Non-invasive markers of airway inflammation in the clinical assessment and management of asthma

Thesis submitted for the degree of Doctor of Medicine at the University of Leicester by Dominick E. Shaw MB ChB MRCP

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1 Statement of work personally performed

I personally helped develop and amend the design of the exhaled nitric oxide case control study. I obtained ethics approval and wrote the detailed trial protocol for this study. I recruited and obtained informed consent for all patients in this study. I personally performed 20% of all the clinical measurements and helped recruit, train and supervise the research nurses who performed the remaining measurements. I performed the initial sputum processing and supervised the technicians who undertook the remaining sputum assays. I also designed the database for this study and entered all the data. I personally undertook all data analysis and interpretation.

I was part of a team that was responsible for patient recruitment and obtaining informed consent for both the observational study and cross sectional study. I helped design the database that these studies used and was involved in both data entry and analysis.

I also designed and submitted a successful application to Asthma UK for funding to set up a new incident airways clinic.
2 Abstract

Asthma is a condition characterised by airway inflammation, variable airflow obstruction and bronchial hyperresponsiveness. Traditionally asthma is assessed by measurements of symptoms and airway function. Recently there has been interest in assessing airway inflammation using non-invasive tests as it has been shown that controlling eosinophilic airway inflammation, as measured in induced sputum in a population of patients with moderate to severe asthma, can lead to a reduction in asthma exacerbations, when compared to current guidelines.

Most patients have mild to moderate asthma and are treated solely in primary care, in a setting not suitable for induced sputum measurements; there exists a need for an easy, safe and inexpensive mechanism for monitoring airway inflammation.

Previous work has demonstrated that the fraction of nitric oxide in the exhaled breath (FE\textsubscript{NO}) is elevated in asthma and that levels decrease after steroid use. These papers led to an explosion of interest in using FE\textsubscript{NO} as a marker for eosinophilic airway inflammation in asthma. However, few studies have evaluated FE\textsubscript{NO} in a clinical setting and compared its use to management protocols.

This thesis explores the relationship between airway inflammation and asthma, and focuses on induced sputum and FE\textsubscript{NO}. I explore the relationship between sputum eosinophil counts and FE\textsubscript{NO} in an observational study, and use these findings to calculate levels for FE\textsubscript{NO} which best identify the presence and absence of a sputum eosinophilia. These levels are then used in a randomised clinical trial, assessing whether FE\textsubscript{NO} measurements can help predict and prevent asthma exacerbations when compared to current clinical guidelines.

Lastly, a large cross sectional study explores the relationship between pre- and postbronchodilator FEV\textsubscript{1} and measures of airway inflammation, allowing for the effect of confounding factors, using a multivariate analysis.
3 Acknowledgements

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4 Publications arising from this thesis

4.1 Publications


Treatment of Stable Asthma; An atlas of investigation and management. Shaw DE, Haldar P, Pevord ID. Editor; Johnston SL. Clinical Publishing 2007


Non- Invasive assessment of airway inflammation in asthma; an overview. Shaw DE, Pevord ID. Current Respiratory Medicine Reviews 2006; 2(2): 189-196

Measurement of markers of inflammation in induced sputum and exhaled air. Shaw DE, Pevord ID. Allergy and Allergic Diseases. Editors Kay AB, Kaplan AP, Bousquet J, Holt P. 2006

Induced Sputum in Asthma. Shaw DE, Berry MA, Green RH, Pavord ID.
Monitoring Asthma. Editor; Gibson PG. Lung Biology in Health and Disease 2005.
Vol 207; 325-348

Observational study of the natural history of eosinophilic bronchitis. Berry M, 
Hargadon B, McKenna S, Shaw DE, Green RH, Brightling CE, Wardlaw AJ, Pavord 

Asthma exacerbations: prevention is better than cure. Shaw DE, Green RH, 
Bradding P. Therapeutics and clinical risk management. Dec 2005 1(4) 273-277

Alveolar nitric oxide in adults with asthma: evidence of distal lung inflammation 
in refractory asthma. Berry M, Hargadon B, Morgan A, Shelley M, Richter J, Shaw 
986-991

Treatment and assessment of acute asthma; BMJ learning article. Shaw DE, 
Wardlaw AJ published online February 2005

4.2 Abstracts

Inflammometry and improved outcomes with discordant asthma. Haldar P, Shaw 

Eligibility for treatment with Omalizumab among patients attending the Glenfield 
Hospital Refractory Asthma Clinic. Hargadon B, Haldar P, Shaw DE, Berry MA, 


Discordance between sputum eosinophil counts and exhaled nitric oxide levels in asthma; Haldar P, Berry MA, Shaw DE, Green RH, Brightling CE, Wardlaw AJ, Povord ID, Birring SS. ERS 2006 Vol 28 supplement 50; 113s:743


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7 Introduction

Asthma is defined as a “chronic inflammatory disorder of the airways...in susceptible individuals, inflammatory symptoms are usually associated with widespread but variable airflow obstruction and an increase in airway response to a variety of stimuli. Obstruction is often reversible, either spontaneously or with treatment” (1992). Whilst this definition covers the usual combination of symptoms and pathophysiological findings, it tells us little about the clinical course or risk of exacerbation. This is pertinent as in practice only variable airflow obstruction is quantified and measured. Thus, there is a need to develop a disease marker that informs the clinician about a patient’s response to corticosteroid treatment or risk of exacerbation. This is especially important here in the UK, as the UK population suffers from one of the highest rates of asthma in the world: there are 5.2 million people with asthma, affecting one in five households and accounting for at least 12.7 million work days lost each year (Asthma UK 2005). In 2002 asthma was responsible for over 1,400 deaths (one-third of which were in people aged under 65) and one hospital admission every 7.5 minutes. Asthma costs the NHS £889 million/year; the estimated bill for lost productivity is £1.2 billion/year and social benefit costs alone are £260 million/year.

There has been a steady improvement in the pharmacological treatment available for asthma over the last 40 years. In 1969 Ventolin® (salbutamol), a short acting β2 agonist was introduced. This was followed in 1972 by the first of the inhaled corticosteroids, Becotide® (beclomethasone dipropionate). More recently longer acting β2 agonists have been introduced, safer inhaled corticosteroids have been developed and in the last two years a monoclonal anti-IgE antibody has been marketed.
Conversely methods of monitoring asthma, used to diagnose, predict and prevent exacerbations, as well as evaluate response to therapy, have not changed since the first peak flow meter was introduced by Wright and McKerrow in 1959. Although peak expiratory flow (PEF) monitoring is used to help predict and identify exacerbations there remains uncertainty about who should undertake PEF monitoring, and how the results should be interpreted. For example, a recent trial has demonstrated that a combination of a falling peak flow and a doubling dose of inhaled corticosteroid cannot prevent an asthma exacerbation (Harrison et al. 2004). Asthma exacerbations are important as asthma exacerbations cause patients great concern, cost a disproportionate amount (Barnes, Jonsson, & Klim 1996; Hoskins et al. 2000; Van Ganse et al. 2002), and can lead to sudden death. Airway inflammation is prominent in post-mortem studies of asthma related deaths (Dunnill 1960), and the question arises as to whether assessment of airway inflammation using non-invasive techniques might lead to better recognition of at risk patients. This question has become particularly pertinent as it has become clear that the pattern of treatment responsiveness of airway inflammation in asthma is heterogeneous. Moreover, the relationship between airway inflammation, disordered airway function, and symptoms is weak (Pauwels et al. 1997). Recent advances in the non-invasive assessment of airway inflammation have provided the opportunity to try and predict and prevent asthma exacerbations, as well as further clarify the heterogeneity of asthma.

In this thesis I will discuss this heterogeneity, and the importance of airway inflammation in asthma. I will cover methods used to measure airway inflammation, specifically induced sputum and exhaled nitric oxide. The second section will concentrate on a trial evaluating the correlation between eosinophilic airway inflammation and exhaled nitric oxide. The third part of this thesis contains a randomised controlled trial testing the hypothesis that an asthma management plan based on exhaled nitric oxide results in fewer asthma...
exacerbations and less inhaled corticosteroid use, when compared to normal asthma treatment guidelines. The final section will concentrate on the relationship between airway inflammation and lung function in a cross sectional multi-variate analysis.
8 Asthma as a heterogeneous disease

The definition of asthma depends upon four cardinal features: airway inflammation, airway hyperresponsiveness, variable airflow obstruction, and associated symptoms (1992). Within asthma these features can overlap, occur independently or change over time, in response to treatment or other external factors such as allergen and irritant exposure, or viral infection. Some of these features also occur in other airways diseases, notably eosinophilic bronchitis (Gibson et al. 1989) and chronic obstructive pulmonary disease (COPD) (Jeffery 1998), leading to difficulties with diagnosis and treatment decisions. The following section will concentrate upon the importance of these features in the asthma phenotype.

8.1 Airway inflammation

Asthma has been traditionally viewed as a condition where airway inflammation causes airway hyperresponsiveness, which in turn leads to variable airflow obstruction and symptoms. This hypothesis is deeply embedded, to the point where it is incorporated into recent definitions of asthma (2003a). However cross-sectional and longitudinal studies of airway inflammation using sputum induction in large populations with a diverse range of presentations suggest that this hypothesis requires modification.

The role of eosinophilic airway inflammation in the pathogenesis of asthma has been heavily influenced by bronchoscopy studies performed over the last 20 years (Djukanovic et al. 1997). These, by necessity, were largely limited to young volunteers with mild disease. The development of a non-invasive technique to assess airway inflammation has made it possible to relate the presence of airway inflammation to objective measures of disordered airway
function in larger and more heterogeneous populations than was possible with bronchoscopy studies. In general these studies have contradicted findings in the earlier bronchoscopy studies and they have not found a correlation between the sputum eosinophil count and various markers of airway dysfunction (Crimi et al. 1998; Green et al. 2002b; Jatakanon et al. 1999; Rosi et al. 1999).

One surprising observation has been that a subset of patients with symptomatic asthma do not have sputum evidence of eosinophilic airway inflammation (Gibson, Simpson, & Saltos 2001; Green, Brightling, Woltmann, Parker, Wardlaw, & Pavord 2002b; Pavord et al. 1999). Many have a sputum neutrophilia. This sputum profile is evident in corticosteroid-naïve (Green, Brightling, Woltmann, Parker, Wardlaw, & Pavord 2002b) as well as corticosteroid-treated subjects (Jatakanon, Uasuf, Maziak, Lim, Chung, & Barnes 1999; Wenzel 2003) suggesting it is not always an artefact related to treatment. Importantly patients with non-eosinophilic asthma respond less well to inhaled budesonide than a group with more typical sputum features (Green, Brightling, Woltmann, Parker, Wardlaw, & Pavord 2002b). Similar sputum findings have been reported in more severe asthmatics (2003b; Gibson, Simpson, & Saltos 2001; Jatakanon, Uasuf, Maziak, Lim, Chung, & Barnes 1999); Wenzel and colleagues (Wenzel 2003) have identified a subgroup of patients with refractory asthma who have bronchoscopic evidence of neutrophilic airway inflammation, normal eosinophil counts and a normal basement membrane thickness. These findings suggest the presence of a distinct asthma phenotype characterised by a predominantly neutrophilic airway inflammatory response and relative corticosteroid resistance. However, these findings are based on single observations, and in a variable disease there is a clear need to establish whether this asthma phenotype and the associated impaired response to corticosteroid treatment persists in the longer-term.

Thus cross-sectional studies suggest that to a large extent disordered airway function and eosinophilic airway inflammation appear to be independently
regulated, suggesting that the earlier paradigm of a simple causal relationship between them needs to be modified. The patient examples shown in Figures 8.1 and 8.2 illustrate this point.

Within patients there is a relationship between change in airway function and eosinophilic airway inflammation following interventions such as allergen challenge (Pin et al. 1992) and treatment with corticosteroids (Pavord, Brightling, Woltmann, & Wardlaw 1999) suggesting that the relationship between changes in these markers within patients might be closer than the relationship between patients. However, whether changes in eosinophilic airway inflammation are causally linked to changes in airway function has been called into question by recent findings with humanised monoclonal antibodies to IL-5 (Leckie et al. 2000). One study has shown that the antibody causes a profound and long lasting reduction in blood and induced sputum eosinophil counts but has no effect on airway responsiveness, lung function or symptoms before or after allergen challenge (Leckie 2003). In another study there was no evidence of improvement in traditional markers of asthma control in a cohort of patients with more severe asthma who were symptomatic and had disordered airway function despite treatment with high dose inhaled corticosteroids (2003b). One problem in interpreting these studies is that the anti IL-5 antibody only partially reduces the tissue eosinophilia (Leckie 2003) although the effects seen were significant. One view is that the findings with anti-IL-5 monoclonal antibodies suggest that changes in airway function and eosinophilic airway inflammation are independent and that the abnormalities of airway function seen in asthma are causally linked to other aspects of the inflammatory response which, although closely linked to eosinophilic airway inflammation, can be disassociated from it (Brightling & Pavord 2004).

A dissociation between eosinophilic airway inflammation and airway hyperresponsiveness can also be clearly observed in patients with eosinophilic
Figure 8.1

Induced sputum cytospin preparation (top) and diary card recordings (bottom) from a 42-year old asthmatic with severe corticosteroid-dependent but currently stable asthma who had 3 severe exacerbations requiring ventilation in the past 3 years. Despite apparently good clinical control at the time of study, this patient has markedly raised numbers of eosinophils in the sputum (note the eosinophilic staining of the cytoplasm and characteristic bi-lobed nucleus).

<table>
<thead>
<tr>
<th>Week 2 date</th>
<th>12/10</th>
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<tr>
<td>Peak flow AM/PM</td>
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<td>440/490</td>
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Figure 8.2

Induced sputum cytospin preparation (top) and diary card recordings (bottom) from a 22 year old female asthmatic with unstable asthma despite use of inhaled corticosteroids. The majority of cells seen are macrophages. Although this patient does not have a sputum eosinophilia she has unstable asthma.

Week 2 date | 22/11 | 23 | 24 | 26 | 27 | 28 | 29
---|---|---|---|---|---|---|---
Daytime asthma | 2 | 2 | 2 | 2 | 3 | 1 | 1
Night time wakening | 3 | 3 | 2 | 3 | 3 | 2 | 3
Peak flow AM/PM | 290/300 | 210/250 | 200/230 | 300/250 | 280/200 | 350/350 | 400/350
No. of puffs of ventolin | 11 | 15 | 13 | 12 | 16 | 8 | 6

0% eosinophil
bronchitis, a condition characterised by corticosteroid-responsive cough and the presence of a sputum eosinophilia occurring in the absence of variable airflow obstruction or airway hyperresponsiveness (Birring et al. 2003; Brightling et al. 1999a; Carney et al. 1997; Gibson, Dolovich, Denburg, Ramsdale, & Hargreave 1989). Closer study of this condition may be particularly informative since any difference in pathology between the two conditions is likely to give important clues to the features that are relevant to the different functional associations. Recently it was established that several of the traditional characteristics of the immunopathology of asthma, including a submucosal eosinophilia and thickening of the basement membrane and lamina reticularis, are also features of eosinophilic bronchitis and are therefore unlikely to be critical factors causing airway hyperresponsiveness or variable airflow obstruction. Indeed the only difference that was observed in the detailed comparison of the two conditions was increased mast cells within the airway smooth muscle in asthma (Brightling et al. 2002). These findings suggest that localisation of mast cells within the airway wall, rather than the presence of eosinophils in the airway mucosa is the crucial determinant of the functional associations of airway inflammation (Brightling et al. 2003).

Both eosinophilic bronchitis and asthma are associated with cough and it is possible that eosinophilic airway inflammation is directly responsible for this aspect of the asthmatic process. The previous demonstration of a significant correlation between the improvement in cough reflex sensitivity and fall in induced sputum eosinophil count following treatment of subjects with eosinophilic bronchitis with inhaled corticosteroids would be consistent with a causal association (Brightling et al. 2000b). Other reports suggest an increased rate of decline in FEV₁ with the development of fixed airflow obstruction in eosinophilic bronchitis (Brightling et al. 1999b); it is possible that this
complication of chronic asthma is also related to eosinophilic airway inflammation.

8.2 Airway hyperresponsiveness

Airway hyperresponsiveness is considered one of the characteristic features of asthma (Lotvall, Inman, & O'Byrne 1998). Airway hyperresponsiveness is defined as increased sensitivity to an inhaled constrictor agonist and a steeper slope of the dose response curve. Two main forms of bronchoconstrictor stimuli exist: direct and indirect. Direct bronchoconstrictors such as histamine or methacholine stimulate receptors on the airway smooth muscle, whilst indirect ones cause bronchoconstriction by secondary release of bronchoconstrictor mediators from mast cells (i.e. inhaled adenosine monophosphate) or activation of neural pathways (i.e. inhaled sodium metabisulphate).

Airway responsiveness is usually measured as the provocative dose of methacholine causing a 20% fall in FEV₁ by linear interpolation of the log dose response curve (PC₂₀). In the general population the distribution of airway hyperresponsiveness follows a continuous unimodal log-normal distribution, with asthma sufferers representing the hyperresponsive part of the distribution curve. A PC₂₀ is not usually measurable in normals suggesting a large difference in airway responsiveness between normals and patients with asthma. The cut off used to identify asthma is normally a methacholine concentration of <8mg/ml; this value had a sensitivity of 100%, a specificity of 93% and a negative predictive value of 100% in a study on a population of 500 college students with a diagnosis of current symptomatic asthma. The authors concluded that a PC₂₀ of greater than 8mg/ml ruled out current asthma and a PC₂₀ of less than 1mg/ml was almost certainly diagnostic of current asthma. Values in between 1 and 8mg/ml were regarded as intermediate (Cockcroft et al. 1992). In patients with asthma airway responsiveness remains stable over time but can increase during
exacerbations or allergen exposure (Lotvall, Inman, & O'Byrne 1998). Airway responsiveness may occasionally normalise after withdrawal from allergen exposure or after corticosteroid therapy, but persists in the majority of patients even after prolonged appropriate treatment (van Grunsven et al. 1999).

The use of methacholine PC$_{20}$ to diagnose asthma has been evaluated: Hunter and colleagues demonstrated that when asthma was defined as consistent symptoms with objective evidence of abnormal variable airflow obstruction, a positive methacholine challenge was more sensitive than PEF amplitude % mean and the acute bronchodilator response in diagnosis (Hunter et al. 2002). Airway responsiveness has been also been shown to correlate with asthma symptoms, need for medication and peak flow variability (Brand et al. 1991; Cockcroft et al. 1977; Xu et al. 1997). The use of airway responsiveness in addition to symptoms to guide asthma treatment has been shown to result in a reduction in asthma exacerbations, albeit at the expense of increased inhaled corticosteroid use (Sont et al. 1999).

### 8.2.1 Pathophysiology

Various mechanisms are involved in development of airway hyperresponsiveness in asthma. Airway wall thickening is implicated: patients with asthma demonstrate subepithelial thickening (Jeffery et al. 1992) and exudation of plasma (Lotvall et al. 1990). This leads onto airway wall thickening, as the airway luminal resistance induced by a certain degree of airway smooth muscle shortening is then enhanced (Hogg, Pare, & Moreno 1987). Secondly epithelial damage may allow greater exposure of bronchial smooth muscle to bronchoconstrictor mediators and decrease the amount of bronchodilating mediators released (Flavahan et al. 1985). Other possible mechanisms include both loss of sympathetic innervation to the lung (Singas et al. 1996), and loss of
the bronchoprotective effect of deep inspiration in asthma (Scichilone & Togias 2004).

8.2.2 Airway hyperresponsiveness and eosinophilic airway inflammation

The relationship between eosinophilic airway inflammation and airway hyperresponsiveness is complex. Crimi and coworkers performed measurements of airway hyperresponsiveness and eosinophilic airway inflammation (using bronchial wash, bronchoalveolar lavage and induced sputum) in 71 subjects. They found no significant correlations between eosinophilic airway inflammation and airway hyperresponsiveness (Crimi, Spanevello, Neri, Ind, Rossi, & Brusasco 1998). Some studies in patients with atopic asthma have found weak correlations between eosinophilic airway inflammation and airway hyperresponsiveness. There was a weak inverse correlation \( r=-0.4 \) in a study involving 35 patients with mild asthma (Jatakanon et al. 1998). Similar relationships have been described in patients receiving inhaled corticosteroids (Pizzichini et al. 1996; Polosa et al. 2000). The current view is that although interrelated, eosinophilic airway inflammation and airway hyperresponsiveness are to a large extent independently regulated. Support for this view comes from a factor analysis of 99 patients with mild asthma (Rosi, Ronchi, Grazzini, Duranti, & Scano 1999), and from the recognition that eosinophilic airway inflammation can occur without airway hyperresponsiveness in patients without functional airway abnormalities seen in asthma, but with a corticosteroid responsive cough and associated eosinophilic airway inflammation. (Gibson, Dolovich, Denburg, Ramsdale, & Hargreave 1989).
8.3 Variable airflow obstruction

Variable airflow obstruction has long been considered a hallmark of asthma (Fletcher 1971; Fletcher & Pride 1984). Airflow obstruction and its reversibility to treatment, or variability in response to stimuli, is incorporated in all the asthma guidelines (1992; 2003a; Bousquet 2000). Although variable airflow obstruction forms only part of the asthma phenotype due to its ease of measurement it predominates in diagnosis and assessment. Both peak flow and spirometry accurately reflect changes in large airway calibre and can be easily measured. Variable airflow obstruction, defined as a 12% improvement in FEV₁ either spontaneously, or following administration of a bronchodilator or glucocorticoid, is considered diagnostic of asthma and is currently the most commonly used diagnostic test (1991).

8.3.1 Pathophysiology

Airflow obstruction is normally induced by a variety of stimuli including allergen exposure, exercise, cold air and dust. Four mechanisms all thought to relate to underlying airway inflammation have been implicated. Firstly allergen exposure leads to an IgE-dependent release of mediators from airway mast cells, including histamine and prostaglandins, leading onto the early asthmatic response. Other stimuli cause combinations of direct contraction of smooth muscle, mediator release from cytokine “primed” inflammatory cells, and stimulation of local and central neural reflexes. All these responses lead to contraction of airway wall smooth muscle and airflow obstruction (Holgate 1993). Secondly airway wall oedema can independently cause airflow obstruction. An increase in microvascular permeability and leakage leads to mucosal thickening and swelling of the airway wall outside the smooth muscle. This swelling of the airway wall and loss of elastic recoil pressure contribute to increased resistance to flow in the airway. This component of asthma is referred to as the late asthmatic response and is similar to the reduction in airway calibre that characteristically occurs 6 to
24 hours following allergen challenge (Holgate 1993). Thirdly, increased exudation of serum protein and cell debris combined with mucus production leads to plugging of small bronchi with a corresponding increase in airflow resistance and airflow obstruction (James, Pare, & Hogg 1989). Lastly chronic inflammation can cause structural changes in the airway wall matrix and airway wall remodelling (Kips & Pauwels 1999; Pepe et al. 2005).

