The use of induced sputum in the clinical assessment and management of asthma

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Ruth H. Green MB ChB MRCP
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The use of induced sputum in the clinical assessment and management of asthma

Ruth H Green

Abstract

Asthma is a disease characterised by airway inflammation, which is predominantly eosinophilic. Recent developments in the technique of sputum induction have provided a safe non-invasive method of measuring airway inflammation that can be applied to wide populations of patients with asthma and other airway diseases. This thesis explores the use of induced sputum to measure airway inflammation in the assessment and management of adults with asthma. It provides the first evidence that the use of this technique in the management of asthma leads to improved patient outcomes. I have employed induced sputum to assess lower airway inflammation in a large population of patients with symptomatic mild to moderate asthma and have demonstrated considerable heterogeneity of the inflammatory response. I have identified a population of patients with isolated neutrophilic airway inflammation and have provided evidence that such patients respond poorly to inhaled corticosteroid treatment. I have described a management strategy directed at normalising the sputum eosinophil count, as well as controlling symptoms and peak flow readings. I have shown that this management strategy leads to a dramatic reduction in severe asthma exacerbations and prevents hospital admissions compared to a traditional clinical approach and that eosinophilic inflammation is an important risk factor for severe asthma exacerbations. Finally, I report that amongst patients with asthma who remain symptomatic despite low dose inhaled corticosteroids, high dose inhaled corticosteroids and long acting β2-agonists have contrasting effects on symptoms, lung function and the sputum eosinophil count, suggesting that there is a dissociation between eosinophilic airway inflammation, day-to-day symptoms and variable airflow obstruction in asthma. These findings suggest that the regular monitoring of airway inflammation may be required for optimal asthma management.
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Statement of work personally performed

I personally designed and obtained funding for the cross-over study and developed and amended the design of the cross-sectional and sputum management studies. I obtained ethical approval for the sputum management and cross-over studies and wrote the detailed trial protocols for each study. I was responsible for patient recruitment for each study and obtained all the informed consent. I personally undertook approximately 50% of all the clinical measurements and recruited, trained and supervised research nurses who performed the remaining measurements. I wrote the laboratory protocols, performed the initial laboratory measurements and recruited, trained and supervised the technicians who undertook the subsequent measurements. The sputum cell counts and leukotriene assays were done by others. I designed the database for each study, performed 75% of data entry and trained and supervised the research nurses who completed the remaining data entry. I personally undertook the data analysis and interpretation.
Publications arising from this thesis

Papers


Pavord ID, Green RH, Berry MA, Brightling CE, Wardlaw AJ. The significance of eosinophilic airway inflammation in asthma. CPD Bulletin Immunology and Allergy 2002; 2(3): 71-74


Abstracts

Green RH, Brightling CE, McKenna S, Hargadon B, Parker D, Bradding P, Wardlaw AJ, Pavord ID. The cost effectiveness of an asthma management strategy directed at normalising the induced sputum eosinophil count. Thorax 2002;57(S III):iii1

Green RH, Brightling CE, McKenna S, Hargadon B, Parker D, Wardlaw AJ, Pavord ID. A placebo controlled comparison of formoterol, montelukast or higher dose of inhaled corticosteroids in subjects with symptomatic asthma despite treatment with low dose inhaled corticosteroid. Thorax 2002;57(S III):iii11

Berry MA, Green RH, Wardlaw AJ, Pavord ID. Factors influencing cross sectional and longitudinal associations between exhaled nitric oxide and induced sputum eosinophil count in adults with asthma. Thorax 2002;57(S III):iii37

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1. Introduction

Asthma is a common disorder, is increasing in prevalence in industrialised societies and attracts considerable research interest. Despite this, a precise, universally accepted definition of the disease remains elusive. Clinically, asthma is characterised by breathlessness, wheeze and cough which is usually worse at night, relieved by bronchodilators and variable in intensity. Since none of these features are specific to asthma, the condition is commonly defined by the presence of abnormal airway function encompassing 3 related features: variable airflow obstruction, airway hyperresponsiveness and chronic airway inflammation, which is usually, eosinophilic. The rather non-specific clinical features of asthma highlight the need for accurate confirmation of the diagnosis by the objective demonstration of abnormal airway physiology.

Within this broad definition of asthma different clinical phenotypes occur. For example, over 80% of patients have atopic disease, often in association with hayfever or eczema, and in these subjects positive skin prick tests or elevated IgE levels can be demonstrated. An important minority of patients however, have non-atopic asthma with no evidence of sensitisation to aeroallergens. These patients tend to present later in life, often with asthma that is resistant to treatment and are more likely to develop fixed airflow obstruction (Ulrik, Backer, & Dirksen 1992). Similarly, the majority of patients with asthma have mild or moderate disease which can usually be well controlled by currently available therapies, but an estimated 5-10% of patients have more severe or “refractory” asthma (American Thoracic Society 2000). Patients considered to have refractory disease include those requiring high doses of anti-inflammatory and bronchodilator medication to maintain their asthma control and those who, despite treatment, have persistent airflow obstruction or recurrent exacerbations of asthma. Each of these phenotypes raises important management issues: high medication use may be associated with undesirable adverse effects, fixed airflow obstruction may cause considerable disability and recurrent asthma exacerbations are likely to result in significant morbidity, a high demand on healthcare resources and even death.
The recognition of patients with refractory disease despite current treatment strategies provides obvious challenges for the improvement of asthma management and for the identification of clinical or pathophysiological features that may identify individuals at particular risk of developing severe disease. Important limitations in the management of patients with milder asthma also remain. For example, whilst most patients who have persistent symptoms despite regular low dose inhaled corticosteroids can be adequately controlled with currently available therapies, a range of options are available and current treatment guidelines recommend ad hoc therapeutic trials for individual patients. Asthma is increasingly recognised as an inflammatory disease of the airway and it is important to question whether advances in the understanding of airway pathophysiology, particularly chronic airway inflammation, in asthma may remove the current limitations of asthma management and lead to improved patient outcomes.

Advances in the non-invasive measurements of airway inflammation have provided the opportunity to address these issues. This thesis assesses the value of the use of the technique of induced sputum to measure airway inflammation, particularly eosinophilic inflammation, in the clinical assessment and management of patients with asthma. It specifically questions the hypotheses that the use of induced sputum will demonstrate that eosinophilic airway inflammation is not invariably present in patients with asthma, that the response to treatment differs according to the extent of eosinophilic inflammation and that targeting treatment to the degree of eosinophilic inflammation will improve clinical outcomes. The introductory section will discuss the importance of eosinophilic inflammation in asthma and will describe the development and validity of sputum induction as a tool to measure airway inflammation. Current asthma management guidelines and their limitations will then be reviewed and potential roles for the use of sputum induction in improving asthma management leading to our hypotheses will be discussed. The results of three studies designed to test these hypotheses will then be presented. The first study examines the nature and severity of airway inflammation in a large population of patients with mild to moderate asthma and relates the findings to treatment response in a
subset of patients studied before and after treatment with inhaled corticosteroids. The second study describes a randomised controlled trial designed to test the hypothesis that a management strategy directed at normalising the induced sputum eosinophil count will reduce exacerbations compared to a traditional clinical approach. The final study describes a placebo controlled cross-over trial designed to compare the effects of higher dose inhaled corticosteroids, additional long acting β₂ agonists and additional leukotriene antagonists with placebo on airway hyperresponsiveness, symptoms and airway inflammation in an unselected population of patients with asthma.
2. Asthma

2.1 Eosinophilic airway inflammation in asthma

Pathologically, asthma is associated with chronic mucosal inflammation, predominantly characterised by infiltration of the airway by activated eosinophils, CD4+ lymphocytes, macrophages and degranulated mast cells. Epithelial activation and fragility, basement membrane thickening, and goblet cell and smooth muscle hypertrophy/hyperplasia are also usually found (Djukanovic et al. 1990; Kay 1996). These changes are associated with disordered airway physiology manifest as mucous hypersecretion, airway hyperresponsiveness and variable airflow obstruction.

In asthma, eosinophilic inflammation and airway hyperresponsiveness generally occur together, such that eosinophils have often been thought to play a causal role in the development of variable airflow obstruction and airway hyperresponsiveness, although much research has failed to fully establish this. Recently, however, the role of the eosinophil in the pathophysiology and clinical manifestations of asthma has been increasingly called into question (Brightling et al. 2002; Leckie et al. 2000). The development of safe non-invasive measures of airway inflammation, particularly the analysis of induced sputum, has provided the opportunity to study wide populations of patients with asthma of varying severity and has great promise in clarifying the importance of eosinophilic inflammation in this disease. The current evidence for the role of the eosinophil in asthma will now be reviewed, along with the development of induced sputum, which has helped to provide much of the more recent evidence.
2.1.1 The biology of eosinophils

The term eosinophil was first introduced in the late 1870s by Paul Ehrlich (Ehrlich 1878), who used it to describe a densely granular leukocyte that demonstrated intense staining with the fluorescein derivative, eosin. Subsequently, the eosinophil was found to be associated with helminth infections (Brown 1898) asthma (Gollasch 1889) and anaphylaxis (Schwarz 1914). The importance of the eosinophil in the pathophysiology of these diseases, however, has proved difficult to determine and important questions remain. The observation that eosinophils were present in animal tissues after episodes of anaphylaxis lead to the initial suggestion that eosinophils ameliorated the allergic process, possibly by ingesting foreign protein, by presenting antigen to lymphocytes or by the inactivation of histamine (Archer 1968). There is increasing evidence, however, to support a pro-inflammatory role for the eosinophil in asthma and other allergic diseases. Eosinophils derive from CD 34+ progenitor cells in the bone marrow under the influence of GM-CSF, IL-3 and IL-5 which is specific for eosinophils and basophils (Denburg 1999). In the absence of allergic disease, eosinophils circulate briefly before migrating to the gastro-intestinal tract where they are thought to have an important role in the host defence against helminthic parasites (Butterworth et al. 1975). Eosinophil specific basic proteins such as major basic protein (MBP), eosinophilic cationic protein (ECP), eosinophil peroxidase (EPO) and eosinophil derived neurotoxin (EDN), which are toxic to the larval stage of helminths are contained within secondary granules. Eosinophils also produce significant amounts of sulphidopeptide leukotrienes and platelet aggravating factor (PAF) and smaller quantities of a range of other cytokines including GM-CSF, TGF-α and TGF-β. In asthma and other allergic diseases, Th2 producing lymphocytes are thought to result in the selective migration of eosinophils into extra-intestinal tissues (Wardlaw 1999). Firstly, IL-5 and eotaxin increase the production and release of eosinophils from the bone marrow. Secondly, IL-4 and IL-13 generated locally in the target organs lead to increased eosinophil adhesion and survival. Finally, eosinophilic degranulation results in the release of eosinophil basic proteins, which have been shown to be toxic to bronchial epithelial cells, and other
inflammatory mediators, particularly the cysteinyl-leukotrienes and PAF, which are thought to induce bronchoconstriction. The mechanisms which cause eosinophilic degranulation and mediator release are not fully understood but include priming with eosinophil active growth factors such as IL-5 (Kita et al. 1992). These findings suggest that eosinophils may be causally associated with many of the pathophysiological features of asthma, rather than having a beneficial effect. Further evidence of such an association has been derived from post mortem studies of asthma deaths and from bronchoscopy studies of patients with symptomatic asthma.

2.1.2 Post-mortem studies of eosinophilic airway inflammation in asthma

Histopathological examination of post mortem specimens from patients with fatal asthma have provided further evidence of an inflammatory response characterised in the majority of cases by the presence of airway eosinophilia. Typically this eosinophilic infiltration can be found throughout the airway wall, within thick viscid plugs that occlude the airway lumen and often extend into the lung parenchyma and alveolar spaces and even into adjacent blood vessels. In addition, extensive eosinophilic degranulation with deposition of major basic proteins occurs. (Azzawi et al. 1992; Copeland 1986; Filley et al. 1982) (Carroll, Cooke, & James 1997). Associated findings include widespread shedding of the airway surface epithelium, thickening of the reticular basement membrane, and enlargement of airway smooth muscle and submucosal glands. A minority of patients dying from asthma, particularly those with sudden onset fatal asthma do not have an airway eosinophilia but demonstrate a relative excess of neutrophils (Sur et al. 1993). Post mortem studies have obvious limitations in that small numbers are included, appropriate controls are often lacking and the effects of treatment are difficult to control for. Additionally, fatal asthma represents the extreme end of a wide spectrum.
2.1.3 Bronchoscopy studies of eosinophilic airway inflammation in asthma

The introduction of fibreoptic bronchoscopy, bronchoalveolar lavage (BAL) and endobronchial biopsies to the investigation of patients with asthma in the 1980s have enabled the characterisation of the immunopathology of patients with milder disease. The predominant finding is of increased numbers of eosinophils in BAL fluid even in patients with very mild disease (Wardlaw et al. 1988). In keeping with the observations in fatal asthma, the eosinophils appear to be activated, with increased concentrations of major basic proteins and leukotrienes found in BAL fluid (Broide et al. 1991; Wardlaw et al. 1989). There is a degree of correlation between the findings in BAL and other markers of asthma severity. Wardlaw et al observed that BAL eosinophils were present in symptomatic asthma but were absent in those in remission (Wardlaw et al. 1988) and others have demonstrated a broad correlation between BAL eosinophils and clinical asthma scores and lung function (Bousquet et al. 1990). Whilst airway hyperresponsiveness has been associated with the presence of a BAL eosinophilia (Walker et al. 1991), the severity of airway hyperresponsiveness does not relate closely to the number of eosinophils in BAL fluid (Wardlaw et al. 1988). There are suggestions that closer clinical correlates are seen with activated, rather than absolute, numbers of eosinophils. In the study by Wardlaw et al, a significant inverse correlation between MBP positive eosinophils and airway hyperresponsiveness was seen (Wardlaw et al. 1988) and Adelroth et al demonstrated that treatment with inhaled corticosteroids lead to a reduction in the amount of ECP in BAL fluid but did not affect the total numbers of eosinophils (Adelroth et al. 1990).

Endobronchial biopsies, have also consistently demonstrated a selective increase in the number of eosinophils in the airway submucosa (Djukanovic et al. 1990; Poston et al. 1992). This usually occurs along with increased numbers of CD4 T-cells (Azzawi et al. 1990). Similar findings have been demonstrated in patients with non-atopic asthma (Bentley et al. 1992) and in some cases of occupational asthma (Frew et al. 1995). Even greater numbers of eosinophils are
seen in patients with aspirin-sensitive asthma (Nasser et al. 1996). Again, the
eosinophils appear to be activated, being partially degranulated and
demonstrating positive staining with EG2 (Djukanovic et al. 1992a). Since most
bronchoscopy studies are confined to patients with mild disease, firm
conclusions about the relationships between mucosal eosinophils and markers of
clinical severity are difficult to draw but broad associations have been shown
(Lim et al. 2000b). Bronchoscopic studies are invasive, expensive and are
therefore generally confined to small numbers of subjects, usually young patients
with mild atopic asthma. Furthermore, the cross-sectional nature of the majority
of these studies is a particular weakness since asthma is a disease characterised
by variability. Assessment of airway inflammation across a range of asthma
phenotypes or in a longitudinal fashion may provide important information about
the relationship between airway pathology and clinical manifestations of disease
and identify factors influencing disease progression and response to treatment.

