: 127-32
48. Pizzichini MM, Pizzichini E, Parameswaran K, et al. Nonasthmatic chronic cough: no effect of treatment with an inhaled corticosteroid in patients without sputum eosinophilia. Can Respir J 1999; 6: 323-30
49. National Institutes of Health. Expert Panel Report II. Guidelines for the diagnosis and management of asthma. National Asthma Education and Prevention Program. Bethesda (WA), 1997
50. Jones SL, Kittelson J, Cowan JO, et al. The predictive value of exhaled oxide measurements in assessing changes in asthma control. Am J Respir Crit Care Med 2001; 164: 738-43

51. Leuppi JD, Salome CM, Jenkins CR, et al. Predictive markers of asthma exacerbation during stepwise dose reduction of inhaled corticosteroids. Am J Respir Crit Care Med 2001; 163: 406-12
52. Barnes PJ, Kharitonov S. Exhaled nitric oxide: a new lung function test? Thorax 1996; 51: 233-7
53. Hamid Q, Springall DR, Riveros-Mareno V, et al. Induction of nitric oxide synthetase in asthma. Lancet 1993; 342: 1510-3
54. Ferreira HH, Madeiros MV, Lima CS. Inhibition of eosinophil chemotaxis by chronic blockade of nitric oxide synthetase. Eur J Pharmacol 1994; 358: 253-9
55. Byrnes CA, Dinarevic S, Shinebourne EA, et al. Exhaled nitric oxide measurements in normal and asthmatic children. Paediatr Pulmonol 1997; 24: 312-8
56. Wildhaber JH, Dore ND, Uhlig T, et al. Measurements of nitric oxide using the single breath technique with positive expiratory pressure in infants [abstract]. Am J Respir Crit Care Med 1998; 157: A469
57. Salome CM. , Roberts AM, Brown NJ, et al. Exhaled nitric oxide in a population sample of young adults. Am J Respir Crit Care Med 1999; 159: 911-6
58. Gerlach H, Rossaint R, Pappert D, et al. Auto-inhalation of nitric oxide after endogenous synthesis in nasopharynx. Lancet 1994; 343: 518-9
59. Kharitonov S, Alving K, Barnes PJ. Exhaled and nasal nitric oxide measurement: recommendations. Eur Respir J 1997; 10: 1683-93
60. Silkoff PE. Recommendations for standardised procedures for the online and offline measurement of exhaled lower respiratory nitric oxide in adults and children -1999. Am J Respir Crit Care Med 1990; 160: 2104-17
61. Yates DH. Role of exhaled nitric oxide in asthma. Immunol Cell Biol 2001; 79: 178-90
62. Massaro AF, Gaston B, Kita D, et al. Expired nitric oxide levels during treatment of acute asthma. Am J Respir Crit Care Med 1995; 152: 800-3
63. Kharitonov S, O'Connor BJ, Evans DJ, et al. Allergen induced late asthmatic reactions are associated with elevations of exhaled nitric oxide. Am J Respir Crit Care Med 1995; 151: 1894-9
64. Kharitonov S, Barnes PJ, O'Connor BJ. Reduction in exhaled nitric oxide after a single dose of nebulised budesonide in patients with asthma [abstract]. Am J Respir Crit Care Med 1996; 153: A799
65. Kharitonov S, Yates DH, Barnes PJ. Regular inhaled budesonide decreases nitric oxide concentration in the exhaled air of asthmatic patients. Am J Respir Crit Care Med 1996; 153: 454-7
66. Piacentini GL, Bodini A, Costella S, et al. Exhaled nitric oxide, serum ECP and airway responsiveness in mild asthmatic children. Eur Respir J 2000; 15: 839-43
67. Leuppi J D, Salome CM, Jenkins CR, et al. Markers of airway inflammation and airway hyperresponsiveness in patients with well controlled asthma. Eur Respir J 2001; 18: 444-50
68. Berlyne GS, Parameswaran K, Kamada D, et al. A comparison of exhaled nitric oxide and induced sputum as markers of airway inflammation. J Allergy Clin Immunol 2000; 106: 638-44
69. Janson C, Herala M. Blood eosinophil count as a risk factor for relapse in acute asthma. Respir Med 1992; 86: 101-4
70. Venge P. Serum measurements of eosinophil cationic protein [ECP] in bronchial asthma. Clin Exp Allergy 1993; 23 Suppl. 2: 3-7
71. Niimmi A, Amitani R, Suzuki K, et al. Serum measurement of eosinophil cationic protein. Clin Exp Allergy 1998; 28: 233-40
72. Shields MD, Brown V, Stevenson C, et al. Serum eosinophilic cationic protein and blood eosinophil counts for the prediction of the presence of airways inflammation in children with wheezing. Clin Exp Allergy 1999; 29: 1382-9
73. Wever AMJ, Wever-Hess J, Hensgens HE, et al. Serum eosinophil cationic protein [ECP] in chronic asthma: relationship to spirometry, flow-volume curves, PC20, and exacerbations. Respir Med 1994; 88: 613-21
74. Ferguson AC, Vaughan R, Brown H, et al. Evaluation of serum eosinophil cationic protein as a marker of disease activity in chronic asthma. J Allergy Clin Immunol 1995; 95: 23-8
75. Matsumoto H, Niimmi A, Minakuchi M, et al. Serum eosinophil cationic protein levels measured during exacerbation of asthma: characteristics of patients with low titres. Clin Exp Allergy 2001; 31: 637-43
76. Lonnkvist K, Hellman C, Lundahl J, et al. Eosinophil markers in blood, serum and urine for monitoring the clinical course in childhood asthma: impact of budesonide treatment and withdrawal. J Allergy Clin Immunol 2001; 107: 812-7
77. Pizzichini E, Pizzichini MM, Efthimiadis A, et al. Measuring airway inflammation in asthma: eosinophils and eosinophilic cationic protein in induced sputum compared with peripheral blood. J Allergy Clin Immunol 1997; 99: 539-44
78. Sont JK, Willems LN, Bel EHJ, et al. Clinical control and histopathological outcome of asthma when using airway hyperesponsiveness as an additional guide to longterm treatment. Am J Respir Crit Care Med 1999; 159: 1043-51
79. in't Veen JC, Smits HH, Hiemstra PS, et al. Lung function and sputum characteristics of patients with severe asthma during an induced exacerbation by double-blind steroid withdrawal. Am J Respir Crit Care Med 1999; 160: 93-9
80. Jayaram L, Parameswaran K, Sears M. Induced sputum cell counts: their usefulness in clinical practice. Eur Respir J 2000; 16: 150-8
81. Brightling CE, Ward R, Goh KL, et al. Eosinophilic bronchitis is an important cause of chronic cough. Am J Respir Crit Care Med 1999; 160: 406-10
82. Parameswaran K, Anvari M, Efthimiadis A, et al. Lipid laden macrophages in induced sputum are markers of gastric oropharyngeal reflux and possible aspiration. Eur Respir J 2000; 16: 1119-22
83. Wark PAB, Gibson PG, Fakes K. The role of induced sputum eosinophils in the assessment of asthma and chronic cough. Respirology 2000; 5: 51-7

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