8.3.2 Airflow obstruction and eosinophilic airway inflammation
A relationship between FEV$_1$ and sputum eosinophils has been demonstrated: Woodruff et al used multivariate analysis of data collected during screening and enrolment of 205 adults with asthma (Woodruff et al. 2001). After controlling for confounding factors, their analysis demonstrated that the induced sputum differential eosinophil count was independently associated with a lower FEV$_1$ and a lower PC$_{20}$; an increased sputum neutrophil percentage was independently associated with lower FEV$_1$ but not with the PC$_{20}$. These results suggest that both eosinophilic inflammation and neutrophilic inflammation independently contribute to abnormalities of FEV$_1$ in asthma. Ten Brinke et al found that the only independent factor associated with persistent airflow limitation was a sputum eosinophilia. Other factors examined included, age at onset, smoking history, atopic status, bronchodilator reversibility, PC$_{20}$ histamine, exhaled nitric oxide, blood eosinophils and total IgE. This was a smaller homogeneous population with a more limited analysis of dichotomous variables; 132 non-smoking asthmatics receiving high dose inhaled corticosteroids were studied and persistent airflow limitation was defined as a postbronchodilator FEV$_1$ or FEV$_1$/FVC less than 75% predicted. The association was not apparent in the subgroup taking oral corticosteroids suggesting that the patients on inhaled corticosteroids may have been undertreated (ten Brinke et al. 2001b). Balzano examined 46 patients diagnosed with a mixture of airways disease; there was a
significant inverse correlation between both FEV₁ and sputum neutrophils, eosinophils and eosinophilic cationic protein. There was also a significant correlation between FEV₁/FVC and the same sputum markers of airway inflammation (Balzano et al. 1999).
9 Asthma control versus severity

One of the difficulties faced by physicians in treating and designing trials for asthma is differentiating between asthma control, severity and exacerbations, terms which are often used interchangeably but mean different things. This difficulty is heightened as patients respond differently to treatment and asthma symptoms can change spontaneously over time. Cockcroft defined good control as minimal symptoms with minimal use of rescue β₂ agonist with near normal lung function, little resting bronchoconstriction and a small response to bronchodilator (Cockcroft & Swystun 1996); this definition fits broadly with the Global Initiative for Asthma (GINA) guidelines for optimal management of asthma. He defined asthma severity as the minimum amount of medication needed to achieve adequate control, rather than defining it purely on symptoms. Thus it would be possible to have a severe but well controlled asthmatic or a poorly controlled mild asthmatic. The former would be on high dose or oral corticosteroids and experience few symptoms, whilst the latter would have symptoms whilst requiring only low dose corticosteroids to alleviate them. These differences become important when recruiting for drug trials, as entrance criteria often require the patient to demonstrate poor control; as asthma is an episodic condition, spontaneous improvement is likely. Reddel also evaluated the differences between asthma control and severity using changes in peak flow variability. The hypothesis that asthma exacerbations had the same degree of peak flow variability as episodes of poor asthma control was tested (Reddel et al. 1999). The results showed that poor asthma control and asthma exacerbations differed in their response to short acting β₂ agonist. During periods of poor asthma control (before treatment with inhaled corticosteroids) the average post-bronchodilator peak expiratory flow was 28% higher than the prebronchodilator value, whilst during an asthma exacerbation there was no response to bronchodilator and therefore no difference between pre- and postbronchodilator
peak flow. Most exacerbations were associated with evidence of viral infection; this lead Reddel et al to suggest that asthma exacerbations were not the same as poor asthma control. This study had a high rate of viral exacerbations and did not take into account asthma severity so the view that exacerbations are linked to control still persists.
10 Asthma exacerbations

10.1 Introduction

The incidence of asthma exacerbations in studies varies with the definition used and the baseline severity and control of the population. Criteria used to define an exacerbation have included a drop in peak flow from a pre-determined baseline, need for rescue oral corticosteroids, increase in the use of rescue \( \beta_2 \) agonist, night time awakening and increased symptom scores. Exacerbations are an important feature of asthma and exacerbation frequency is increasingly seen as an important outcome measure in clinical trials (Cockcroft & Swystun 1996).

In the formoterol and corticosteroids establishing therapy (FACET) study, designed to evaluate the benefits of adding a long acting \( \beta_2 \) agonist to different doses of inhaled corticosteroid, a severe exacerbation was defined as an episode requiring treatment with oral corticosteroids, as judged by the investigator, or a decrease in the morning peak flow on 2 consecutive days to more than 30% below the baseline value (established during the run in period) (Pauwels, Lofdahl, Postma, Tattersfield, O'Byrne, Barnes, & Ullman 1997). Mild exacerbations were defined as at least 2 consecutive days with a peak flow 20% less than baseline, or nocturnal awakening, or 3 additional inhalations of terbutaline, when compared to the run in period. Approximately 850 patients entered the study and were randomised into one of four groups. The total number of severe exacerbations was 425 over a 12-month period, giving an overall exacerbation rate of 0.5 exacerbations/patient/year. The total number of mild exacerbations was 16463. In the Gaining Optimal Asthma Control study (GOAL) the combination of salmeterol/ fluticasone (as Seretide\textsuperscript{®}) was compared to fluticasone alone in 3 different groups of patients over the course of one year; an exacerbation was defined as hospitalisation or as requiring antibiotics or oral
corticosteroids. The baseline demographics revealed that 3416 patients experienced 1832 exacerbations, giving an exacerbation rate of 0.54 exacerbations/patient/year before entry into the study proper (Bateman et al. 2004). These figures demonstrate that severe asthma exacerbations are common and that the addition of a long acting $\beta_2$ agonist reduces asthma exacerbations. The FACET study also revealed that higher dose inhaled corticosteroids have a marked beneficial effect on exacerbation frequency but relatively less effect on symptoms and peak expiratory flow, whereas with the addition of long acting $\beta_2$-agonists the opposite was true (Pauwels, Lofdahl, Postma, Tattersfield, O'Byrne, Barnes, & Ullman 1997; Tattersfield et al. 1999). This indicates that exacerbation frequency does not relate closely to symptoms and measures of disordered airway function, suggesting that the mechanisms responsible for these features of asthma are different (Rosi, Ronchi, Grazzini, Duranti, & Scano 1999). This demonstrates that different strategies are needed to reduce asthma exacerbations, as well as optimise asthma control.

Studies have consistently shown that poorly controlled asthma and asthma exacerbations cost a great deal more than well-controlled asthma; Hoskins et al found the average cost per patient was 3.5 times higher for a patient having an asthma exacerbation, compared to a patient who did not (Hoskins, McCowan, Neville, Thomas, Smith, & Silverman 2000). Similar figures have been presented by Van Ganse and colleagues (Van Ganse, Laforest, Pietri, Boissel, Gormand, Ben Joseph, & Ernst 2002). Barnes and coworkers suggested there was significant scope for cost reduction by improving disease control, as a third of the direct cost of asthma was related to emergency room use, hospitalisation and death (Barnes, Jonsson, & Klim 1996).
10.2 Aetiology

The precise aetiology of asthma exacerbations is still unclear (Hays & Fahy 2003). Viral infections have been associated with asthma exacerbations; in a community based longitudinal study of children with asthma viruses were detected in 80% of the episodes of decreased PEF of increased symptoms (Johnston et al. 1995). This is true in adults; 26% of patients admitted with an exacerbation of asthma had detectable viral load by polymerase chain reaction, as compared to 18% of matched patients with stable asthma (Green et al. 2002c). Bacterial respiratory infections play a less important role in the genesis of asthma exacerbations, although the role of chronic *Mycoplasma pneumoniae* and *Chlamydia pneumoniae* is still debated (Blasi et al. 2002; Cunningham et al. 1998; Kraft et al. 2002). Other recognised factors causing exacerbations include environmental air pollution (Sunyer et al. 1997) and allergen exposure (Green, Custovic, Sanderson, Hunter, Johnston, & Woodcock 2002c). Smoking not only causes exacerbations but cigarette smoke can also reduce the efficacy of inhaled corticosteroids (Chaudhuri et al. 2003).

10.3 Prevention of exacerbations

The current strategy recommended by the British Thoracic Society/ Scotish Intercollegiate Group Network suggests a stepwise approach to control asthma symptoms and exacerbations (2003a). However patients who appear clinically well controlled on inhaled corticosteroids can still have evidence of airway inflammation and airway hyperresponsiveness (Boulet, Turcotte, & Brochu 1994) and be vulnerable to exacerbations, airway remodelling and possibly fixed airways obstruction (Beckett & Howarth 2003). Self management plans advocate doubling the dose of inhaled corticosteroid if the peak flow drops. This approach has been questioned; Harrison et al found that doubling the dose of inhaled corticosteroid, based on a fall in peak flow of >15% from baseline or an increase
in the symptom score from baseline, did not prevent the need for oral corticosteroids (Harrison, Oborne, Newton, & Tattersfield 2004). The authors surmised that a higher dose of inhaled corticosteroid might be needed to prevent an asthma exacerbation. Foresi et al demonstrated that quadrupling the inhaled corticosteroid dose at the onset of an asthma exacerbation had a beneficial clinical effect and reduced the requirement for oral corticosteroids compared to placebo (Foresi, Morelli, & Catena 2000). This suggests that once peak flow or symptoms deteriorate it is not too late for an exacerbation to be prevented. New treatment regimes have been suggested to improve asthma control and prevent asthma exacerbations. O'Byrne et al evaluated the use of budesonide and formoterol (combined as Symbicort®) as both maintenance and reliever therapy (SMART) (O'Byrne et al. 2005) and compared it with budesonide/formoterol fixed dose therapy and high dose budesonide in patients with moderate persistent asthma and poor symptom control despite inhaled corticosteroid therapy. Overall SMART reduced the number of exacerbations when compared to fixed dose therapy. However as noted by Gibson (Gibson 2005) this approach might lead to over or under treatment of asthma so caution is needed when using SMART beyond the study entry criteria.
11 Measuring airway inflammation in asthma

11.1 Background

The goals of asthma management are the accurate diagnosis and effective control of symptoms, including nocturnal symptoms and exercise induced asthma; prevention of exacerbations, and the achievement of best pulmonary function with minimal side effects (2003a). Whilst this is achieved in the majority of patients, there remains a significant number who are misdiagnosed (Robinson et al. 2003) or who suffer from troublesome symptoms and frequent exacerbations (Tattersfield, Postma, Barnes, Svensson, Bauer, O'Byrne, Lofdahl, Pauwels, & Ullman 1999). The routine diagnosis and treatment of asthma in primary care and most secondary care settings in the U.K. involves evaluating variable airflow obstruction with spirometry and peak flow measurement, and assessing symptom control, but does not assess the two other cardinal features of asthma: airway inflammation and airway hyperresponsiveness. None of the currently available diagnostic tests are sufficiently sensitive to rule out asthma (Hunter, Brightling, Woltmann, Wardlaw, & Pavord 2002) with the result that treatment trials are often instigated without good evidence of variable airflow obstruction, airway hyperresponsiveness or airway inflammation. One study has shown that out of 263 subjects referred to a tertiary referral centre with suspected asthma 160 received an alternative diagnosis (Joyce, Chapman, & Kesten 1996). Many of these had received prolonged treatment with potentially toxic therapy before the correct diagnosis was reached. Even in tertiary referral centres the diagnosis of refractory asthma can be difficult to make with certainty (Robinson, Campbell, Durham, Pfeffer, Barnes, & Chung 2003).

As discussed above patients who appear clinically well controlled on inhaled corticosteroids can still have evidence of airway inflammation and airway
hyperresponsiveness (Boulet, Turcotte, & Brochu 1994; Sont et al. 1996) and consequently be vulnerable to exacerbations, airway remodelling and possibly fixed airways obstruction (Beckett & Howarth 2003). The development of feasible and valid non-invasive methods to assess airway inflammation has made it possible to examine whether more accurate diagnoses or better identification of vulnerable patients who need more intensive treatment can be achieved. However in order to be useful, the method used to assess airway inflammation needs to be feasible in a clinical setting and the results need to inform the physician about clinically important aspects of the disease that cannot be discerned by a simpler method.

### 11.2 Feasibility of measuring airway inflammation

Various methods exist to measure markers of airway inflammation. These include the measurement of cells and biomarkers found in induced sputum, the blood eosinophil count (Horn et al. 1975), eosinophilic cationic protein (in blood and sputum), assessment of exhaled gases (including exhaled nitric oxide and carbon monoxide), breath condensate, bronchoalveolar lavage and bronchial biopsy.

Table 11.1 summarises the feasibility of the various approaches to assessing airway inflammation. Sputum induction has been shown to be easy and safe and sputum differential cell counts and mediator concentrations have been demonstrated to be repeatable and responsive in a variety of clinical situations (Green et al. 2002a; Pavord et al. 1997). Sputum induction has the advantage of providing measurements of the type and degree of airway inflammation (eosinophilic vs. neutrophilic). The technique is inexpensive, although is labour intensive and does require training and experience to obtain reliable results. There are well-validated methods for induction and processing of induced sputum. Exhaled nitric oxide has the advantage of being simple to measure and
Table 11.1

Feasibility of measuring airway inflammation

<table>
<thead>
<tr>
<th>Procedure</th>
<th>Safety and ease of performing technique</th>
<th>Ease of analysing result</th>
<th>Time to result</th>
<th>Cost</th>
<th>Influence on outcome proved</th>
<th>Potential use</th>
</tr>
</thead>
<tbody>
<tr>
<td>induced sputum</td>
<td>+++</td>
<td>++</td>
<td>3-4 hours</td>
<td>moderate</td>
<td>yes</td>
<td>Secondary care</td>
</tr>
<tr>
<td>Blood eosinophil count</td>
<td>++++</td>
<td>+++</td>
<td>30 min</td>
<td>inexpensive</td>
<td>Possible</td>
<td>Secondary care</td>
</tr>
<tr>
<td>eosinophil cationic protein</td>
<td>++++</td>
<td>+++</td>
<td>3-4 hours</td>
<td>moderate</td>
<td>Not proven conclusively</td>
<td>Research</td>
</tr>
<tr>
<td>exhaled nitric oxide</td>
<td>++++</td>
<td>+++</td>
<td>immediate</td>
<td>expensive</td>
<td>Not proven</td>
<td>Research</td>
</tr>
<tr>
<td>carbon monoxide</td>
<td>+++</td>
<td>+++</td>
<td>immediate</td>
<td>inexpensive</td>
<td>Studies awaited</td>
<td>Research</td>
</tr>
<tr>
<td>breath condensate (hydrocarbons)</td>
<td>++</td>
<td>+</td>
<td>moderate</td>
<td>inexpensive</td>
<td>Studies awaited</td>
<td>Research</td>
</tr>
<tr>
<td>BAL, bronchial wash and biopsy</td>
<td>+</td>
<td>+</td>
<td>2 days</td>
<td>moderate</td>
<td>Not proven</td>
<td>Tertiary care</td>
</tr>
</tbody>
</table>
provides an immediate result but does not provide information on the nature of the inflammatory response.
12 Methods of monitoring airway inflammation

12.1 Induced sputum

Induced sputum is safe in both children (Gibson et al. 2002) and adults (Paggiaro et al. 2002), repeatable and inexpensive. It provides a clinically important marker of airway inflammation in the diagnosis of asthma. It can help guide treatment dosing and can reliably predict exacerbations. It can be used to measure a variety of cells, effector mediators, cellular markers and cytokines, leading to new insights into the pathophysiology of airways disease. The development of this technique has been a significant advance in asthma management and has lead to important new insights into the disease.

12.1.1 Methodology

Sputum induction using nebulised hypertonic saline is used to collect respiratory secretions from the airways of patients who do not expectorate spontaneously. It is generally agreed that the central airways are sampled with induced sputum: this view is supported by studies showing a greater proportion of granulocytes in both sputum and bronchial samples compared with bronchoalveolar lavage (Alexis et al. 2000; in V et al. 1998; Moodley, Krishnan, & Laloo 2000) and by the demonstration that sputum induction results in greater clearance of radiolabelled aerosol from the central airways than the peripheral airway (Alexis et al. 2001). There is evidence that increasing the duration of sputum induction leads to sampling of more distal airways (Gershman et al. 1999) although, as yet, the clinical utility of this technique has not been explored. The precise mechanism leading to production of secretions is not known, but it may involve both direct and indirect mechanisms. The increased osmolarity of the airway lining fluid during induction is thought to precipitate production of mucus by the submucosal glands and also increase the vascular permeability of the bronchial mucosa,
resulting in release of pro-inflammatory mediators and further increased mucus production.

A variety of protocols for sputum induction have been published and shown to be safe, provided patients are pre-treated with bronchodilators and monitored carefully (Paggiaro, Chanez, Holz, Ind, Djukanovic, Maestrelli, & Sterk 2002). Risk factors for bronchoconstriction include a low baseline FEV₁ % predicted (de la Fuente et al. 1998), overuse of short acting β₂ agonists (Pizzichini et al. 2002) and poor asthma control (ten Brinke et al. 2001a). Theoretically higher nebuliser output, higher concentration of inhaled saline, a longer duration of saline inhalation and reduced frequency and timing of safety assessment might also influence safety. The use of higher output nebulisers has also been associated with the development of a sputum neutrophilia 24 hours after sputum induction. Whether this is seen with the low output nebulisers is unknown. Current practice is to use a relatively low output ultrasonic nebuliser (output 0.7-0.9 ml/min) since this method has been shown to be successful in various settings (Green, Brightling, McKenna, Hargadon, Parker, Bradding, Wardlaw, & Pavord 2002a; Pavord, Pizzichini, Pizzichini, & Hargreave 1997). Furthermore there are theoretical reasons to suggest that that the risk of bronchoconstriction and the effect of sputum induction on neutrophil counts might be less (Pavord 1998).

Once expectorated, sputum should be processed within 2 hours. There is evidence that sputum can be stored for up to 9 hours in a refrigerator at 4°C or that sputum can be snap frozen for longer without affecting cell counts (Efthimiadis et al. 2002), although experience with these techniques is limited. The whole expectorate or selected sputum plugs can be processed. The latter approach has the advantage of producing better quality cytospins which may result in more repeatable counts (Gershman et al. 1996; Ward et al. 1999). Sputum plugs are selected and centrifuged with dithiothrietol. The total cell count, cell viability and squamous cell contamination are assessed using a
haemocytometer. Differential cell counts are determined by counting 400 leukocytes on an appropriately stained cytospin. Some of the other biomarkers of airway inflammation that have been successfully measured in sputum are shown in Table 12.1.

### 12.1.2 Measurement characteristics of induced sputum

Induced sputum is well validated (Pavord, Pizzichini, Pizzichini, & Hargreave 1997) and normal ranges have been published for large adult populations (Belda et al. 2000;Spanevello et al. 2000). Table 12.2. Age has been shown to influence differential sputum neutrophil counts, with higher values occurring in the older age groups (Thomas et al. 2004). Up to 80% of corticosteroid-naïve patients (Pizzichini, Pizzichini, Efthimiadis, Evans, Morris, Squillace, Gleich, Dolovich, & Hargreave 1996) and 50% of corticosteroid treated patients (Louis et al. 2000) with current asthmatic symptoms have a sputum eosinophil count above the normal range. There is good evidence that the sputum differential eosinophils, macrophage and neutrophil counts and the sputum supernatant concentration of eosinophilic cationic protein, cysteinyl-leukotrienes, prostanoids and IL-8 can be measured repeatably in asthma (Gershman, Wong, Liu, Mahlmeister, & Fahy 1996;in, V et al. 1996;Pizzichini, Pizzichini, Efthimiadis, Evans, Morris, Squillace, Gleich, Dolovich, & Hargreave 1996) and COPD (Brightling et al. 2001). The differential lymphocyte and epithelial cell count and the total cell count are less repeatable. The sputum eosinophil count is responsive in that it increases when asthma worsens (after allergen challenge and following relevant occupational exposures (Lemiere et al. 1999;Pin, Freitag, O'Byrne, Girgis-Gabardo, Watson, Dolovich, Denburg, & Hargreave 1992;Wark et al. 2002)), and decreases when asthma improves with inhaled corticosteroid treatment (Pavord, Brightling, Woltmann, & Wardlaw 1999). There is a suggestion that the sputum eosinophil count is more responsive to change than tissue eosinophil counts following treatment with corticosteroids (Bentley et al. 1996;Pavord, Brightling, Woltmann,
Table 12.1
Cell types and molecular markers that have been successfully measured in induced sputum

<table>
<thead>
<tr>
<th>Cells</th>
<th>Effector Mediators</th>
<th>Cellular Markers</th>
<th>Cytokines</th>
</tr>
</thead>
<tbody>
<tr>
<td>eosinophils</td>
<td>leukotrienes C/D/E_4</td>
<td>eosinophilic cationic protein</td>
<td>Interleukin-8</td>
</tr>
<tr>
<td>neutrophils</td>
<td>prostaglandin D_2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>macrophages</td>
<td>histamine</td>
<td></td>
<td></td>
</tr>
<tr>
<td>lymphocytes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>epithelial Cells</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 12.2
Mean (SD) differential cell counts (%) and total cell count (x 10^6/ml sputum) for all subjects and when sub-grouped by age (from Thomas et al) and in two other studies of normal subjects- Belda et al and Spanevello et al.

<table>
<thead>
<tr>
<th></th>
<th>Total population</th>
<th>0-29 years</th>
<th>30-39 years</th>
<th>40-49 years</th>
<th>50-59 years</th>
<th>60+ years</th>
<th>Belda</th>
<th>Spanevello</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number (male)</td>
<td></td>
<td>66 (24)</td>
<td>17 (9)</td>
<td>12 (4)</td>
<td>13 (3)</td>
<td>13 (2)</td>
<td>11 (6)</td>
<td>96 (53)</td>
</tr>
<tr>
<td>Neutrophil</td>
<td></td>
<td>47.0 (27.0)</td>
<td>26.9 (19.8)</td>
<td>38.4 (19.2)</td>
<td>40.4 (25.2)</td>
<td>69.3 (20.8)</td>
<td>68.5 (20.6)</td>
<td>37.5 (64.0)</td>
</tr>
<tr>
<td>Lymphocyte</td>
<td></td>
<td>1.0 (1.4)</td>
<td>1.0 (1.0)</td>
<td>1.1 (1.3)</td>
<td>0.8 (1.1)</td>
<td>0.6 (0.7)</td>
<td>1.5 (2.8)</td>
<td>1.0 (2.6)</td>
</tr>
<tr>
<td>Eosinophil</td>
<td></td>
<td>0.3 (0.6)</td>
<td>0.5 (0.8)</td>
<td>0.2 (0.5)</td>
<td>0.3 (0.3)</td>
<td>0.2 (0.3)</td>
<td>0.3 (0.6)</td>
<td>0.4 (1.1)</td>
</tr>
<tr>
<td>Macrophage</td>
<td></td>
<td>49.0 (25.2)</td>
<td>65.6 (17.8)</td>
<td>59.5 (16.7)</td>
<td>56.6 (25.2)</td>
<td>27.6 (20.2)</td>
<td>28.5 (18.5)</td>
<td>58.8 (86.1)</td>
</tr>
<tr>
<td>Epithelial Cells</td>
<td></td>
<td>2.5 (3.2)</td>
<td>3.3 (3.2)</td>
<td>1.5 (1.6)</td>
<td>2.3 (1.6)</td>
<td>2.5 (4.4)</td>
<td>2.4 (4.4)</td>
<td>1.6 (4.4)</td>
</tr>
<tr>
<td>Total cell count</td>
<td></td>
<td>2.1 (2.36)</td>
<td>2.9 (2.5)</td>
<td>2.1 (1.5)</td>
<td>1.5 (1.0)</td>
<td>1.6 (1.5)</td>
<td>2.3 (4.1)</td>
<td>4.1 (9.7)</td>
</tr>
<tr>
<td>x 10^6 /ml</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
& Wardlaw 1999) or anti IL-5 (Flood-Page et al. 2003; Leckie, ten Brinke, Khan, Diamant, O'Connor, Walls, Mathur, Cowley, Chung, Djukanovic, Hansel, Holgate, Sterk, & Barnes 2000). Currently no intervention has been consistently shown to decrease the sputum differential neutrophil count. There are theoretical reasons to suggest that the total neutrophil count may be a more responsive measure than the differential count when the sputum differential neutrophil count is high, since the relationship between them becomes relatively flat above a differential neutrophil count of 80% (Neale N et al. 2002).
13  The use of induced sputum in asthma

13.1 Diagnosis
The presence of a sputum eosinophilia in asthma is sufficiently common to suggest that it may have a role in diagnosis. Two studies have directly addressed this question. Hunter et al showed that the validity of a sputum eosinophil count outside the normal range in identifying asthma (defined as consistent symptoms with objective evidence of abnormal variable airflow obstruction) approached the sensitivity and specificity of measurement of airway responsiveness and was better than PEF amplitude % mean and the acute bronchodilator response (Hunter, Brightling, Woltmann, Wardlaw, & Pavord 2002). Smith et al have reported similar findings, although in this study a high exhaled nitric oxide concentration achieved a similarly high diagnostic accuracy (Smith et al. 2004). There is evidence that incorporation of induced sputum into the routine diagnostic work up of patients with asthma might identify patients with a high risk of adverse outcomes more reliably since a high sputum eosinophil count has been associated with exacerbation risk (Jatakanon, Lim, & Barnes 2000). It might also facilitate the recognition of patients with non-eosinophilic asthma allowing the clinician to avoid the unnecessary use of high dose corticosteroids.

13.2 Identification of corticosteroid responsive disease
There is increasing evidence that the presence of a sputum eosinophilia may predict corticosteroid responsiveness. This was first clearly demonstrated in the 1950’s by Morrow-Brown who showed that patients with asthma and an eosinophilia improved with systemic corticosteroids whereas those without a sputum eosinophilia did not (Brown 1958). It has been shown that patients with non-eosinophilic asthma respond less well to inhaled budesonide than a group
with more typical sputum features (Green, Brightling, Woltmann, Parker, Wardlaw, & Pavord 2002b; Pavord, Brightling, Woltmann, & Wardlaw 1999); this is also the case with longer-term corticosteroid treatment in patients with more severe asthma (Green, Brightling, McKenna, Hargadon, Parker, Bradding, Wardlaw, & Pavord 2002a). A sputum eosinophilia is a predictor of a steroid response irrespective of the clinical context: patients with chronic cough and a sputum eosinophilia respond well to inhaled corticosteroids compared to those without an eosinophilia, and similarly patients with COPD and a sputum eosinophilia respond better to corticosteroids than those without (Brightling, Ward, Wardlaw, & Pavord 2000b; Brightling et al. 2000a; Pizzichini et al. 1998; Pizzichini et al. 1999c).