2.1.4 The action of glucocorticoids on eosinophilic airway inflammation in
asthma

Glucocorticoids have wide-ranging effects on airway inflammation in asthma
mediated through a variety of mechanisms. They bind to the glucocorticoid
receptor located in the cytoplasm of target cells leading to the disassociation of a
heat shock protein allowing the activated glucocorticoid-steroid complex to bind
to DNA in the nucleus. This leads to the direct or indirect transcription of target
genesis (Beato, Herrlich, & Schutz 1995) resulting in the induction or repression of
the gene. Glucocorticoids are thus thought to suppress inflammation by
increasing the synthesis of a range of anti-inflammatory proteins including
lipoctin-1 and secretory leucocyte inhibitory protein and by decreasing the
transcription of a number of mediators, particularly the cytokines IL-1B, TNF-α,
IL-4, IL-5, IL-8 and others. Additionally, they appear to inhibit a number of
inflammatory enzymes such as inducible nitric oxide synthase (INOS) and cyclo-
oxygenase 2 (COX-2) and to decrease the transcription of inflammatory
receptors (Barnes 1998). Direct interaction of glucocorticoids with other
activated transcription factors such as NF-κB is also thought to contribute to their
anti-inflammatory activity (Adcock et al. 1995). Through a combination of these
mechanisms, glucocorticoids are extremely effective in controlling inflammation in asthmatic airways and whilst they clearly have multiple cellular effects their suppression of eosinophilic airway inflammation is particularly striking. Inhaled corticosteroids have been shown to reduce the number and activation of eosinophils in bronchial biopsies (Djukanovic et al. 1992c) and in induced sputum (Jatakanon et al. 1999a), although the effects on cells and mediators in bronchoalveolar lavage fluid appear less consistent (Lim et al. 1999; Olivieri et al. 1997). Glucocorticoids dramatically reduce eosinophil survival by inhibiting the effects of key cytokines including IL-5 and GM-CSF leading to increased eosinophil apoptosis (Lamas, Leon, & Schleimer 1991). They are also able to directly inhibit mediator release from eosinophils and may reduce circulating eosinophil numbers by a direct action on the production of eosinophils in the bone marrow. It has recently been suggested that the anti-inflammatory actions of glucocorticoids may be potentiated by long acting β-2 agonists with which they are often used. In vitro studies have shown evidence of a number of potentially relevant synergistic mechanisms including inhibition of the proliferation of airway smooth muscle cells mediated by the activation of transcription factors (Roth et al. 2002), by the inhibition of TNF-α induced IL-8 release from airway smooth muscle cells (Pang & Knox 2000), inhibition of adhesion molecule expression (Spoelstra et al. 2002) and reduced secretion of GM-CSF by bronchial epithelial cells (Korn, Jerre, & Brattsand 2001).

2.1.5 Neutrophilic airway inflammation in asthma

The neutrophil is the characteristic cell of the acute inflammatory response providing an important defence against infection primarily by phagocytosis. Neutrophils are recruited to the site of inflammation by the release of chemoattractants such as IL-8, complement C5a and tumour necrosis factor-α (TNF-α), and are often the predominant inflammatory cell in the airways of patients with chronic bronchitis, bronchiecstasy and chronic obstructive pulmonary disease (COPD) (Stockley & Hill 2000). Activated neutrophils release proteases, oxygen-delivered free radicals and also a number of cytokines
and chemokines including IL-1β, IL-6 and IL-8. Traditionally, the neutrophil was not thought to be of significance in patients with mild to moderate asthma, with most studies reporting similar numbers of neutrophils in the airways of such patients as in healthy controls whether measured in induced sputum (Keatings et al. 1996), bronchoalveolar lavage fluid (Lacoste et al. 1993) or in bronchial biopsies (Wenzel et al. 1999). More recently, however, the presence of non-eosinophilic inflammation associated with increased neutrophil numbers and elevated IL-8 levels has been recognised in patients with mild or stable asthma by a number of groups (Gibson et al. 1998; Tarodo et al. 1999; Wark, Gibson, & Fakes 2000) (Wilson et al. 2000). In contrast, increased neutrophils have been more consistently demonstrated in the airways of patients with severe asthma (Lacoste et al. 1993; Wenzel et al. 1997) and an association between neutrophilic airway inflammation and the development of fixed airflow obstruction in asthma has been suggested (Woodruff et al. 2001). Intense neutrophilic airway inflammation has also been demonstrated in patients ventilated due to status asthmaticus (Lamblin et al. 1998) and in those who die suddenly of asthma (Sur et al. 1993).

In contrast to their action on eosinophils, corticosteroids do not inhibit neutrophilic inflammation and actually increase peripheral neutrophil counts, possibly by preventing neutrophil apoptosis (Cox 1995). Since most patients with severe asthma have been treated with high doses of inhaled and/or oral corticosteroids, the significance of neutrophilic airway inflammation in these groups remains unclear. Further studies assessing the incidence of neutrophilic inflammation, particularly in the airways of patients with mild or newly diagnosed asthma are therefore warranted.
2.2 The use of induced sputum to investigate airway inflammation in asthma

The development of induced sputum as a safe non-invasive measure of airway inflammation has great potential in assessing the nature of airway inflammation across the spectrum of asthma severity and thus furthering our understanding of asthma and other airway diseases. Evidence supporting the methodology and application of this technique to clinical practice shall now be discussed.

2.2.1 The development of sputum analysis as a non-invasive marker of airway inflammation

Interest in the analysis of induced sputum in asthma has increased dramatically over recent years but the association between sputum inflammatory markers and asthma is far from new. Charcot-Leyden crystals (Charcot & Robin 1853; Leyden 1872) and Curschmann's spirals (Curschmann 1882) were identified in sputum from patients with asthma in the late 19th century and were found to be associated with the presence of eosinophils. A clinical use for the examination of asthmatic sputum was first suggested by Morrow Brown in the late 1950s who suggested that the presence of eosinophils in a crude Leishman stained sputum smear identified patients who responded to treatment with oral corticosteroids (Morrow Brown 1958). Twenty years later inflammatory mediators such as histamine and slow reacting substance of anaphylaxis were identified in sputum (Turnbull et al.) and since then sputum has been used to measure a whole range of inflammatory mediators.

2.2.2 The use of hypertonic saline in sputum induction

Initial analysis of sputum was made using smears of spontaneously expectorated sputum stained with May-Grunwald-Giemsa (Gibson et al. 1989b). The obvious limitation of this technique was the inability to obtain samples from a number of patients and control subjects. This has been largely overcome by the introduction
of ultrasonically nebulised hypertonic saline to facilitate sputum production (Pin et al. 1992). The mechanism of action of hypertonic saline remains uncertain: stimulation of cough receptors, increased airway vascular permeability (Umeno, McDonald, & Nadel 1990) and direct stimulation of the mucociliary escalator have all been postulated (Daviskas et al. 1996). The use of a standard protocol involving sequential 5 minute inhalations of 3, 4 and 5% hypertonic saline delivered via a relatively low output ultrasonic nebuliser (Pin et al. 1992) has been shown to result in successful sputum induction in our laboratory in over 90% of patients with asthma and other airway diseases and 100% of healthy control subjects (Hunter et al. 1999). Induced sputum results in similar cell counts and inflammatory mediators as spontaneous samples with the exception of fibrinogen which is present in higher concentrations in spontaneous sputum (Pizzichini et al. 1996c). The duration of nebulisation of hypertonic saline has been shown to affect the cellularity and biochemical content of sputum samples, with later samples having lower neutrophil counts and mediator concentrations in particular (Belda et al. 2001; Gershman et al. 1999; Richter et al. 1999). This suggests that the early part of induction samples central airways with peripheral airways/alveoli being sampled later. Adherence to the full induction protocol wherever possible is important to allow comparisons between samples. Methacholine challenge testing performed prior to sputum induction does not affect the cellular and biochemical profile of sputum and the two procedures can be performed on a single visit if required (Spanevello et al. 1999).

2.2.3 Safety of sputum induction

Hypertonic saline is a potential bronchoconstrictor (Smith & Anderson 1990) and its safety in patients with asthma is clearly important. Salbutamol premedication and careful monitoring of forced expiratory volume in one second (FEV₁) during sputum induction are recommended to minimise the risk of significant bronchoconstriction (Pizzichini et al. 2002). A review of the published studies which have reported the safety of sputum induction when these precautions have been applied has estimated that the mean % fall in FEV₁ varies between 5.6% in mild asthma to 7.2% in patients with uncontrolled symptoms or asthma.
exacerbations (Pizzichini et al. 2002). The mean % fall in FEV₁ among patients with mild asthma in our laboratory was 5.4% with a maximum fall of 23% (Hunter et al. 1999). Such excessive airway constriction, as defined by a fall in FEV₁ of >20%, occurred in 8.3% of the patients studied in our laboratory and in 5.8%–32% of other reported series (Pin et al. 1992; Pizzichini E. et al. 1998; Pizzichini et al. 1997; Vlachos-Mayer et al. 2000; Wong & Fahy 1997). Factors which have been shown to predict hypertonic saline induced bronchoconstriction include baseline airway hyperresponsiveness (Fahy et al. 2001; ten Brinke et al. 2001; Wong & Fahy 1997) baseline eosinophilic airway inflammation (Fahy et al. 2001; ten Brinke et al. 2001; Wong & Fahy 1997) and the excessive use of rescue short-acting β₂-agonists in the days preceding the induction (Pizzichini et al. 1996c; ten Brinke et al. 2001). However, the greatest falls in FEV₁ have occurred in patients with mild airflow obstruction (Pizzichini et al. 2002) emphasising the need to perform inductions carefully in all patient groups.

In a study of the safety of sputum induction in clinical practice, patients who had a baseline FEV₁ of <70% predicted, significant bronchodilator reversibility or a clinical exacerbation, were given normal saline to initiate sputum induction, shorter inhalation times were used and inhalations were discontinued when an adequate sputum sample was expectorated. These modifications did not reduce the success of the procedure and a >20% fall in FEV₁ occurred in only 6% of patients with a baseline FEV₁ of <40% predicted (Vlachos-Mayer et al. 2000). As discussed earlier, however, the duration of induction does alter the cellularity of sputum samples, particularly the neutrophil differential cell count and should be borne in mind when interpreting results. Isotonic saline results in less bronchoconstriction (Bacci et al. 1996; Cataldo et al. 2001; Popov et al. 1995), produces sputum samples with comparable cellular and biochemical profiles (Bacci et al. 1996; Cataldo et al. 2001) but reduces the success rate of the procedure compared to hypertonic saline (Popov et al. 1995). Similarly, the use of hypertonic saline delivered by ultrasonic nebulisers with a higher output results in higher success rates at the expense of increased adverse effects (Boushey et al. 1996; Fahy et al. 1993; Popov et al. 1995). Finally, repeated
inductions performed over periods of eight to twenty-four hours using relatively high output nebulisers have been shown to increase the sputum neutrophil count in normal controls and patients with asthma suggesting that the induction itself may act as an inflammatory stimulus (Holz et al. 1998; Nightingale, Rogers, & Barnes 1998). Whilst these findings limit the value in the short term serial assessment of neutrophilic airway inflammation, their functional importance is not clear since airway hyperresponsiveness has been shown to be increased 30 minutes after sputum induction (Bacci et al. 1996) but returns to normal by 24 hours (Kips J.C. et al. 1995).

### 2.2.4 Sputum processing

**Selection of sputum from saliva**

Two methods of sputum analysis are widely used: analysis of selected sputum plugs and analysis of the whole expectorate of sputum. Processing the whole expectorate has the disadvantages of dilution of the sample by saliva, which is variable, difficult to quantify and results in increased squamous cell contamination. Selection of sputum plugs by the use of blunt forceps results in a median squamous cell contamination of 1.6% leading to improvements in cytospin quality (Pizzichini et al. 1996a). Compared to the residual sample, the analysis of selected plugs does not appear to significantly alter differential cell counts but increases total cell counts, cellular viability and levels of ECP in the supernatant (Pizzichini et al. 1996a). One study has suggested that selection of sputum plugs results in higher percentage eosinophil counts and lower percentage neutrophil counts compared to analysis of entire unselected samples, but the magnitude of the differences between techniques were felt to be insufficient to be of clinical importance (Spanevello et al. 1998). Nevertheless, processing of sputum samples in longitudinal studies should be performed according to a standard protocol.

**Homogenisation of sputum using dithiothriethiol (DTT)**

Initial attempts at analysing sputum samples were limited by poor repeatability due to inadequate cell dispersal. The use of dithiothriethiol (DTT), which produces
mucolysis by opening disulphide bonds which cross-link glycoprotein fibres has improved this, making total and differential cell counts easier, quicker, and more reproducible (Popov et al. 1995; Wooten & Dulfano 1978). Cell definition is improved in sputum treated with DTT, although identification of lymphocytes and epithelial cells may be less accurate (Lemiere et al. 2001a). The use of DTT appears to result in greater total cell counts, a slight reduction in cellular viability, but has no effect on differential cell counts (Efthimiadis et al. 1997; Popov et al. 1994).

2.2.5 Sputum analysis

**Total and differential cell counts**

Total cell counts are usually performed manually in a haemocytometer along with a determination of cell viability using the trypan blue exclusion method (Fahy et al. 1993; Pizzichini et al. 1996a). Differential cell counts were initially obtained by staining with May-Grünwald-Giemsa followed by additional staining with toluidine blue for accurate metachromatic cell counts (Gibson et al. 1989b). Despite this process, differentiation of eosinophils from neutrophils often remained difficult requiring additional staining with chromotrope 2R on occasions (Hargreave et al. 1993). Improved cell definition resulting from the use of DTT has subsequently enabled accurate differential cell counts to be performed on cytopspins stained with Wright’s stains, without the need for specific eosinophil stains although additional toluidine blue stains are still required for accurate metachromatic cell counts (Popov et al. 1994).

**Differential cell counts versus absolute cell counts**

Sputum cell counts can be reported as the differential count of the total cells present or as the absolute cell count. A differential cell count is often preferred as it is repeatable (Pin et al. 1992; Pizzichini et al. 1996a) but there are concerns that it may not adequately reflect the intensity of cellular inflammation. Estimations from our own laboratory have shown a close linear relationship between differential and absolute cell counts for both neutrophils and eosinophils up to a differential cell count of around 80%. Thereafter the differential cell count reached a plateau despite increasing total cell counts suggesting that absolute cell
counts might be a more responsive measure in situations where differential cell counts are anticipated to be high, for example in patients with an exacerbation of chronic obstructive pulmonary disease (Neale N et al. 2002).

**Flow cytometry**
Attempts at using flow cytometry to perform automated cell counts have been hindered by the difficulty in separating eosinophils from neutrophils by this method (Loppow et al. 2000). Flow cytometry has also been used to provide more detail information about cell subtype and activation (in't Veen et al. 1998) but is generally limited by poor cell preservation. An alternative approach-using laser scanning cytometry has shown promising results in obtaining accurate differential eosinophil counts although remains currently restricted by cost and the need for operator expertise (Woltmann et al. 1999).

**Immunocytochemistry**
Immunocytochemistry has provided a further technique suitable for the analysis of induced sputum samples. Cell suspensions require adequate fixing followed by immunocytochemical staining commonly with alkaline-phosphatase-antialkaline phosphatase complexes. Specific monoclonal antibodies are then applied and incubated overnight at -4°C. Secondary antibodies are added and the antibody/antigen complex visualised using the alkaline phosphatase-linked substrate with appropriate counterstains (Gauvreau et al. 2000). The agreements between these techniques and Wright’s staining is good for eosinophils and neutrophils but poor for lymphocytes and epithelial cells, leading to the suggestion that immunocytochemistry should be the preferred technique for the identification of these latter cell types (Lemiere et al. 2001a).

**The measurement of fluid phase mediators**
There is increasing interest in the measurement of inflammatory mediators in induced sputum supernatant including cytokines, chemokines, eicosanoids, proteases and a number of other markers. Radioimmunoassays (RIA), where the concentration of a mediator present in a sample is inversely proportional to the level of emitted radioactivity, have been used to measure mediators such as Eosinophil Cationic Protein (ECP) (in't Veen et al. 1996), Eosinophil Protein X
(EPX) (Koller et al. 1994), histamine and tryptase (Fahy et al. 1993). An alternative approach employing enzyme linked immunosorbant assays (ELISA) has been used to measure a wide range of inflammatory mediators in induced sputum including the cytokines (eg IL-5), chemokines (eg IL-8, eotaxin), proteases (eg MMP-1) and eicosanoids (eg the leukotrienes) (Kelly et al. 2002; Pavord et al. 1999b). Here the mediator concentrations are measured in proportion to the optical density of the product formed by the conjugated enzymes.

