Occasional reviews

Association of sputum parameters with clinical and functional measurements in asthma

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Asthma is a clinical entity characterised by a combination of three features: airway obstruction with spontaneous and/or pharmacological reversibility, increased airway responsiveness (BHR), and airway inflammation.[1 2] Asthma originates with airway inflammation which results in the pathological process and ultimately culminates in symptoms.[2]

Airway inflammation has been considered the primary event leading to airway obstruction and hyperresponsiveness.[2] The principal pathological features of asthma include thickening and disorganisation of the tissues of the airway wall with epithelial shedding, deposition of collagen under the basement membrane, hypertrophy/hyperplasia of the smooth muscle, epithelial damage, occlusion of airways by secretion, and infiltration of eosinophils and T lymphocytes.[1] The investigation into the mechanisms involved in the pathogenesis of asthma has been hampered by difficulties in gaining direct access to asthmatic airways for evaluating the inflammatory process. Until recently it has not been possible in clinical practice to measure inflammation directly, and the presence or absence of airway inflammation has been assumed from symptoms, measurement of BHR, and the effect of treatment. The introduction of a method of sputum analysis[3 4] which is a non-invasive safe tool for analysing the cellular and biochemical component of the airway secretions has allowed the direct measurement of airway inflammation in asthma.[3–5] In fact, measurement of induced sputum has been shown to be a repeatable,[6 7] valid,[7 8] and feasible[4 5] method for assessing eosinophilic airway inflammation.

Despite many studies of the possible association between the three features that characterise chronic bronchial asthma using sputum parameters as a measure of airway inflammation,[4 7–34] the interrelations between the sputum cytological and biochemical profile and clinical or functional parameters in asthma remain a matter of debate. This raises the question whether sputum analysis is a useful tool in evaluating the integrated effect (consequences) of inflammation on the functional and clinical phenotypes of asthma. In this review we attempt to address this question.

Association between airway obstruction and sputum parameters

The functional abnormality in asthma is essentially a variable airflow obstruction. The reduction in forced expiratory volume in one second (FEV_1) with a reduced ratio of FEV_1 to vital capacity (VC) is generally sufficient for confirmation of airflow obstruction, but recognition of its variability is based on a substantial improvement in the FEV_1 following an inhaled β adrenergic agent. The association of FEV_1 or FEV_1/VC with sputum parameters is still controversial. In an initial report Gibson *et al*[3] were not able to find any significant correlation between the percentage of sputum eosinophils or metachromatic cells and FEV_1 or FEV_1/VC in asthmatic subjects. Later, Pin *et al*[4] found a significant correlation between baseline FEV_1 and the percentage of sputum eosinophils in a group of asthmatic patients. Furthermore, Virchow *et al*[9] showed that sputum levels of eosinophil cationic protein (ECP) were more closely related to lung function parameters than were serum concentrations of ECP and/or microscopic sputum analysis. Virchow *et al*[9] were able to demonstrate a relationship between sputum ECP concentrations and the degree of airway obstruction in patients with chronic inflammatory airway disease who had not been pretreated with corticosteroids. In the same paper FEV_1, airway resistance, and improvement in FEV_1 after inhalation of 0.2 mg albuterol as a percentage of the baseline values were reported to be related to the sputum ECP concentration in asthmatic subjects.[9] However, this study relied upon a small number of asthmatic patients in whom the indices of asthma severity—such as clinical scores, duration of disease, and bronchial hyperresponsiveness—were not defined. More recently Pizzichini *et al*[10] confirmed the findings of Virchow *et al*,[9] demonstrating that the strength of the correlations between sputum or blood parameters and functional measurements was higher for sputum than for blood. In a study by Keatings *et al*[11] the increased number of sputum eosinophils correlated with

Table 1 Correlation coefficients (r) for relationships between functional data and sputum eosinophil levels in published studies

$PC_{20}FEV_1$	r	FEV_1	r
Foresi *et al*[20]	−0.52	Pin *et al*[22]	−0.71
Pizzichini *et al*[10]	−0.4	Pizzichini *et al*[8]	−0.68
Polosa *et al*[30]	−0.67	Ronchi *et al*[13]	−0.24
Crimi *et al*[14]	NS	Rosi *et al*[17]	NS
Jatakanon *et al*[18]	−0.24	Lim *et al*[15]	−0.63
Ronchi *et al*[13]	−0.34		
Rosi *et al*[17]	NS		
Lim *et al*[15]	−0.66		

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the maximal fall in FEV_1 during the late response to allergen and was accompanied by increased levels of interleukin (IL)-5 and tumour necrosis factor α (TNF-α) in sputum. Many other studies, however, have shown wide ranges of significance and predictability (table 1) for the correlation between sputum eosinophils and baseline FEV_1.[7 8 12–16] Pretreatment with corticosteroids, narrow ranges of FEV_1, or inclusion of healthy subjects or patients with other diseases might be responsible for these discrepancies.