13.3 Management of asthma exacerbations
Several studies have shown that the sputum eosinophil count is an independent variable predicting the occurrence of an asthma exacerbation after inhaled corticosteroid withdrawal (Jatakanon, Lim, & Barnes 2000; Jones et al. 2001; Leuppi et al. 2001). Moreover significant increases in the sputum eosinophil count occur well before the onset of exacerbations (Pizzichini et al. 1999b). Recently a study tested the hypothesis that a management approach that measures and attempts to normalise eosinophilic airway inflammation, as well as minimise symptoms and maximise lung function, might be particularly effective in preventing exacerbations. In a randomised controlled trial, 74 subjects attending outpatients with moderate to severe asthma subjects were randomised to treatment either according to the British Thoracic Society guidelines or to a management strategy where treatment was adjusted according to the sputum eosinophil counts (Green, Brightling, McKenna, Hargadon, Parker, Bradding, Wardlaw, & Pavord 2002a). In the sputum management group decisions about anti-inflammatory treatment were made in accordance with an algorithm based on control of symptoms and maintenance of the sputum eosinophil count at or
below 3% with a minimum dose of anti-inflammatory treatment. The 3% cut-off was chosen because this was previously shown to identify individuals with corticosteroid-responsive asthma (Pavord, Brightling, Woltmann, & Wardlaw 1999). If the sputum eosinophil count was less than 1%, anti-inflammatory treatment was reduced irrespective of asthma control. If the eosinophil count was 1-3%, no changes to anti-inflammatory treatment were made, and if the eosinophil count was greater than 3%, anti-inflammatory treatment was increased. Decisions about changes in bronchodilator treatment were based on individual patients' symptoms, peak expiratory flow readings, and use of rescue β₂ agonists using the same criteria as in the BTS management group. Management decisions were made by an independent individual who was unaware of the clinical characteristics of the patient, and who recorded separate treatment plans to be followed depending on whether the patients' asthma was poorly or well controlled. The strategy based on sputum eosinophil counts achieved significantly better control of eosinophilic related airway inflammation over the twelve-months of the trial (Figure 13.1). There was also an improvement in methacholine PC_{20}. Both management strategies achieved equivalent control of symptoms, quality of life and disordered airway function (Figure 13.1). However, in the sputum management group there was a marked reduction in severe asthma exacerbations and significantly fewer hospital admissions due to asthma exacerbations (Figure 13.2).

This study therefore supports the view that assessment of eosinophilic airway inflammation provides additional important information, necessary for the optimum management of more severe asthma. It also supports the view that there is a causal association between eosinophilic airway inflammation and asthma exacerbations, although it remains possible that airway eosinophilia is a surrogate marker of another airway abnormality, and that these other corticosteroid-responsive abnormalities are more important in the pathogenesis of asthma exacerbations.
Figure 13.1
Comparison of effects of two treatment strategies on symptoms score (assessed by visual analogue score (VAS)). Asthma quality of life questionnaire (AQLQ), $\beta_2$-agonist use to relieve asthma symptoms, peak expiratory flow, and post-bronchodilator FEV$_1$. One strategy (BTS management group) utilised standard guidelines of the British Thoracic Society (BTS) and the other (sputum management group) adjusted the anti-inflammatory treatment with corticosteroids based on the eosinophil counts.
Figure 13.2
Comparison of effects of two treatment strategies on rates of severe exacerbations of asthma. One strategy (BTS guidelines) utilised standard guidelines of the British Thoracic Society and the other (sputum guidelines) adjusted the anti-inflammatory treatment with corticosteroids based on the eosinophil counts.
14 Exhaled nitric oxide

One of the major limitations of sputum induction is that the induction and processing are labour intensive and need to be done relatively quickly. New advances in the preservation of sputum may help circumvent this problem (Kelly, Hargreave, & Cox 2003). A further problem is that it is not possible to get an immediate result, limiting the clinical utility of the technique. There is an important need for simpler measures that could be applicable in primary care settings.

14.1 Background

Nitric oxide (NO) is a widely distributed, highly reactive endogenous regulatory molecule. It was discovered that this free radical was the previously uncharacterised endothelial derived relaxing factor (Ignarro et al. 1987; Palmer, Ferrige, & Moncada 1987). NO has various important functions in the respiratory system, including promoting vascular and bronchial dilatation (Belvisi et al. 1992; Fischer & Hoffmann 1996; Guo et al. 1995; Ialenti et al. 1992; Jain et al. 1993; Maa et al. 2003). It acts through activating cystosolic guanylate cyclase, converting GTP to cyclic GMP. As NO is highly reactive, measures have been indirect and based on determination of L-citrulline (produced when NO is formed from L-arginine) or by determination of nitrite and nitrate, oxidized metabolites of NO. In gas phase NO is stable at low concentrations and in 1991 Gustafsson and colleagues found that endogenous nitric oxide can be measured in exhaled breath (Gustafsson et al. 1991). This was followed by the observations that levels are high in asthma (Alving, Weitzberg, & Lundberg 1993) and decrease after steroid use (Kharitonov, Yates, & Barnes 1996).
14.2 Formation of nitric oxide

The synthesis of NO is mediated by nitric oxide synthases (NOS). Two forms exist: constitutive (cNOS) and inducible (iNOS). The constitutive forms exist as endothelial (eNOS) and neural (nNOS). iNOS has been shown in bronchial epithelial cells, alveolar macrophages (Kobzik et al. 1993; Liu et al. 1998; Tracey et al. 1994; Wang et al. 1998), nasal vascular endothelial cells (Furukawa et al. 1996) and nasal ciliated epithelial cells (Furukawa, Harrison, Saleh, Shennib, Chagnon, & Giaid 1996; Rosbe et al. 1996). iNOS can produce much greater amounts of NO than cNOS (nanomolar concentrations) and increased iNOS is found in airway epithelial cells and in asthma. Guo et al demonstrated that patients with asthma exhibit increased expression of iNOS mRNA in the airways compared with healthy controls (Guo et al. 2000), and that patients using corticosteroids had decreased expression of iNOS protein and mRNA compared with those not receiving corticosteroids.

iNOS (or Type II NO synthase) is coded for by chromosome 17 cen-q. iNOS is not normally expressed in most tissues but is induced in inflammatory, endothelial, epithelial and smooth muscle cells by a variety of pro-inflammatory cytokines including tumour necrosis factor α, interferon-γ and interleukin-1β. (Denis 1991; Ding, Nathan, & Stuehr 1988; Morris, Jr. & Billiar 1994; Stuehr et al. 1991). Unlike the constitutive isoforms of NOS, iNOS is not dependent upon calmodulin for its action. iNOS binds calmodulin so avidly that its activity is independent of calcium fluxes within the physiological range and its activity is influenced by transcriptional induction from cytokines and other immunological stimuli. STAT-1, a transcription factor, may also be involved (Guo, Comhair, Zheng, Dweik, Eissa, Thomassen, Calhoun, & Erzurum 2000). NF-kappaB (another transcription factor) is up-regulated in allergic inflammation and down-regulated by corticosteroids (Barnes & Adcock 1998).
Each of the three NOS isoforms is present in the airways and can contribute to the formation of NO. In a study on healthy, atopic and asthmatic children NOS2 (iNOS) mRNA was detected in bronchial epithelial cells from all groups, whereas NOS1 (nNOS) mRNA was not detectable and NOS3 (eNOS) mRNA was found in only 36 of the 43 samples obtained. The levels of iNOS correlated with the fractional concentration of nitric oxide in exhaled breath ($F_{ENO}$) measured at a flow of 200mls/sec, adding evidence that the raised NO in the airways is due to iNOS production (Lane et al. 2004).

Other factors may be important in the formation of NO in the airways. At least 2 other mechanisms for the formation of NO have been postulated, including the release of NO from S-nitrosothiols, (this may account for approximately 80% of NO release) (Sheu, Zhu, & Fung 2000) and nitrite protonation to form nitrous acid which releases NO gas with acidification (Stamler et al. 1992).

Genetic factors are known to affect the production of NO. van's Gravesande et al demonstrated a strong relationship between a known functional NOS3 missense sequence variant in the endothelial nitric oxide gene (G894T) and NO level in a cohort of subjects with asthma (van's Gravesande KS et al. 2003). NOS1 polymorphisms also appear to be associated with asthma symptoms and IgE levels (Gao et al. 2000); further work has shown the number of AAT repeats in intron 20 of this gene correlate with NO levels, with a higher number of repeats correlating with lower NO levels (Henriksen et al. 1999).

**14.3 Function of nitric oxide**

NO is a free radical with one unpaired electron and a short half life of 1-5 seconds. NO is also an ubiquitous messenger molecule that is involved in the homeostasis of multiple biological functions. NO is also a pro-inflammatory molecule in the lung. It is produced by alveolar macrophages in response to
stimulation by endotoxins and cytokines (Liu et al. 1997; Robbins et al. 1994). NO also has a toxic effect in the lung where it is oxidised to peroxynitrite, a potent epithelial toxin found in asthmatic airways after allergen exposure. NO has other important functions in the respiratory system, including promoting vascular and bronchial dilatation, mediating ciliary beat frequency, promoting mucus secretion and acting as a neurotransmitter for non-adrenergic, non-cholinergic neurons (Belvisi, Stretton, Yacoub, & Barnes 1992; Belvisi et al. 1995; Fischer & Hoffmann 1996; Guo, De Raeve, Rice, Stuehr, Thunnissen, & Erzurum 1995; Ialenti, Ianaro, Moncada, & Di Rosa 1992; Jain, Rubinstein, Robbins, Leise, & Sisson 1993). Other roles for NO include promoting TH2 lymphocyte proliferation (Barnes & Liew 1995) and acting as a potent mediator of neurogenic oedema in animal models (Ialenti, Ianaro, Moncada, & Di Rosa 1992; Kuo, Liu, & Barnes 1992). The balance of NO activity is controlled by uptake by antioxidant molecules such as haemoglobin and glutathione.

14.4 Measurement of nitric oxide

The fraction of exhaled nitric oxide present in exhaled breath ($F_{ENO}$) can be measured by chemiluminescence. This is the most sensitive method and uses ozone to react with NO and produce nitrogen dioxide. This reaction emits photons in a stoichiometric relationship correlating with the amount of NO present. This allows measurements down to 1 part per billion (ppb) (Lundberg et al. 1996). $F_{ENO}$ can be measured either “offline” or “online”. Online measurement involves the inhalation of NO free air immediately followed by exhalation at a steady flow directly into the measuring apparatus, whereas in offline measurements the exhaled air is collected in a Mylar® balloon and then transported to the NO analyser.

$F_{ENO}$ measurements are influenced by a number of variables. The most crucial is the exhaled flow. As NO is produced continuously in the airways, the
concentration of NO will vary with the flow of exhaled air. Various models have been proposed to elucidate the relationship between flow and NO concentration. Initially a two-compartmental model of NO production was proposed (Silkoff et al. 2000; Tsoukias & George 1998) but this has been superseded by a recent trumpet shaped model. These studies incorporated axial diffusion into a one dimensional model of NO gas exchange in the lungs and predict a significant back diffusion of NO from the airways into the alveolar region, resulting in loss of NO that would therefore not appear in exhaled breath; this may cause an underestimation of both the maximum airway flux and the airway diffusing capacity for NO. This outcome depends upon on a significant production of NO being produced by the small airways (Shin et al. 2004).

The American Thoracic Society/ European Respiratory Journal guidelines 2005 recommend measuring $\text{FeNO}$ at a flow of 50ml/sec (2005). Prior to this joint statement, the different organisations recommended different flows, making study comparison difficult. Other factors are known to affect $\text{FeNO}$ readings. Causes of lung inflammation increase $\text{FeNO}$ levels, including bronchiectasis, viral infection, fibrosing alveolitis, allergic rhinitis, pulmonary tuberculosis, COPD and pulmonary sarcoidosis; however pneumonia (Adrie et al. 2001) and cystic fibrosis (Balfour-Lynn, Laverty, & Dinwiddie 1996) have both been shown to reduce $\text{FeNO}$ levels. Caffeine ingestion and smoking both reduce $\text{FeNO}$ levels, and a nitrate-rich diet increases them. There has been debate about diurnal variation in $\text{FeNO}$ levels, but this aspect is now thought to be minimal (Kharitonov et al. 2003). Atopy is also associated with high $\text{FeNO}$ levels. The relationship is such that in trials using $\text{FeNO}$ as a marker of airway inflammation in asthma, the influence of atopy can be difficult to exclude (Steerenberg et al. 2003). This is complicated by the influence of rhinitis with both asthma and atopy. Levels of NO generated in the nose are much greater than those generated in the lower airway (Tornberg et al. 2002), and nasal contamination could theoretically cause difficulties with measurement. In general exhaled oral NO is thought to reflect lower airway
inflammation rather than upper airway inflammation (caused by atopy or allergic rhinitis) as the flow used when performing $F_{E_{NO}}$ measurements generates an excess pressure in the oral cavity; this is assumed to close the velum and prevent contamination from NO produced in the paranasal sinuses (Isono et al. 1993; Isono et al. 1998).
15 Nitric oxide in asthma

Numerous studies have shown that $F_{ENO}$ is raised in patients with asthma (Kharitonov, Gonio, Kelly, Meah, & Barnes 2003; Pedroletti et al. 2002; Zanconato et al. 2002). $F_{ENO}$ measurements are safe, non-invasive, reproducible and accurate, easy to perform, and have the ability to change in response to therapeutic interventions. Despite immediate optimism that $F_{ENO}$ would prove to be a useful non-invasive marker in asthma, over 10 years later clinical trials are still awaited that demonstrate the benefit of $F_{ENO}$ measurements conclusively in a clinical setting. $F_{ENO}$ measurement is highly reproducible using the NIOX (Aerocrine, Sweden) machine. A recent paper described a standard deviation of 2.11 ppb in a group of 59 patients suggesting that a change in $F_{ENO}$ levels of 4 ppb would indicate a change in the status of airway inflammation (Kharitonov, Gonio, Kelly, Meah, & Barnes 2003).

15.1 Relationship to eosinophilic airway inflammation

The relationship between measurements of eosinophilic airway inflammation and $F_{ENO}$ depends on the population studied. Children have been most extensively studied as $F_{ENO}$ offers obvious advantages over more invasive tests such as induced sputum, bronchoalveolar lavage and endobronchial biopsy. Payne and colleagues examined the relationship between $F_{ENO}$ and eosinophil inflammation in endobronchial biopsies from 31 children with difficult asthma and in 7 control patients, before and after a 2 week course of oral prednisolone. There was a correlation between $F_{ENO}$ and eosinophil score, the relationship being strongest in patients with persistent symptoms after prednisolone (Payne et al. 2001). Similar relationships between $F_{ENO}$ and airway eosinophil counts have been demonstrated in bronchoalveolar lavage fluid (Warke et al. 2002) and induced sputum from children with asthma (Mattes et al. 1999; Piacentini et al. 1999).
Jatakanon et al demonstrated a similar relationship in adults with mild asthma; sputum eosinophil counts correlated with $\text{FE}_{\text{NO}}$ measurements, and both factors had a negative correlation with methacholine $\text{PC}_{20}$ (Jatakanon, Lim, Kharitonov, Chung, & Barnes 1998). Berlyne et al demonstrated a significant but weak correlation between $\text{FE}_{\text{NO}}$ and eosinophil differential counts in subjects with steroid naïve asthma, in subjects with eosinophilic bronchitis without asthma, and in healthy atopic subjects. $\text{FE}_{\text{NO}}$ levels were significantly lower in the subjects with asthma taking steroids compared with those not, despite there being no difference in the sputum cell counts. Their conclusion was that $\text{FE}_{\text{NO}}$ has limited utility as a surrogate clinical measurement for either the presence or severity of eosinophilic airway inflammation, except in steroid-naive subjects (Berlyne & Barnes 2000; Berlyne et al. 2000). Blood eosinophil levels have been found to correlate with $\text{FE}_{\text{NO}}$ in several studies (Covar et al. 2003; Silvestri et al. 1999; Steerenberg, Janssen, de Meer, Fischer, Nierkens, van Loveren, Opperhuizen, Brunekreef, & van Amsterdam 2003; Strunk et al. 2003).

The relationship between $\text{FE}_{\text{NO}}$ and eosinophils is complicated by two studies which found no relationship between airway eosinophils on bronchial biopsy and $\text{FE}_{\text{NO}}$ levels (Lim et al. 2000; Turktas et al. 2003).

### 15.2 Asthma diagnosis

Smith and colleagues suggested that compared to measures of airflow limitation, and using bronchial hyperresponsivness as the gold standard test for asthma, measuring $\text{FE}_{\text{NO}}$ to diagnose asthma had a 88% sensitivity at a level of 20ppb (and a flow of 50ml/sec) in steroid naïve subjects with symptoms suggestive of asthma. This compared favourably with 86% sensitivity for a presence of a differential sputum eosinophil count above 3% and sensitivities of between 0-47% for traditional tests for asthma ($\text{FEV}_1$, $\text{FEV}_1/\text{FVC}$, peak flow variability and peak flow corticosteroid response) (Smith, Cowan, Filsell, McLachlan, Monti-
Sheehan, Jackson, & Taylor 2004). There was a strong positive association between $\text{FE}_{\text{NO}}$ and sputum eosinophils ($r=0.67$, $p<0.001$) and a strong negative association between $\text{FE}_{\text{NO}}$ and $\text{PD}_{15}$ ($r=-0.56$, $p<0.001$).

### 15.3 Asthma treatment

To date, only two studies have successfully used $\text{FE}_{\text{NO}}$ measurements to titrate corticosteroid dosage in subjects with asthma (Pijnenburg et al. 2005; Smith et al. 2005). Smith and coworkers used $\text{FE}_{\text{NO}}$ measurements (at 250ml/sec) to downtitrate inhaled corticosteroids in 92 subjects with asthma. In a single blind trial, subjects were treated according to their $\text{FE}_{\text{NO}}$ measurements or current guidelines. Following a run in period, (of between 3 and 12 months) during which all subjects received 750μg of inhaled corticosteroid, the steroid dose was reduced in the $\text{FE}_{\text{NO}}$ group if the $\text{FE}_{\text{NO}}$ was $<15$ppb. In the control group, steroid reduction was based on current guidelines and only occurred when the subjects had over the course of the previous week achieved all of the following; less than 2 night-time awakenings, a mean peak flow amplitude of $<20\%$, bronchodilator use $<4$ times on 1 or 2 days, minimal asthma symptoms and a $\text{FEV}_1 >90\%$ predicted. If the patients did not fulfil all these criteria, an episode of loss of control was counted. In the $\text{FE}_{\text{NO}}$ group the optimal dose was one dose above the dose at which the subjects $\text{FE}_{\text{NO}}$ was $>15$ppb. In the control group the optimal dose was one dose above the dose at which a loss of control had occurred. These became the optimal doses at which subjects entered the final year long stage of the study. During this stage the steroid dose was increased in the $\text{FE}_{\text{NO}}$ group if the $\text{FE}_{\text{NO}}$ was $>15$ppb. In the control group the optimal dose was increased if a loss of control (defined as for the dose optimisation period) occurred. The steroid dose was reduced on predetermined criteria in both groups, but never below the optimal starting dose. Although there were fewer exacerbations in the $\text{FE}_{\text{NO}}$ group, this did not reach statistical significance; however, overall the $\text{FE}_{\text{NO}}$ managed group used 45% less inhaled corticosteroid.
At first glance the results seem impressive, but closer scrutiny reveals that the dosage difference was mainly due to an increased dose of inhaled steroids in the control group (de Jongste 2005). The study does confirm however that a management plan based on \( \text{FE}_{\text{NO}} \) is safe and practical and may allow steroid reduction without a concomitant increase in asthma exacerbations.

Pijnenburg and colleagues compared a \( \text{FE}_{\text{NO}} \) based algorithm to a symptom based algorithm to titrate inhaled corticosteroid doses in 39 and 45 children respectively. After a one year follow up there was no significant difference in symptom scores, lung function, corticosteroid doses or exacerbation rates between the two groups, but there was a significant improvement in the methacholine \( \text{PD}_{50} \) in the \( \text{FE}_{\text{NO}} \) group compared with the control group (Pijnenburg, Bakker, Hop, & de Jongste 2005).

### 15.4 Asthma exacerbation

Apart from the study by Smith et al, few studies have assessed the use of \( \text{FE}_{\text{NO}} \) in asthma exacerbation prediction in adults. Leuppi et al evaluated potential markers of asthma exacerbations, including \( \text{FE}_{\text{NO}} \), differential sputum eosinophil count and airway hyperresponsiveness to both mannitol and histamine in 50 patients with well controlled asthma (Leuppi, Salome, Jenkins, Anderson, Xuan, Marks, Koskela, Brannan, Freed, Andersson, Chan, & Woolcock 2001). The authors steadily reduced the inhaled steroid dose until the patient had an asthma exacerbation, then looked back to see which measure best predicted the exacerbation. Both measures of airway hyperresponsiveness and sputum eosinophil counts predicted the exacerbation. Although there was an increase in the \( \text{FE}_{\text{NO}} \) prior to an asthma exacerbation, this did not reach statistical significance. In a similar study Deykin and co-authors assessed the ability of sputum eosinophils, airway hyperresponsiveness and \( \text{FE}_{\text{NO}} \) to predict an asthma exacerbation following substitution of a patient’s inhaled steroid for either
salmeterol or placebo (Deykin et al. 2005). Neither FENO nor measures of airway hyperresponsiveness successfully predicted an asthma exacerbation, but both actual and change in baseline differential sputum eosinophil counts did. These studies contrast with a smaller study by Harkins et al who also assessed the ability of FENO to predict an asthma exacerbation. They measured FENO in 22 patients and then followed them for 2 weeks (Harkins, Fiato, & Iwamoto 2004); patients who experienced an exacerbation had a significantly higher FENO.

Another study recently evaluated if patients with asthma, but currently in remission or not experiencing symptoms, had evidence of persistent airways inflammation. Bronchial biopsies were obtained from subjects in clinical remission, subjects with asthma, and healthy control subjects. The presence and/or activation state of eosinophils, mast cells, macrophages, T lymphocytes, IL-5, eotaxin, and inducible nitric oxide synthase were analysed and compared with less invasive indicators of airway inflammation including FENO. Eosinophils, T cells, mast cells, and IL-5 were significantly elevated in the airway mucosa of subjects in remission compared with control subjects; blood eosinophil cell counts, FENO levels, and bronchial response to adenosine-5'-monophosphate all correlated significantly with the quantity of tissue eosinophils. This led the authors to suggest that significant airway remodelling can be found in subjects in clinical remission (van den Toorn et al. 2001), and that FENO may help reflect this process in asymptomatic individuals.

15.5 Nitric oxide as a marker of distal airway inflammation in asthma

The ability to measure cellular inflammation in the distal lung is limited by the clinical tools currently available. Both transbronchial biopsy (Kraft et al. 1996) and post mortem studies (DUNNILL 1960) have revealed inflammation in the distal lung in patients with asthma. Recently various two compartmental models
(Shin, Condorelli, Rose-Gottron, Cooper, & George 2004; Silkoff, Sylvester, Zamel, & Permutt 2000; Tsoukias & George 1998) of exhaled nitric oxide have been developed which allow calculation of the alveolar and bronchial contribution to the overall exhaled nitric oxide airway concentration. These models input FENO concentrations from different flows into complex mathematical algorithms. These calculations provide information on alveolar nitric oxide, bronchial nitric oxide and the airway wall transfer of nitric oxide. These models can be understood in more simple terms if the relationship between FENO flux and concentration is plotted at different flows. Figure 15.1. FENO flux (overall amount) increases with increasing flow; however, at the same time the FENO concentration decreases as the flow increases. This is analogous to water flowing through a heater; as the flow of water increases through the heater the total amount of energy transferred (flux) increases, but the final temperature of the water decreases (concentration). In approximate terms the gradient of the flux at any flow equals the alveolar nitric oxide concentration; if a regression line from this gradient is extrapolated backwards to a point where the flow is assumed to be zero, the bronchial FENO can be calculated. Figure 15.2. More simply put, FENO at a high flow is thought to sample more distal (alveolar) lung, and FENO performed at lower flows is thought to sample proximal (bronchial) lung.