Interpretation of the results of mediator measurements in induced sputum should pay particular care to an assessment of the validity of the technique applied since a number of factors may influence the results. Consideration should be given to the validity, sensitivity, specificity, precision, cross-reactivity and stability of the assay and internal laboratory controls are required to determine intra- and inter-assay variability (Stockley & Bayley 2000). In addition, the detection of a mediator in sputum suspension may be limited by it’s binding with other molecules: IL-8 for example, may bind to DNA or heparin found within sputum thus limiting its function and subsequent detection by ELISA techniques (Kelly et al. 2002). Saliva contains lower concentrations of ECP, tryptase and elastase but higher levels of histamine and endothelin-1 than sputum (Fahy et al. 1993) and the contamination of sputum supernatants by saliva is therefore an important consideration. Finally the use of DTT in sputum processing may affect results by reducing disulphide bonds present in several mediators. It does not appear to interfere with the assay of ECP, IL-5, IL-8 fibrinogen, albumin, or tryptase, but may slightly decrease staining of EG-2, Eosinophil peroxidase (EPO) and myeloperoxidase (MPO) (Grebski, Peterson, & Medici 2001; Pizzichini et al. 1996a; Popov et al. 1994). The measurement of mediators from sputum supernatant should therefore include appropriate controls to monitor the effects of DTT.
2.2.6 The reliability of induced sputum as a marker of airway inflammation in asthma

Validity
The validity of sputum induction as a marker of airway inflammation in asthma has been well established. The technique has been performed in relatively large numbers of healthy volunteers and normal ranges have been determined (Belda et al. 2000; Spanevello et al. 2000). In particular the differential eosinophil count in sputum from healthy volunteers is very low (90th percentile 1.1%) (Belda et al. 2000). In contrast, patients with asthma often demonstrate an elevated induced sputum eosinophil count. For example, up to 80% of corticosteroid naïve subjects and almost half of patients with persistent asthma taking inhaled corticosteroids have a sputum eosinophil count out of the normal range (Gibson, Simpson, & Saltos 2001). Particularly high sputum eosinophil counts are often observed during acute severe exacerbations of asthma (Pizzichini et al. 1997). The relative numbers of eosinophils in induced sputum correlates well with eosinophil numbers in bronchial washes and less well with counts in bronchoalveolar lavage (BAL) and bronchial biopsies (Fahy et al. 1995b; Grootendorst et al. 1997; Maestrelli et al. 1995). Sputum samples, however, are more cellular, particularly with regards to granulocytes, and have higher concentrations of inflammatory mediators than samples obtained during bronchoscopy presumably as a result of less dilution and an origin in larger, central airways (Keatings et al. 1997a). To investigate the likely site of origin of induced sputum samples, Alexis and colleagues collected induced sputum from healthy volunteers who had inhaled radiolabelled sulphur particles administered to different components of the lung. They employed a novel technique to deliver a radiolabelled aerosol bolus to the central airways and as a comparison used an ultrasonic nebuliser to deliver smaller particles of similarly radiolabelled solution to the peripheral airways. Their results showed that deposition of the aerosol to the central airways resulted in more than 16 times more radioactivity in induced sputum along with 10 times greater clearance from the whole lung compared to peripheral deposition. These results support the suggestion that induced sputum
derives from the central airways with minimal contribution from peripheral airways (Alexis et al. 2001).

**Repeatability**

Induced sputum differential cell counts and measurements of soluble inflammatory mediators in the sputum supernatant have been shown to be highly repeatable (Girgis-Gabardo et al. 1994; in't Veen et al. 1996; Pizzichini et al. 1996a; Spanevello et al. 1997). In particular, 95% of repeated sputum eosinophil measures lie within a twofold range of the original measurement (Pizzichini et al. 1996a). Additionally, between-observer repeatability of the differential eosinophil, neutrophil and macrophage cell counts in induced sputum samples is high. Lymphocyte and epithelial cell counts are less consistent and poor cell viability and high squamous contamination increase between-observer variability (Ward et al. 1999) emphasising the need for careful induction and processing of samples.

**Responsiveness**

Induced sputum cellular and fluid phase markers of inflammation have also been shown to be responsive to change. Sputum eosinophils, metachromatic cells, tryptase, ECP, Interleukin-5, TNF-α and cysteinyl leukotrienes all increase after allergen challenge (Fahy et al. 1994; Keatings et al. 1997b; Macfarlane et al. 2000; Pizzichini et al. 1996b). In contrast, sputum eosinophil differential counts, ECP, fibrinogen and IL-5 fall after treatment with oral corticosteroids (Claman et al. 1994; Pizzichini et al. 1997). Administration of inhaled corticosteroids is also associated with significant reductions in sputum eosinophil counts (Pavord et al. 1999a; van Rensen et al. 1999) even in subjects with mild asthma (Jatakanon et al. 1998a) and a dose-dependent reduction in eosinophils has been demonstrated (Jatakanon et al. 1999a).
2.2.7 The relationship between eosinophils in induced sputum and other markers of asthma severity

**Symptoms and lung function**

Early bronchoscopy studies reported a broad correlation between clinical asthma severity and the degree of airway eosinophilia (Bousquet et al. 1990; Wardlaw et al. 1988). In contrast, studies using induced sputum, which have allowed the inclusion of diverse patient populations, have not consistently demonstrated close relationships between eosinophilic airway inflammation and symptoms or lung function. For example, Pin et al found an inverse correlation between FEV₁ and sputum eosinophil counts (Pin et al. 1992). In contrast another study of 20 asthmatics after an eight-week course of high dose inhaled steroids found no correlation between sputum eosinophils and clinical markers of severity although there was a weak correlation with airway hyperresponsiveness (Gibson, Saltos, & Borgas 2000). Vignola et al (1998) compared the degree of airways inflammation in mild and moderate asthma and found that the number of eosinophils were more marked in the more severe disease group (Vignola et al. 1998). In a study of induced sputum in 74 asthmatics ranging from mild to severe persistent disease, asthma severity as assessed by lung function, symptoms scores and airway hyperresponsiveness, correlated with the degree of airway eosinophilia. A weak correlation was also seen between sputum neutrophilia and symptom scores (Louis et al. 2000b). A study of 43 mild to severe asthmatics demonstrated higher sputum eosinophil counts in severe compared to mild and moderate asthmatics as defined by the clinical Aas score, but only weak correlations with FEV₁ and airway hyperresponsiveness were observed (Ronchi et al. 1997). The weak relationships between clinical markers of asthma severity limit the ability of clinicians to identify the extent of underlying airway inflammation. Parameswaran and colleagues assessed the extent of agreement between clinical judgements of sputum cell counts and actual cell counts in 76 patients with asthma and found that the overall agreement was poor, even with experienced clinicians (Parameswaran et al. 2000).
Studying the relationship between various markers of asthma severity and airway inflammation is difficult. Symptoms are not objective and peak flow measurements are unreliable (Hunter et al. 2002). Cross-sectional studies involving a single measurement of FEV$_1$ and eosinophil count, in a disease like asthma which is defined in terms of its variable severity are crude, particularly when the sampling errors involved in measuring the degree of airway eosinophilia are taken into account. There is a paucity of longitudinal studies correlating asthma severity with airway inflammation on an individual basis.

**Airway hyperresponsiveness**

Airway hyperresponsiveness is usually considered to be a hallmark of the pathophysiology seen in asthma. It can be demonstrated by challenge testing, either with stimuli such as methacholine and histamine, which have a direct effect on airway smooth muscle, or by using indirect stimuli such as adenosine, exercise and hypertonic saline which lead to bronchoconstriction via a number of inflammatory cells and mediators. Challenge tests are performed by a number of different methods, but usually by inhaling the stimulus during tidal breathing from a Wright’s nebuliser or by administering the stimulus via a dosimeter. Spirometry is performed at baseline and after each inhalation and the concentration or dose of the stimulus is increased, usually until the FEV$_1$ is seen to fall by 20% or more. High levels of agreements have been demonstrated between the Wright’s nebuliser and dosimeter methods (Rasmussen et al. 1999; Yan, Salome, & Woolcock 1983), but the Wright’s nebuliser method is the most widely used and is recommended by current guidelines (Crapo et al. 2000). This method requires the calculation of the provocative concentration that results in a 20% fall in FEV$_1$ (PC$_{20}$) by linear interpolation of the log dose-response curve (Crapo et al. 2000).

Recent definitions of asthma (Barnes et al. 1997) (Global Initiative for Asthma 1995) have suggested that there is a causal association between eosinophilic airway inflammation and airway hyperresponsiveness. Studies using induced sputum have challenged this view, suggesting a more complex relationship. In a study of 71 asthmatics no relationship was seen between sputum eosinophilia and airway hyperresponsiveness to methacholine although the eosinophil count did
inversely correlate with lung function (Crimi et al. 1998). Some studies have seen a correlation. For example Jatakanon et al found a weak inverse correlation (r=-0.4) between the sputum eosinophil count and PC_{20} methacholine in 35 stable asthmatics taking only \( \beta_{2} \) agonists (Jatakanon et al. 1998b). Similar results have been seen in other studies which have included patients taking inhaled corticosteroids (Pizzichini et al. 1996a; Polosa et al. 1998). It is interesting to note that the studies demonstrating a significant correlation between sputum eosinophils and methacholine PC_{20} have largely been confined to subjects with atopic asthma (Claman et al. 1994; Jatakanon et al. 1998b; Lim et al. 1999; Polosa et al. 1998). Studies have suggested that airway hyperresponsiveness to the indirect stimulus adenosine 5'-monophosphate (AMP) correlates more closely with sputum eosinophils both in patients with asthma (van den et al. 2001) and with allergic rhinitis without asthma (Polosa et al. 2000). In contrast, airway hyperresponsiveness to hypertonic saline has been shown to be dissociated from sputum eosinophilic inflammation in asthma (Iredale et al. 1994).

The current evidence supports the idea that airway hyperresponsiveness and eosinophilic airway inflammation are independently regulated but closely interrelated, a view supported by a factor analysis undertaken by Rosi et al in 99 mild asthmatics (Rosi et al. 1999). This would predict that in a cross-section of patients, for a given degree of inflammation, marked differences in airway hyperresponsiveness could result. This is consistent with the observation that marked airway hyperresponsiveness can occur in the context of minimal airway eosinophilia (Turner et al. 1995). Conversely patients with eosinophilic bronchitis have a sputum eosinophilia identical to that seen in asthma, but with none of the functional abnormalities associated with asthma (Gibson et al. 1989a). A detailed comparison of the immunopathology of asthma and eosinophilic bronchitis found that the two conditions are associated with identical airway inflammation with an airway eosinophilia, activation of Th2 lymphocytes and basement membrane thickening. The only difference was that in asthma but not in eosinophilic bronchitis or normal controls the airway smooth muscle is infiltrated by mast cells (Brightling et al. 2002) thus suggesting that airway
hyperresponsiveness is caused by mast cell derived mediators rather than the presence of an airway eosinophilia. Further evidence for a disassociation between airway hyperresponsiveness and eosinophilic airway inflammation comes from recent studies using a monoclonal antibody to Interleukin-5, which was able to reduce the sputum eosinophilia after allergen challenge, but had no effect on the fall in FEV₁, or on airway responsiveness (Leckie et al. 2000).

Markers of airway inflammation in exhaled breath

Exhaled nitric oxide

Exhaled nitric oxide (NO) is an additional non-invasive marker of airway inflammation that has been studied in asthma and other airway diseases. NO is commonly measured by a single breath exhalation against resistance into a chemiluminescence analyser (Kharitonov, Alving, & Barnes 1997). This measures the concentration of exhaled NO by recording the intensity of photons of light emitted when NO and ozone react to form energised NO₂. Increased levels of exhaled NO have been clearly demonstrated in asthma (Kharitonov et al. 1994b; Persson et al. 1994). This increase is believed to result from the activation of inducible nitric oxide synthases by pro-inflammatory cytokines and is thought to arise predominantly from the lower airway (Kharitonov et al. 1996). A significant, albeit weakly positive correlation between exhaled NO and sputum eosinophils has been shown in patients with steroid naive asthma (Berlyne et al. 2000; Jatakanon et al. 1998b). Exhaled NO and induced sputum eosinophil counts have also been shown to be significantly correlated following inhalation challenges in patients with occupational asthma (Obata et al. 1999). A similar correlation in the change in NO and mucosal eosinophils has been shown following allergen challenge (Ricciardolo et al. 2002). Despite these associations, important questions about the relationship between exhaled NO and airway inflammation remain. Studies have shown poor correlations between exhaled NO and eosinophils in bronchial biopsies (Lim et al. 2000b) or in bronchoalveolar lavage (Lim et al. 1999). Cross sectional studies in patients taking inhaled corticosteroids have given mixed results. In a preliminary study of 23 subjects taking inhaled steroids we found a significant correlation between log exhaled NO and the log sputum eosinophil count (r=0.77, p<0.001) (Green et al. 2000), but others have not demonstrated a close relationship (Berlyne et al. 2000; Leuppi
et al. 2001). Furthermore, longitudinal studies of the effects of inhaled corticosteroids on the two markers have also produced contrasting findings (Jatakanon et al. 1999a; Jones et al. 2002; van Rensen et al. 1999), and low dose theophylline results in a fall in airway eosinophils with no change in exhaled NO in steroid naïve asthmatic patients (Lim et al. 2001). Despite these differences, both the induced sputum eosinophil count and exhaled NO are important predictors of corticosteroid response in asthma (Little et al. 2000). It is likely that exhaled NO and sputum eosinophils reflect distinct components of the inflammatory pathway in asthma, albeit related to each other. Further work, particularly in the form of longitudinal studies, is needed to address the relationship between these two non-invasive markers of inflammation.

**Exhaled carbon monoxide**

Carbon monoxide (CO) is also excreted by the lungs and therefore detectable in exhaled breath. It can be measured by a number of techniques including the use of electrochemical sensors, near-infrared analysers and laser spectrophotometers (Kharitonov & Barnes 2001). Exhaled CO levels have been reported in stable asthma (Zayasu et al. 1997), in patients with acute exacerbations of asthma and in patients with severe asthma (Yamaya et al. 2001). CO levels appear to be less sensitive than exhaled NO or induced sputum eosinophil counts to treatment with corticosteroids, however (Zanconato et al. 2002), and there are no studies prospectively evaluating the use of this measurement in the management of asthma.

**Exhaled breath condensate**

Analysis of exhaled breath condensate, collected by cooling or freezing exhaled air, is a totally non-invasive method enabling the detection of non-volatile mediators. Exhaled condensates may be stored and subsequently tested using gas chromatography, spectrophotometry or ELISA, although contamination from the upper airway is a concern. A range of inflammatory mediators including hydrogen peroxide, eicosanoids, products of lipid peroxidation, vasoactive amines and nitrite species have been identified in exhaled breath condensates. Increased concentrations of each of these have been reported in asthmatic subjects, but the clinical significance of the technique remains unclear and its...
value in clinical practice has not yet been determined (Kharitonov & Barnes 2001).

**Inflammatory mediators in blood and urine**

Measurements of circulating levels of eosinophils in the blood or of markers of eosinophil activation, such as serum ECP or urine EPO or EPX have been proposed as useful non-invasive methods of measuring airway inflammation (Koller et al. 1995). These techniques appear to be less sensitive to changes in asthma control than induced sputum or exhaled NO measurements, however and do not appear to closely relate to disease activity (Payne et al. 2001). These limitations are likely to preclude their use in clinical practice although further studies are required.