More recently, a statistical approach by factor analysis performed by Rosi *et al*[17] showed that airway obstruction and sputum parameters (eosinophils and ECP) are independent factors or dimensions that separately characterise the status of patients with chronic stable asthma.[17]

Association between bronchial hyperresponsiveness (BHR) and sputum parameters

Several studies have cited evidence that BHR is secondary to epithelial cell damage mediated through products of damage being released by inflammatory cells into the airways during the inflammatory process.[35 36] In fact, the degree of eosinophil activation and degranulation has been considered important in the development of BHR.[35] Changes in BHR have been associated with changes in sputum inflammatory indices even if, as for measures of airway obstruction, the significance of correlations between BHR and sputum parameters has a wide range of variation (table 1).[8 10 12–18 20] The increase in the percentage of eosinophils and in the concentration of ECP after experimental rhinovirus infection in asthmatic subjects was accompanied by a corresponding increase in airway hyperresponsiveness to histamine.[21] Moreover, Pin *et al*[22] found a significant correlation between the magnitude of increase in both eosinophils and metachromatic cells and the decrease in histamine $PC_{20}FEV_1$ 32 hours after allergen inhalation in patients with mild asthma. More recently Shi *et al*[23 24] have reported an increase in BHR accompanying the increase in sputum eosinophils in asthmatic patients after inhalation of IL-4[23] and IL-5,[24] which suggests a direct contribution of eosinophils to the development of BHR. Sulakvelidze *et al*[25] observed that repeated inhalation of low doses of allergen causes airway eosinophilia and an increase in IL-5 concentrations associated with hyperresponsiveness. Gibson *et al*[26] found a strong association between an increased concentration of sputum eosinophils, sputum mast cells, and nasal eosinophils and hyperresponsiveness to hypertonic saline in childhood.

Other studies have found a weaker relationship between sputum parameters and BHR.[7 8 16 18 27] In patients with mild asthma Jatakanon *et al*[18] found a relationship between sputum eosinophils and methacholine $PC_{20}FEV_1$ over a wide range, suggesting not only that a varying degree of airway inflammation may be found in patients with mild asthma, but also that methacholine $PC_{20}FEV_1$ cannot be considered a sensitive marker of airway inflammation in this kind of asthmatic subject. Pizzichini *et al*[8] showed a significant relationship between methacholine $PC_{20}FEV_1$ and eosinophils, eosinophil-derived neurotoxin (EDN), and major basic protein (MBP), but not ECP. The inclusion of healthy subjects and smokers with bronchitis in the correlation could in part explain its high significance; in fact, the correlation was weaker when only asthmatic subjects were analysed.

On the other hand, Spanevello *et al*[7] did not find any correlation between the percentage of eosinophils and airway responsiveness to methacholine. Similarly, Iredale *et al*[27] showed no correlation between eosinophilia of induced sputum and bronchial responsiveness to hypertonic saline. In adolescents with severe asthma[16] the relationship between sputum eosinophils and an asthma severity score which included symptoms and use of medication, baseline FEV_1, and histamine $PC_{20}FEV_1$ was clinically meaningless ($p = 0.06$, $r = 0.46$).

All the above correlations implied the use of simple regression analysis which might have biased the results.[14] By using multiple regression analysis, which is appropriate when the dependency of a variable on a series of independent variables (inflammatory cells) is sought, Crimi *et al*[14] were not able to find any significant relationship between sputum cytological parameters and methacholine $PD_{15}FEV_1$. Rosi *et al*[17] used factor analysis to examine the independence of respiratory function, BHR, and bronchial inflammation in the assessment of patients with chronic stable asthma. The results of that study indicated the lack of correlation between baseline BHR and sputum parameters. Such evidence would further support the usefulness of routine measurement of all these dimensions for the correct assessment of asthma.

Mechanical factors might in part confound the association between airway inflammation and BHR. Skloot *et al*[37] have found that dose-response curves to methacholine may be affected by breathing manoeuvres such as deep inhalation. However, Brusasco *et al*[28] did not find any correlation between methacholine $PD_{50}FEV_1$, with deep inhalation prohibited, and sputum inflammatory cells. Consistent with these data of Brusasco *et al* are the recent observations by Pacini *et al*.[29] It is therefore still uncertain whether breathing manoeuvres are an important confounding factor in the assessment of the relationship between BHR and airway inflammation.

The different responses to individual agonists such as histamine, methacholine, exercise and bradykinin, which are not necessarily closely associated, might be another important confounding factor. Polosa *et al*[30] observed a weak correlation between methacholine $PC_{20}FEV_1$ and the percentage of eosinophils in the sputum ($r = –0.56$, $p = 0.04$) while the correlation between bradykinin $PC_{20}FEV_1$ and the percentage of eosinophils or absolute eosinophils was marked ($r = –0.76$ and $–0.72$). Polosa *et al*[30] therefore hypothesised that the mechanisms by which bradykinin exerts its

effects are particularly sensitive to acute inflammation of the bronchial mucosa, whereas methacholine responsiveness might predominantly be dependent on structural changes of the airways.