Alveolar NO is elevated in conditions associated with distal lung inflammation, including pulmonary fibrosis (Lehtimaki et al. 2001a) and chronic obstructive pulmonary disease (Hogman et al. 2002). Support for the view that alveolar nitric oxide represents airway inflammation in the more distal and alveolar region of the lung, rather than more proximal and bronchial region, comes from several studies which have evaluated FENO in patients with different severities of asthma and the effect of steroid treatment in patients with asthma. Lehtimaki et al originally demonstrated that patients with nocturnal asthma (i.e. more severe) had elevated alveolar nitric oxide concentrations compared to patients with asthma who suffered from daytime symptoms only (Lehtimaki et al. 2002). The
Figure 15.1
Relationship between NO flux and exhalation flow.
Figure 15.2
Calculation of alveolar and bronchial nitric oxide using linear regression.
authors also demonstrated that inhaled fluticasone decreased bronchial but not alveolar nitric oxide output in patients with mild asthma (Lehtimaki et al. 2001b). Evidence that alveolar nitric oxide is elevated in patients with more severe (refractory asthma) and is modified by oral rather than inhaled corticosteroids has been published (Berry et al. 2005a). Berry and colleagues demonstrated a relationship between bronchoalveolar lavage eosinophil counts and alveolar nitric oxide concentration, but found no relationship between either induced sputum eosinophil counts or bronchial wash eosinophil counts, and alveolar nitric oxide. The authors demonstrated an elevated alveolar nitric oxide concentration in patients with refractory asthma, when compared to patients with less severe asthma; importantly there was no reduction in the alveolar nitric oxide concentration in patients with refractory asthma who received a doubling dose of their inhaled corticosteroids. There was a significant reduction in the alveolar nitric oxide concentration in patients with refractory asthma who received a course of oral prednisolone. Gelb and coworkers also demonstrated a reduction in the alveolar nitric oxide concentration following a course of oral prednisolone in patients with moderate asthma (Gelb et al. 2004). Taken together these studies suggest that alveolar nitric oxide does reflect distal airway inflammation; it also raises the possibility that the pathology of refractory and mild to moderate asthma differ in both site of inflammation and response to steroid therapy.
16 Managing asthma

16.1 Introduction

National and international guidelines recommend a step wise approach to the management of asthma (1992; 2003a). In the British Thoracic Society/Scottish Intercollegiate Group Network (BTS/SIGN) guidelines, increasing or decreasing treatment is dependent upon asthma control. Asthma control is defined as minimal symptoms during the day and night, minimal need for reliever medication, no exacerbations, no limitation of physical activity and normal lung function (in practical terms FEV₁ and/or PEF >80% predicted or best). The overall aim of the guidelines is to step up treatment in order to achieve control.

The approach taken by the Global Initiative for Asthma (GINA) guidelines is more complicated. The definition of asthma severity varies according to whether a patient is on treatment or not. Pre-treatment the severity of a patient's asthma may be classified into one of four steps based on the clinical features present. When a patient is already on treatment, the classification of severity is based on the clinical features present and the step of the daily medication regimen that the patient is currently on. Thus the combination of the current level of symptoms and the current treatment step should enable the establishment of the patient's asthma severity and the corresponding appropriate maintenance treatment. The aim of treatment in the GINA guidelines is to achieve asthma control, which is defined by GINA as minimal chronic symptoms, including nocturnal symptoms, minimal exacerbations, no emergency visits, minimal (ideally no) need for as-needed β₂ agonist, no limitation on activities, PEF circadian variation of less than 20 percent, (near) normal PEF, and minimal (or no) treatment related adverse effects.

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16.2 Treatment Regimes

Although the guidelines differ in terms of defining asthma control and severity, levels of treatment are similar. In essence patients with mild intermittent asthma (BTS/SIGN) or intermittent asthma (GINA) are treated with an inhaled short-acting $\beta_2$ agonist. As severity or symptoms worsen treatment is added in a stepwise fashion, with inhaled corticosteroids forming the backbone of both guidelines.

16.2.1 Short acting $\beta_2$ agonists

Short acting $\beta_2$ agonists (more properly known as intermediate $\beta_2$ agonists) (Cockcroft 2005), such as salbutamol or terbutaline, are widely used for the relief of both symptoms and bronchospasm in asthma. Short acting $\beta_2$ agonists work by attenuating airway smooth muscle contraction; $\beta_2$ receptor activation leads to an increase in cyclic adenosine monophosphate which leads to activation of protein kinase. This in turn inhibits smooth muscle contraction by phosphorylating and inhibiting myosin light chain kinase, leading to airway smooth muscle relaxation. The pharmacokinetics of short acting $\beta_2$ agonists allow the inhaled form of the drug to work faster, at a lower dose, and with fewer side effects when compared to intravenous or oral therapy (Williams, Winner, & Clark 1981). Relatively low doses lead to bronchodilatation (Henriksen & Dahl 1983).

Debate has focused on the safety of short acting $\beta_2$ agonists. Well recognised side effects such as palpitations and headache do occur, but some studies have suggested a link between short acting $\beta_2$ agonist use and mortality. In a nested case control study involving 129 cases and 655 control subjects selected from a cohort of 12301 users of asthma medication, the mortality rate increased significantly with the use of all $\beta_2$ agonists; more so for fenoterol than for albuterol (Suissa et al. 1994). A second study examined 532 asthma deaths and 532 asthma admissions. Mortality was linked to short acting $\beta_2$ agonist use in the
preceding 1-5 years, although not to short acting β2 agonist use in the preceding 4-12 months (Anderson et al. 2005). This apparent excess mortality may be due to patients with more unresponsive severe disease taking greater amounts of short acting β2 agonist, or it may be due to the drug itself. Recently the effects of short acting β2 agonist on the cardiovascular system have been postulated as a possible cause of the observed increased mortality (Salpeter, Ormiston, & Salpeter 2004a). The same authors also suggested that tolerance to the drug’s effect may occur, leading to increased use of the drug in order to achieve the same effect (Salpeter, Ormiston, & Salpeter 2004b).

The therapeutic response to short acting β2 agonists in relation to genetic polymorphisms has recently been examined. Israel et colleagues found that patients with a genetic polymorphism of arginine/arginine, as opposed to glycine/glycine at the amino acid residue 16 of the β2 adrenergic receptor, had worse peak flows. Not only did the arg/arg group have lower peak flows with albuterol use compared with the gly/gly group, but the arg/arg group also had lower peak flows when using albuterol compared to placebo (Israel et al. 2004), raising the possibility that treatment is making a proportion of patients worse (Tattersfield & Hall 2004).

16.2.2 Inhaled glucocorticoids
Glucocorticoids are the most effective anti-inflammatory agents currently available. Glucocorticoids mediate their anti-inflammatory effect via glucocorticoid receptors that modulate inflammatory gene expression by acting as ligand activated transcription factors. Glucocorticoids are lipophilic and enter the cell cytoplasm by means of passive diffusion. After activation the glucocorticoid receptor, which is located predominantly in the cell cytoplasm, enters the nucleus of the cell and binds to specific glucocorticoid response elements in glucocorticoid receptor target genes, resulting in direct or indirect
transcription of target genes. Glucocorticoids therefore suppress inflammation by both increasing the synthesis of a wide range of anti-inflammatory proteins including lipocytin-1 and secretory leukocyte inhibitory protein, and by decreasing transcription of various mediators including the cytokines IL-1β, TNF-α, IL-4, IL-5 and IL-8. Direct interaction of glucocorticoids with other activated transcription factors is also thought to occur.

Inhaled glucocorticoids have been shown to minimise symptoms, improve methacholine PC_{20}, decrease exacerbations and improve lung function (Georgitis 1999). Only low to moderate doses of inhaled glucocorticoids are needed to give a maximal improvement in lung function and PC_{20}. Debate has focused on whether daily inhaled glucocorticoid treatment is needed for the largest population of patients with asthma, those with mild persistent asthma. Boushey and colleagues examined the effects of daily budesonide, zafirlukast and placebo on 225 patients in a year long study (Boushey et al. 2005). All patients were instructed to take a course of inhaled or oral corticosteroids as guided by a symptom based asthma action plan. This meant that the placebo group were regarded as receiving "intermittent" therapy. Primary endpoints were pulmonary function, exacerbation frequency, symptoms and parameters of airway inflammation. Although budesonide improved prebronchodilator FEV₁, the changes in FEV₁ were similar after salbutamol administration between the groups. Budesonide also reduced FE_{NO}, sputum eosinophils and bronchial reactivity; however there was no difference between all 3 groups in terms of exacerbation frequency, asthma control or asthma-related quality of life. The study concluded that patients with mild persistent asthma did not require daily inhaled corticosteroid, and that intermittent therapy was safe. The conclusions of the study were confirmed by a more recent study comparing as required combination therapy (beclomethasone 250microg and albuterol 100microg) with regular inhaled glucocorticoid treatment (beclomethasone 250microg b.d.) (Papi et al. 2007). The combination group had similar peak flow readings, exacerbation
rates and used less inhaled corticosteroid over the 6 months of the study. Although these results challenge the current status quo of therapy for mild persistent asthma, several problems remain, particularly with the study by Boushey et al.: when the data is compared to other studies with similar recruitment criteria there is a significant difference in exacerbation rates between the placebo controlled groups (O'Byrne 2005). Secondly, as these patients have mild asthma there is unlikely to be major changes seen in the end points (peak flow, symptom scores and asthma control questionnaires) in any of the groups. Thirdly, the decrease in markers of airway inflammation may point to a longer term improvement in lung function or exacerbation rate which the trial would be too short to show.

In general inhaled glucocorticoids are extremely safe in adults at the doses at which they are commonly used (Pedersen & O'Byrne 1997). Local side effects do occur: dysphonia (Williamson et al. 1995) and oral candidiasis (Kennedy et al. 2000) can occur due to deposition of inhaled corticosteroid in the oropharynx. Other possible side effects include bruising, skin atrophy (Mak, Melchor, & Spiro 1992) and reduced bone mineral density (Israel et al. 2001). Drugs with high first pass metabolism in the liver such as budesonide and fluticasone have fewer systemic side effects than beclomethasone, but at high doses systemic absorption through the buccal cavity and airway mucosa is an important factor. Newer drugs such as Ciclesonide® may offer better local side effect profiles. As ciclesonide contains an inactive prodrug that liberates the active desisobutyryl-ciclesonide in response to esterases in the airways, it gives fewer local side effects and an extended lung retention time, allowing once daily dosing (Barnes 2004).
16.2.3 Long acting $\beta_2$ agonists

Long acting $\beta_2$ agonists such as salmeterol or formoterol are recommended for patients who have symptoms that persist despite inhaled glucocorticoid therapy. Long acting $\beta_2$ agonists work primarily by relaxing airway smooth muscle, with additional effects on mast cells and vascular permeability (Nelson 1995). The mechanism of the additive beneficial clinical effects of long acting $\beta_2$ agonists and inhaled glucocorticoids together (see below) is yet to be fully clarified. In vitro and in vivo animal data suggests that long acting $\beta_2$ agonists have an anti-inflammatory potential either through $\beta_2$ stimulation or by membrane stabilisation (Kips & Pauwels 2001). Long acting $\beta_2$ agonists may also enhance the effects of steroids by inducing ligand-independent nuclear translocation and activation of glucocorticoid receptors (Eickelberg et al. 1999). Inhaled glucocorticoids can also upregulate $\beta_2$ receptor expression (Baraniuk et al. 1997); this may explain why concomitant therapy with inhaled glucocorticoids is required to preserve long acting $\beta_2$ agonists activity and explain why monotherapy with long acting $\beta_2$ agonists is far less effective (Leblanc et al. 1996).

The beneficial clinical effects of adding long acting $\beta_2$ agonists to inhaled glucocorticoids have been well demonstrated. Greening and colleagues found that in a group with mild to moderate asthma adding salmeterol to beclomethasone 200microg bd improved symptom and peak flow control when compared to a dose of beclomethasone 500microg bd (Greening et al. 1994). Woolcock et al studied patients with moderate to severe asthma; they found that when compared to doubling the dose of baseline inhaled corticosteroid, adding in a long acting $\beta_2$ agonists improved symptoms and lung function (Woolcock et al. 1996). The effect on asthma exacerbations of adding a long acting $\beta_2$ agonist to inhaled glucocorticoids has also been assessed. In the FACET study the addition of formoterol to both low and higher dose inhaled glucocorticoid reduced exacerbation rates when compared to the baseline dose of inhaled glucocorticoid alone (Pauwels, Lofdahl, Postma, Tattersfield, O'Byrne, Barnes, & Ullman 1997).
Another study had similar results with a group of milder patients; adding formoterol resulted in fewer exacerbations when compared to doubling the dose of inhaled glucocorticoids (O'Byrne et al. 2001).

Most of the long acting $\beta_2$ agonists trials require at least 15% improvement in FEV$_1$ as a standard enrolment criterion whereas the true incidence of this degree of reversibility is nearer a third in primary care (Jamison & McKinley 1993), and 5-10% in secondary care (Hunter, Brightling, Woltmann, Wardlaw, & Pavord 2002). This raises important questions about the generalisability of these studies (Green & Pavord 2001).

The side effects of long acting $\beta_2$ agonists are similar to those of short acting $\beta_2$ agonists. Long acting $\beta_2$ agonists have been previously implicated in excess asthma deaths; this increased risk was ascribed to sole therapy with long acting $\beta_2$ agonists. Following a recent review of the Salmeterol Multicentre Research Trial (SMART) the US Food and Drug Administration labelled Serevent® with a box warning of a small but significant increase in asthma related deaths, and encouraged concomitant inhaled corticosteroid use (Lurie & Wolfe 2005). The SMART study demonstrated a small but statistically significant increased risk respiratory or asthma related deaths in the total population receiving salmeterol (Nelson et al. 2006). Further examination of the data reveals that the differences were most significant in patients of African-American origin. Most importantly, this group of patients had poorly controlled asthma prior to study entry, and more severe asthma overall with less than 50% receiving treatment with an inhaled corticosteroid; it is therefore impossible to state whether the increased risk of respiratory or asthma related death was due to treatment with salmeterol (Nelson 1995). In reality, two large multicentre studies of combination therapy (salmeterol/ fluticasone and formoterol/budesonide) have demonstrated a reduction in asthma exacerbations (Bateman, Boushey, Bousquet, Busse, Clark,
16.2.4 Methylxanthines

Methylxanthines (theophylline) function chiefly as bronchodilators, and work via phosphodiesterase inhibition. Bronchodilator effects are seen at serum concentrations of greater than 10mg/ml whilst possible anti-inflammatory effects are thought to occur at lower concentrations (Barnes & Pauwels 1994). Low dose theophylline treatment has a minor influence on chronic airway inflammation (Sullivan et al. 1994), but no effect on airway hyperresponsiveness. Sustained release theophylline and aminophylline have beneficial effects on lung function and asthma symptoms. Sustained release preparations are particularly useful for prevention of nocturnal symptoms, due to their long half life (Weinberger & Hendeles 1996). Theophylline is currently used as an add on therapy in patients who are not controlled on a combination of inhaled corticosteroids and long acting β2 agonist; although theophylline is less expensive than long acting β2 agonists, it is less efficacious and has a worse side effect profile (Davies, Brooks, & Devoy 1998). At higher doses (>10 mg/kg body weight/day), theophylline can cause significant adverse effects, including nausea and vomiting. Serious toxic effects do not normally occur at serum concentrations below 15 μg per ml. The normal approach to dosing and monitoring is to aim for a steady-state serum concentration of between 5 and 15 μg per ml during long-term theophylline treatment. Monitoring of serum concentrations is advised when high-dose theophylline therapy (>10 mg/kg body weight/day) is started.

16.2.5 Leukotriene modifiers

Leukotriene modifiers include cysteinyi leukotriene 1 receptor antagonists (montelukast, pranlukast, zafirlukast) and a 5-lipoxygenase inhibitor (zileuton).
Leukotriene receptor antagonists block the CysLT1 receptors on airway smooth muscle and other cells and thus inhibit the effects of cysteinyl leukotrienes that are released from mast cells and eosinophils; 5-lipoxygenase inhibitors block the synthesis of all leukotrienes. These mechanisms result in a small bronchodilator effect, reduction in allergen and exercise induced bronchoconstriction (Drazen, Israel, & O’Byrne 1999; Lipworth 1999), a small anti-inflammatory effect on eosinophilic airway inflammation (Pizzichini et al. 1999a), and a reduction in asthma exacerbations (Barnes & Miller 2000). The physiological effect seen with leukotriene modifiers is less impressive than that seen with low doses of inhaled glucocorticoids and patients cannot take leukotriene modifiers instead of their normal inhaled glucocorticoid without risking loss of asthma control (Bleecker et al. 2000); however the addition of leukotriene modifiers to inhaled glucocorticoids may improve asthma control (Virchow, Jr. et al. 2000). Leukotriene modifiers are less effective in improving asthma control when compared to long-acting β₂-agonists as add on therapy (Busse et al. 1999). Leukotriene modifiers are well tolerated, and few class-related effects have so far been reported. The occurrence of Churg-Strauss syndrome in association with leukotriene modifier therapy has been explained by the concomitant reduction in the dose of systemic glucocorticosteroids.

16.2.6 Systemic corticosteroids

Systemic corticosteroids (glucocorticoids) have the same mechanism of action as inhaled glucocorticoids. Long term treatment with systemic glucocorticoids is used in a small population of patients with severe persistent asthma despite therapy with combined high dose inhaled glucocorticoids, long acting β₂-agonists, theophyllines and leukotriene antagonists. Oral glucocorticoids such as prednisone, prednisolone, and methylprednisolone are preferred because of their minimal mineralocorticoid effect, relatively short half-life, and limited effects on striated muscle. Long-term therapy with oral glucocorticoids should be given
once in the morning every day or every other day (Dunlap & Fulmer 1984). Side effects of long-term systemic glucocorticoid treatment include osteoporosis, hypertension, diabetes mellitus, adrenal axis suppression, cataracts, glaucoma, obesity, skin thinning and muscle weakness. Oral glucocorticoid therapy should always be combined with continued inhaled glucocorticoid as this may allow a reduction in the systemic dose of glucocorticoid needed to provide asthma control (Adams et al. 2005).

### 16.2.7 Glucocorticoid resistance

Some patients exhibit resistance to glucocorticoid therapy, although true glucocorticoid resistance is extremely rare and is thought to be due to inactivating mutations of the glucocorticoid receptor gene (Leung & Bloom 2003). Resistance to therapy may be relative and respond to a higher dose of glucocorticoid; this may be due to genetic polymorphisms resulting in overproduction of cytokines, or from allergen or concurrent infection induced T cell activation. True and apparent resistance can be distinguished by the presence or absence of glucocorticoid induced side effects. True glucocorticoid resistance affects all tissues, as there is only one glucocorticoid receptor gene, and is not associated with steroid induced side effects. Patients with apparent resistance still exhibit steroid induced side effects as the glucocorticoid resistance is only at the level of the T cell. The commonest explanation for treatment resistance is poor treatment concordance (Cochrane, Horne, & Chanez 1999).

### 16.2.8 Steroid sparing agents

Steroid sparing agents are reserved for patients with refractory asthma whom experience steroid related systemic side effects. Various steroid sparing agents can be used, but evidence for them is weak and they are not included in current guidelines. Most experience has been with methotrexate. A recent Cochrane
review (Davies, Olson, & Gibson 2000) evaluated 10 trials using methotrexate in 185 people. There was a mean reduction in steroid dose, by between 3-4mg, but there was a high incidence of hepatotoxicity; the review concluded that the small potential reduction in steroid used was probably insufficient to reduce steroid related side effects, and routine use of methotrexate could not be warranted. Cochrane reviews have also reached similar conclusions on gold (Evans, Cullinan, & Geddes 2001b), cyclosporin (Evans, Cullinan, & Geddes 2001a), azathioprine (Dean et al. 2004) and troleandomycin (Evans, Cullinan, & Geddes 2001c). These conclusions are based on sparse data and anecdotal evidence continues to suggest that carefully selected patients, with evidence of airway inflammation, may benefit from appropriate steroid sparing agents (Redington et al. 1998).
17 Methods

17.1 Allergen sensitisation

Atopy was assessed by skin prick tests to *Dermatophagoides pteronyssinus*, cat and dog fur, grass and tree pollen, and *Aspergillus fumigatus* with normal saline and histamine controls (Alk-Abello, Berkshire, UK). A positive response to an allergen on the skin prick tests was recorded in the presence of a weal >2mm more than the negative control.

17.2 Spirometry

Spirometry was performed with a Vitalograph spirometer (Vitalograph, Buckinghamshire, UK). The spirometer was calibrated daily by a qualified lung function technician. Bronchodilator reversibility was assessed 15 minutes after administration of 200microg salbutamol inhaled via a Volumatic®. FEV\textsubscript{1} was recorded as the best of 3 successive readings within 100ml.

17.3 Airway hyperresponsiveness

Using the standard Juniper tidal breathing method the concentration of methacholine causing a 20% fall in FEV\textsubscript{1} was recorded as the PC\textsubscript{20} FEV\textsubscript{1} (Juniper et al. 1978). In brief, following the measurement of the baseline FEV\textsubscript{1} subjects inhaled normal saline followed by doubling concentrations of methacholine 0.03-16mg/ml via a Wright’s nebuliser (flow 0.13ml/min driven by dry compressed air). The subject was instructed to breathe quietly (tidal breathing) for 2 min with a nose clip. The FEV\textsubscript{1} was measured 30 and 90s after nebulisation. If the FEV\textsubscript{1} fell less than 20% the procedure was repeated with the next highest concentration. If the FEV\textsubscript{1} fell more than 20% from baseline (or the highest
concentration had been given), no further methacholine was given. Methacholine 
PC_{20} \text{ FEV}_1 concentration was calculated by linear interpolation of the log dose 
response curve. The output of the Wright’s nebuliser was assessed at baseline by 
a qualified lung function technician using the following protocol: 3ml of saline 
was placed into the nebuliser at room temperature. The solution was then 
weighed and the solution nebulised at a flow of 71/min for 2 minutes. This 
process was repeated three times for a range of flow and the average output at 
each flow calculated. The necessary flow was determined to give an output of 
0.13ml/min. The process was repeated at one monthly intervals by the same 
individuals.

17.4 Sputum induction

17.4.1 Instructions for patients

Prior to commencing the induction the procedure is fully explained to the patient 
with the emphasis on the following:
Instruction on spitting out saliva generated during inhalation of saline into a 
“discard“ vessel.
Instruction about blowing their nose, rinsing their mouth and swallowing the 
water prior to trying to expectorate sputum.
Instruction on how to expectorate effectively. It is necessary to demonstrate the 
technique for coughing up sputum and moving sputum form the back of the 
throat, forward to the specimen container.
A reminder not to swallow sputum as it comes up the bronchial tree.
Guidance on posture: sitting straight upright during nebulisation, and leaning 
forward during expectoration.

17.4.2 Protocol

The following protocol was used to obtain sputum from patients:
Measure baseline FEV₁ on 3 occasions.
Give 200µg of salbutamol by MDI and spacer.
After 20 minutes, measure post bronchodilator FEV₁ 3 times. Use the best post-bronchodilator FEV₁ value to calculate any subsequent fall in FEV₁ during the procedure.
Do not proceed if the FEV₁ after inhalation of the short acting β₂ agonist is less than 1 litre.
Fill the nebuliser cup with 5ml of 3% pyrogen-free hypertonic saline. Hold the nebuliser upright and do not adjust from the default maximum output setting.
Ask the patient to breathe tidally, whilst taking a slightly deeper breath every minute. Do not use a nose clip. Discontinue if significant symptoms occur or if the patient experiences undue discomfort. (A discard vessel should be available for the patient to spit out any excessive saliva generated during the induction).
After 5 minutes, ask the patient to rinse their mouth and throat with water and to blow their nose in order to reduce squamous cell contamination and post-nasal drip.
Ask the patient to cough any sputum into a plastic sputum pot using a deep cough. Several attempts at coughing should be made until the sound of the cough becomes dry and unproductive.
Measure FEV₁ (3 measurements will be made if FEV₁ falls by greater than 10% or 200mls (whichever is greater) compared with the best post-bronchodilator FEV₁)
Repeat the above steps on two occasions with 4% and 5% pyrogen-free hypertonic saline, respectively, if the FEV₁ has not fallen by more than 10% or 200ml (which ever is greater) of the best postbronchodilator value. If the FEV₁ falls by more than 10% or 200ml (whichever is greater) but less than 20% or 400ml (whichever is greater), repeat the steps with the same concentration of saline. Patients should not breathe saline for > 15 minutes in total.
If the FEV₁ falls by more than 20% or 400ml (whichever is greater) of the best post-bronchodilator value, or if significant symptoms occur, stop nebulisation and administer repeat short acting β₂ agonist.

17.4.3 Calibration

The manufacturer performed the initial calibration of the mass median diameter and output. Subsequent calibration checks of nebuliser output were performed by a qualified lung function technician using the following protocol: 5ml of 3% hypertonic saline was placed into the nebuliser at room temperature. The nebuliser was then weighed and the solution nebulised for 5 minutes. The nebuliser was reweighed and emptied. The process was repeated 3 times for a range of flows and the average output calculated. The same individual repeated the calibration at monthly intervals.

17.4.4 Safety procedures during sputum induction

As inhaled hypertonic saline is a bronchoconstrictor the process of sputum induction was carried out in a careful safety first manner. Resuscitation equipment and nebulised salbutamol were available and a doctor nearby at all times.

17.4.5 Protocol for sputum processing

The following protocol was used to process and count the sputum samples. Sputum is collected on ice and processed at 4°C within 2 hours of expectoration. Select sputum plugs from saliva and transfer to Petri dish. Transfer sputum free from salivary contamination into an empty (pre-weighed) polypropylene centrifuge tube (opaque) with screw top.
Subtract the weight of the empty centrifuge tube from the weight of the centrifuge tube plus selected sputum to obtain the weight of sputum portion to be processed.