**Exacerbations**

Exacerbations of asthma cause considerable morbidity, impact on health care costs (Hoskins et al. 2000) and in rare cases asthma deaths. There is increasing evidence to suggest that patients across the spectrum of asthma severity remain at risk of a severe exacerbation and that even patients with apparently stable well controlled asthma remain vulnerable to a severe attack (Hoskins et al. 2000; Reddel et al. 1999). Definitions of an asthma exacerbation usually refer to an acute deterioration in asthma symptoms and/or demonstration of acute airflow obstruction usually as a fall in peak expiratory flow (PEF) (Pauwels et al. 1997). The variation in peak expiratory flow seen during an exacerbation has been shown to be strikingly different from that seen during periods of poor asthma control, however, suggesting that reliance on PEF in diagnosing asthma exacerbations may be unreliable (Reddel et al. 1999). The aetiology of asthma exacerbations is diverse and not completely understood but probable triggers include viral infections (Beasley et al. 1988) (Johnston et al. 1995), increased allergen exposure (Meijer et al. 1996), air pollution (Linaker et al. 2000) and reduction in inhaled corticosteroid dose (Jatakanon, Lim, & Barnes 2000). In addition, there is evidence to suggest an excess of asthma exacerbation in women compared to men, although the cause of this is unknown (Tattersfield et al. 1999).
The pathophysiology of asthma exacerbations is complex and incompletely understood, but, based on the findings in asthma deaths, is likely to involve severe eosinophilic inflammation along with airflow obstruction caused primarily by the impaction of the bronchi with mucous and cellular debris (Carroll, Cooke, & James 1997). The majority of asthmatics studied within 48 hours of the start of treatment for an acute severe asthma exacerbation in our laboratory demonstrated sputum evidence of marked eosinophilic airway inflammation (Wardlaw et al. 2000) It is increasingly recognised that the 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 (Kips 2001). This view is supported by the findings of the FACET study, which showed that higher dose inhaled corticosteroids had a marked beneficial effect on exacerbation frequency but relatively little effect on symptoms and peak expiratory flow whereas with the addition of long acting $\beta_2$-agonists the opposite was true (Pauwels et al. 1997). The sputum findings at the time of an asthma exacerbation together with the reduction in exacerbation frequency seen with additional corticosteroid treatment suggests that eosinophilic airway inflammation might be particularly important in the genesis of exacerbations. In keeping with this, a recent study showed that the sputum eosinophil count was the only independent variable predicting the occurrence of an asthma exacerbation after inhaled corticosteroids were withdrawn (Jatakanon, Lim, & Barnes 2000). A similar study assessing predictive markers of asthma exacerbations during reduction in the dose of inhaled corticosteroids showed that the sputum eosinophil count was significantly higher before a failed dose reduction than before a successful dose reduction (Leuppi et al. 2001). In another study where oral corticosteroids were withdrawn in subjects with prednisolone dependent asthma, a significant increase in the sputum eosinophil count occurred well before the onset of increased symptoms and worsening airflow obstruction (Pizzichini et al. 1999b). These finding suggest that a sputum eosinophilia may be an important early predictor of subsequent exacerbations and question whether the induced sputum eosinophil count may be an important surrogate marker of exacerbation frequency.
2.2.8 Clinical applications of sputum induction in patients with asthma

**Induced sputum as a diagnostic test for asthma**

A sputum eosinophil count outside the normal range in patients with symptoms consistent with asthma has 72% specificity and 80% sensitivity in conforming a diagnosis of asthma (defined as consistent symptoms with objective evidence of variable airflow obstruction). This is superior to peak flow variability and measurements of the acute bronchodilator response and is almost as valid as the identification of abnormal airway hyperresponsiveness (Hunter et al. 2002). In a preliminary community based study sputum eosinophil counts were not able to differentiate between subjects reporting asthma symptoms and healthy controls since the majority fell within the normal range, suggesting that induced sputum has a limited role in identifying patients with asthma in epidemiological surveys (Lemiere et al. 2001b). Occupational asthma is generally associated with similar sputum characteristics as non-occupational asthma, and occupational challenges are associated with an increase in the sputum eosinophil count (Lemiere et al. 1999; Obata et al. 1999). A recent study, however, identified eosinophilic airway inflammation in only 37% of patients with a diagnosis of occupational asthma (Anees et al. 2002) and the validity of identifying a sputum eosinophilia in the diagnosis of occupational asthma has not been confirmed.

**The use of induced sputum in the management of asthma**

The development of induced sputum as a safe non-invasive marker of airway inflammation has lead to much interest in the use of this technique in the clinical management of asthma. To place this in context, I will discuss the evidence supporting the use of induced sputum in clinical practice in section 2.3, in the light of current asthma management guidelines.
2.3 The concept of asthma as a heterogeneous inflammatory disease

Asthma is a heterogeneous disease with considerable variation in its aetiology, presentation, severity, physiology and response to treatment. Whilst this diverse clinical picture is widely recognised, the heterogeneous nature of the underlying airway inflammation in asthma has only recently been recognised and will now be discussed.

2.3.1 A historic perspective

Whilst developments in the understanding of allergic mechanisms in asthma have lead to an emphasis on the importance of eosinophilic inflammation, asthma has long been recognised as a heterogeneous disease. As early as 1918 Francis Rackemann identified clear clinical subgroups on the basis of history, skin tests and response to "a clinical experiment such as a change in residence, a restriction in diet or an elimination of some supposedly offending substance." (Rackemann F.M. 1918). In his 1927 "Studies in asthma" he published data on over 1500 patients, 1000 of which he had followed for at least 2 years. Almost half of this group were said to have "extrinsic asthma" due to "hypersensitivity to some foreign substance outside of the body and who have asthma on exposure to, or contact with it" (Rackemann F.M. 1927). The majority of the remainder were classified as having "intrinsic asthma...implying the essential cause of the trouble is inside of the body." This group included patients thought to have "bacterial asthma" with symptoms precipitated by infection, "reflex asthma", "cardiac asthma" and "chronic bronchitis and emphysema." A further minority, including those with "chronic severe" or "fatal" asthma, were recorded as "unclassified". Whilst some of these subtypes are now clearly recognised as distinct diseases, many of Rackemann's observations appear particularly insightful and the concept of extrinsic (atopic) and intrinsic (non-atopic) asthma remains valid.
2.3.2 The immunopathology of atopic and non-atopic asthma

Using this immunological distinction between extrinsic and intrinsic asthma as a starting point, several studies have explored the possibility that distinct immunopathological subtypes also occur. A series of bronchial biopsy studies demonstrating that both phenotypes are associated with eosinophilic airway inflammation, activated CD4+ lymphocytes and increased IL5 and possibly IL4 expression has lead to the suggestion that the atopic and non-atopic asthma may not in fact, represent distinct entities (Bentley et al. 1992; Humbert et al. 1996; Humbert et al. 1997; Ying et al. 1997). More recently, however, Amin and colleagues have reported that whilst both patients with atopic and non-atopic asthma had increased numbers of mast cells in bronchial biopsy specimens, atopic asthma was associated with higher eosinophil and T-cell numbers, greater epithelial damage and more frequent IL-4 and IL-5 positive cells whereas patients with non-atopic asthma had greater numbers of neutrophils and IL-8 positive cells (Amin et al. 2000). Despite the uncertainty about the extent of the pathological differences in atopic and non-atopic asthma there is increasing evidence that a significant proportion of asthma is not associated with classical eosinophilic inflammation. The employment of induced sputum has been largely instrumental in gathering much of this data.

2.3.3 Heterogeneity of airway inflammation in acute asthma exacerbations

The first evidence for the predominance of non-eosinophilic inflammation in some patients with asthma came from studies of patients with acute severe exacerbations of the disease. In a small early study of asthma deaths, Sur et al suggested that a distinction could be made between patients dying from sudden-onset and slow-onset fatal asthma, with the former having a paucity of eosinophils but an excess of neutrophils in the airway submucosa at post-mortem (Sur et al. 1993). Using a sputum eosinophil count ≥4% as a cut off, Turner et al observed that 16 out of 34 patients with a mild exacerbation (defined as a two week deterioration in asthma symptoms) had no eosinophilia in spontaneous or induced sputum (Turner et al. 1995). Fahy et al found that neutrophils were frequently the prominent inflammatory cell in the sputum of 18 adults studied at
the time of an episode of acute severe asthma and that particularly high levels of IL-8 and free neutrophil elastase were also present. Many of these patients were receiving oral corticosteroids, however, and a treatment effect can therefore not be excluded (Fahy et al. 1995a). In a study of tracheal aspirates collected from 10 patients at the time of intubation for respiratory failure due to acute severe asthma, Ordonez and colleagues also reported particularly high neutrophil numbers and IL-8 levels although eosinophil numbers were also significantly greater than normal (Ordonez et al. 2000).

2.3.4 Heterogeneity of airway inflammation in chronic persistent and severe asthma

The presence of non-eosinophilic inflammation has also been reported in patients with chronic severe asthma. Wenzel et al have characterised the airway immunopathology of patients with severe refractory asthma using bronchial biopsies and have observed two distinct subgroups. The majority of patients had increased airway eosinophils and a thickened basement membrane in common with earlier studies in milder disease. An important subgroup, however, were found to have predominantly neutrophilic airway inflammation, absence of eosinophils and normal basement membrane thickness (Wenzel et al. 1999). Gibson et al used induced sputum to study 56 non-smoking adults with chronic persistent asthma taking high dose inhaled corticosteroids and also found two different inflammatory patterns. Classical eosinophilic inflammation with elevated ECP levels was seen in 41% of subjects whereas the remainder had normal induced sputum counts. There were no obvious differences in clinical markers of asthma severity between the two groups but the non-eosinophilic patients had elevated sputum neutrophils and IL-8 levels and were more likely to be non-atopic (Gibson, Simpson, & Saltos 2001). In a study of the sputum characteristics of patients with mild, moderate or severe but stable asthma, Jatakanon et al observed that sputum eosinophils were elevated in patients with mild and severe (but not moderate) asthma compared to normal controls, and that neutrophil counts were elevated only in those with severe asthma. An important number of patients, however, particularly those with moderate and severe disease, had normal sputum eosinophil counts (Jatakanon et al. 1999c). An
important concern with all of these studies is that the majority of patients were taking high doses of inhaled or even oral corticosteroids and the influence of high doses of anti-inflammatory treatment on airway inflammation, particularly neutrophilic inflammation, is unclear.

2.3.5 Heterogeneity of airway inflammation in mild intermittent asthma

Relatively few studies have addressed the issue of heterogeneity of airway inflammation in patients with mild or corticosteroid naïve asthma. In a study of 23 patients with asthma treated with as required \(\beta_2\)-agonists only in our clinic, 9 had eosinophil counts of <3%. The eosinophilic and non-eosinophilic groups had similar lung function, peak flow variability and symptom severity but non-eosinophilic patients were less likely to be atopic, less hyperresponsive and more likely to be current smokers. Furthermore, non-eosinophilic asthma was associated with a significantly poorer response to treatment with inhaled corticosteroids (Pavord et al. 1999a). In their study of 38 workers with a diagnosis of occupational asthma, Anees et al found that the majority of subjects had normal induced sputum eosinophil counts with evidence of sputum neutrophilia (Anees et al. 2002). Two further small studies have described the absence of eosinophilic inflammation in patients with mild asthma (Tarodo et al. 1999; Wark, Gibson, & Fakes 2000), but the true prevalence of non-eosinophilic asthma remains uncertain. There is therefore an important need for further studies, which assess the inflammatory profile of large numbers of a diverse range of patients including those who are corticosteroid-naïve.

2.4 Asthma management

I will now summarize current asthma management guidelines, discuss the evidence for the various treatment options currently available for each stage of asthma severity, and highlight some of the limitations of current management. I will also outline promising new areas of development. The use of induced
sputum as a marker of treatment response and in targeting the appropriate therapy for individual patients will then be discussed.

2.4.1 Introduction

Recent national and international asthma management guidelines recommend a stepwise approach, with treatment increased until asthma control is achieved and stepped down once control has been maintained for several months (British Thoracic Society & Scottish Intercollegiate Network 2003; Global Initiative for Asthma 1995). Currently available anti-inflammatory and bronchodilator drugs are very effective and good asthma control can be achieved for most patients. A significant minority, however, will have more severe persistent asthma which is difficult to manage and which may necessitate alternative approaches. New drugs, which improve control for patients with severe disease, minimise side effects or improve patient compliance, are required. Several new classes of treatment, which may fill these roles, are currently under investigation in asthma. I will review the evidence supporting current asthma therapies including non-pharmacological treatments, suggest alternative approaches where appropriate and finally discuss novel classes of drugs which may be useful in the future management of asthma. I will discuss the pharmacological options for each category of severity, which are defined in Table 2.1. A summary of the main pharmacological treatments for asthma at each stage of severity is given in table 2.2.
Table 2.1. Classification of asthma severity

The presence of any of the features of severity is sufficient to place a patient in that category. Patients in any category are at risk of severe exacerbations. (Global Initiative for Asthma 1995)

<table>
<thead>
<tr>
<th>Severity</th>
<th>Day time symptoms</th>
<th>Night time symptoms</th>
<th>Peak Flow</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mild intermittent</td>
<td>Less than once per week</td>
<td>Less than twice per month</td>
<td>≥ 80% predicted, variability &lt; 20%</td>
</tr>
<tr>
<td>Mild persistent</td>
<td>Between once per week and once daily</td>
<td>More than twice per month</td>
<td>≥ 80% predicted, variability 20%-30%</td>
</tr>
<tr>
<td>Moderate persistent</td>
<td>Daily, attacks affect activity</td>
<td>More than once weekly</td>
<td>60%-80% predicted, variability &gt;30%</td>
</tr>
<tr>
<td>Severe persistent</td>
<td>Continuous, limited activity</td>
<td>Frequent</td>
<td>≤60% predicted, variability &gt;30%</td>
</tr>
</tbody>
</table>
Table 2.2. Summary of pharmacological treatment for asthma of varying severity

<table>
<thead>
<tr>
<th>Mild intermittent asthma:</th>
<th>short acting B&lt;sub&gt;2&lt;/sub&gt; agonists as required</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mild persistent asthma:</td>
<td>add low dose inhaled corticosteroids</td>
</tr>
<tr>
<td>Moderate persistent asthma:</td>
<td>select one of the following options:</td>
</tr>
<tr>
<td></td>
<td>- low dose inhaled corticosteroids plus long acting B&lt;sub&gt;2&lt;/sub&gt; agonist</td>
</tr>
<tr>
<td></td>
<td>- higher dose inhaled corticosteroids</td>
</tr>
<tr>
<td></td>
<td>- low dose inhaled corticosteroids plus leukotriene antagonist</td>
</tr>
<tr>
<td></td>
<td>- low dose inhaled corticosteroids plus oral theophylline</td>
</tr>
<tr>
<td>Severe persistent asthma:</td>
<td>high dose inhaled corticosteroids plus one or more of the following:</td>
</tr>
<tr>
<td></td>
<td>- long acting B&lt;sub&gt;2&lt;/sub&gt; agonist</td>
</tr>
<tr>
<td></td>
<td>- leukotriene antagonist</td>
</tr>
<tr>
<td></td>
<td>- oral theophylline</td>
</tr>
<tr>
<td></td>
<td>- oral B&lt;sub&gt;2&lt;/sub&gt; agonist</td>
</tr>
<tr>
<td></td>
<td>add oral corticosteroids if control still not achieved</td>
</tr>
<tr>
<td></td>
<td>consider corticosteroid sparing agents</td>
</tr>
</tbody>
</table>
2.4.2 Mild intermittent asthma

As required short acting $\beta_2$ agonists

Inhaled short acting $\beta_2$ agonists such as salbutamol and terbutaline are effective bronchodilators and should be prescribed to all patients with symptomatic asthma (BTS 1997; Global Initiative for Asthma 1995). They are also useful in preventing symptoms of exercise-induced asthma when given before the start of exercise (Anderson et al. 1976) and are important in the treatment of acute severe asthma. Their mechanism of action is thought to occur primarily by the relaxation of airway smooth muscle cells, but they also increase mucociliary clearance. They do not have any effective anti-inflammatory activity. Although sympathomimetic agents, short acting $\beta_2$ agonists have few side effects when inhaled, but tremor, palpitations and tachycardia can occur with high doses. They should be used for symptom relief on an as required basis, since studies have shown that their regular use provides no additional benefit (Dennis et al. 2000) (Walters & Walters 2000) and may even be harmful. Furthermore, individual patients’ requirements for short acting $\beta_2$ agonists provides a useful guide to the need for a step-up in treatment: current guidelines suggest that if they are used on a daily basis for symptom control then regular anti-inflammatory agents are indicated (Global Initiative for Asthma 1995). The use of more than one canister of short acting $\beta_2$ agonists per month has been particularly associated with poorly controlled disease and should therefore alert the prescriber to the need for increased regular anti-inflammatory treatment (Suissa et al. 1994). Tolerance to the effects of short acting $\beta_2$ agonists can occur, particularly to the protection against bronchoconstriction induced during indirect challenges (O’connor, Aikman, & Barnes 1992). In addition, polymorphisms of the $\beta_2$-receptor that may confer a reduced response to these agents have been identified, although the clinical significance of these observations is as yet unclear (Liggett 1997).
2.4.3 Mild persistent asthma

Low dose inhaled corticosteroids

Corticosteroids are currently the most effective anti-inflammatory agents for the treatment of asthma and inhaled corticosteroids are currently recommended for all patients with persistent asthma who require short acting β₂ agonists more than once per day (BTS 1997) or those with intermittent asthma who experience severe exacerbations (Global Initiative for Asthma 1995). They exert their anti-inflammatory effects through a diverse range of mechanisms as discussed earlier. Studies have consistently shown that treatment with regular inhaled corticosteroids results in significant improvements in airway inflammation in asthma, an effect demonstrated on bronchial biopsies (Djukanovic et al. 1992b) and also on non-invasive markers of airway inflammation such as the differential eosinophil count in induced sputum or nitric oxide concentrations in exhaled breath (Jatakanon et al. 1999a). Furthermore, there is evidence that corticosteroid treatment is not helpful in the absence of eosinophilic airway inflammation (Pavord et al. 1999a). In conjunction with these improvements in airway inflammation, inhaled corticosteroids improve symptoms (Djukanovic et al. 1992c), health status (Mahajan et al. 1997), airway hyperresponsiveness and lung function (Haahtela et al. 1991), and reduce asthma exacerbations (O’Byrne et al. 2001). There is also epidemiological evidence from cohort and case-control studies showing that regular low dose inhaled corticosteroids reduce both hospital admissions (Donahue et al. 1997) and asthma deaths (Suissa et al. 2000). A recent study of patients with mild, apparently well controlled asthma showed that the addition of regular low dose inhaled corticosteroids resulted in significant reductions in asthma exacerbations compared to placebo (O’Byrne et al. 2001). These marked benefits, coupled with the low incidence of side effects have lead some to argue that inhaled corticosteroids should be given to all but the mildest patients. It is not yet fully known whether long term treatment with inhaled corticosteroids alters the natural history of the condition and protects against accelerated declines in lung function. A prospective study of the effects of inhaled corticosteroids on pulmonary function showed that children who
started inhaled corticosteroid treatment more than 5 years after the onset of asthma had a significantly lower FEV₁ than children who started treatment within 2 years of the diagnosis (Agertoft & Pedersen 1994). In a placebo controlled trial in children with mild to moderate asthma, the use of regular inhaled corticosteroids over four to six years resulted in improvements in symptoms, exacerbation rates and airway hyperresponsiveness compared to placebo, but no differences in lung function (The childhood asthma management program research group 2000). Further, long-term prospective studies of the effects of regular inhaled corticosteroids on the decline in lung function in adults are needed to address this important issue.