Finally, morphological and functional studies have shown that airway wall remodelling[38] and the inability to dilate constricted airways[37] may be responsible for BHR. So far it has been stated that structural changes in the airway walls are probably driven by mediators released by inflammatory cells as a consequence of chronic inflammation, but it is not clear how each of the many changes that occur in the airway wall contributes to altering airway function in asthma.[39]

Association between symptoms and sputum parameters

Pizzichini *et al*[8] found a significantly higher proportion of eosinophils and an increased concentration of ECP, MBP, EDN, and albumin in the sputum of symptomatic asthmatic subjects than in those with few or no symptoms. Eosinophils and fluid phase ECP, EDN, MBP, and albumin were inversely correlated with the symptom score. Fujimoto *et al*[31] and Ronchi *et al*[13] found sputum eosinophilia significantly higher in patients with severe asthma than in those with mild to moderate asthma. However, symptoms of mildly uncontrolled asthma have not always been found to be associated with eosinophilic airway inflammation as measured by sputum analysis. Turner *et al*[12] described an unexpected finding of symptom exacerbation in patients without sputum eosinophilia. Although airway obstruction was significantly more severe in the eosinophilic subjects, the prevalence of symptoms (chest tightness, wheezing, dyspnoea, and cough) did not differ significantly between the eosinophilic and non-eosinophilic patients.

Breathlessness is an important outcome measure in asthma. The association between perception of breathlessness and airway inflammation has been shown by Roisman *et al*[40] who measured eosinophilic infiltration and epithelial shedding in bronchial biopsy specimens from asthmatic patients. The slope of the relationship between the visual analogue score (VAS) and FEV_1 during bradykinin inhalation related negatively to eosinophilic airway inflammation.[40] Similar findings have been reported during hypertonic challenge in steroid treated subjects with severe asthma by measuring sputum eosinophilia.[32] In't Veen *et al*[32] suggested that, despite treatment with inhaled corticosteroids, eosinophilic airway inflammation directly impairs the perception of dyspnoea in patients with severe asthma even if the central effect of a direct inhaled corticosteroid on acuity of perception could not be excluded.[32 41]

Management and treatment

Several authors have investigated the effect of anti-inflammatory treatment on sputum parameters. Claman *et al*[19] found that treatment with prednisone induced an improvement in peak flow related to the percentage decrease of eosinophils in induced sputum. Despite the fact that the magnitude of improvement in FEV_1 with prednisone related to the magnitude of the fall in sputum eosinophils and fluid phase IL-5, Pizzichini *et al*[33] found that treatment with prednisone improved the symptoms, FEV_1 and blood eosinophil and ECP concentrations within one day whereas the sputum indices attenuated more slowly, which suggests that the airway inflammatory response to prednisone does not accurately reflect the clinical and functional responses. Moreover, Fahy *et al*[34] found a dissociation between the beneficial effects of relatively low doses of inhaled corticosteroids on indicators of asthma control and the lack of their effect on indicators of airway inflammation such as sputum eosinophils, ECP, fibrinogen, and mucin-like glycoprotein.

The anti-inflammatory effect of inhaled prostaglandin E_2 (PGE_2) has recently been demonstrated by measuring changes in sputum inflammatory cells after allergen challenge. Gauvreau *et al*[42] showed that pretreatment with inhaled PGE_2 resulted in a suppression of the increase in sputum parameters (eosinophils, $EG2^+$ cells, and metachromatic cells), BHR, and the fall in FEV_1 after allergen challenge.

The anti-inflammatory effect of theophylline has recently been investigated by Minoguchi *et al*[43] who found that withdrawal of theophylline resulted in both an increase in the percentage of eosinophils with its activated ($EG2^+$) form and asthma symptoms and a significant decrease in morning peak expiratory flow (PEF) and FEV_1.

Cysteinyl leukotrienes (LTC_4, LTD_4 and LTE_4) are known to play an important part in the pathogenesis of asthma[44] but the effects of cysteinyl leukotrienes and their receptor antagonists on sputum eosinophils are still a matter of debate. Neither the inhalation of LTD_4[45] nor the administration of montelukast (MK-0476), a cysteinyl leukotriene receptor antagonist,[46] induced changes in the number of sputum eosinophils.

From these results it can be argued that, because it is a non-invasive, relatively safe and repeatable measurement, the analysis of induced sputum may be a practical and useful tool in monitoring the effect of a drug on airway inflammation. The dissociation between the therapeutic effect on clinical or functional parameters and the effect on the indicators of airway inflammation reinforces once again the necessity for a multiple assessment of the features of asthma.

Conclusions

Sputum eosinophilia represents a characteristic feature of asthma and the analysis of induced sputum has now acquired a relevant role in the identification and monitoring of airway inflammation in asthma.

Nonetheless, a wide variation in the clinical meaning of the relationships between sputum parameters and clinical or functional parameters has been observed. In fact, neither symptoms nor lung function nor bronchial hyperresponsiveness can be predicted by meas-

uring sputum cellular or biochemical parameters. This, on the one hand, reinforces the role of sputum analysis in the assessment and monitoring of asthma and, on the other, suggests that no inference can be made about the clinical or functional status from measurement of sputum parameters.

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