Add dithiothrietol (DTT) freshly diluted to 0.1% (from a stock solution of 1%) using phosphate buffered saline using 4x weight/volume (e.g. 4 ml DTT per gram of selected sputum).

Disperse sputum by repeated gentle aspiration into a plastic pipette, 15 seconds vortex and 15 minutes rocking on a bench rocker on ice.

Add an equal volume of Dulbecco’s phosphate buffered saline (D-PBS). Vortex for a further 15 seconds, filter the sputum suspension through a 48 mm nylon gauze pre-wet flat with D-PBS, shake off excess and centrifuge at 2000 rpm (790 g) for 10 minutes. Aliquot all of the supernatant in 0.5 ml portions into 2 ml microtubes, leaving behind a covering of fluid and the undisturbed pellet. There should be sufficient supernatant for 2-4 microtubes of supernatant.

Resuspend the cell pellet in 0.5 ml to 1 ml of D-PBS (depending on size of cell pellet) and mix gently with a wide bore plastic pipette.

Assess total cell count and cell viability using a Neubauer haemocytometer and the trypan blue exclusion method:

Flood haemocytometer with 10 µl of cell suspension mixed thoroughly with 10µl of 0.4% trypan blue.

Count all cells in the centre square and in the four 1mm corner squares of chamber 1 of the haemocytometer. Cells should be classified as viable, non-viable and squamous.

Calculate the mean number of cells per square and the portion of viable and squamous cells.

Calculate the total number of cells and the total cell count (cells/ml sputum).

Total number of cells = mean number of cells/square x 2 x 10,000 x volume cells resuspended in (ml)

Total cell count (cells/g sputum) = mean number of cells/square x 2 x 10,000 x volume cells resuspended in (ml) / weight of selected sputum (g).
Adjust the cell suspension to 0.5-0.75 x 10^6 cells/ml with D-PBS.
Use 50µl to prepare two cytospins, and centrifuge at 450rpm (18.1 g) for 6 minutes using a Shandon III cytocentrifuge.
Air dry four slides for at least 15 min at room temperature, then fix with methanol for 10 minutes.
Perform a 400 cell count (non-squamous cells) differentiating between eosinophils, neutrophils, macrophages, epithelial cells and lymphocytes.

**17.4.6 Cell counting**

**17.4.6.1 Romanowski stain preparation**

1.5g Azure-B-thiocyanate in DMSO was dissolved at 37°C and 0.5g Eosin was dissolved in 300ml methanol at room temperature. The Azure blue solution was slowly added to the Eosin and stored away from light.

Dilute Romanowski stain:

62ml 10mM HEPES buffer pH 7.2

3.5ml DMSO

4.6ml Romanowski stain

**17.4.6.2 Differential cell counts**

A sputum differential cell count was obtained by counting >400 non-squamous cells on a Romanowski stained cytospin. Figure 17.1. Cell counts were performed blinded to the clinical characteristics of the patients and, where applicable, to the randomisation group. A proportion of the slides were recounted without reference to the original results to assess repeatability.

**17.5 Exhaled nitric oxide measurement**

The earliest studies used mass spectrometry to measure FE_{NO}. Currently chemiluminescence analysers are used; these measure the light emitted when
Figure 17.1
Induced sputum cytospin.
Black arrows denote eosinophils
nitric oxide reacts with ozone. Measurements can be online (the patient exhales directly into the analyser) or offline (NO is collected in Mylar® bags and then transported to the machine where the exhaled NO is analysed). Most modern analysers incorporate NO scrubbers to remove NO from the inhaled air and minimise the effect of environmental pollution for online analysis. NO measurement is normally performed with the patient inhaling NO free air through the mouth to total lung capacity, followed by immediate expiration with exhalation against a pressure of at least 5cm H₂O to ensure closure of the velopharyngeal aperture to help prevent nasal contamination. Nose clips can increase nasal contamination and are not worn.

In the case control study exhaled nitric oxide concentration was measured using an online chemiluminescence analyser (NIOX; Aerocrine, Stockholm, Sweden). Air was inhaled via a scrubber to ensure nitric oxide free air, up to tidal volume and then exhaled at constant pressure (>10cmH₂O) to aid closure of the velopharynx. The rate of exhalation was set by a standard valve at 50ml/sec and controlled by means of visual feedback. The exhaled nitric oxide concentration was recorded as the mean of 3 recordings of the plateau phase. The analyser was calibrated twice weekly against a standard gas containing 200ppb of nitric oxide, according to the manufacturers instructions. In the earlier observational study a Logan-Sinclair analyser was used to measure FENO at an exhalation flow of 250ml/s; the mean of three recordings of the plateau phase of NO was again taken. The discrepancy in machines arose as the guidelines changed (1999;Kharitonov, Alving, & Barnes 1997) and as newer equipment became available.

This difference in machines led to a change in the NO level used to identify an eosinophil count of greater than 3%. The original observational study suggested that a NO level of 8.3ppb at a flow of 200ml/sec was the most sensitive and specific value in identifying a differential sputum eosinophil count of greater than
3%. We performed a comparison of the Logan and Niox chemiluminescence analysers and used this correction factor to determine the NO level of 26ppb at a flow of 50ml/sec that we used in the case control study (see abstract list). Similar work was later replicated and published (Menzies et al. 2005).

17.6 Asthma diary records

Patients completed daily diary cards recording daytime and night time symptoms, twice daily peak expiratory flow (PEF), short acting β₂ agonist use and courses of oral prednisolone. Symptom scores ranged from 0 to 3:
For daytime symptoms 1= occasional symptoms, 2= symptoms most of the day, 3= asthma very bad, unable to perform normal activities at all.
For night time symptoms 0=none, 1=awoke once due to asthma, 2=awoke 2-3 times due to asthma, 3= awake most of the night due to asthma.

17.7 Peak flow variability

The maximum peak expiratory flow (PEF) from the best of 3 attempts was recorded twice daily using a Mini-Wright peak flow meter (Clement Clarke International Ltd., Harlow, UK). From this the highest, lowest, mean and amplitude percent of mean were calculated after each study visit.

17.8 Juniper asthma control questionnaire

The Juniper asthma control questionnaire (ACQ) was used to assess asthma control in the study. This is a validated questionnaire that was designed in consultation with 100 international experts. Each symptom was scored for its importance in evaluating asthma control. From the 91 responses, the five highest scoring symptoms were selected for the ACQ. In addition, one question on β₂ agonist use and one on airway calibre were added. The ACQ was tested in a 9-
week observational study of 50 adults with symptomatic asthma. The ACQ and other measures of asthma health status were assessed at baseline, 1, 5 and 9 weeks. In patients whose asthma was stable between clinic visits, reliability of the ACQ was high (intraclass correlation coefficient (ICC)=0.90). The questionnaire was very responsive to change in asthma control (p<0.0001). Cross-sectional and longitudinal validity were supported by correlations between the ACQ and other measures of asthma health status (Juniper et al. 1999). See Appendix.
The use of exhaled nitric oxide concentration to identify eosinophilic airway inflammation: an observational study in adults with asthma

Abstract

Background
Assessment of eosinophilic airway inflammation may be helpful in the management of asthma. Nitric oxide (NO) has potential advantages as a tool to monitor airway inflammation although little is known about the relationship between NO and eosinophilic airway inflammation and the factors which influence it.

Hypothesis
We set out to define the relationship between exhaled NO and the sputum eosinophil count, identify the exhaled NO concentration that best identified a sputum eosinophil count >3% and investigate the impact of several potential confounding factors in 566 consecutive patients with varying severity of asthma. Finally we examined the ability of exhaled NO concentrations measured at differing exhalation flows to identify the presence of a sputum eosinophilia.

Results
We found a significant positive relationship between exhaled NO and sputum eosinophil count \( R^2 = 0.26, p<0.001 \) which was best described using a non-linear model. There were no clinically important confounding factors to this model. In non-smokers an exhaled NO concentration of >8.3 p.p.b. at 250ml/s gave 71% sensitivity and 72% specificity for identifying a sputum eosinophil count of >3%.
Conclusions
This value of exhaled NO would seem to be the best for identifying significant eosinophilic airway inflammation. It is applicable to a wide range of non-smoking patients with asthma; exhalation flow does not alter the ability of exhaled NO concentration to detect a sputum eosinophilia.

Introduction
There is increasing evidence that monitoring airway inflammation is clinically helpful: a sputum eosinophilia identifies patients who respond well to corticosteroid treatment in isolated chronic cough (Pizzichini, Pizzichini, Parameswaran, Clelland, Efthimiadis, Dolovich, & Hargreave 1999c), asthma (Green, Brightling, Woltmann, Parker, Wardlaw, & Pavord 2002b) and COPD (Brightling, Monteiro, Ward, Parker, Morgan, Wardlaw, & Pavord 2000a) and a management strategy directed at symptoms and maintenance of a sputum eosinophil count below 3% results in a marked reduction in asthma exacerbations in patients with moderate to severe asthma (Green, Brightling, McKenna, Hargadon, Parker, Bradding, Wardlaw, & Pavord 2002a). Assessment of exhaled nitric oxide (NO) concentration is also potentially useful for measuring lower airway inflammation (Alving, Weitzberg, & Lundberg 1993; Kharitonov et al. 1994) and has the advantage of being simple, repeatable and providing an immediate result, making it particularly well suited to repeated measurement in the clinic.

There is some evidence that monitoring exhaled NO concentration might be clinically useful since Jones et al. found an exhaled NO concentration of 15 p.p.b. (at 250ml/s) or an increase in exhaled NO concentration of 10 p.p.b predicted failure of withdrawal of inhaled corticosteroids (Jones, Kittelson, Cowan, Flannery, Hancox, McLachlan, & Taylor 2001). However, in a similar study Leuppi did not find a difference in exhaled NO concentration between patients with...
asthma who failed corticosteroid withdrawal and those who did not (Leuppi, Salome, Jenkins, Anderson, Xuan, Marks, Koskela, Brannan, Freed, Andersson, Chan, & Woolcock 2001). In the absence of consistent data linking exhaled NO concentration with outcome, a reasonable basis for a study investigating whether measurement of exhaled NO is useful in the assessment and management of asthma is to determine the concentration which best identifies the presence or absence of eosinophilic airway inflammation.

A number of studies have examined the relationship between exhaled NO concentration and sputum eosinophil counts in small homogeneous populations of children (Warke, Fitch, Brown, Taylor, Lyons, Ennis, & Shields 2002) and adults with asthma (Jatakanon, Lim, Kharitonov, Chung, & Barnes 1998); inhaled corticosteroids (van Rensen et al. 1999), cigarette smoking (Verleden et al. 1999), female sex hormones (Kirsch et al. 1999; Zervou, Klentzeris, & Old 1999), and atopy (Gratiou et al. 1999; Ho et al. 2000); all have been shown to affect the concentration of exhaled NO in adults and might conceivably alter the relationship between exhaled NO and eosinophilic airway inflammation. The aim of this study was to investigate the relationship between exhaled NO concentration and induced sputum eosinophil count in a large heterogeneous population of adult subjects with asthma and to identify important factors influencing this relationship. We have used these data to determine the concentration of exhaled NO that best identified a sputum eosinophil count above 3%. This cut-off value was used to increase anti-inflammatory treatment in an earlier study which showed that an asthma management strategy that aimed to normalize eosinophilic airway inflammation was associated with a reduction in asthma exacerbations (Green, Brightling, McKenna, Hargadon, Parker, Bradding, Wardlaw, & Pavord 2002a). Finally, we have examined whether measurement of exhaled NO concentration at different flows alters the relationship between exhaled NO concentration and sputum eosinophil count.
Methods

Patients
Five hundred and sixty-six consecutive patients who were seen at the Glenfield hospital outpatients with stable asthma were recruited. All subjects had symptoms of asthma and objective evidence of airway hyper-responsiveness and/or airflow variability as demonstrated by one or more of the following: an inhaled concentration of methacholine causing a fall of more than 20% in forced expiratory volume in 1 s (FEV₁) (PC₂₀) of less than 8mg/ml; an increase in FEV₁ of 15% or greater following inhalation of 200 μg of Salbutamol; or peak flow variability of greater than 20% recorded twice daily over a 2 week period. Atopy was defined as a weal 2mm greater than control on skin prick testing or specific IgE (Pharma CAP test) test to one or more of dust mite, grass, tree, cat, dog or Aspergillus allergens. Subjects were classified as smokers (current smokers), ex-smokers (current non-smokers with >5 pack year smoking history) or non-smokers (never smokers or ex-smokers with <5 pack year history). All patients gave informed consent before entering the study and ethical approval was given by the Leicester Research and Ethics Committee.

Study design
The data were obtained from a single visit at which exhaled NO concentration was measured prior to measurement of airway responsiveness, administration of salbutamol and induced sputum collection.

Measurements
Spirometry was performed using a Vitalograph® spirometer (Vitalograph Ltd, Maids Moreton, UK), morning and evening peak flow was measure using Mini-Wright® peak flow meters (Clement-Clark International, Harlow, UK) and skin test was performed using standard techniques taking a weal of 2mm greater than the negative control to be positive. Induced sputum was obtained as
previously described (Pavord, Pizzichini, Pizzichini, & Hargreave 1997). Eosinophil count was expressed as a percentage of non-squamous cells based on a count of 400 inflammatory cells. Exhaled NO was measured using a chemiluminescence analyser (LR2000 Logan Research, Rochester, UK) at an exhalation flow of 250ml/s; the mean of three recordings of the plateau phase of NO was taken as the expired NO concentration (Kharitonov, Alving, & Barnes 1997). Sixty randomly selected patients had exhaled NO concentration measured on two chemiluminescence analysers, LR2000 (Logan-Sinclair, Rochester, UK) and NIOX (Aerocrine, Stockholm, Sweden), in random order at exhalation flows of 250ml/s and 10, 30, 50, 100 and 200ml/s, respectively.

Analysis
Percentage eosinophil count and exhaled NO concentrations were log normally distributed and were therefore log transformed for statistical methods which assume normal distribution. Comparisons between two groups were made using T-tests and between more than two groups with one way ANOVA with Tukey’s post hoc test for between individual group differences. Correlation coefficients were calculated using Pearson’s product–moment correlation coefficient. Multiple independent regression was used to examine the relationship between exhaled NO and sputum eosinophil counts and the effect of smoking, gender, inhaled steroids, age and FEV₁ % predicted on this relationship. Regression analysis examines the strength of a relation between one or more predictor variables and a target variable, by calculating an equation to relate their values. Multiple independent regression simultaneously considers all independent variables in the prediction of a target variable. To assess how well the data fits the model, goodness of fit is calculated, using the root mean square method. Maximal bronchial NO output was calculated as previously described (Tsoukias & George 1998). Receiver–operating characteristic curves were constructed to determine the concentration of exhaled NO which best identified a sputum eosinophil count above 3% separately for smokers and non-smokers and at different flows. Within
the non-smoking group ROC curves were subsequently generated separately for atopic and non-atopic subjects, males and females and steroid-treated and naive subjects. Measurements of 0 were assigned the lowest measurable value for each test (0.25% for sputum eosinophil count, 0.1 p.p.b. for exhaled NO) prior to log transformation. All statistical calculations were made using SPSS 10 for Windows.

**Results**

Complete data were obtained from 566 (90%) patients from 632 volunteers; 58 (9%) patients were unable to complete sputum induction, three (0.4%) patients were unable to do exhaled NO measurements and five (0.8%) were not able to do either. Table 18.1 summarizes the baseline characteristics of the patients who completed both tests. Exhaled NO concentrations were higher in non-smokers than current smokers (6.1 vs. 2.8 p.p.b., mean fold difference 2.2%, 95% confidence intervals (CIs) 1.6, 3.0, p<0.001), but no significant difference between ex-smokers (5.3 p.p.b.) and either of the other groups. There was no significant difference in sputum eosinophil counts between current smokers, ex-smokers and non-smokers (p=0.31). Males had significantly higher exhaled NO concentrations (6.4 vs. 4.3 p.p.b., mean fold difference 1.5%, 95% CI 1.2, 1.9, p<0.002) and sputum eosinophil counts (2.37% vs. 1.6%, mean fold difference 1.4%, 95% CI 1.1, 1.9, p=0.009) than females.

There was a significant association between exhaled NO and sputum eosinophil count in the group as a whole ($R^2=0.26$, p<0.001) which was best described with a nonlinear (quadratic) model. The association was closer in nonsmokers ($R^2=0.28$, p<0.001; Figure 18.1) than current smokers ($R^2=0.15$, p<0.001) or ex-smokers ($R^2=0.08$, p=0.11). In the non-smoking model FEV$_1$ had a significant association with sputum eosinophil count independent of exhaled NO (coefficient -0.18%/L, 95% CI -0.28, -0.08; p<0.001) as did age (coefficient -0.007%/year,
Table 18.1
Summary of patient characteristics for cross sectional data.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number (Female)</td>
<td>566 (319)</td>
</tr>
<tr>
<td>Age*</td>
<td>49 (16 – 82)</td>
</tr>
<tr>
<td>Current smokers (%)</td>
<td>113 (22)</td>
</tr>
<tr>
<td>Ex-smokers (%)</td>
<td>51 (9)</td>
</tr>
<tr>
<td>Pack years history (ex and current smokers only)†</td>
<td>15 (6-80)</td>
</tr>
<tr>
<td>Currently on inhaled steroids (%)</td>
<td>317 (56)</td>
</tr>
<tr>
<td>Beclomethasone equivalent dose of inhaled corticosteroid (treated patients only) †</td>
<td>800 (200-4000)</td>
</tr>
<tr>
<td>Currently on oral corticosteroids (%)</td>
<td>33 (6)</td>
</tr>
<tr>
<td>Atopic (%)</td>
<td>289 (51)</td>
</tr>
<tr>
<td>FEV₁ % predicted (%)</td>
<td>85 (19)</td>
</tr>
<tr>
<td>FEV₁/FVC (%)</td>
<td>72.8 (11.9)</td>
</tr>
<tr>
<td>PC₂₀ (mg/ml)**</td>
<td>0.32 (0.71)</td>
</tr>
<tr>
<td>Sputum eosinophil count (%) **</td>
<td>2.3 (0.73)</td>
</tr>
<tr>
<td>Sputum neutrophil count (%)</td>
<td>60.1 (25.8)</td>
</tr>
<tr>
<td>Exhaled nitric oxide concentration at 250ml/sec (ppb)**</td>
<td>6.7 (0.65)</td>
</tr>
</tbody>
</table>

FEV₁ = forced expiratory volume in one second, FVC = forced vital capacity, PC₂₀ = provocative concentration of methacholine causing a 20% fall in FEV₁. Data is shown as mean (standard deviation) except where marked *mean (range), **geometric mean (Log standard deviation) and †median (range).
Figure 18.1
Scatter plot of sputum eosinophil count vs. exhaled nitric oxide concentration in non-smokers with quadratic regression line, males are shown in black triangles and females grey triangles.
95% CI -0.01, -0.003; p<0.001). Gender, height, inhaled corticosteroids, oral corticosteroids and atopy did not have an association with sputum eosinophil count independent of exhaled NO. The high number of low exhaled NO concentrations in females meant that the linear but not the non-linear relationship differed in men and women.

The area under the ROC curves for smokers (AUC= 0.63%, 95% CI 0.48, 0.78; p=0.10) or ex-smokers (AUC=0.62%, 95% CI 0.47, 0.77; p=0.09) was not significantly different from 0.5 for identifying a sputum eosinophil count >3%, and therefore no further analysis was performed on these groups. Within the non-smoking group the area under the ROC curve for identifying a sputum eosinophil count >3% was 0.77 (95% CI 0.73, 0.82; p<0.001). An exhaled NO concentration of 8.3 p.p.b. identified a sputum eosinophil count >3% with 71% sensitivity and 72% specificity (Figure 18.2). The results of the FENO trial application of these concentrations to subgroups of patients are given in Table 18.2.

With the exception of 10ml/s, exhalation flow did not greatly alter the ability of exhaled NO concentration to identify the presence of a sputum eosinophilia, and correlation coefficients for the relationship between exhaled NO and sputum eosinophil counts were broadly similar at all flows. (Table 18.3).

**Discussion**

This observational study is the first to systematically investigate the relationship between exhaled NO concentration and sputum eosinophil count in a large heterogeneous population of adults with asthma. The study has shown that there is a significant positive association between exhaled NO and induced sputum eosinophil count, a finding that is consistent with findings in smaller, more homogeneous populations (Berlyne, Parameswaran, Kamada, Efthimiadis,
Figure 18.2
ROC curve for non smokers determining an exhaled nitric oxide concentration which identifies a sputum eosinophil count >3%

[Image of ROC curve with sensitivity and 1-specificity axes, indicating an 8.3ppb threshold]
Table 18.2

Table of analysis of ROC curves in subgroups using an Exhaled nitric oxide concentration of 8.3ppb to identify a sputum eosinophil count >3%.

<table>
<thead>
<tr>
<th></th>
<th>Number</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>AUC</th>
<th>95% C.I.</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Smokers</td>
<td>113</td>
<td>-</td>
<td>-</td>
<td>0.63</td>
<td>0.48, 0.78</td>
<td>0.10</td>
</tr>
<tr>
<td>Non smokers</td>
<td>405</td>
<td>71</td>
<td>72</td>
<td>0.77</td>
<td>0.73, 0.82</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Steroid naive</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>100</td>
<td>60</td>
<td>82</td>
<td>0.69</td>
<td>0.54, 0.84</td>
<td>0.01</td>
</tr>
<tr>
<td>Male</td>
<td>82</td>
<td>73</td>
<td>60</td>
<td>0.71</td>
<td>0.51, 0.91</td>
<td>0.037</td>
</tr>
<tr>
<td>Steroid treated</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>122</td>
<td>55</td>
<td>68</td>
<td>0.72</td>
<td>0.64, 0.81</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Male</td>
<td>101</td>
<td>81</td>
<td>76</td>
<td>0.84</td>
<td>0.76, 0.91</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Atopic</td>
<td>206</td>
<td>83</td>
<td>64</td>
<td>0.77</td>
<td>0.64, 0.89</td>
<td>0.001</td>
</tr>
<tr>
<td>Non atopic</td>
<td>199</td>
<td>62</td>
<td>64</td>
<td>0.66</td>
<td>0.54, 0.72</td>
<td>0.032</td>
</tr>
</tbody>
</table>
Table 18.3
Table of analysis of ROC curves of exhaled nitric oxide concentration at different flows to determine a sputum eosinophil count of >3% and correlation co-efficients for the association between exhaled nitric oxide concentration and sputum eosinophil counts at different flows. (* - nlmin\(^{-1}\), ** - Maximal bronchial NO output)

<table>
<thead>
<tr>
<th>Flow (mlsec(^{-1}))</th>
<th>Number</th>
<th>AUC</th>
<th>95% C.I.</th>
<th>p</th>
<th>Value (ppb)</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>Correlations Coefficient</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>60</td>
<td>0.68</td>
<td>0.54, 0.83</td>
<td>0.02</td>
<td>112</td>
<td>70</td>
<td>70</td>
<td>0.32</td>
<td>0.013</td>
</tr>
<tr>
<td>30</td>
<td>60</td>
<td>0.75</td>
<td>0.61, 0.88</td>
<td>0.002</td>
<td>53</td>
<td>74</td>
<td>72</td>
<td>0.37</td>
<td>0.004</td>
</tr>
<tr>
<td>50</td>
<td>60</td>
<td>0.77</td>
<td>0.63, 0.9</td>
<td>0.001</td>
<td>36</td>
<td>78</td>
<td>72</td>
<td>0.39</td>
<td>0.002</td>
</tr>
<tr>
<td>100</td>
<td>60</td>
<td>0.76</td>
<td>0.63, 0.89</td>
<td>0.001</td>
<td>22</td>
<td>78</td>
<td>72</td>
<td>0.39</td>
<td>0.002</td>
</tr>
<tr>
<td>200</td>
<td>60</td>
<td>0.73</td>
<td>0.59, 0.87</td>
<td>0.004</td>
<td>13</td>
<td>74</td>
<td>72</td>
<td>0.30</td>
<td>0.018</td>
</tr>
<tr>
<td>250</td>
<td>405</td>
<td>0.77</td>
<td>0.73, 0.82</td>
<td>&lt;0.001</td>
<td>8.3</td>
<td>71</td>
<td>72</td>
<td>0.47</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>CbrMax**</td>
<td>60</td>
<td>0.76</td>
<td>0.63, 0.89</td>
<td>0.001</td>
<td>87*</td>
<td>83</td>
<td>69</td>
<td>0.43</td>
<td>0.001</td>
</tr>
</tbody>
</table>
& Hargreave 2000; Chan-Yeung et al. 1999; Mattes, Storm van's, Reining, Alving, Ihorst, Henschen, & Kuehr 1999). These data are consistent with the view that in non-smokers exhaled NO and sputum eosinophil counts identify a common airway abnormality.