**Side effects of inhaled corticosteroids**

Patients are often concerned about the possibility of adverse effects of inhaled corticosteroids, and in some parts of the world, notably North America, this has lead to their relative under-use. At low doses, up to 800 mcg daily of beclomethasone dipropionate or budesonide or 500 mcg daily of fluticasone, side effects are not usually significant, but do become an issue at doses beyond this. Dysphonia commonly occurs due to deposition of inhaled corticosteroid particles locally in the oropharynx (Williamson et al. 1995) and oral candidiasis may also develop (Kennedy et al. 2000). Systemic side effects include bruising and atrophy of the skin (Mak, Melchor, & Spiro 1992) and reduced bone mineral density (Israel et al. 2001). Suppression of the adrenocortical axis can occur but this is not usually clinically significant (Clark & Lipworth 1997). These systemic effects occur partly due to gastrointestinal absorption of swallowed particles and partly due to systemic absorption via the airways. The use of spacer devices, dry powder mechanisms and mouth rinsing after inhaler use minimise adverse effects (Brown et al. 1990; Meeran et al. 1995; Selroos & Halme 1991). Drugs with high first pass metabolism in the liver such as budesonide and fluticasone have less systemic side effects than beclomethasone (Derendorf et al. 1998), but at high doses (>800-1000 mcg daily of BDP/budesonide or >500 mcg daily of fluticasone) systemic absorption through the buccal and airway mucosa is an important consideration.
Cromones

The cromones sodium cromoglycate and nedocromil sodium, both given by inhalation, have been used as controller therapies in mild persistent asthma (Anwar et al. 1993). Their mechanism of action is not fully understood, although they are believed to suppress IgE-mediated inflammatory responses and may inhibit inflammatory cells (Diaz et al. 1984). Sodium cromoglycate has been shown to reduce symptoms and exacerbation frequency (Edwards 1994) and nedocromil sodium to improve symptoms, lung function and airway responsiveness (Bel et al. 1990). Overall, however, they appear to be rather less effective than low dose inhaled corticosteroids (Szefler & Nelson 1998) and their long-term effects on airway inflammation are unknown. The use of these agents in adults has therefore largely been superseded by the introduction of low doses of inhaled steroids for the majority of patients with persistent asthma.

2.4.4 Moderate persistent asthma

An important number of patients with asthma treated with low dose inhaled corticosteroids have sufficient symptoms to justify an increase in treatment. The clinician is faced with an increasing number of treatment options for this important group of patients. Unfortunately data from published placebo controlled studies of the different treatments is not always applicable to everyday clinical practice in this area and important questions remain. We will therefore present the current evidence for each of the major treatment options and briefly discuss some of the outstanding issues.

Long acting \(\beta_2\)-agonists

Long acting \(\beta_2\)-agonists (Salmeterol and Formoterol) are currently generally recommended as the first choice for patients who have symptoms that persist despite regular inhaled corticosteroids (British Thoracic Society & Scottish Intercollegiate Network 2003). Salmeterol is a partial agonist of the \(\beta_2\)-receptor whilst formoterol is a full agonist. Both appear to have similar clinical effects, but formoterol has a more rapid onset of action (van Noord et al. 1996). Side effects of tachycardia, tremor and muscle cramps are rarely a problem unless

38
given in high doses. Tolerance to the effects of long acting β2-agonists with loss of bronchodilator activity following the subsequent administration of both short and long acting β2-agonists has been reported (Grove & Lipworth 1995; Newnham et al. 1995). As with short acting β2-agonists, these agents work primarily via the relaxation of airway smooth muscle, with additional effects on mast cells and vascular permeability, but without significant anti-inflammatory activity (Nelson 1995). This lack of anti-inflammatory activity precludes their use as first line agents in asthma (Lazarus et al. 2001) and current guidelines recommend that they are only prescribed alongside regular inhaled corticosteroids (BTS 1997; Global Initiative for Asthma 1995). When used in this way, long acting β2-agonists have been shown to improve day-time and night-time symptoms and the need for rescue β2-agonists (Kesten et al. 1991; Pearlman et al. 1992). In a randomised controlled trial of 852 patients treated with low dose inhaled corticosteroids (the FACET study) the addition of formoterol to inhaled low or high dose budesonide improved symptoms and lung function. In addition, the number of both mild and severe asthma exacerbations was reduced, where mild exacerbations are defined as a fall in PEF of >20% from baseline on 2 or more days, increased use of rescue short acting β2-agonists or nocturnal wakening and severe exacerbations defined as a fall in PEF of >30% from baseline on 2 or more days or a deterioration in symptoms requiring rescue oral corticosteroids (Pauwels et al. 1997). This study also directly compared the addition of formoterol to the alternative strategy of increasing the dose of inhaled corticosteroids. Compared to a fourfold increase in the dose of inhaled corticosteroids, the addition of formoterol resulted in similar improvements in symptom control but smaller reductions in severe asthma exacerbations. In an uncontrolled study of 429 patients with symptomatic asthma followed over six months, the addition of salmeterol to inhaled beclomethasone dipropionate (BDP) was shown to result in a greater increases in peak expiratory flow (PEF) and greater decreases in symptom scores than a 2.5 fold increase in the dose of inhaled BDP, but no differences in exacerbations were seen (Greening et al. 1994). More recently, the OPTIMA study in patients with milder disease suggested that the addition of formoterol resulted in greater reductions in exacerbation frequency than doubling the dose of inhaled corticosteroids.
One important concern with long acting $\beta_2$-agonists is that subjects recruited into many clinical trials are not fully representative of the patients we see in everyday clinical practice. Many of the published studies, for example, only recruited patients who demonstrated acute improvements in FEV$_1$ following inhaled bronchodilators of 15% or more (Greening et al. 1994; Pauwels et al. 1997; Reiss et al. 1997; Spector, Smith, & Glass 1994; Christian et al. 2000). Such degrees of bronchodilator reversibility are distinctly unusual in clinical practice: only 28% of patients with asthma in general practice demonstrated a 15% improvement in FEV$_1$ following 2.5mg nebulised salbutamol (Jamison & McKinley 1993) and only 5-10% of patients with asthma in our clinic demonstrated similar increases in FEV$_1$ following 200$\mu$g inhaled salbutamol (Hunter et al. 2002). Subjects therefore are not only atypical but are particularly likely to respond to bronchodilator therapy. There is a risk that these studies are generalised to wider patient populations when a more reasonable interpretation is that long acting $\beta_2$ agonists are particularly helpful for a subgroup of patients who have marked bronchodilator response.

**Increasing the dose of inhaled corticosteroids**

The traditional approach to patients with persistent symptoms despite low doses of inhaled corticosteroids was to increase the corticosteroid dose, but the evidence for this is somewhat inconsistent. Whilst some studies have demonstrated clear dose related improvements in symptoms and lung function (Dahl et al. 1993; Nathan et al. 2000; Pauwels et al. 1997) others have not demonstrated clinically important benefits with moderate or high doses (Adams, Bestall, & Jones 2000). Overall the beneficial effects of increasing the dose of inhaled corticosteroids appear to be modest and may be largely outweighed by the increased risk of side effects. As discussed earlier, the comparative studies have suggested that higher doses of inhaled steroids are less effective at controlling symptoms and peak flow variability compared to the addition of long acting $\beta_2$ agonists (Greening et al. 1994; Pauwels et al. 1997). Whilst the relationship between improvement in symptoms and inhaled corticosteroid dose reaches a plateau, control of exacerbation frequency is more closely related to inhaled corticosteroid dose. In the FACET study a fourfold increase in the dose...
of budesonide resulted in a significantly greater reduction in the number of asthma exacerbations than the addition of formoterol (Pauwels et al. 1997). Conversely, a two fold increase in the dose of budesonide did not result in similar improvements in the rates of exacerbations in milder patients included in the OPTIMA study (O'Byrne et al. 2001), suggesting that the higher dose ranges are required for the optimal prevention of exacerbations. There is increasing evidence that asthma exacerbations are associated with eosinophilic airway inflammation (Leuppi et al. 2001; Pizzichini et al. 1999b) and the benefits of the high doses of inhaled corticosteroids on exacerbation frequency are therefore likely to reflect dose related anti-inflammatory effects. Turner and colleagues have shown that a doubling of the dose of Beclomethasone in subjects with symptomatic asthma and a persistent sputum eosinophilia despite treatment with inhaled corticosteroids improved symptoms and significantly reduced the sputum eosinophil count whereas the addition of salmeterol led to improvements in symptoms but no change in the sputum eosinophil count (Turner et al. 1998). Similarly, in a study of increasing doses of budesonide in patients with steroid naïve asthma, Jatakanon et al demonstrated a dose dependent reduction in the percentage of eosinophils in induced sputum (Jatakanon et al. 1999a). Whilst low doses of inhaled corticosteroids are therefore probably appropriate for the majority of patients, higher doses of these drugs may be indicated in some patients who experience frequent severe exacerbations of asthma or who have persistent airway inflammation.

**Leukotriene antagonists**

Montelukast and zafirlukast are both effective cysteinyl leukotriene receptor antagonists capable of markedly inhibiting exercise-induced bronchoconstriction (Finnerty et al. 1992; Manning et al. 1990) and the early and late response to inhaled allergen (Diamant et al. 1999; Taylor et al. 1991). When added to as required β2 agonists, clinical trials have shown improvement in lung function, (Reiss et al. 1997; Spector, Smith, & Glass 1994) reduction in the need for rescue bronchodilators (Leff et al. 1998; Spector, Smith, & Glass 1994) and some evidence of a reduction in eosinophilic airway inflammation (Pizzichini et al. 1999a). In the UK, leukotriene antagonists are currently licensed for use in
patients who remain symptomatic despite treatment with inhaled corticosteroids. Clinical trials have shown evidence of efficacy in patients taking high doses of inhaled steroids (Christian et al. 2000) and the introduction of montelukast has been shown to allow a reduction in the dose of inhaled corticosteroid without loss of asthma control (Lofdahl et al. 1999). The effectiveness of the addition of leukotriene antagonists compared to increasing the dose of inhaled corticosteroids in patients with persistent symptoms, however, has not yet been fully addressed. Two studies published in abstract form comparing zafirlukast with higher doses of inhaled corticosteroids did not show any important differences between the two treatment strategies (Nayak et al. 1998; Ringdal, White, & Harris 1999). A recent metanalysis has suggested that the addition of leukotriene antagonists to inhaled corticosteroids does not significantly reduce asthma exacerbations compared to increasing the dose of inhaled corticosteroids (Ducharme 2002) but there is a paucity of adequately powered studies addressing this issue and further work is needed (Ringdal, White, & Harris 1999). The relative effectiveness of leukotriene antagonists compared to long acting β2 agonists as add-on therapy also remains unclear and needs further investigation (Bjermer et al. 2000). Whilst some studies have shown that the addition of long acting β2 agonists results in greater improvements in asthma control than the addition of leukotriene antagonists (Fish et al. 2001; Nelson et al. 2000), others demonstrate that comparable improvements in symptoms and lung function are seen, with leukotriene antagonists providing additional anti-inflammatory effects that long acting β2 agonists do not (Lipworth et al. 2000; Wilson et al. 2001). It is possible that subgroups of patients with asthma may be particularly suited to treatment with leukotriene antagonists, perhaps though genetic variations in the cysteinyl-leukotriene pathways (see below).

Theophylline
Theophylline has been used for many years in relatively high doses as a bronchodilator but due to adverse effects it has often been reserved for use in patients with more severe asthma. Gastrointestinal upset is particularly common (Pollard et al. 1997) but tachycardia and arrhythmia can also occur and measurements of serum concentrations are generally advised with high dose
treatment (Global Initiative for Asthma 1995). Recent interest has been in the use of theophylline at lower doses where the risk of side effects is minimised. The combination of low dose inhaled corticosteroids and theophylline has been shown to result in comparable asthma control as higher doses of inhaled corticosteroids and may provide slightly greater improvements in lung function (Evans et al. 1997; Lim et al. 2000a; Ukena et al. 1997). A meta-analysis has suggested that long acting $\beta_2$ agonists are more effective than theophylline in patients taking low doses of inhaled corticosteroids and result in fewer side effects (Wilson, Gibson, & Coughlan 2000). Unlike long acting $\beta_2$ agonists, however, theophylline has been shown to have possible anti-inflammatory activity and may therefore have a role in some patients (Lim et al. 2001; Sullivan et al. 1994).

### 2.4.5 Severe persistent asthma

A proportion of patients will have persistent symptoms despite appropriate treatment for moderate persistent asthma as outline above. Whilst representing a relatively small minority, these patients experience much morbidity, consume significant healthcare resources (Barnes, Jonsson, & Klim 1996) and are probably best managed in specialist settings. Before additional therapeutic measures are considered it is important to accurately confirm the diagnosis, to ensure that persistent symptoms are due to asthma rather than other aggravating factors such as rhinitis or gastro-oesophageal reflux and to assess compliance with existing therapy. Once these issues have been addressed current guidelines advocate a step up in treatment, usually with high doses of inhaled corticosteroids in combination with long acting $\beta_2$ agonists, leukotriene antagonists, theophylline, oral $\beta_2$ agonists or a combination of these agents. There have been no randomised controlled studies comparing these different treatment options in this group of patients and therefore additional therapy should be instituted on a trial basis and discontinued if there is no objective evidence of benefit (BTS 1997; Global Initiative for Asthma 1995).
Oral corticosteroids and corticosteroid sparing agents

A further group of patients have severe persistent asthma that remains difficult to control despite the measures outlined above. In these circumstances treatment with oral corticosteroids, usually in the form of daily prednisolone, may be required to minimise symptoms and prevent severe asthma exacerbations. Whilst courses of oral corticosteroids are unquestionably a vital part of the management of acute exacerbations, careful consideration should be made before they are administered on a long term basis since there is a high risk of significant adverse effects (Allen, Mullen, & Mullen 1994). Where they are required, the lowest dose which maintains asthma control should be given. Preventative therapy for osteoporosis should be considered and patients should be monitored for the development of hypertension, diabetes, cataracts, glaucoma and adrenal suppression. Obesity, thinning and bruising of the skin and myopathy are also important concerns. High doses of inhaled corticosteroids, up to 2mg daily of beclomethasone or equivalent, should always be continued, as these are likely to allow a reduction in the oral corticosteroid dose (Adams, Bestall, & Jones 2001). Other corticosteroid sparing agents include methotrexate, gold and cyclosporin. Whilst there is some evidence that these agents have steroid-sparing effects in asthma (Aaron, Dales, & Pham 1998; Bernstein et al. 1996; Lock, Kay, & Barnes 1996), each have their own safety concerns and their use should be confined to specialist units. The risk of adverse effects from the use of long term oral corticosteroids and the lack of safe alternatives necessitates careful monitoring of the response to treatment. A small minority of patients with severe asthma demonstrate resistance to corticosteroid treatment despite apparently good compliance. The mechanisms for this resistance are not fully understood but may relate to transcriptional regulation of genes associated with steroid responsive inflammation (Adcock & Caramori 2001).


2.4.6 Non pharmacological therapies in asthma

**Smoking cessation**

Cigarette smoking in adults with asthma is associated with an accelerated decline in lung function (Ulrik & Lange 2001), increased symptom severity and exacerbation frequency (Siroux et al. 2000) and an impaired response to inhaled corticosteroids (Chalmers et al. 2002). Whilst studies confined to populations of patients with asthma have not been done, smoking cessation clearly has a number of important health benefits which are likely to be particularly important to patients with pre-existing respiratory disease. Appropriate advice should therefore be given to all patients with asthma who smoke, and pharmacological treatments such as nicotine replacement therapy or buproprion should also be considered.

**Self management plans**

Combined with regular medical review, asthma self-management plans, particularly those that include written advice for patients to follow should symptoms and/or peak flow readings deteriorate, have been shown to reduce hospital admissions for asthma and are recommended in current guidelines (Gibson et al. 2000). Despite this, there have been some suggestions that neither patients nor primary health care professionals are convinced of their benefits and they may be particularly suited to those patients with poor symptom perception or recurrent asthma exacerbations (Jones, Pill, & Adams 2000).

**Breathing retraining, Buteyko techniques and physical training**

There is increasing interest in breathing retraining techniques in asthma, particularly amongst patients and the lay press. The Buteyko technique, for example, which uses hypoventilation in an attempt to raise blood PCO$_2$ levels, has been advocated as a method to allow reductions in, or even withdrawal of, asthma medication. Unfortunately rigorous trials of these methods have not yet been published and they should therefore be viewed with caution. It has recently been recognised, however, that many patients treated for asthma in primary care also have symptoms suggestive of dysfunctional breathing patterns (Thomas et
al. 2001a). Preliminary results of a physiotherapy based breathing retraining programme in such patients suggested significant improvements in health status in the short term (Thomas et al. 2001b) but more work in this area is clearly needed. It is likely that retraining techniques may improve symptoms and health status where there is dysfunctional breathing, either in the context of mild asthma or where asthma has been misdiagnosed. Physical training methods have been shown to improve cardiovascular fitness but not lung function in patients with asthma but effects on symptoms and quality of life have not been assessed (Ram, Robinson, & Black 2000).

**Allergen avoidance**

The exposure of patients with atopic asthma to the allergens that they are sensitised to has been shown to increase asthma symptoms and airway hyperresponsiveness and to cause bronchoconstriction (Boulet et al. 1993). Studies of measures which aim to control the exposure of house dust mite and pet allergens, however, have not conclusively been shown to improve asthma outcomes and larger trials have been advocated (Gotzsche et al. 2001). Studies of allergen control measures in infancy have shown reductions in respiratory symptoms (Chan-Yeung et al. 2000; Custovic et al. 2001) but it remains to be seen such measures will prevent the development of atopy and asthma in later life.

### 2.4.7 Future developments in the management of asthma

It is likely that new therapies will become available over the next 5-10 years. Some of the more promising agents are discussed below. There is also likely to be increasing interest in the heterogeneous nature of asthma in the future, specifically the heterogeneity of treatment response. Identification of factors predicting a response to treatment will enable therapy to be targeted, may improve outcomes and result in more rational, economical use of treatment. This is likely to be particularly important with the introduction of novel agents which are likely to be expensive, effective against only specific components of a complex inflammatory cascade, and therefore best reserved for subgroups of
patients most likely to respond. New developments in the pharmacogenetics of asthma are likely to play a key role in this area.

2.4.7.1 Novel pharmacological therapies

Phosphodiesterase E4 (PDE4) inhibitors
Potent and selective phosphodiesterase E4 (PDE4) inhibitors that suppress eosinophilic airway inflammation by reducing eosinophil survival, inhibiting chemotaxis and mediator synthesis and impair adhesion molecule expression have been recently developed (Torphy 1998). The two agents in the most advanced stages of development (Cilomilast and Roflumilast) have both been shown to reduce inflammation in asthma models (Bundsich et al. 2001; Underwood et al. 1998). They appear to attenuate exercise induced bronchoconstriction (Timmer et al. 2002) and cilomilast has been shown to improve lung function when given to patients with COPD and asthma on inhaled corticosteroids (Compton et al. 2001). Further clinical trials in patients with asthma are awaited.

Anti-IgE monoclonal antibody
IgE has an important role in the development of allergic diseases in atopic subjects and suppression of IgE is therefore a potential target in the management of atopic asthma. A monoclonal anti-IgE antibody, omalizumab, which blocks the interaction of IgE with mast cells and basophils, has been developed. This has now been studied in patients with moderate and severe allergic asthma treated with inhaled corticosteroids. Compared to placebo omalizumab, given as a subcutaneous injection at doses titrated to serum IgE levels, resulted in improved symptom control (Busse et al. 2001), fewer exacerbations and greater reductions in inhaled corticosteroid doses with no apparent adverse effects (Busse et al. 2001; Soler et al. 2001). It therefore appears to be a potentially useful anti-inflammatory agent in patients with atopic asthma.
Monoclonal Antibody to Interleukin-5

Interleukin-5 (IL-5) is a very selective cytokine which is responsible for the maturation and release of eosinophils in the bone marrow. Since eosinophils are a characteristic pathological feature of asthma, inhibition of IL-5 represents another potential treatment and two monoclonal antibodies to IL-5 are currently under investigation. The first published study showed that the humanised anti-IL-5 monoclonal antibody SB-240563 was able to reduce the sputum eosinophilia after allergen challenge when given intravenously, but had no effect on the early or late fall in FEV₁, or on airway responsiveness (Leckie et al. 2000). Since eosinophilic airway inflammation appears to be related to asthma exacerbations (Jatakanon, Lim, & Barnes 2000), it is possible that agents such as anti-IL-5 will be more useful in preventing asthma exacerbations than minimising day to day symptoms.

Humanised recombinant Interleukin-12

Interleukin-12 (IL-12) is another potential treatment for asthma. It is a macrophage-derived cytokine that is able to suppress eosinophilic inflammation via modulation of T lymphocyte responses. A trial of subcutaneous humanised recombinant IL-12 given to patients with mild asthma was somewhat disappointing. As with anti-IL-5, suppression of eosinophilic inflammation occurred but was not associated with improvements in airway hyperresponsiveness (Bryan et al. 2000). Additionally, significant side effects developed in a number of subjects and this is likely to limit its usefulness.

Interleukin-4 receptor antagonists

Interleukin-4 (IL-4) is another key cytokine in the development of airway inflammation that has been targeted in the search for novel asthma therapies. A nebulised soluble IL-4 receptor which acts as an IL-4 antagonist is under investigation. Initial studies have shown that this drug is well tolerated and may reverse the deterioration in symptoms and lung function that occur following withdrawal of inhaled corticosteroids (Borish et al. 2001). Study withdrawal due to asthma exacerbations following corticosteroid withdrawal was not prevented, however, and larger studies of longer duration are required (Borish et al. 1999).
2.4.7.2 Recent advances in the pharmacogenetics of asthma

Pharmacogenetics, the study of how genetic differences influence the variability of individual patient responses to drugs, aims to distinguish responders from non-responders and thus lead to rationalized drug therapy. The clinical heterogeneity of asthma has lead to increasing interest in the study of the genetic variability of this disease. There has been particular interest in the pharmacogenetics of $\beta_2$-agonists and modifiers of the cysteinyl-leukotriene pathway.

$\beta_2$-Agonist pharmacogenetics

The cell surface $\beta_2$-adrenergic receptor ($\beta_2$AR), via which $\beta_2$-agonists exert their effects, contains a number of genetic variants. Single nucleotide polymorphisms resulting in amino acid substitutions at positions 16 and 27 of the receptor and at position 19 of its upstream peptide are particularly common in Caucasian populations and are related to each other (Dewar et al. 1998; McGraw et al. 1998). The role of these genetic polymorphisms in $\beta_2$-agonist treatment response remains unclear, however. Some studies, for example, have suggested that the $\beta_2$AR position 16 genotype is associated with the response to $\beta_2$-agonist treatment with Gly16 homozygotes having diminished and Arg16 homozygotes exaggerated treatment responses (Aziz et al. 1998; Israel et al. 2000; Lima et al. 1999). Other studies, however have failed to demonstrate such an association (Hancox, Sears, & Taylor 1998; Tan et al. 1997). It is possible that combinations of different alleles (haplotypes) rather than single nucleotide polymorphisms are important in determining treatment responses.

Leukotriene Pharmacogenetics

Cysteinyl leukotrienes are important mediators in the inflammatory response in asthma. They are derived from arachidonic acid via the 5-lipoxygenase pathway. The study of the pharmacogenetics of the leukotrienes has concentrated on two key enzymes of this leukotriene synthesis pathway, 5-lipoxygenase and leukotriene $C_4$ (LTC$_4$) synthase. 5-lipoxygenase catalyses the conversion of arachidonic acid to leukotriene-$A_4$ and is blocked by the drug zileuton, which is
not licensed in the U.K. An early study suggested that the response to a zileuton derivative exhibited considerable genetically determined variability with patients who have two mutant alleles at the promoter sequence of the 5-lipoxygenase gene being resistant to treatment (Drazen et al. 1999). The second key enzyme, LTC\textsubscript{4} synthase is involved in the conversion of LTA\textsubscript{4} to LTC\textsubscript{4}, which subsequently form LTD\textsubscript{4} and LTE\textsubscript{4}. The leukotriene receptor antagonists montelukast and zafirlukast inhibit the binding of these cysteinyl leukotrienes to their receptor. Again, genetic polymorphisms of the LTC\textsubscript{4} synthase gene may relate to variations in clinical response, with one study suggesting that patients with C/C and C/A variants of the LTC\textsubscript{4} synthase promoter respond particularly well to treatment with zafirlukast (Sampson et al. 2000).

Whilst clearly much more work is needed in this field, the study of pharmacogenetics offers great potential in furthering our understanding of the heterogeneous nature of asthma and improving our use of existing asthma therapies. Such advancements, which may enable the use of genotyping to tailor therapy for individual patients, are eagerly awaited.

2.4.7.3 The role of induced sputum in the management of asthma

The induced sputum eosinophil count as a marker of corticosteroid treatment response

Traditionally a treatment response in asthma is defined by improvement in symptoms and simple measures of lung function such as the peak expiratory flow (PEF) (BTS 1997). The observations that these features do not relate closely to the degree of underlying eosinophilic airway inflammation (Crimi et al. 1998; Rosi et al. 1999) and the limited capacity of clinicians to predict the nature and extent of lower airway inflammation in patients with symptomatic asthma (Parameswaran et al. 2000) has lead to increasing interest in the development of an inflammatory marker that could identify patients likely to respond to additional treatment. The induced sputum eosinophil count is a plausible marker of the response to corticosteroid treatment since corticosteroids exert their anti-inflammatory effects at least in part by suppression of eosinophilic airway
inflammation (Barnes 1989). Its use as a marker of response to both oral and inhaled corticosteroids has been assessed. Little et al found that a sputum eosinophil count (>4%) had a 68% positive predictive value with a sensitivity of 59% and specificity of 76% for an improvement in FEV₁>15% after a two-week course of oral corticosteroids in patients with chronic stable asthma (Little et al. 2000). In a preliminary study of 23 patients with asthma treated with as-required β₂-agonists who were studied before and 2 months after treatment with inhaled corticosteroids, the baseline sputum eosinophil count was an important predictor of the response to treatment. Improvement in symptoms, airway responsiveness and sputum eosinophilia were confined to those patients who had a baseline eosinophil count of >3% (Pavord et al. 1999a). More recently, Meijer and colleagues described a significant correlation between sputum and peripheral blood eosinophil counts and the improvement in FEV₁, methacholine PC_{20} and in quality of life following treatment with inhaled or oral corticosteroids in patients with symptomatic atopic asthma (Meijer et al. 2002). These studies raise important questions about the universal use of increased doses of inhaled corticosteroids or oral corticosteroids in uncontrolled asthma.

The use of induced sputum to select the appropriate add-on treatment for patients who remain symptomatic despite low dose inhaled corticosteroids

A significant number of patients with asthma have symptoms that persist despite treatment with regular inhaled corticosteroids (Neville et al. 1999) There is an increasing number of treatment options for this important group of patients but relatively little data from placebo controlled studies comparing different treatments. Some of the better studies include a highly selected population and the data may not be relevant to wider patient groups. The heterogeneous nature of the response to anti-inflammatory treatment and the observation that corticosteroids are ineffective in the absence of a sputum eosinophilia (Pavord et al. 1999a) are important considerations. Furthermore, studies using non-invasive markers of airway inflammation have suggested that the relationship between symptoms, disordered airway function, exacerbations, inflammation and decline in lung function is complex and that these different features of the disease differ in their response to treatment. For example the available evidence suggests that AHR is related to microlocalisation of mast cells within airway smooth muscle.
and responds to treatment with long acting beta agonists, whilst control of exacerbations is best achieved by high dose inhaled corticosteroids (Pauwels et al. 1997), presumably via suppression of eosinophilic airway inflammation. The relative effectiveness of different classes of treatment on these different aspects of the disease is summarised in table 2.3. Current management guidelines do not discriminate between the different components of the disease and therefore may not result in optimal asthma control. Whilst the guidelines suggest that the introduction of a new treatment is carefully monitored and the treatment continued only if there is evidence of benefit, in practice this judgement can be difficult to make. Since new treatment is usually introduced when asthma is unstable, there is always the possibility that improvements in symptoms are spontaneous rather than treatment related. As a result, there is considerable potential for multiple drug treatment in asthma. The implication from these observations is that the response to additional treatment in subjects with symptomatic asthma despite low dose inhaled corticosteroids might be influenced by the amount of persistent eosinophilic airway inflammation. For example, higher doses of inhaled corticosteroids and leukotriene receptor antagonists may be most beneficial in patients with sputum evidence of persistent eosinophilic airway inflammation and long-acting $\beta_2$-agonists most helpful in those without. If this is the case then measuring airway inflammation by induced sputum will be useful in allowing targeting of treatment to those individuals most likely to respond. Studies that directly compare the different treatment options in diverse patients in whom the nature of the underlying disease has been carefully characterised and which assess a range of outcome measures are therefore needed. Such studies may uncover easily identifiable patient characteristics that predict a response to an individual drug and enable more effective, targeted use of treatment. This approach might also result in more economical use of treatment compared to the ad hoc treatment trials that are currently recommended.
Table 2.3. Evidence for the efficacy of each class of treatment on the four major features of asthma.