We found that the relationship between exhaled NO and sputum eosinophil count in the population as a whole was best described using a non-linear model. This may be an artefact related to measurement of exhaled NO concentration at a relatively high flow resulting in concentrations that were near to the detection level in a significant number of patients. Using a non-linear model we found relatively few factors which significantly confounded the relationship between exhaled NO concentration and sputum eosinophil counts. Furthermore, the exhaled NO concentrations that best identified the presence of eosinophilic airway inflammation were similar in different categories of patients. This is important in that it suggests that a management strategy incorporating this value would be applicable across a wide range of patients. An important caveat is that exhaled NO concentration did not relate closely to sputum eosinophil count in current smokers or ex-smokers and did not predict the presence of a sputum eosinophilia in these patients. In the small number of patients who were taking regular oral corticosteroids, the relationship between sputum eosinophil count and exhaled NO concentration was similar, although we acknowledge that the power to detect a difference in this group is limited.

There are a number of methodological issues with our study that require further discussion. There was a closer relationship between exhaled NO concentration and sputum differential cell count, than total cell count so the analysis was limited to differential cell counts. The underlying assumption that sputum eosinophil differential cell counts or exhaled NO concentration reflect eosinophilic airway inflammation measured in other ways, such as mucosal biopsy cell counts, may not be correct (Lim, Jatakanon, Meah, Oates, Chung, & Barnes
2000). However, our justification for regarding sputum eosinophil count as the gold standard comparator with exhaled NO concentration is that, unlike these other measures, sputum eosinophil count has been clearly associated with outcome, specifically corticosteroid responsiveness (Green, Brightling, Woltmann, Parker, Wardlaw, & Pavord 2002b) and exacerbation frequency (Green, Brightling, McKenna, Hargadon, Parker, Bradding, Wardlaw, & Pavord 2002a). Furthermore, a management strategy that aims to normalize eosinophilic airway inflammation as well as symptoms is associated with a reduction in asthma exacerbations (Green, Brightling, McKenna, Hargadon, Parker, Bradding, Wardlaw, & Pavord 2002a). We have used values of >3% sputum eosinophil count for indicating the presence or absence of eosinophilic airway inflammation, because this was the value used in this study. Response to inhaled corticosteroids is also most clearly seen in patients with sputum eosinophil counts at this level (Pavord, Brightling, Woltmann, & Wardlaw 1999). Our study was started before the publication of American and European Thoracic Society guidelines and we have consequently used a higher than recommended exhalation flow (1999; Kharitonov, Alving, & Barnes 1997). However, we measured exhaled NO concentration at a number of different flows and this did not alter the tests ability to detect a sputum eosinophilia.

There is currently a great deal of interest in measuring airway inflammation in clinical practice. As well as being associated with improved outcome in difficult asthma (Green, Brightling, McKenna, Hargadon, Parker, Bradding, Wardlaw, & Pavord 2002a), measurement of airway inflammation may facilitate the identification of unusual disease phenotypes such as non-eosinophilic corticosteroid resistant asthma (Pavord, Brightling, Woltmann, & Wardlaw 1999) and eosinophilic bronchitis (Brightling, Ward, Goh, Wardlaw, & Pavord 1999a). However, collection and analysis of induced sputum is more technically demanding and time consuming than measurement of exhaled NO concentration, which can be carried out quickly and can provide an immediate result. Our data
can be used to determine appropriate concentrations for exhaled NO to identify the presence of a sputum eosinophilia in studies designed to determine the role of measuring exhaled NO concentration in the clinical management of asthma.

Acknowledgements
Physiological measurements were performed by Beverley Hargadon, Sue McKenna and Maria Shelley. Mike Berry, Dominick Shaw and Ruth Green were supported by research grants from Asthma UK. Christopher Brightling is a Department of Health clinical scientist.
19 The Use of Exhaled Nitric Oxide to Guide Asthma Management: A Randomised Controlled Trial

Abstract

Rationale
Current asthma guidelines recommend adjusting anti-inflammatory treatment on the basis of the results of lung function tests and symptom assessment, neither of which are closely associated with airway inflammation.

Objectives
We tested the hypothesis that titrating corticosteroid dose using the concentration of exhaled nitric oxide in exhaled breath (FE\textsubscript{NO}) results in fewer asthma exacerbations and more efficient use of corticosteroids, when compared to traditional management.

Methods
118 participants with a primary care diagnosis of asthma were randomised to a single blind trial of corticosteroid therapy based on either FE\textsubscript{NO} measurements (n=58) or British Thoracic Society guidelines (n = 60). Participants were assessed monthly for 4 months and then two monthly for a further 8 months. The primary outcome was the number of severe asthma exacerbations. Analyses were by intention to treat.

Measurements and Main Results
The estimated mean (SD) exacerbation frequency was 0.33/patient/year (0.69) in the FE\textsubscript{NO} group and 0.42 (0.79) in the control group (mean difference -21% [95% CI -57% to 43%] p=0.43). Overall the FE\textsubscript{NO} group used 11% more inhaled
corticosteroid ([95% CI -17% to 42%] p=0.40), although the final daily dose of inhaled corticosteroid was lower in the FeNO group (557µg vs. 895µg, mean difference 338µg [95% CI -640 to -37] p=0.028).

**Conclusion**

An asthma treatment strategy based on the measurement of exhaled nitric oxide did not result in a large reduction in asthma exacerbations or in the total amount of inhaled corticosteroid therapy used over 12 months, when compared with current asthma guidelines.

**Introduction**

Asthma is defined and characterised by the presence of symptoms associated with variable airflow obstruction, airway inflammation and airway hyperresponsiveness (1992). It is a common disease which is responsible for significant morbidity, mortality and health care cost (Barnes, Jonsson, & Klim 1996). Current guidelines recommend that decisions about treatment are based on assessment of symptoms and airflow obstruction. However, there is no clear relationship between symptom control, variable airflow obstruction and the extent of eosinophilic airway inflammation (Crimi, Spanevello, Neri, Ind, Rossi, & Brusasco 1998; Rosi, Ronchi, Grazzini, Duranti, & Scano 1999) and a response to corticosteroids is most closely associated with the presence of eosinophilic airway inflammation (Pavord, Brightling, Woltmann, & Wardlaw 1999). There is also evidence that management with the additional aim of decreasing eosinophilic airway inflammation reduces exacerbation frequency in patients managed in secondary care, and that a sputum eosinophilia is a marker of preventable, corticosteroid responsive asthma exacerbations (Green, Brightling, McKenna, Hargadon, Parker, Bradding, Wardlaw, & Pavord 2002a). In the U.K. 80% of patients with asthma are managed in primary care (Asthma UK 2005); assessment of eosinophilic airway inflammation using induced sputum is not
applicable in this setting (Green, Brightling, McKenna, Hargadon, Parker, Bradding, Wardlaw, & Pavord 2002a).

Recently the concentration of nitric oxide present in exhaled breath (F\textsubscript{ENO}) has been evaluated as a tool for assessing asthma (Smith, Cowan, Filsell, McLachlan, Monti-Sheehan, Jackson, & Taylor 2004). F\textsubscript{ENO} is elevated in patients with asthma (Alving, Weitzberg, & Lundberg 1993; Kharitonov, Yates, Robbins, Logan-Sinclair, Shinebourne, & Barnes 1994), is reduced by treatment with inhaled corticosteroids (Kharitonov, Yates, & Barnes 1996) and correlates with eosinophilic airway inflammation measured using bronchial biopsies and induced sputum (Jatakanon, Lim, Kharitonov, Chung, & Barnes 1998; Payne, Adcock, Wilson, Oates, Scallan, & Bush 2001). It is particularly applicable for monitoring asthma in primary care as the test is easy to perform (Kharitonov, Gonio, Kelly, Meah, & Barnes 2003), it provides an immediate result, and inexpensive portable monitors are now available. Two recent studies have investigated the use of F\textsubscript{ENO} to guide treatment in asthma (Pijnenburg, Bakker, Hop, & de Jongste 2005; Smith, Cowan, Brassett, Herbison, & Taylor 2005). Neither study showed an improvement in exacerbation frequency although in one study the daily dose of inhaled steroids was lower (Smith, Cowan, Brassett, Herbison, & Taylor 2005).

Our aim was to test the hypothesis that the use of F\textsubscript{ENO} for titrating corticosteroid dose results in fewer exacerbations and more efficient use of corticosteroid therapy. We designed a single-blind randomised controlled trial comparing exacerbation frequency and corticosteroid dosage in patients whose asthma management was based on measurements of F\textsubscript{ENO} to one where management was based on the British Thoracic Society and Scottish Intercollegiate Guidelines Network treatment guidelines (2003a). Results from this work have previously been published as an abstract (Shaw et al. 2006).
Setting and Participants

Participants were identified from registers held in General Practices around Leicester. All participants were aged over 18 and had a diagnosis of asthma recorded in their GP notes. Participants were eligible if they had received at least one prescription for any anti-asthma medication in the last 12 months. The study was restricted to current non-smokers with a past smoking history of less than 10 pack years. Participants were also excluded if they were considered by their physician to be poorly compliant or had had a severe asthma exacerbation, requiring a course of prednisolone, within 4 weeks of study entry. All suitable participants on the registers who responded to an invitation from their GP to be contacted by the research team were invited to participate in the study. Ethical approval for the study was given by ethics committees from both the University Hospitals of Leicester and the Leicester Primary Research Care Alliance; all participants gave written informed consent.

Clinical Methods

Participants attended Glenfield Hospital for tests to characterise their asthma. Long acting β2 agonists or antihistamines were withheld for 48 hours prior to testing; short acting β2 agonists were withheld for 8 hours. Tests were performed in the following order and at the same time of day for each patient: exhaled nitric oxide levels measured at a flow of 50ml/sec (2005) using a Niox chemiluminescence analyser (Aerocrine, Stockholm, Sweden); forced expiratory volume (FEV1) and forced vital capacity (FVC) using a Gold Standard Vitalograph® spirometer, as the best of 3 measurements within 100mls of each other; methacholine challenge test to determine the concentration of methacholine required to provoke a 20% fall in the FEV1 (PC20) using a Wrights® nebuliser and the tidal breathing method (Juniper, Frith, Dunnett, Cockcroft, & Hargreave 1978). Subjects with a FEV1 <70% predicted and FEV1/FVC <0.7 had bronchodilator reversibility after inhaling 400μg salbutamol via a volumatic
instead of a $PC_{20}$. Induced sputum analysis was carried out as previously described (Pavord, Pizzichini, Pizzichini, & Hargreave 1997). Eosinophil counts were expressed as a percentage of non-squamous cells based on a count of 400 inflammatory cells. Skin prick tests (ALK Abello®) to house dust mite, grass, tree, cat, dog and aspergillus allergens were performed, with atopy defined as a wheal 2mm greater than control. Post bronchodilator FEV$_1$ was performed 20 minutes after 400µg salbutamol at the end of every visit; values recorded after methacholine challenge testing were not analysed in the results. After the first visit the patient’s inhaler technique was checked, and the patient was issued with a peak flow diary and meter (Mini-Wright®) and asked to record their peak expiratory flow as the best of 3 recordings both morning and evening. The peak expiratory flow amplitude percent of mean was calculated by subtracting the lowest from the highest recording and dividing by the mean.

Participants were seen 2 weeks later and then every month for 4 months followed by every 2 months for a further 8 months in order to be consistent with our earlier study of inflammation guided management (Green, Brightling, McKenna, Hargadon, Parker, Bradding, Wardlaw, & Pavord 2002a). Each visit occurred at the same time of day and consisted of assessment of exhaled nitric oxide, spirometry and post bronchodilator FEV$_1$, 20 minutes after 400µg salbutamol at the end of every visit. Figure 19.1. Peak flow and symptom diaries were analysed and compliance assessed by monitoring adherence to script collection. Participants were issued with self management plans based on their best baseline peak flow from the first 2 weeks of the study; if their peak flow fell to less than 70% of their best for 48 hours during the study, or their asthma deteriorated, they were asked to attend hospital where they were assessed by a physician (MB). At the 6 and 12 month visit induced sputum and methacholine challenge testing were also performed.
Figure 19.1

Study Design

$F_{ENO}$ - Fraction of exhaled nitric oxide at 50ml/sec

SPT- Skin prick tests

$PC_{20}$ - Methacholine challenge test

Sputum- Differential sputum eosinophil count

JACS- Juniper asthma control score
Intervention

After the first visit participants were randomly allocated to receive treatment either on the basis of their $\text{FE}_{\text{NO}}$ measurements ($\text{FE}_{\text{NO}}$ group) or according to a conventional stepwise asthma management plan (control group) (2003a). Randomisation was done by an independent individual (CB) with the method of minimisation (Altman & Bland 2005), and was stratified by the baseline sputum eosinophil count, $\text{FE}_{\text{NO}}$ and rescue steroid courses in the last year. Participants were seen monthly for the first 4 months and 2 monthly thereafter. Figure 19.1; all visits were at the same time of day. At each visit the patient’s asthma control was determined using the validated Juniper asthma control questionnaire which scores asthma control from 0-6; a score of greater than 1.57 was used to identify poorly controlled asthma (Juniper, O'Byrne, Guyatt, Ferrie, & King 1999; Juniper et al. 2005). In the control group treatment was doubled if the score was $>1.57$, and treatment was halved if the score was $<1.57$ for 2 consecutive months. Figure 19.2. In the $\text{FE}_{\text{NO}}$ group treatment was adjusted following a set protocol according to both the $\text{FE}_{\text{NO}}$ and Juniper scores. Figure 19.3. If the $\text{FE}_{\text{NO}}$ was greater than 26ppb inhaled corticosteroid treatment was increased, if it was less than 16 ppb or less than 26ppb on 2 consecutive occasions it was decreased. Bronchodilator therapy was increased if symptoms were uncontrolled, despite a $\text{FE}_{\text{NO}}$ of $<26\text{ppb}$. We chose these cut offs as they have been shown to best identify a sputum eosinophil count of $>3\%$ and $<1\%$ respectively (Berry et al. 2005b), after correction for expiratory flow (Menzies, Fardon, Burns, & Lipworth 2005). Assessment of asthma control was made per protocol by investigators who were unaware of the participants’ randomisation status (BH/SM/MS/HP). At each visit two different treatment decisions, one for each randomisation group, were made by an independent physician who was also unaware of the randomisation status (RG/IP). The correct treatment
Figure 19.3
Management protocol for control group (based on BTS guidelines)
JACS- Juniper asthma control questionnaire

**Step 1**
Inhaled short-acting β agonist as required

**Step 2**
Add inhaled steroid 200-800 mcg/day BDP equivalent

**Step 3**
Add inhaled long-acting β₂ agonist (LABA)

**Step 4**
Increase inhaled steroid up to 2000 mcg/day
Addition of a fourth drug
e.g. leukotriene modifier, theophylline, β agonist tablet

**Step 5**
Oral prednisolone (use lowest dose providing adequate control)
Maintain high dose inhaled steroid at 2000 mcg/day
Refer patient for specialist care

*Step up*
if asthma JACS >1.57

*Step down*
if JACS <1.57 for 2 months

*Step up*
if asthma JACS >1.57

*Step down*
if JACS <1.57 for 2 months
Figure 19.3
Management protocol for FE\textsubscript{NO} group

Exhaled NO < 16 ppb on first occasion or exhaled NO 16-26 ppb on second occasion

**YES**
Juniper Asthma Control Score \leq 1.57?
**NO**

- Step down anti-inflammatory treatment *
- Step down bronchodilator treatment once off inhaled steroids **

- Step down anti-inflammatory treatment *
- Step up bronchodilator treatment **

Exhaled NO > 26 ppb

**YES**
Juniper Asthma Control Score \leq 1.57?
**NO**

- Step up anti-inflammatory treatment *
- No change in bronchodilator treatment **

- Step up anti-inflammatory treatment *
- Step up bronchodilator treatment once on maximum anti-inflammatory treatment **

* Hierarchy of Anti-Inflammatory Treatment:
1) Low dose inhaled steroid (100-200\mu g BDP bd)
2) Moderate dose inhaled steroid (200-800\mu g BDP bd)
3) High dose inhaled steroid (800-2000\mu g BDP bd)
4) High dose inhaled steroid (800-2000\mu g BDP bd) plus leukotriene antagonist
5) Higher dose inhaled steroid (2000\mu g BDP bd) plus leukotriene antagonist
6) Higher dose inhaled steroid (2000\mu g BDP bd) plus leukotriene antagonist plus oral Prednisolone 30mg 2/52, then titrating dose reducing by 5mg/week

** Hierarchy of Bronchodilator Treatment
1) PRN short acting $\beta_2$-agonists
2) Long acting $\beta_2$ agonist
3) Long acting $\beta_2$ agonist plus theophylline
4) Long acting $\beta_2$-agonist plus theophylline plus nebulised bronchodilator
decision, according to the participants' group, was communicated to the patient by a separate unblinded physician (DS).

**Safety Measure**

As there was evidence before study commencement that a high \( \text{FeNO} \) would not always reflect a high differential sputum eosinophil count (Berry, Shaw, Green, Brightling, Wardlaw, & Pavord 2005b), we determined *a priori* that patients in the \( \text{FeNO} \) group who had their anti-inflammatory treatment increased to the equivalent dose 2000\( \mu \)g beclomethasone dipropionate (BDP) per day, and whose \( \text{FeNO} \) was still greater than 26ppb, and had not fallen to 60% (Jones, Kittelson, Cowan, Flannery, Hancox, McLachlan, & Taylor 2001) of baseline, would have their differential sputum eosinophil count checked. If there was no eosinophilic airway inflammation present, treatment was reduced in a stepwise fashion, unless the \( \text{FeNO} \) increased by greater than 60% from baseline. An inhaled corticosteroid dose of 2000\( \mu \)g BDP was chosen as this is the normal point for referral from primary care for secondary care evaluation.

An asthma exacerbation was defined *a priori* as an episode of increasing asthma symptoms requiring a course of oral steroids or antibiotics; participants were asked to contact the research nurses if their asthma deteriorated. Participants were assessed and treated according to the BTS guidelines by a physician (MB) not involved in the regular treatment decisions and blinded to the participants’ randomisation status. At the end of the study participants were asked to record which group they thought they had been assigned to as an assessment of the success of blinding.

**Analysis**

We estimated that we needed 53 participants in each group to give 80% power to detect a 50% reduction in the rate of asthma exacerbations, based on a
Poisson regression analysis and a two-sided test at the 5% level. Our power calculation was based on findings from the FACET study (Pauwels, Lofdahl, Postma, Tattersfield, O'Byrne, Barnes, & Ullman 1997) which found an exacerbation frequencies of 0.91/patient/year and our own audit data suggesting that exacerbation frequency approximates to a Poisson distribution. We confirmed goodness of fit to a Poisson distribution prior to doing the analysis. If participants withdrew from the study we analysed their data by intention to treat; the last value recorded was carried forward for inhaled corticosteroid dose and $FE_{NO}$ reading, and for methacholine responsiveness and sputum eosinophils, the mean of the previous values was used. $FE_{NO}$ was log transformed, in order to assume a normal distribution, and expressed as a geometric mean; it was compared over the 12 months as area under the curve using an independent t-test. Steroid dose (expressed as equivalent dose to beclomethasone dipropionate (BDP) (Green, Brightling, McKenna, Hargadon, Parker, Bradding, Wardlaw, & Pavord 2002a) was also compared over the 12 months of the study as area under the curve using an independent t-test. Differential sputum eosinophil count and methacholine $PC_{20}$ were log transformed in order to assume a normal distribution, and their changes at 6 and 12 months from baseline were compared using an independent t-test. All data was analysed using SPSS for Windows (version 12) and Intercooled Stata for Windows (version 7).

**Results**

900 participants were contacted by their own GP. Of these, 146 participants declined the invitation and 636 failed to respond. Figure 19.4. We recruited 119 participants between January 2004 and December 2004; one patient could not perform the measurement of exhaled nitric oxide; 58 were allocated to the $FE_{NO}$ group and 60 to the control group. 6 participants withdrew from the $FE_{NO}$ group and 9 participants from the control group. None of the participants withdrew because of poorly controlled asthma. The two treatment groups were well
Figure 19.4
Consort profile
JACS- Juniper asthma control questionnaire
Sputum- Induced sputum differential cell count
SPT- Skin prick tests
PC$_{20}$ - Methacholine challenge test

900 patients contacted
781 declined, or failed to respond to initial letter from GP or contact letter from research team
119 patients recruited
1 patient could not perform F$_{ENO}$ test
118 patients randomised
FEV$_1$, F$_{ENO}$, JACS, PC$_{20}$, Sputum, SPT

58 F$_{ENO}$ group
6 withdrew during follow up;
1 intercurrent illness
2 moved away
3 changed mind
52 completed 12 month follow up

60 Control group
9 withdrew during follow up;
2 intercurrent illness
2 moved away
4 changed mind
1 pregnancy
51 completed 12 month follow up
matched at baseline for demographic and clinical features. Table 19.1. Measurement of exhaled nitric oxide was successful on every occasion. Assessed as area under the curve over the 12 months of the study, the $\text{FENO}$ was 24% lower ([95% CI -8% to 55%] $p=0.14$) in the $\text{FENO}$ group when compared to control group. There was no difference in the Juniper asthma control score, peak expiratory flow readings and $\text{FEV}_1$ between the groups over the duration of the study. Figure 19.5. Nine participants in the $\text{FENO}$ group needed a reassessment of management goals because of corticosteroid resistant persistent elevation of $\text{FENO}$ which was not reflective of eosinophilic airway inflammation.

There were 18 exacerbations in 12 participants in the $\text{FENO}$ group and 26 exacerbations in 19 participants in the control group. The rate of asthma exacerbation experienced by the $\text{FENO}$ group was 0.33/patient/year (SD 0.69) compared with 0.42 (SD 0.79) in the control group (mean difference -21% [95% CI -57% to 43%] $p=0.43$; Figure 19.6. The total amount of inhaled corticosteroid used during the study was 11% greater ([95% CI -15% to 37%] $p=0.40$) in the $\text{FENO}$ group compared with the control group. However, the final daily dose of inhaled corticosteroid was significantly lower in the $\text{FENO}$ group compared to the control group (557μg vs. 895μg, (mean difference 338μg, [95% CI -640 to -37]; $p=0.028$; Figure 19.5. At 6 months there was a 0.5 doubling dose improvement in the methacholine $\text{PC}_{20}$ in the $\text{FENO}$ group and a 0.7 doubling dose worsening in the control group (mean difference 1.14 [95% CI -0.09 to 2.36] $p=0.07$). At 12 months there was a 0.2 and 0.6 doubling dose improvement in the $\text{PC}_{20}$ in the $\text{FENO}$ group and control group respectively (mean difference -0.34 [95% CI -1.37 to 0.69] $p=0.51$). The differential sputum eosinophil count had reduced at 6 months by 1.6 fold and 1.4 fold in the $\text{FENO}$ group and control group respectively ($p=0.43$) and at 12 months the eosinophil count had increased by 1.01 fold and 1.31 fold in the $\text{FENO}$ group and control group respectively ($p=0.48$). Overall 5 patients (8%) had long acting $\beta_2$ agonists
Table 19.1
Baseline Demographics by Group

Figures are mean (SD) except for * geometric mean (68% confidence intervals), # median, (range)

<table>
<thead>
<tr>
<th>FE_{NO} Group</th>
<th>Control Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>58</td>
</tr>
</tbody>
</table>

### BTS Step

<table>
<thead>
<tr>
<th></th>
<th>Group</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Step 1</td>
<td>9 (16%)</td>
<td>9 (15%)</td>
</tr>
<tr>
<td>Step 2</td>
<td>24 (41%)</td>
<td>22 (37%)</td>
</tr>
<tr>
<td>Step 3</td>
<td>9 (16%)</td>
<td>13 (22%)</td>
</tr>
<tr>
<td>Step 4</td>
<td>14 (24%)</td>
<td>14 (23%)</td>
</tr>
<tr>
<td>Step 5</td>
<td>2 (3%)</td>
<td>2 (3%)</td>
</tr>
</tbody>
</table>

### Demographic

<table>
<thead>
<tr>
<th></th>
<th>Group</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Female</td>
<td>31</td>
<td>33</td>
</tr>
<tr>
<td>Age, years*</td>
<td>50 (20-75)</td>
<td>52 (24-81)</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>27.5 (5.02)</td>
<td>28.1 (5.43)</td>
</tr>
<tr>
<td>% former smokers</td>
<td>22</td>
<td>25</td>
</tr>
<tr>
<td>Oral steroid courses</td>
<td>1.2 (2.0)</td>
<td>1.3 (1.8)</td>
</tr>
<tr>
<td>in last year/patient</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Daily dose inhaled corticosteroid, μg</td>
<td>697 (708)</td>
<td>652 (533)</td>
</tr>
</tbody>
</table>

### Clinical

<table>
<thead>
<tr>
<th></th>
<th>Group</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Atopy %</td>
<td>62</td>
<td>70</td>
</tr>
<tr>
<td>Family history of asthma (%)</td>
<td>55</td>
<td>60</td>
</tr>
<tr>
<td>Rhinitis (%)</td>
<td>57</td>
<td>40</td>
</tr>
<tr>
<td>Nasal Polyps (%)</td>
<td>11</td>
<td>8</td>
</tr>
<tr>
<td>FEV₁, litres</td>
<td>2.5 (0.92)</td>
<td>2.57 (0.99)</td>
</tr>
<tr>
<td>FEV₁ as % predicted</td>
<td>81.4 (20.9)</td>
<td>84.9 (20.1)</td>
</tr>
<tr>
<td>FEV₁/FVC</td>
<td>71 (10.7)</td>
<td>72 (9.9)</td>
</tr>
<tr>
<td>% change post salbutamol</td>
<td>6.2 (9.4)</td>
<td>5.4 (8.6)</td>
</tr>
<tr>
<td>Peak expiratory flow</td>
<td></td>
<td></td>
</tr>
<tr>
<td>amplitude % mean</td>
<td>23.9 (17.1)</td>
<td>18.7 (10.4)</td>
</tr>
<tr>
<td>Sputum eosinophil count, %*</td>
<td>1.3 (0.3, 5.7)</td>
<td>1.7 (0.3, 9.8)</td>
</tr>
<tr>
<td>Sputum neutrophil count, %</td>
<td>65.7 (27.7)</td>
<td>62.0 (21.6)</td>
</tr>
<tr>
<td>Total cell count*(10⁹/ml)</td>
<td>1.4 (0.3, 7.0)</td>
<td>1.4 (0.3, 6.3)</td>
</tr>
<tr>
<td>Methacholine PC₂₀*(mg/ml)</td>
<td>1.4 (0.1, 16.0)</td>
<td>2.2 (0.2, 16.0)</td>
</tr>
<tr>
<td>Juniper asthma control score</td>
<td>1.32 (0.65)</td>
<td>1.26 (0.75)</td>
</tr>
<tr>
<td>FE_{NO} ppb*</td>
<td>29.2 (14.0, 61.0)</td>
<td>31.2 (13.3, 73.1)</td>
</tr>
</tbody>
</table>
Figure 19.5
Changes in Juniper Asthma Control Score, $F_{ENO}$ and mean daily dose of inhaled corticosteroid. Points are mean (SEM) except for *geometric mean (Log SE).
Figure 19.6
Cumulative exacerbations in the control and $F_{ENO}$ group.