<table>
<thead>
<tr>
<th>Treatment class</th>
<th>Symptoms/VAO</th>
<th>Exacerbations</th>
<th>Inflammation</th>
<th>Decline in FEV₁</th>
</tr>
</thead>
<tbody>
<tr>
<td>Short acting B₂ agonists</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>?</td>
</tr>
<tr>
<td>Long acting B₂ agonists</td>
<td>++</td>
<td>+</td>
<td>-</td>
<td>?</td>
</tr>
<tr>
<td>inhaled corticosteroids</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>leukotriene antagonists</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>?</td>
</tr>
<tr>
<td>Theophylline</td>
<td>+</td>
<td>?</td>
<td>+</td>
<td>?</td>
</tr>
</tbody>
</table>

VAO = Variable airflow obstruction
The use of a treatment strategy targeting the induced sputum eosinophil count in the long term management of patients with asthma

There have been no longitudinal studies assessing the use of repeated measures of eosinophilic airway inflammation in induced sputum to guide anti-inflammatory therapy. There is, however, indirect evidence to suggest that such an approach may lead to improved asthma control. In a prospective randomised controlled trial, Sont and colleagues recruited 75 mainly mild to moderate asthmatics attending hospital clinics and randomised them in a single-blind fashion to receive treatment targeted at reducing airway hyperresponsiveness (AHR strategy) or traditional management (reference strategy) (Sont et al. 1999). Patients were then followed at three monthly intervals for two years. In both treatment strategies anti-inflammatory treatment was guided by symptoms, the use of rescue bronchodilator medication, diurnal variability in peak flow and measurements of FEV₁. In the AHR strategy anti-inflammatory treatment was also targeted to the degree of airway hyperresponsiveness, measured as the methacholine PC₂₀ (mg/ml). The degree of improvement in airway inflammation was assessed in a subset of patients who underwent bronchoscopy at the beginning and end of the study. The results showed that there was a 1.8-fold decrease in the rate of mild exacerbations amongst the patients treated according to the AHR strategy compared to controls. In addition, patients in the AHR strategy group demonstrated greater decreases in the thickness of the sub-epithelial reticular layer in bronchial biopsies. The improvements in outcome in the AHR strategy group, however, were accompanied by a significant increase in the use of anti-inflammatory treatment, with a median difference in dose of inhaled corticosteroids of 400 µg per day. This finding, along with the improvements in bronchoscopic measures of airway inflammation seen in the AHR group at the end of the study suggest that the improved outcomes seen may have been primarily due to better control of airway inflammation. Indeed, the improvements in airway hyperresponsiveness demonstrated in patients managed by the AHR strategy were not significantly better than those seen in the control group.
Since AHR and eosinophilic airway inflammation are not closely associated the use of more direct markers of eosinophilic airway inflammation such as induced sputum may well result in greater improvement in clinical outcomes than that seen by Sont et al. As already discussed, eosinophilic airway inflammation appears to be more closely associated with asthma exacerbations than with symptoms and peak flow variability and the identification of a sputum eosinophilia serves as an early predictor of future exacerbations (Pizzichini et al. 1999b). Furthermore, the presence of eosinophilic airway inflammation appears to be associated with diminished symptom perception in asthma (Veen et al. 1998). Taken with the finding that a sputum eosinophilia is a marker of response to treatment with inhaled corticosteroids (Pavord et al. 1999a) these observations lead to the suggestion that a management strategy that uses the induced sputum eosinophil count as a guide to the need for anti-inflammatory therapy will lead to reduced asthma exacerbations compared to a traditional clinical approach. There is a pressing need to test this theory in a prospective controlled study. Such an approach may also elucidate important risk factors for the development of asthma exacerbations and provide further information about the longitudinal relationships between eosinophilic airway inflammation, symptoms and disordered airway function in asthma.
2.5 Hypothesis

I hypothesise that widely heterogeneous patterns of airway inflammation are found in asthma. More specifically, I postulate that a pattern of predominantly neutrophilic airway inflammation is present in a subset of patients with mild and moderate asthma and that this phenotype is associated with a poor response to inhaled corticosteroids.

I hypothesise that eosinophilic airway inflammation in asthma is closely related to severe exacerbations and that a management strategy that aims to control lower airway eosinophilic inflammation as well as symptoms will result in fewer exacerbations than a traditional management approach.

I hypothesise that where patients with asthma remain symptomatic despite treatment with low dose inhaled corticosteroids, the response to additional treatment varies according to the extent of lower airway eosinophilic inflammation. Finally I propose that different treatments result in different effects on symptoms, variable airflow obstruction and lower airway inflammation in this patient group.
3. Methods

3.1 Clinical methods

3.1.1 Allergen sensitisation

Atopy was assessed either by radioallergosorbent tests or skin prick tests to *Dermatophagoides pteronyssinus*, cat fur, grass pollen and *Aspergillus fumigatus* solutions with normal saline and histamine controls (Alk-Abelló, 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.

3.1.2 Spirometry

Spirometry was performed with a Compact Vitalograph spirometer (Vitalograph, Buckinghamshire, UK). Bronchodilator reversibility was assessed 15 minutes after administration of 200µg salbutamol inhaled via a Volumatic. FEV₁ was recorded as the better of two successive readings within 100 mL. Spirometers were calibrated weekly by a qualified lung function technician.

3.1.3 Airway responsiveness

Using the standard tidal breathing method the concentration of methacholine causing a 20% fall in FEV₁ was recorded as the PC₂₀FEV₁ (Juniper, Cockcroft, & Hargreave 1994). In brief, following the measurement of the baseline FEV₁ subjects inhaled saline followed by doubling concentrations of methacholine 0.03-16 mg/ml via a Wright’s nebuliser (flow 0.13ml/min driven by dry compressed air) (gift from Fisons, Leicestershire, UK). The subject was instructed to breathe quietly (tidal breathing) for 2 min with a nose clip. The FEV₁ was measured 30 and 90 s after the nebulisation is completed. If the FEV₁ falls less than 20% the procedure was repeated with the next highest
concentration. If the FEV₁ fell more than 20% from baseline (or the highest concentration had been given), no further methacholine was given. Methacholine PC_{20}FEV₁ concentration was calculated by linear interpolation of 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. 3 ml of saline was placed into the nebuliser at room temperature. The nebuliser was then weighed and the solution nebulised at a flow of 7L/min for 2 minutes. The nebuliser was reweighed and emptied. The process was repeated three times for a range of flow rates and the average output at each flow rate calculated. The necessary flow was then determined to give an output of 0.13 ml/min (6.0 L/min.) The process was repeated at one monthly intervals by the same individual.

3.1.4 Sputum induction

Instructions for Patients
Prior to commencing the induction the procedure was fully explained to the patient with emphasis on the following:
(i) Instruction on spitting out saliva generated during inhalation of saline into a "discard" vessel.
(ii) Instruction about blowing their nose and rinsing their mouth and swallowing the water prior to trying to expectorate sputum. (It is important that the subject moves quickly through this procedure to prevent loss of sputum due to swallowing).
(iii) Instruction on how to expectorate effectively. It is necessary to explain and demonstrate the technique for coughing up sputum and moving sputum from the back of the throat, forward to the specimen container.
(iv) A reminder not to swallow the sputum as it comes up the bronchial tree.
(v) Guidance on posture: sitting straight upright during nebulisation, and leaning forward during expectoration.

Protocol
Subjects were pre-treated with inhaled salbutamol 200 μg 10-30 minutes before sputum induction to minimise bronchoconstriction. Sputum was induced using 3,
4 and 5% saline inhaled in sequence for five minutes via an ultrasonic nebuliser (Medix, Harlow, UK; output 0.9 ml/min; mass median diameter 5.5 μm). Tidal breathing was employed, taking a slightly deeper breath every minute. After each inhalation patients blew their noses and rinsed their mouths to minimise nasal contamination and expectorated sputum into a sterile pot. FEV₁ was measured after each inhalation. If the FEV₁ fell by more than 10% but less than 20%, the same concentration of saline was administered. If the FEV₁ fell by more than 20% of the best post-bronchodilator value, or if significant symptoms occurred, then nebulisation was stopped and a further dose of short-acting β-agonist was administered. (Figure 3.1) The manufacturer performed the initial calibration of mass median diameter and output. Subsequent calibration checks of nebuliser output were performed by a qualified lung function technician using the following protocol. 5 ml 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 three times for a range of flow rates and the average output calculated. The same individual repeated the calibration procedure at monthly intervals.

Safety Procedures During the Induction

Inhaled hypertonic saline is a bronchoconstrictor stimulus so sputum induction using ultrasonically nebulised hypertonic saline was carried out with care. The usual laboratory resuscitation apparatus plus nebulised salbutamol was readily available. A doctor either performed the procedure or was nearby during each procedure.
Figure 3.1 Sputum induction protocol

Measure FEV$_1$

Salbutamol 200 µg inhaled or 2.5mg nebulised

Remeasure FEV$_1$ after 20 minutes

Administer 3% saline nebulised for 5 minutes

Blow nose, rinse mouth and swallow water

Expectorate sputum

≥10%, <20% fall in FEV$_1$

Remeasure FEV$_1$

≥20% fall in FEV$_1$ or troublesome symptoms

<10% fall in FEV$_1$

Repeat procedure with 4 and 5%

Discontinue
3.1.5 Measurement of exhaled Nitric Oxide (NO)

End-exhaled NO was measured with a chemiluminescence analyser (Logan Research, Rochester, UK) with subjects exhaling at a flow rate of 250ml/second from total lung capacity. The analyser was fitted with a biofeedback display to provide visual guidance for the patient to maintain the required flow rate. NO was sampled from a sidearm attached to the mouthpiece and the NO value was taken from the point corresponding to the plateau of the end-exhaled CO₂ reading (5 to 6% CO₂). The mean value of three successive readings was recorded.

3.1.6 Symptom visual analogue scores

Symptom scores were recorded using a 100mm visual analogue scale fixed at both ends by no symptom to the worst symptom ever for each of the symptoms of dyspnoea, cough, and wheeze (appendix I). On subsequent visits patients referred to their previous responses as advocated by Guyatt et al (Guyatt et al. 1985) and recorded their current symptom severity on the same scale.

3.1.7 Asthma quality of life questionnaire

Health status was assessed using the asthma quality of life questionnaire (Juniper et al. 1992). This consists of 32 questions measuring four domains: symptoms, limitation of activities, emotional dysfunction and the response to environmental stimuli. The limitation of activities domain was examined by the subject specifying five frequent activities in which they experienced asthma symptoms and scoring the degree of limitation of each on a seven-point Likert scale. The same activities were used at each subsequent visit. The other domains were similarly assessed using a seven-point Likert scale.
3.1.8 Asthma diary records

Patients completed daily diary cards recording daytime and night-time symptoms, twice daily peak expiratory flow (PEF), medication use including rescue β₂ agonist and oral prednisolone, days off work and emergency care visits (appendix II). PEF was recorded as the best of three successive readings using a Mini-Wright peak flow meter (Clement Clarke International Ltd., Harlow, UK). Symptom scores ranged from 0 to 3 (for daytime symptoms: 0 = none, 1 = occasional symptoms, 2 = symptoms most of the day, 3 = asthma very bad, unable to do normal activities at all; for night-time symptoms 0 = none, 1 = awoken once due to asthma, 2 = awoken 2-3 times due to asthma, 3 = awake most of the night due to asthma).

3.1.9 Peak flow variability

The maximum PEF amplitude % mean was derived from the maximum within day variability observed over a 14 day period as the ratio of the difference between the highest and lowest PEF and the mean PEF.
3.2 Laboratory methods

3.2.1 Protocol for sputum processing

Sputum free from salivary contamination was selected and weighed. To the selected sputum was added four times the volume/weight of 0.1% dithiothreitol (DTT) (Sigma, Poole Dorset). The sputum was dispersed by gentle aspiration into a Pasteur pipette, vortexed for 15 s and 15 mins rocking on a bench spiromix. After the addition of an equal volume of Dulbecco’s phosphate buffered saline (D-PBS) (Sigma, Poole, Dorset) the sputum suspension was filtered through 48μm nylon gauze and centrifuged 2000rpm (790g) for 10 mins. The sputum supernatant was removed and stored at -80°C for future mediator assay. The cell pellet was resuspended in a small volume of PBS. An aliquot was removed and a total cell count, squamous cell contamination and viability were assessed using a neubauer haemocytometer by the trypan blue exclusion method. The cell suspension was adjusted with PBS to 0.5-0.75 x10^6 cells/ml and cytospins were prepared from 75μl aliquots at 450rpm (18.1g) for 6 mins using a Shandon III cytocentrifuge (Shandon, UK). The cytospins were stained in neat Romanowski stain for 5 mins and fixed in dilute stain for 25 mins.

**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:

- 62 ml 10mM HEPES buffer pH 7.2
- 3.5 ml DMSO
- 4.6 ml Romanowski stain
Differential cell counts

A sputum differential cell count was obtained by counting ≥400 non-squamous cells on a Romanowski stained cytospin (figure 3.2). Cell counts were performed blinded to the clinical characteristics of the patients and, where applicable, to the randomisation group. A proportion of slides were recounted without reference to the original results and intraclass correlation coefficients were calculated for each cell type to assess intra-observer repeatability (appendix III).
Figure 3.2  Examples of induced sputum cytopsins (a) eosinophils (b) neutrophils (c) macrophages (x200)
3.2.2 Cysteinyl leukotrienes enzyme immunoassay

The cysteinyl leukotrienes LTC₄/LTD₄/LTE₄ were measured using a commercial ELISA (Cayman Chemical, Ann Arbor, MI). Although the addition of DTT did not significantly affect the standard curve, all standards and controls were reconstituted in 0.009% DTT. Buffer, cysteinyl-leukotriene standard, acetylcholinesterase tracer and antiserum were otherwise reconstituted as per the protocol. 100 µl and 50 µl of buffer solution were added to non-specific binding and maximum binding wells respectively. 50µl of cysteinyl leukotriene standards and 50µl of samples diluted to 1:5 were aliquoted to appropriate wells. 50µl of cysteinyl-leukotriene acetylcholinesterase tracer and 50µl of cysteinyl-leukotriene antiserum were added to each well with the exception of total activity and non-specific binding wells. The plates were then covered and incubated for 18 hours at room temperature, developed with Ellman’s reagent reconstituted in UltraPure water and then read. Sample results were then calculated from the standard curve. The intra- and interassay coefficient of variability were <10% and lower limit of detection was 13 pg/ml of sample.
4. Studies

4.1 Analysis of induced sputum in adults with asthma. Identification of a subgroup with an isolated sputum neutrophilia and a poor response to inhaled corticosteroids.

Abstract

The debate as to whether asthma is a single or heterogeneous disease remains unresolved although pathological studies, mostly using fibreoptic bronchoscopy on small numbers of subjects, have emphasised the similarities between different clinical phenotypes. We have non-invasively assessed lower airway inflammation using induced sputum in 34 normal controls and 259 adults with symptomatic asthma receiving treatment at steps 1-3 of the British Thoracic Society (BTS) guidelines. A subgroup of 49 patients treated with as required β2-agonists only who met BTS criteria for a step-up in treatment were studied before and 2 months after treatment with inhaled budesonide 400μg bd. There was considerable heterogeneity in induced sputum cell counts, particularly in non-atopic patients. A subgroup of 60 patients had a distinctive sputum cell profile with a sputum neutrophil count higher than our normal range (> 65.3%) and a normal sputum eosinophil count (<1.9%). These patients were older, predominantly female and were more likely to be non-atopic but otherwise had similar clinical and physiological features to the group as a whole. Amongst the 49 subjects studied before and after inhaled budesonide, 11 patients had an isolated sputum neutrophilia. Following treatment, these patients demonstrated significantly less improvements in visual analogue symptom scores (-5.5 v -19.4 mm; mean difference 13.9; 95% CI 0.7, 27.0), FEV₁ (-0.08 v 0.13 l; mean difference 0.21; 95% CI 0.03, 0.39) and PC₂₀ (0.15 v 1.29 doubling doses; mean difference 1.11; 95% CI 0.13, 2.15) than the remaining 38 patients. Our results suggest the presence of a distinct subgroup of patients with mild to moderate asthma who have predominantly neutrophilic airway inflammation and who respond less well to treatment with inhaled corticosteroids.
Introduction

Clinicians have long regarded asthma as a heterogeneous disease (Rackemann F.M. 1921) (Aas 1981) although detailed clinicopathological studies have tended to emphasise the similarities in the underlying airway pathology and disordered function between patients (Bentley et al. 1992; Bentley, Durham, & Kay 1994; Folkard, Westwick, & Millar 1997; Humbert et al. 1996; Humbert et al. 1997; Humbert et al. 1999; Tang et al. 1997). Airway inflammation in asthma has usually been assessed invasively using bronchoscopic techniques so studies are largely confined to a population of young adults with mild atopic asthma. Whether the findings can be generalised to a wider, more heterogeneous population analogous to that seen in clinical practice is unclear.

More recent studies where airway inflammation has been assessed non-invasively using induced sputum in a more diverse range of patients have shown predominant neutrophilic airway inflammation in some patients with severe asthma (Jatakanon et al. 1999c; Louis et al. 2000b) and in others studied during acute exacerbations (Fahy et al. 1995a). Whether these changes reflect the severity of the disease or the effect of treatment is unclear. We have measured airway inflammation in 34 normal and 259 subjects with symptomatic asthma receiving treatment at British Thoracic Society (BTS) Steps 1-3 (BTS 1997) and have related sputum cell counts to the response to inhaled corticosteroids in 49 subjects. We have used this data to test the hypothesis that a predominant neutrophilic airway inflammation is present in a subset of patients with milder asthma and that this phenotype is associated with a poor response to inhaled corticosteroids.