![Graph showing cumulative exacerbations](image)

- Control group: 19 patients, 26 exacerbations
- $F_{ENO}$ group: 12 patients, 18 exacerbations

$p=0.43$
started in the FE\textsubscript{NO} group and 7 (12\%) patients had them started in the control group.

In a separate subgroup analysis, both groups were split into subjects with and without evidence of variable airflow obstruction, defined as one or more of the following: PC\textsubscript{20} <8mg/ml at the first visit; peak expiratory flow amplitude percent of mean >20\%; improvement in FE\textsubscript{V1} >15\% following 400\mu g salbutamol at the second visit. The rates of exacerbation were lower in the subgroups without variable airflow obstruction within both the FE\textsubscript{NO} group (n=44) and control group (n=39) respectively, but the differences were not significant. In the FE\textsubscript{NO} group exacerbation rates were 0.36 compared to 0.23 exacerbations/patient/year for participants with and without variable airflow obstruction, respectively (p=0.57); in the control group the exacerbation rates were 0.49 and 0.29 exacerbations/patient/year for participants with and without variable airflow obstruction, respectively (p=0.35). There was no significant difference in exacerbation rates in subjects with variable airflow obstruction between the FE\textsubscript{NO} group and control group (p=0.44).

Baseline log FE\textsubscript{NO} correlated with log sputum eosinophil count ($r^2=0.455$, $p<0.001$; Figure 19.7. A FE\textsubscript{NO} of <26ppb was associated with a differential sputum eosinophil count of <3\% for 85\% of all visits when both were measured. However, on over half the occasions when both were measured, an FE\textsubscript{NO} of >26ppb was associated with a sputum eosinophil count of <3\%. In participants with sputum eosinophils >3\% and FE\textsubscript{NO} of >26ppb exacerbation frequency was 0.38 versus 0.67 exacerbations/patient/year in the FE\textsubscript{NO} group compared with the control group respectively. In participants with sputum eosinophils <3\% and a FE\textsubscript{NO} of >26ppb exacerbation frequency was 0.09 versus 0 exacerbations/patient/year in the FE\textsubscript{NO} group compared with the control group respectively. The demographic details of each group were not different. The assessment of blinding revealed that 49\% of participants were not sure which
Figure 19.7

Scatter plot of baseline $F_{E\text{NO}}$ against baseline differential sputum eosinophil count. Corresponding cut off points for $F_{E\text{NO}} = 26$ ppb and eosinophils$= 3\%$ are drawn as lines.
group they had been assigned to, 33% correctly identified their group and 18% incorrectly identified their group.

**Discussion**

Our study was designed to evaluate the use of FE\textsubscript{NO} to guide asthma management in primary care, a setting where the technique is likely to be particularly applicable. The use of FE\textsubscript{NO} measurements to guide treatment decisions did not result in lower exacerbation frequency or in a lower maintenance dose of inhaled corticosteroid when compared to traditional asthma management. Although participants in the FE\textsubscript{NO} group finished the study on a significantly lower dose of inhaled corticosteroid, use of inhaled corticosteroid over the 12 months of the study was not different between the groups.

Participants randomised to both groups experienced a considerably lower exacerbation frequency compared to that initially estimated and to that experienced over the previous year. This improvement was not seen in the control arm of an earlier study in participants with more severe asthma recruited from secondary care (Green, Brightling, McKenna, Hargadon, Parker, Bradding, Wardlaw, & Pavord 2002a), suggesting that the improvement in asthma control was because of more intensive monitoring in a secondary care setting. As a result of this improvement, our study was underpowered to exclude a 50% reduction in exacerbation frequency.

Our findings are consistent with those of two recent studies (Smith, Cowan, Brassett, Herbison, & Taylor 2005), neither of which found a significant reduction in exacerbation frequency with FE\textsubscript{NO} directed management. The study of Smith et al found that FE\textsubscript{NO} directed management was associated with a significant decrease in inhaled corticosteroid dose (Smith, Cowan, Brassett, Herbison, & Taylor 2005); the number of prednisolone courses administered was 0.48 and
0.6/patient/year in the $\text{FeNO}$ and control groups respectively (Smith, Cowan, Brassett, Herbison, & Taylor 2005). Pijnenburg et al. reported an improvement in airway hyperresponsiveness, but no reduction in corticosteroid dosage or exacerbation rates using $\text{FeNO}$ directed management in a population of 85 children (Pijnenburg, Bakker, Hop, & de Jongste 2005); 7 children experienced an exacerbation (defined as course of oral prednisolone) in the $\text{FeNO}$ group and 10 in the control group respectively; both groups experienced a significant increase in inhaled corticosteroid dose during the study. Comparison across studies is not straightforward as there were important differences in management protocols and $\text{FeNO}$ target ranges. In particular use of long acting $\beta$ agonists, which has been associated with a lower exacerbation frequency, was not allowed in the study by Smith et al (Smith, Cowan, Brassett, Herbison, & Taylor 2005). However, the effect of $\text{FeNO}$ guided management on exacerbation rates is consistent across studies. This increases our confidence that a large effect of $\text{FeNO}$ guided management on exacerbation frequency is unlikely. We cannot exclude a smaller albeit clinically relevant effect. Longer and larger studies will be required to do this.

We chose our $\text{FeNO}$ cut off values on the basis of earlier work identifying them as the best indicators of the presence or absence of a raised sputum eosinophil count (Berry, Shaw, Green, Brightling, Wardlaw, & Pavord 2005b) a measure that has been consistently shown to be useful in monitoring asthma (Green, Brightling, McKenna, Hargadon, Parker, Bradding, Wardlaw, & Pavord 2002a; Jayaram et al. 2002). The fact that $\text{FeNO}$ guided management was most effective in participants where $\text{FeNO}$ and sputum eosinophil counts were concordant is consistent with the view that $\text{FeNO}$ acts as a marker of eosinophilic airway inflammation. The absence of effect of $\text{FeNO}$ guided management on exacerbation frequency makes it unlikely that $\text{FeNO}$ is identifying additional aspects of the inflammatory response that are important in the pathogenesis of preventable exacerbations of asthma; it also implies that $\text{FeNO}$ is an imperfect
marker of eosinophilic airway inflammation. A post hoc analysis indicated that our cut off for increasing inhaled corticosteroid dose was a sensitive, but not specific, marker of eosinophilic inflammation. This meant that in a significant proportion of participants inhaled corticosteroid therapy was increased in the absence of eosinophilic airway inflammation; exacerbation frequency in these participants was low whether randomised to the $F_{ENO}$ or control groups. Our study had a built in safety measure where participants whose $F_{ENO}$ remained raised despite a daily dose of 2000μg BDP equivalent had a more detailed evaluation with reference to previously measured induced sputum eosinophil counts. We did this because we reasoned that clinicians would be uncomfortable with increasing therapy beyond this level without specialist review and because current guidelines recommend a review in participants whose asthma is uncontrolled at BTS treatment step 4 (2003a). As a result of this evaluation, the goals of management were changed in a significant proportion (16%) of participants randomised to $F_{ENO}$ guided management; this is the most likely explanation for the initial increase and then decrease in the inhaled corticosteroid dose seen in the $F_{ENO}$ group. The presence of a significant proportion of participants with an elevated $F_{ENO}$ associated with a normal sputum eosinophil count who have a good prognosis is an important limitation of the technique. Our study did not identify any obvious clinical characteristics associated with this pattern of inflammatory markers; further work is required to investigate this. Low $F_{ENO}$ values were reliably associated with absence of eosinophilic inflammation, supporting suggestions (Zacharasiewicz et al. 2005) that a strategy of using of $F_{ENO}$ to guide reduction of inhaled corticosteroid dose might be more effective than the strategy adopted by us.

Our study has several limitations. Firstly, it was not possible to design this study in a double-blinded method. However, the potential for bias was reduced by ensuring that the subjects were blind to their randomisation status, treatment decisions were made in strict accordance with the protocol and rescue oral
corticosteroids or antibiotics were started by a physician who was also unaware of the participants' randomisation status. Secondly, our study could be criticised as we recruited participants with a clinical diagnosis of asthma. It is possible that a clearer reduction in exacerbations would have been seen in participants recruited on the basis of the results of physiological or pathological tests. However, the diagnosis of asthma in the UK remains a largely clinical one (2003a) and we were keen to recruit participants who were representative of those currently seen in primary care. Furthermore, a subgroup analysis on participants with objective evidence of variable airflow obstruction did not show a significant reduction in exacerbation rates within the FE\textsubscript{NO} group. Thirdly, it is possible that more frequent monitoring of FE\textsubscript{NO} might have led to a better outcome. Future studies should investigate whether this is the case and whether a protocol involving more frequent monitoring of FE\textsubscript{NO} is achievable in primary care. Finally, there is a concern about the generalisability of our findings since our population had more severe asthma than that seen previously in a primary care population (Walsh et al. 1999). This may be because recruitment of participants was constrained by limitations imposed by the ethics committees meaning that participants were particularly committed, as they had to respond to both an initial invitation from their primary care practitioner to be contacted and then an invitation to participate. This factor is unlikely to be responsible for the absence of effect of FE\textsubscript{NO} guided management as there is evidence that management guided by markers of eosinophilic inflammation works best in participants with more severe asthma, and in those in whom long acting β\textsubscript{2} agonists are used (Pauwels, Lofdahl, Postma, Tattersfield, O'Byrne, Barnes, & Ullman 1997). However, we cannot discount the possibility that we recruited a population who were particularly aware of their asthma symptoms and who responded particularly well to traditional management.

In conclusion we have found that a management strategy using FE\textsubscript{NO} to guide asthma treatment is feasible in participants with asthma managed in primary
care, but does not lead to a large reduction in either asthma exacerbations or inhaled corticosteroid use when compared to the current treatment strategy.

**Role of the funding source**

This study was funded by a grant from Asthma UK. The study sponsor had no role in study design, data collection, data analysis, data interpretation, or in the writing of the report.

**Acknowledgments**

The authors would like to acknowledge the expert input of the respiratory research nurses, Beverley Hargadon, Sue McKenna, Maria Shelley, Hilary Pateman and the laboratory staff, Debbie Parker, Will Monterio and Natalie Neale; without their hard work, dedication and skill the study would not have been possible. We would also like to thank the doctors, nurses and practice managers of the following GP practices: Markfield, Anstey, Groby and Glenfield. Above all we would like to thank the volunteers.
20 Association between Neutrophilic Airway Inflammation and Airflow Limitation in Adults with Asthma

Abstract

Background
There is debate about the mechanisms of persistent airflow limitation in asthma. Chronic inflammation is assumed to be important although there is limited and contradictory information about the relationship between airway inflammation and post bronchodilator FEV₁ (forced expiratory volume in 1 second).

Methods
We have assessed the cross sectional relationship between pre- and postbronchodilator FEV₁ and measures of airway inflammation, after allowing for the effects of potential confounding factors. Multivariate analysis was performed on data collected from 1197 consecutive patients with asthma seen in the respiratory outpatients at Glenfield hospital between 1997 and 2004. Relationships between induced sputum total neutrophil and differential eosinophil cell counts, and pre- and postbronchodilator lung function were examined.

Results
Sputum total neutrophil but not differential eosinophil count was associated with a lower postbronchodilator FEV₁. Both differential eosinophil and total neutrophil count were associated with lower prebronchodilator FEV₁. These effects were independent after adjustment for age, smoking, ethnicity, asthma duration and inhaled corticosteroid use. A 10-fold increase in neutrophil count was associated
with a 92ml (95% C.I. 29,158: p=0.007) reduction in the postbronchodilator FEV₁.

Conclusions
In this large heterogeneous population of adults with asthma we have shown that prebronchodilator FEV₁ is associated with both neutrophilic and eosinophilic airway inflammation whereas sputum total neutrophil counts alone are associated with postbronchodilator FEV₁. This supports the hypothesis that neutrophilic airway inflammation has a role in the progression of persistent airflow limitation in asthma and raises the possibility that this progression and the development of COPD share a common mechanism.

Introduction
Asthma has been associated with an increased rate of decline in lung function: in a 15 year follow up study by Lange et al, adults with asthma showed a greater decline in lung function than those without the disease, with an unadjusted loss of 38ml per year occurring in the patients with asthma compared to a 22ml loss per year in the controls (Lange et al. 1998). One goal of asthma management is to stop long-term respiratory disability by preventing this loss of lung function so a clearer understanding of the mechanisms involved in the development of fixed airflow obstruction is important. Factors that have been associated with an increased rate of decline include smoking (Sears 2000), duration of asthma (Sears 2000) and absence of atopy (Ulrik, Backer, & Dirksen 1992). Chronic airway inflammation is widely assumed to be important in the genesis of fixed airflow obstruction, although previous studies examining the relationship between airway inflammation and postbronchodilator FEV₁ have produced conflicting results (Bousquet et al. 1990;Djukanovic et al. 1990;ten Brinke, Zwinderman, Sterk, Rabe, & Bel 2001b;Wenzel et al. 1999).
Ten Brinke et al showed that the only independent factor associated with persistent airflow limitation was a differential sputum eosinophilia (ten Brinke, Zwinderman, Sterk, Rabe, & Bel 2001b), whereas Woodruff et al demonstrated that raised differential sputum eosinophil and neutrophil counts were both associated with a lower prebronchodilator FEV₁ (Woodruff, Khashayar, Lazarus, Janson, Avila, Boushey, Segal, & Fahy 2001). One difficulty of studies of this kind is that definition of best FEV₁ is imprecise in a condition that is associated with variable airflow obstruction, although increasing study numbers might allow important relationships to become apparent. We set out to investigate the relationship between pre- and postbronchodilator FEV₁ and measurements of eosinophilic and neutrophilic airway inflammation in a large, well-characterized population of adults with asthma.

**Methods**

**Subjects**

1197 consecutive patients seen in the respiratory outpatient clinic at Glenfield hospital between November 1997 and March 2004 were included in the study. Glenfield hospital is a secondary care facility covering a population of 1 million people (of mixed ethnicity and social class). The main indications for referral for the assessment of patients’ airway disease were diagnostic uncertainty and poor symptom control. Informed consent for the assessment of airway inflammation was obtained for all patients as part of the clinical assessment of airway disease, and the ethics committee from the University Hospitals of Leicester gave ethical approval for the study. All subjects had symptoms of asthma and objective evidence of airway hyper-responsiveness and/or variable airflow obstruction as demonstrated by one or more of the following: an inhaled concentration of methacholine causing a fall of more than 20% in FEV₁ (PC₂₀) of less than 8mg/mL; an increase in FEV₁ of 15% or greater 20 minutes after inhalation of 200µg of albuterol; or peak expiratory flow (PEF) variability of greater than 20%
mean based on PEF recorded twice daily over a two week period. A methacholine
challenge was not performed if the patients FEV₁ was <70% of predicted. In this
situation patients were included if they had a greater than 15% improvement in
their FEV₁ 20 minutes after bronchodilator. Atopy was defined as a wheal 2mm
greater than control on skin prick testing or specific IgE (Pharma CAP test) to
one or more of dust mite, grass, tree, cat, dog or Aspergillus allergens. Smoking
history was recorded as pack years, and was validated against other hospital or
primary care records or exhaled carbon monoxide monitoring if there was doubt
about its veracity.

Measurements
Spirometry was performed using a Vitalograph® spirometer as the best of
consecutive readings within 100ml and skin prick tests were performed using
standard techniques (Dreborg 1989). Pre- and postbronchodilator measurements
were recorded, 20 minutes after the inhalation of 200µg albuterol via spacer.
Induced sputum was obtained and processed as previously described (Pavord,
Pizzichini, Pizzichini, & Hargreave 1997). Methacholine challenge was performed
using a Wright’s nebuliser and the Juniper tidal breathing method (Sterk et al.
1993).

Protocol
At the first visit patients underwent: Spirometry, methacholine challenge test,
sputum induction, skin prick testing and measurement of specific IgE to common
aeroallergens; history of cigarette smoking and duration of symptoms were
recorded. At the next visit pre- and postbronchodilator FEV₁ was recorded.

Analysis
Eosinophil counts were expressed as a percentage of non-squamous cells since
eosinophil differential counts are log normally distributed (Belda, Leigh,
Parameswaran, O'Byrne, Sears, & Hargreave 2000;Pavord, Pizzichini, Pizzichini,
Hargreave 1997; Spanevello, Confalonieri, Sulotto, Romano, Balzano, Migliori, Bianchi, & Michetti 2000). Neutrophil counts were expressed as the total number of neutrophils per gram of sputum. Neutrophil counts were expressed in this way as sputum differential neutrophil counts increase with age (Thomas, Green, Brightling, Birring, Parker, Wardlaw, & Pavord 2004), have a biphasic distribution in our population and because increases in neutrophilic airway inflammation are better reflected by the total neutrophil count rather than the differential (Neale N, Parker D, Barlow S, Green RG, Brightling CE, & Pavord ID 2002). Multiple independent regressions were used to identify predictors of post bronchodilator and pre-bronchodilator FEV₁. Age, height, gender and ethnic origin are known to be associated with these outcomes and were therefore included in the model. Total neutrophil counts and percent eosinophil counts were log transformed prior to analysis to fulfil the model assumption of normal distribution. Atopy and inhaled corticosteroid use were considered potential confounders and were entered into the models as binary variables. Duration of asthma symptoms was also considered a possible cofactor and was entered as a continuous variable. All analysis was performed using SPSS 10 for windows.

Results
Patient demographics are given in Table 20.1.

Postbronchodilator FEV₁
Postbronchodilator FEV₁ was significantly predicted by this model (R squared = 0.43, p<0.001, Table 20.2). Inhaled corticosteroid use was associated with a lower postbronchodilator FEV₁ by on average 223ml (95% confidence interval (CI) 354ml, 93ml, p<0.001) compared with corticosteroid naïve patients. Postbronchodilator FEV₁ was 15ml lower per pack year smoked (95% CI 20ml, 9ml, p<0.001) and 7ml lower per year of asthma (95% CI 11ml, 3ml, p<0.001). Sputum neutrophil count was associated with a 92ml reduction in post
Table 20.1  
Study demographics

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number (Female)</td>
<td>1197 (706)</td>
</tr>
<tr>
<td>Age*(years)</td>
<td>47 (18-83)</td>
</tr>
<tr>
<td>FEV₁ as a % of predicted</td>
<td>82.4 (0.8)</td>
</tr>
<tr>
<td>FEV₁/FVC</td>
<td>72 (0.5)</td>
</tr>
<tr>
<td>Smokers (number)</td>
<td>407</td>
</tr>
<tr>
<td>Pack years (smokers only)</td>
<td>13.2 (0.7)</td>
</tr>
<tr>
<td>Duration of symptoms (years)</td>
<td>22 (0.5)</td>
</tr>
<tr>
<td>Inhaled steroids (number)</td>
<td>969</td>
</tr>
<tr>
<td>Sputum neutrophil count (x10⁶/g of sputum)**</td>
<td>0.96 (0.03)</td>
</tr>
<tr>
<td>Sputum eosinophil count (%)**</td>
<td>1.8 (0.03)</td>
</tr>
</tbody>
</table>

Figures are expressed as mean (standard error mean) unless marked * mean (range) and ** geometric mean (log standard error mean)
Table 20.2
Mutiple independent regression: Dependent Variable: Postbronchodilator FEV₁

<table>
<thead>
<tr>
<th>Change in FEV₁ in ml per change in variable</th>
<th>Unstandardized Coefficients</th>
<th>Significance 95% Confidence Interval for Variable</th>
</tr>
</thead>
<tbody>
<tr>
<td>Constant</td>
<td>-4928</td>
<td>.723 .000 -6.348 -3.509</td>
</tr>
<tr>
<td>Age (year)</td>
<td>-14.5</td>
<td>.002 .000 -.018 -.011</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>52.9</td>
<td>.004 .000 .045 .061</td>
</tr>
<tr>
<td>Gender(m/f) (if female)</td>
<td>-305</td>
<td>.057 .000 -.416 -.194</td>
</tr>
<tr>
<td>Ethnic origin (if female)</td>
<td>-448</td>
<td>.097 .000 -.637 -.258</td>
</tr>
<tr>
<td>Inhaled steroid (yes/no) (if on ICS)</td>
<td>-223</td>
<td>.066 .001 -.354 -.093</td>
</tr>
<tr>
<td>Atopy (yes/no)</td>
<td>98.7</td>
<td>.083 .234 -.064 .262</td>
</tr>
<tr>
<td>Pack years (per pack year)</td>
<td>-14.7</td>
<td>.003 .000 -.020 -.009</td>
</tr>
<tr>
<td>Duration of asthma (year)</td>
<td>-7.04</td>
<td>.002 .001 -.011 -.003</td>
</tr>
<tr>
<td>Log neutrophils</td>
<td>-91.6</td>
<td>.034 .007 -.158 -.026</td>
</tr>
<tr>
<td>Log eosinophils</td>
<td>-50</td>
<td>.033 .127 -.116 .014</td>
</tr>
</tbody>
</table>

A 10 fold increase in total neutrophil count was associated with a 92ml reduction in post bronchodilator FEV₁.
bronchodilator FEV₁ per 10 fold rise in total sputum neutrophil count per mg (95% CI 158ml, 26ml, p<0.007). Sputum eosinophil count and atopy did not independently predict postbronchodilator FEV₁.

**Prebronchodilator FEV₁**
Prebronchodilator FEV₁ was significantly predicted by this model (R squared = 0.39, p<0.001, Table 20.3.). Inhaled corticosteroid use was associated with a lower prebronchodilator FEV₁ when compared with corticosteroid naïve patients. Prebronchodilator FEV₁ was reduced by 14ml per pack year smoked (95% CI 19ml, 8ml, p<0.001) and 9ml per year of asthma symptoms (95% 13ml, 5 ml, p<0.001). Sputum neutrophil count was associated with an 83ml reduction in prebronchodilator FEV₁ per 10 fold rise in total neutrophil count per gram (95% CI 152ml, 15ml, p<0.017) and sputum eosinophil count predicted a 116ml lower prebronchodilator FEV₁ per 10 fold increase in percent eosinophil count (95% CI 183ml, 49ml, p<0.001). Atopy did not independently predict prebronchodilator FEV₁.

**Discussion**
We have shown a relationship between pre- and postbronchodilator FEV₁ and sputum measures of airway inflammation in a large heterogeneous population of adults with asthma. Postbronchodilator FEV₁ was associated with sputum total neutrophil count. Smoking, inhaled corticosteroid use and asthma duration were also associated with a lower postbronchodilator FEV₁. A raised sputum neutrophil count was associated with a lower prebronchodilator FEV₁ but to a lesser extent than the differential sputum eosinophil count where a 10-fold increase in eosinophil percentage was associated with a 116ml lower prebronchodilator FEV₁.
### Table 20.3

Multiple independent regression: Dependent Variable: Prebronchodilator FEV$_1$

<table>
<thead>
<tr>
<th>Change in FEV$_1$ in ml per change in variable</th>
<th>Unstandardized Coefficients</th>
<th>Significance 95% Confidence Interval for B</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Change in variable</td>
<td>Standard Error</td>
</tr>
<tr>
<td>Constant</td>
<td>-4698</td>
<td>.748</td>
</tr>
<tr>
<td>Age (year)</td>
<td>-14.4</td>
<td>.002</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>51.1</td>
<td>.004</td>
</tr>
<tr>
<td>Gender (male/female)</td>
<td>-272</td>
<td>.058</td>
</tr>
<tr>
<td>Ethnic origin (if female)</td>
<td>-419</td>
<td>.100</td>
</tr>
<tr>
<td>Inhaled steroid (yes/no) (if on ICS)</td>
<td>-255</td>
<td>.069</td>
</tr>
<tr>
<td>Atopy (yes/no)</td>
<td>64.24</td>
<td>.086</td>
</tr>
<tr>
<td>Pack years (years)</td>
<td>-13.76</td>
<td>.003</td>
</tr>
<tr>
<td>Duration of asthma (year)</td>
<td>-8.93</td>
<td>.002</td>
</tr>
<tr>
<td>Log neutrophils</td>
<td>-83.25</td>
<td>.035</td>
</tr>
<tr>
<td>Log eosinophils</td>
<td>-116</td>
<td>.034</td>
</tr>
</tbody>
</table>

A 10 fold increase in percent eosinophil count predicted a 116ml lower prebronchodilator FEV$_1$.

A 10 fold rise in total neutrophil count was associated with an 83ml reduction in prebronchodilator FEV$_1$. 
Woodruff et al have also demonstrated a relationship between airway neutrophilia and persistent airflow limitation in asthma (Woodruff, Khashayar, Lazarus, Janson, Avila, Boushey, Segal, & Fahy 2001). They used multivariate analysis of data collected during screening and enrolment of 205 adults with asthma. After controlling for confounding factors, their analysis demonstrated that eosinophil percentage in induced sputum was independently associated with a lower FEV₁ and a lower PC₂₀. In the same models an increased sputum neutrophil percentage was independently associated with lower FEV₁ but not with PC₂₀. These results suggest that both eosinophilic inflammation and neutrophilic inflammation independently contribute to abnormalities of FEV₁ in asthma. A study by Little et al also demonstrated that maximal FEV₁ was inversely associated with both duration of disease and differential sputum neutrophil count. Our findings and the findings of earlier studies would be consistent with a model where eosinophilic airway inflammation contributes to variable airflow obstruction and airway hyperresponsiveness, and neutrophilic inflammation contributes to irreversible airflow obstruction in asthma (Little et al. 2002). However one small longitudinal bronchoscopy study found that eosinophil counts in bronchial biopsies did not correlate with either pre- or postbronchodilator FEV₁ (van Rensen et al. 2005). This may reflect the different lung compartment sampled in bronchial biopsy as compared to induced sputum.

Our findings differ from those of Ten Brinke et al who found that out of age at onset, smoking history, atopic status, bronchodilator reversibility, PC₂₀ histamine, exhaled nitric oxide, blood eosinophils and total IgE, the only independent factor associated with persistent airflow limitation was sputum eosinophilia (ten Brinke, Zwinderman, Sterk, Rabe, & Bel 2001b). This was a smaller homogeneous population with a more limited analysis of dichotomous variables; 132 non-smoking asthmatics on high dose inhaled corticosteroids were studied and persistent airflow limitation was defined as a postbronchodilator FEV₁ or FEV₁/FVC less than 75% predicted. The association was not apparent in the
subgroup taking oral corticosteroids suggesting that the patients receiving inhaled corticosteroids may have been undertreated.

Our findings apply to patients referred to secondary care for investigation of poor symptom control or diagnostic uncertainty. Our study should be interpreted within light of these constraints. Our patients were not assessed at the time of exacerbation although we did not specifically collect information on the time of the last exacerbation, so it is possible that effect of a prior exacerbation may affect the assessment of airway inflammation. This was a large study so it was not possible to collect data on the variables of potential importance including occupational dust exposure or time of last cigarette or exact treatment dose, but the use of corticosteroids or long acting $\beta_2$ agonists have been shown not to influence sputum neutrophil counts significantly (Green, Brightling, Woltmann, Parker, Wardlaw, & Pavord 2002b). It was also not possible to perform complex analysis of cell activation markers on induced sputum supernatant. Further studies should evaluate this. We were careful to standardize sputum induction time, so this is unlikely to have biased our neutrophil counts. Similarly there is no evidence that prior methacholine challenge affects sputum cell counts (Spanevello et al. 1999).

Our findings are consistent with the view that neutrophilic airway inflammation contributes to the development of fixed airflow obstruction in asthma although we recognize that a cross sectional analysis such as this cannot prove a causal relationship. However there are several lines of evidence to suggest that the relationship we have seen is real. Firstly our findings are consistent with those of several previous, smaller studies (Little, MacLeod, Chalmers, Love, McSharry, & Thomson 2002; Woodruff, Khashayar, Lazarus, Janson, Avila, Boushey, Segal, & Fahy 2001; Woodruff & Fahy 2002). Secondly the relationship between neutrophilic airway inflammation and progressive airflow obstruction is biologically plausible since neutrophils can secrete a variety of inflammatory...
factors including cytokines, proteases and lung parenchymal reactive oxygen species that can cause mucus hypersecretion and airway damage. Thirdly, our findings are consistent with increasing evidence implicating neutrophilic inflammation in severe asthma (2003b), a phenotype that is particularly associated with fixed airflow obstruction (Wenzel, Schwartz, Langmack, Halliday, Trudeau, Gibbs, & Chu 1999).

One of the difficulties of a study investigating progressive airflow obstruction in asthma is the definition of best achievable FEVi. We recognize that some patients may have had important improvement in FEVi with more intensive corticosteroid therapy. However, improvement in FEVi following corticosteroid treatment is associated with an increase in the sputum eosinophil count but not sputum neutrophils (Pavord, Brightling, Woltmann, & Wardlaw 1999) so this factor is unlikely to have influenced the relationship between sputum neutrophils and postbronchodilator FEVi. In keeping with this view Little et al have shown that an increase in sputum neutrophils is associated with lower post bronchodilator, post oral corticosteroid FEVi (Little, MacLeod, Chalmers, Love, McSharry, & Thomson 2002).

Previous studies have demonstrated a consistent cross sectional relationship between sputum neutrophils and post bronchodilator FEVi in chronic obstructive pulmonary disease (COPD) (Perng et al. 2004). Moreover, the rate of decline in FEVi in subjects with COPD is associated with the sputum neutrophil count (Stanescu et al. 1996). These findings raise the possibility that the mechanisms of development of progressive fixed airflow obstruction in asthma and COPD have some similarities. Further study of these mechanisms is potentially clinically important since there is no evidence that the neutrophilic small airway inflammatory response thought to be important in the pathogenesis of COPD is corticosteroid responsive (Brightling, Monteiro, Ward, Parker, Morgan, Wardlaw, & Pavord 2000a).
21 Summary

To establish if a treatment protocol based on $\text{FE}_{\text{NO}}$ measurements is better at predicting and preventing asthma exacerbations than clinical guidelines, several assumptions have to be made. Firstly, it must be assumed that $\text{FE}_{\text{NO}}$ measurements reflect an important aspect of the disease process acting either directly or as a surrogate marker, secondly that $\text{FE}_{\text{NO}}$ is modifiable and responds to treatment, and thirdly that $\text{FE}_{\text{NO}}$ measurements tell the clinician something that cannot be discerned by simpler methods. The model for using $\text{FE}_{\text{NO}}$ measurements as a marker of airway inflammation was induced sputum differential eosinophil counts.

Although the use of differential eosinophil counts has been shown to be better at reducing asthma exacerbations than a symptom based approach in moderate to severe asthma (Green, Brightling, McKenna, Hargadon, Parker, Bradding, Wardlaw, & Pavord 2002a), this has not been the case in mild to moderate asthma (Jayaram et al. 2006), although new symptom based algorithms show promise (Papi, Canonica, Maestrelli, Paggiaro, Olivieri, Pozzi, Crimi, Vignola, Morelli, Nicolini, & Fabbri 2007). This may be because a further assumption, that there is discordance between airway inflammation and symptoms, is incorrect in mild to moderate asthma. While evidence from the FACET study suggested that in moderate to severe asthma higher dose inhaled corticosteroids have a marked beneficial effect on exacerbation frequency, but relatively less effect on symptoms and peak expiratory flow (indicating that exacerbation frequency does not relate closely to symptoms and measures of disordered airway function) (Pauwels, Lofdahl, Postma, Tattersfield, O'Byrne, Barnes, & Ullman 1997), evidence in mild asthma is lacking. Although cross-sectional studies also suggest that to a large extent disordered airway function and eosinophilic airway inflammation may be independently regulated, the relationship between changes...
in these markers within patients might be closer than the relationship between patients.

21.1 FE\textsubscript{NO} and sputum eosinophilia in the observational study

The relationship between FE\textsubscript{NO} and sputum eosinophil counts was significant but not strong ($R^2 = 0.26$, $p<0.01$), and differed depending upon the cohort studied: the association was closer in nonsmokers ($R^2 = 0.28$, $p<0.001$) than current smokers ($R^2 = 0.15$, $p<0.001$) or ex-smokers ($R^2 = 0.08$, $p=0.11$), and there was a gender difference. There was also a weaker relationship at lower FE\textsubscript{NO} values, which may reflect the sampling flow used (250ml/sec). In terms of identifying a sputum eosinophil count of $>3\%$ a FE\textsubscript{NO} of 8.3 p.p.b. had 71$\%$ sensitivity and 72$\%$ specificity meaning that an individual with this FE\textsubscript{NO} value was 2.6 times more likely to have a sputum eosinophil count of $>3\%$. The best sensitivity and specificity at 50ml/sec (in a much smaller number of patients) was 78$\%$ and 72$\%$ respectively at 36 p.p.b. (Taylor et al. 2006). This may suggest that the cut offs used in the intervention study were incorrect; we initially used 26p.p.b. as a cut off as this was the value at which we had the most data. Since our study there has been a suggestion that the clinical significance of a FE\textsubscript{NO} value between 25-50p.p.b. is unclear. Future studies need to consider not only using a different cut off, but also individually tailoring FE\textsubscript{NO} values, possibly in a similar manner to peak flow.

21.2 FE\textsubscript{NO} and sputum eosinophilia in the intervention study

The relationship between baseline log FE\textsubscript{NO} and log sputum eosinophil count was similar to that seen in the observational study, at 50ml/sec ($r^2 = 0.455$, $p<0.001$). A FE\textsubscript{NO} of $<26$ppb was associated with a differential sputum eosinophil count of $<3\%$ for 85$\%$ of all visits when both were measured. However a FE\textsubscript{NO} of $>26$ppb
was frequently associated with a sputum eosinophil count of <3%, so in a significant proportion of participants inhaled corticosteroid therapy was increased in the absence of eosinophilic airway inflammation; these patients had a good prognosis. This discrepancy may arise because $F_{ENO}$ represents a different part of the inflammatory phenotype, especially in response to treatment. Other factors including nasal contamination and atopy may have influenced $F_{ENO}$ measurements.

21.3 Study Weaknesses

Aside from the weaknesses already discussed, several other problems became apparent. In the interventional study the treatment algorithm led itself to a large increase in inhaled corticosteroid dose in the $F_{ENO}$ group, as the dose was doubled at each step. When the corticosteroid dose of 9 patients, with a poor relationship between $F_{ENO}$ measurements and sputum eosinophil counts, was down titrated there was a large fall in the overall amount of corticosteroid used. It may have been prescient to only increase each step by 200-400 micrograms.

The decision to only use measurement of $F_{ENO}$ at 50ml/sec, based on the guidelines, may have disadvantaged the interventional study. A broader range of flows may give more discriminating information, especially in patients with different severities of asthma.

The exacerbation frequency was much lower in the control group during the study when compared to previous studies, and to the exacerbation rate pre-study. This greatly affected our power calculation but study recruitment was difficult and made harder by the ethics committees.

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21.4 Sputum neutrophilia and postbronchodilator FEV₁

This large cross sectional study showed a significant relationship between neutrophilic airway inflammation and postbronchodilator FEV₁. This supports the hypothesis that neutrophilic airway inflammation has a role in the progression of persistent airflow limitation in asthma and raises the possibility that this progression and the development of COPD share a common mechanism. This emphasizes the importance of developing a treatment that modulates neutrophilic airway inflammation, as this could potentially prevent decline in lung function.

21.5 Unanswered questions

Using a management algorithm based on $F_{ENO}$ was no worse than current clinical guidelines; could it therefore be improved? Focus must fall on several areas. Firstly, is it possible to generate a best $F_{ENO}$ measurement for each individual? Change around this value may better reflect underlying change in airway inflammation than an $F_{ENO}$ cut off based on population data.

Secondly, is the step up/ step down model the best algorithm to follow? It may make more sense to use low $F_{ENO}$ measurements as a negative predictive tool for airway inflammation, mirroring troponin use in myocardial ischaemia.

Thirdly, a significant number of patients with a label of asthma had no evidence of airways disease, yet were receiving treatment. This may suggest that earlier more intensive assessment of airway physiology may prevent unnecessary treatment. Further work is needed to answer these questions, but the non-invasive assessment of airway inflammation in asthma continues to shed light on a condition which despite its prevalence, is still poorly understood.
22 Appendix

22.1 Juniper asthma control questionnaire

On average, during the past period, how often were you woken by your asthma during the night?
0 Never
1 Hardly ever
2 A few times
3 Several times
4 Many times
5 A great many times
6 Unable to sleep because of asthma

On average, during the past period, how bad were your asthma symptoms when you woke up in the morning?
0 No symptoms
1 Very mild symptoms
2 Mild symptoms
3 Moderate symptoms
4 Quite severe symptoms
5 Severe symptoms
6 Very severe symptoms

In general, during the past period, how limited were you in your activities because of your asthma?
0 Not limited at all
1 Very slightly limited
2 Slightly limited
3 Moderately limited
4 Very limited
5 Extremely limited
6 Totally limited

In general, during the past period, how much shortness of breath did you experience because of your asthma?

0 None
1 A very little
2 A little
3 A moderate amount
4 Quite a lot
5 A great deal
6 A very great deal

In general, during the past period, how much of the time did you wheeze?

0 Not at all
1 Hardly any of the time
2 A little of the time
3 A moderate amount of the time
4 A lot of the time
5 Most of the time
6 All the time

On average, during the past period, how many puffs of short acting bronchodilator (e.g., Ventolin) have you used each day?
| 0 None                                          | 0 >95% predicted |
| 1 1–2 puffs most days                          | 1 95–90%        |
| 2 3–4 puffs most days                          | 2 89–80%        |
| 3 5–8 puffs most days                          | 3 79–70%        |
| 4 9–12 puffs most days                         | 4 69–60%        |
| 5 13–16 puffs most days                        | 5 59–50%        |
| 6 More than 16 puffs most days                 | 6 <50% predicted |

7. FEV₁ prebronchodilator: ........................ 0 >95% predicted

FEV₁ predicted: .................................... 1 95–90%

FEV₁% predicted: ................................. 2 89–80%

FEV₁% predicted: ................................. 3 79–70%

FEV₁% predicted: ................................. 4 69–60%

FEV₁% predicted: ................................. 5 59–50%

FEV₁% predicted: ................................. 6 <50% predicted
22.2 Letter of invitation

A more intensive approach to diagnosis and management of asthma in primary care. A randomised controlled trial.

A research study is being carried out at the Glenfield Hospital by Dr D Shaw.

a. The study has been designed to examine/investigate methods of monitoring airway inflammation in asthma. Patients are being asked to complete questionnaires/answer questions about asthma.

b. It is hoped that the results of this study will improve/help the future management of patients with asthma.

If you would like to take part in this study, details of which are given on the information leaflet enclosed, please complete the reply slip enclosed with this letter and return it in the pre-paid envelope. The research nurse/investigator will then contact you to arrange a convenient time to obtain your consent and ask you to fill in a questionnaire/conduct a short interview, which should last no more than one hour.

I would like to thank you for taking time to read this letter and hope to hear from you soon. If you have any queries, please feel free to contact me on the telephone number below.

Yours Sincerely,

Dr D Shaw, Research Fellow, Glenfield Hospital
Institute of Lung Health

Telephone No: 0116 2871471 ext: 2724
22.3 Consent form

A more intensive approach to diagnosis and management of asthma in primary care. A randomised controlled trial.

Name of Researcher: Dr Dominick Shaw

Please initial box

1. I confirm that I have read and understand the information sheet dated 15/05/03, (version 1) for the above study and have had the opportunity to ask questions.

2. I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason, without my medical care or legal rights being affected.

3. I understand that sections of any of my medical notes may be looked at by responsible individuals from Glenfield Hospital or from regulatory authorities where it is relevant to my taking part in research. I give permission for these individuals to have access to my records.

4. I agree to take part in the above study.

Name of Patient  Signature  Date

Name of Person taking consent (if different from researcher)  Signature  Date

Researcher  Signature  Date

1 for patient; 1 for researcher; 1 to be kept with hospital notes
22.4 Patient information form

Principal Investigator Supervising Consultant

Dr. Dominick Shaw Dr. Ian Pavord
Specialist Registrar Consultant Physician

You are being invited to participate in a research study.

Before you decide it is important for you to understand why the research is being done and what it will involve.

Please take time to read this information and discuss it with others if you wish.

Please ask us if anything is not clear or if you need more information.

Take time to decide whether or not you wish to take part.

Purpose of the study

The breathing tubes in asthma become very congested and inflamed with different types of cells. This inflammation in the breathing tubes is thought to lead to sudden deteriorations in the breathing of patients with asthma. Inflammation in the lower breathing tubes can be measured by different methods, including measuring a gas present in your breath called nitric oxide. This is a new test and whether it provides useful information over and above the tests that are traditionally done in asthma (i.e. peak flow) is unclear.
Our study proposes to evaluate whether measuring nitric oxide in exhaled air using a simple breathalyser kit is helpful in the long-term management of asthma.

**Why have I been chosen?**
You have been invited to participate if you have been diagnosed with asthma and are receiving 1 or more inhaler prescriptions per year.

**Do I have to take part?**
No, taking part in this study is entirely up to you. If you decide not to take part it will not influence your current or future treatment. If you decide to take part you will be asked to sign a consent form. You will still be free to withdraw at any time, without giving a reason. Again, this will not affect the standard of care that you receive.

**What will happen to me if I take part?**
On your first visit, you will be seen by a specialist respiratory nurse at the Glenfield hospital, where you will be asked to fill out a questionnaire. You will then have a variety of breathing tests, designed to test how well your lungs work and how twitchy your breathing tubes are.

1) **Spirometry**
Spirometry is the name of the test that is done to measure how fast you can blow out and how much air you can blow out in total. During this test you will be asked to blow into a tube connected to a recording device as fast as you can for as long as you can. We usually ask you to repeat this test two or three times but sometimes more. This test takes about 2 minutes to complete.
If your spirometry results are slightly lower than normal you will not have a methacholine challenge test (see test 3) but will have a bronchodilator reversibility test.

2) Bronchodilator reversibility test
This test is designed to see whether your breathing tubes dilate to an inhaled drug called salbutamol. This is the main drug used in the treatment of asthma. After inhaling a small dose of salbutamol, your spirometry results will be reassessed to see whether you breathing tubes are working more efficiently.

3) Methacholine challenge test
This test is to find out how "twitchy" your breathing tubes are. It is a simple and safe test widely used in the assessment of asthma. We ask you to breathe in some mist, which contains a substance called methacholine, for 2 minutes at a time, after which your spirometry breathing tests are repeated. Methacholine is a mild irritant to the breathing tubes and may make you feel tight or wheezy. For safety we would start the test at a very low dose which would gradually be increased if your breathing tests do not change significantly. If your breathing tests do go down, or you feel unable to continue, the test would be stopped and we would give you an inhaler to take, which rapidly reverses the effects of the methacholine and also makes it safe to continue to the next test. The length of this test depends on how long it takes for your breathing tubes to become twitchy but usually takes between 15 and 30 minutes.

4) Sputum induction
The aim of this test is to measure the amount of inflammation in the breathing tubes. We would ask you to inhale some salty mist, this loosens up the secretions in the breathing tubes which we would ask you to cough into a pot. We would always treat you with an inhaler or nebuliser before this test to prevent you from getting wheezy during it, sometimes however people can feel a
bit tight or twitchy following this test, this usually resolves very quickly with further inhaler or nebuliser treatment. This test takes about twenty minutes.

5) Skin Atopy
This will assess your potential to develop an allergy to various substances including grass and pollen. A small scratch will be made on your forearm and the degree of redness and swelling will be measured. Occasionally the swelling and redness requires an anti-histamine to settle.

6) Peak flow
You will be given a peak flow diary to keep. This will mean doing a simple breathing test at home every morning and night and recording the result. You will also be asked to keep a record of your asthma symptoms.

7) Exhaled nitric oxide
This is a quick and easy measurement of the amount of inflammation in the breathing tubes. It involves breathing into a rubber tube connected to an analyser for a few seconds. This is repeated a few times. This test takes about four minutes to complete.

After this two-week visit you will be asked to re-attend monthly for 4 months and then two monthly for 8 months, a total of 8 further visits. These visits will be brief and at your own convenience as far as possible. You will also be asked to attend for sputum induction at 3, 6 and 12 months. This test should not take longer than 15 minutes.

You will be allocated to one of two groups- a group receiving treatment based on measurement of exhaled nitric oxide and a group receiving treatment based on traditional markers of asthma such as peak flow.
Depending on your results and symptoms your asthma medication will be increased or decreased, to try and improve your asthma control.

**Which group will I be allocated to?**
The groups are selected by a computer which has no information about the individual – i.e. by chance.

**What do I have to do?**
You should carry on as normal. This study should not affect your lifestyle. You should carry on taking your regular medication. The study will not affect you or your baby if you are pregnant, or become pregnant during the trial.

**What is the procedure being tested?**
We are assessing a new way of monitoring asthma treatment. Normally asthma medication is increased or decreased depending on symptoms. We are comparing this old method with a new method based on the assessment of exhaled nitric oxide.

**What are the possible disadvantages of taking part?**
The challenge test and the sputum test can sometimes make you feel a bit tight chested but this can be rapidly reversed with an inhaler or nebuliser. As part of the study you may receive steroids as part of your treatment. Steroids are drugs which reduce inflammation in the airways. They can be taken in tablet or inhaled form. Rarely steroids can cause side effects. You will only receive steroids in this study if your breathing tests are worse than predicted. Occasionally they can cause weight gain and easy bruising.

**What are the possible benefits of taking part?**
This trial looks at ways of assessing asthma severity and its treatment. It is hoped that we will be able to assess and control asthma symptoms more
accurately and sensitively. This will hopefully lead to a decrease in asthma exacerbations and inappropriate medication use, as well as identifying some patients whose symptoms may not be due to asthma.

**What if something goes wrong?**
The chance of this is remote. However if you are harmed by taking part in this research study, there are no special compensation arrangements. If you are harmed due to someone’s negligence, then you may have grounds for a legal action but you may have to pay for it. Regardless of this, if you wish to complain, or have any concerns about any aspect of the way you have been approached or treated during the course of this study, the normal National Health Service complaints mechanisms will be available to you.

**Will my taking part in this study be kept confidential?**
All information which is collected about you during the course of this study will be kept strictly confidential. Any information about you, which leaves the hospital, will have your name and address removed so you cannot be identified from it. Your G.P. will be informed of your participation in the study.

**Will travel expenses be reimbursed?**
Taxi fares or a refund for mileage allowance will be available. (maximum £15)

**What will happen to the results of the research study?**
The results will hopefully be published in a respiratory journal. They may also be presented at respiratory meetings. Once published you will be able to obtain a copy of the results if you wish. Please ask us if you would like a copy.

**Who is funding and organising the research?**
The National Asthma Campaign, a charity dedicated to improving the treatment of asthma, is funding the research.
Neither the doctors nor nurses involved stand to gain financially from involvement in this study.

**Who has reviewed the study?**
All research that involves NHS patients or staff, information from NHS medical records or uses NHS premises or facilities must be approved by an NHS Research Ethics Committee before it goes ahead. Approval does not guarantee that you will not come to any harm if you take part. However, approval means that the committee is satisfied that your rights will be respected, that any risks have been reduced to a minimum and balanced against possible benefits and that you have been given sufficient information on which to make an informed decision.

**Contact for further information**
Dr Dominick Shaw, Institute for Lung Health, Glenfield Hospital.
Thank you for reading this information sheet.
Please keep a copy of this information sheet, and a copy of the consent form.
23 References

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