Methods

Subjects

Patients and controls were recruited from patients, staff and volunteers attending the Department of Respiratory Medicine at the Glenfield Hospital. Normal controls had no symptoms suggestive of asthma, were non-smokers or ex-
smokers who had not smoked within 12 months of study entry and had a past history of less than ten pack years, had normal spirometric values (FEV₁ >80% predicted and FEV₁/FVC >80%) and normal methacholine airway responsiveness (PC₂₀ > 16mg/ml.). Subjects with asthma had consistent symptoms and one or more of the following: a methacholine PC₂₀ of <8mg/ml; a >15% increase in FEV₁ 10 minutes after 200μg salbutamol or a >20% maximum within day variability in peak expiratory flow (PEF) measured twice daily over 14 days. Patients had no clinical or radiological evidence of bronchiectasis and no symptoms suggesting acute lower respiratory tract infection within a month of entering the study. All patients had an FEV₁ % predicted of >65% and a smoking history of less than 10 pack years. Clinical records were used to corroborate patients' smoking histories and exhaled carbon monoxide was measured where there was any doubt. All patients with asthma treated at BTS steps 1-3 attending our respiratory out-patients clinic who fulfilled the entry criteria and who agreed to participate were included. Assessments were carried out following informed consent as part of a project examining the validity, repeatability and responsiveness of induced sputum differential inflammatory cell counts which was approved by the Leicestershire Hospitals Research Ethics committee.

Study design and protocol

All subjects

Patients and controls attended on two occasions. On the first occasion allergen sensitivity was measured by radioallergosorbent tests for specific IgE or skin prick testing to *Dermatophagoides pteronyssinus*, cat fur, grass pollen and *Aspergillus fumigatus* and atopy was defined as one or more positive skin test (weal>2mm larger than negative control) or elevated specific IgE (>0.34ku/l) to one or more antigens. Spirometry before and after inhaled salbutamol and chest radiography was performed. Subjects recorded PEF twice daily as the best of three blows over a 14 day period. On the second visit Methacholine airway responsiveness was measured using the Tidal breathing method (Juniper, Cockcroft, & Hargreave 1994) followed after recovery by sputum induction and
processing as previously described (Pavord et al. 1997). The duration of inhalation of hypertonic saline was standard. (Methods 3.1.3, 3.1.4, 3.2.1).

Investigation of a subgroup before and after treatment with inhaled corticosteroids

A subgroup of patients taking as required β2-agonists only who met British Thoracic Society criteria for a step-up in treatment (using rescue β2 agonists more than once per day, having nocturnal waking or limitations in activities, peak flow variability ≥ 20% or PEF ≤ 80% of predicted or best) (BTS 1997) were given inhaled budesonide 400 μg twice daily for two months. These patients identified their predominant symptom (breathlessness, wheeze or cough) and the severity of this was assessed using 100mm visual analogue scales (VAS) from no symptom (0mm) to the worst ever symptom (100mm). This scale was the most responsive outcome measure in our earlier study (Pavord et al. 1999a) and has been validated (Brightling et al. 2001). They then attended for a third occasion, when the spirometry, methacholine inhalation test and VAS symptom scores were repeated, 12 hours after the last dose of treatment. Data from some of these patients has been presented previously (Pavord et al. 1999a).

Analysis of data

Normal ranges were derived from the eosinophil and neutrophil counts of the control subjects as the mean plus 2.0 standard deviations and the mean plus 1.7 standard deviations using one-tailed and two-tailed tests respectively. One tailed tests were used for eosinophil counts since many normal subjects have eosinophil counts of 0% and it is therefore not possible to identify a lower reference limit. Spirometric values, induced sputum macrophage, neutrophil, lymphocyte and epithelial differential cell counts and maximum PEF amplitude % mean were described as mean (SEM). Methacholine PC20 results were log normally distributed and were log transformed and described as geometric mean (log SEM). Sputum eosinophil counts were expressed as median and interquartile range (IQR). Differences between groups were analysed for normally distributed
variables using the independent t-test and for variables not observing a normal distribution using the Mann-Whitney U test. The correlation between sputum eosinophils and methacholine PC$_{20}$, PEF A%M and FEV$_1$ were assessed using the Spearman Rank test. Differences in methacholine PC$_{20}$ were expressed as doubling doses. The chi-squared test was used to compare the percentage of patients using inhaled steroids and the percentage of atopic patients between groups.

**Results**

**All subjects**

Normal ranges derived from normal subjects were <65.3% for sputum neutrophil counts and <1.9% for sputum eosinophil counts. 143 patients had intermittent asthma treated with as required β$_2$-agonists only (Step 1 of the British Thoracic Society guidelines) (BTS 1997) 116 had more persistent symptoms requiring regular inhaled corticosteroids (steps 2 and 3) (BTS 1997) (table 1). 20 patients (11 steroid naïve, 9 atopic) were current smokers and 78 (42 steroid naïve, 26 atopic) were ex smokers but all had a history of < 10 pack years. Patient details categorised according to atopic status and use of inhaled corticosteroids were as shown (table 4.1). The mean (SEM) daily dose of inhaled steroid (in Beclomethasone equivalent doses) for atopic and non-atopic subjects was 424(56)μg and 416(50)μg respectively. Non-atopic asthma was associated with less methacholine airway responsiveness (methacholine PC$_{20}$ 1.34 v 0.68 mg/ml; geometric mean difference 1.0 doubling doses; 95% CI of difference 0.4,1.6; p=0.002) and higher mean neutrophil count (54.1 v 45.0% mean difference 9.1%; 95% CI of difference 2.3,15.8; p=0.008).

Overall, sputum evidence of eosinophilic airway inflammation was the most common abnormality in the group as a whole with 135 patients (52%) having an induced sputum eosinophil count outside our normal range. The median sputum eosinophil count was significantly lower in atopic subjects receiving inhaled corticosteroids (1.1%) compared to similarly treated non-atopic subjects. (3.3%,
p<0.05) (table 4.1). Among the whole study population there was no correlation between the sputum eosinophil count and the methacholine PC$_{20}$ ($r=-0.03$; p>0.05), the maximum PEF amplitude % mean ($r=-0.02$, p>0.05) or the %predicted FEV$_1$ ($r=-0.03$, p>0.05). Among the 114 atopic patients, a weakly negative correlation between the sputum eosinophil count and the methacholine PC$_{20}$ was observed ($r = -0.30$, p<0.01). In contrast, the 145 non-atopic patients demonstrated a weakly positive correlation between these two measurements ($r=0.22$, p<0.05).
Table 4.1. Patient details and sputum cell counts in normal controls, atopic and non-atopic subjects.

Mean (s.e.m.) † Geometric mean (log s.e.m.) ‡ Median (range)
*p<0.05 atopic v non-atopic subjects, **p<0.01 atopic v non-atopic subjects

<table>
<thead>
<tr>
<th></th>
<th>Normal Controls</th>
<th>All patients</th>
<th>ATOPIC</th>
<th>Inhaled steroids</th>
<th>NON-ATOPIC</th>
<th>ATOPIC</th>
<th>Inhaled steroids</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>34</td>
<td>114</td>
<td>61</td>
<td>53</td>
<td>145</td>
<td>82</td>
<td>63</td>
</tr>
<tr>
<td>Male (%)</td>
<td>46</td>
<td>49**</td>
<td>62**</td>
<td>34</td>
<td>34**</td>
<td>38**</td>
<td>30</td>
</tr>
<tr>
<td>Age</td>
<td>34(16)</td>
<td>39(21)**</td>
<td>40(21)**</td>
<td>38(38)**</td>
<td>53(18)**</td>
<td>54(25)**</td>
<td>50(20)**</td>
</tr>
<tr>
<td>Age at onset</td>
<td>-</td>
<td>16(31)**</td>
<td>27(38)**</td>
<td>11(19)**</td>
<td>47(25)**</td>
<td>48(26)**</td>
<td>45(24)**</td>
</tr>
<tr>
<td>FEV₁ % predicted</td>
<td>98.8(5.6)</td>
<td>86.5(1.4)</td>
<td>91.6(1.6)</td>
<td>80.7(2.4)</td>
<td>85.7(1.4)</td>
<td>88.8(1.8)</td>
<td>81.7(1.9)</td>
</tr>
<tr>
<td>FEV₁/FVC %</td>
<td>85.3(1.3)</td>
<td>75.1(0.9)</td>
<td>76.2(1.0)</td>
<td>73.8(1.7)</td>
<td>73.0(0.1)</td>
<td>74.1(0.9)</td>
<td>71.6(1.2)</td>
</tr>
<tr>
<td>Methacholine PC₂₀ mg/ml†</td>
<td>&gt;16</td>
<td>0.68(0.07)**</td>
<td>0.89(0.11)*</td>
<td>0.50(0.11)*</td>
<td>1.34(0.06)**</td>
<td>1.58(0.07)*</td>
<td>1.06(0.09)*</td>
</tr>
<tr>
<td>PEF amplitude % mean</td>
<td>8.5(0.9)</td>
<td>26.0(2.0)</td>
<td>22.2(2.1)</td>
<td>30.1(4.0)</td>
<td>21.7(1.3)</td>
<td>18.7(1.4)</td>
<td>25.0(2.2)</td>
</tr>
<tr>
<td>Blood eosinophils %</td>
<td>2.2(0.6)</td>
<td>4.6(0.4)</td>
<td>4.1(0.4)</td>
<td>5.1(0.8)</td>
<td>3.7(0.3)</td>
<td>3.6(0.4)</td>
<td>3.9(0.4)</td>
</tr>
<tr>
<td>Sputum TCC (x 10⁶/ml)</td>
<td>2.8(0.5)</td>
<td>2.3(0.3)</td>
<td>2.3(0.4)</td>
<td>2.2(0.5)</td>
<td>2.0(0.4)</td>
<td>1.4(0.2)</td>
<td>2.6(0.7)</td>
</tr>
<tr>
<td>Squamous cells (%)‡</td>
<td>5.1(14.0)</td>
<td>4.4(15.3)</td>
<td>3.8(14.8)</td>
<td>4.7(15.8)</td>
<td>3.8(10.0)</td>
<td>4.4(11.3)</td>
<td>3.8(8.4)</td>
</tr>
<tr>
<td>Viability (%)</td>
<td>60.8(2.8)</td>
<td>61.7(2.0)</td>
<td>63.5(2.7)</td>
<td>59.6(3.5)</td>
<td>62.6(1.7)</td>
<td>60.6(2.1)</td>
<td>65.0(2.7)</td>
</tr>
<tr>
<td>Eosinophils (%)‡</td>
<td>0.0(0.3)</td>
<td>2.5(7.0)</td>
<td>4.4(9.0)*</td>
<td>1.1(1.4)*</td>
<td>2.0(12.6)</td>
<td>1.5(6.9)*</td>
<td>3.3(20.4)*</td>
</tr>
<tr>
<td>Neutrophils (%)</td>
<td>30.8(3.3)</td>
<td>45.0(2.5)**</td>
<td>44.2(3.3)**</td>
<td>45.9(4.4)</td>
<td>54.1(2.3)**</td>
<td>57.1(2.9)**</td>
<td>49.9(3.6)</td>
</tr>
<tr>
<td>Macrophages (%)</td>
<td>61.0(3.0)</td>
<td>43.5(2.4)**</td>
<td>42.4(3.0)**</td>
<td>44.7(4.2)**</td>
<td>32.8(2.0)**</td>
<td>33.0(2.5)**</td>
<td>32.5(3.1)**</td>
</tr>
<tr>
<td>Lymphocytes (%)</td>
<td>1.4(0.4)</td>
<td>1.0(0.1)</td>
<td>1.0(0.1)</td>
<td>0.9(0.3)</td>
<td>0.8(0.1)</td>
<td>0.8(0.2)</td>
<td>0.8(0.2)</td>
</tr>
<tr>
<td>Epithelial cells (%)</td>
<td>3.8(0.9)</td>
<td>3.2(0.5)</td>
<td>3.2(0.8)</td>
<td>3.3(0.8)</td>
<td>2.7(0.4)</td>
<td>2.8(0.5)</td>
<td>2.7(0.6)</td>
</tr>
</tbody>
</table>
Demonstration of a subgroup with isolated neutrophilic inflammation

There was considerable heterogeneity in induced sputum eosinophil and neutrophil cell counts, even among those patients treated with as required β₂-agonists alone. A subgroup of 60 patients, including 35 who were steroid naive, had a distinctive sputum cell profile with a sputum neutrophil count outside the normal range and a normal sputum eosinophil count. 5 of these were current smokers and 20 were ex smokers. Compared to the remaining group, these patients were older, tended to develop asthma later and were more likely to be female and non-atopic than the whole group. Otherwise clinical and physiological features were similar (table 4.2).
Table 4.2. Characteristics of patients with isolated sputum neutrophilia and all other patients studied.

<table>
<thead>
<tr>
<th></th>
<th>Neutrophilic(^1)</th>
<th>Others(^2)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(\beta) agonist only</td>
<td>Inhaled steroids</td>
</tr>
<tr>
<td>n</td>
<td>35</td>
<td>25</td>
</tr>
<tr>
<td>Male (%)</td>
<td>34</td>
<td>28</td>
</tr>
<tr>
<td>age(\dagger)</td>
<td>54(24)</td>
<td>48(13)</td>
</tr>
<tr>
<td>age onset(\dagger)</td>
<td>41(30)</td>
<td>42(28)</td>
</tr>
<tr>
<td>FEV(_1) % pred</td>
<td>88(2.8)</td>
<td>86(3.2)</td>
</tr>
<tr>
<td>FEV(_1)/FVC ratio</td>
<td>75(1.5)</td>
<td>74(2.4)</td>
</tr>
<tr>
<td>% atopic</td>
<td>22.9</td>
<td>32</td>
</tr>
<tr>
<td>% taking inhaled steroids</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>PEF A%M</td>
<td>20.9(3.2)</td>
<td>25.2(2.8)</td>
</tr>
<tr>
<td>PC(_{20}) (mg/ml)(\ddagger)</td>
<td>1.4(0.09)</td>
<td>1.2(0.10)</td>
</tr>
<tr>
<td>Sputum eosinophil count(%)(\dagger)</td>
<td>0.4(0.9)</td>
<td>0.7(0.9)</td>
</tr>
<tr>
<td>Sputum neutrophil count(%)</td>
<td>81.0(1.6)</td>
<td>85.3(2.0)</td>
</tr>
</tbody>
</table>

\(^1\)Patients with sputum neutrophils >65.3% and eosinophils <1.9%
\(^2\)Patients with sputum eosinophils >1.9% or neutrophils <65.3%

*p<0.05; **p<0.01
Patients studied before and after treatment with inhaled corticosteroids

92 of the patients treated with as required β2-agonists only met the BTS criteria for a step up in treatment. 49 such patients were randomly selected and agreed to attend again two months after treatment with inhaled budesonide 400μg bd. Of these subjects, 11 were included in the subgroup described above, having an isolated sputum neutrophilia with no evidence of eosinophilic airway inflammation. (table 4.3). Compared to the other 38 patients studied before and after treatment, these subjects demonstrated significantly less improvements in visual analogue symptom scores (-5.5 v -19.4 mm; mean difference 13.9; 95%CI 0.7, 27.0; p =0.04), FEV1 (-0.08 v 0.13 l; mean difference 0.21; 95% CI 0.03, 0.39; p = 0.026) and PC20 (0.15 v 1.29 doubling doses; mean difference 1.11; 95% CI 0.13,2.15; p = 0.029) (figure 4.1).
Table 4.3. Baseline characteristics of patients studied before and after treatment with budesonide 400μg bd for two months.

<table>
<thead>
<tr>
<th></th>
<th>Neutrophilic¹</th>
<th>Others²</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>11</td>
<td>38</td>
</tr>
<tr>
<td>Male (%)</td>
<td>27.3%</td>
<td>55.3%</td>
</tr>
<tr>
<td>age†</td>
<td>57(21)</td>
<td>45(22)*</td>
</tr>
<tr>
<td>age onset†</td>
<td>56(13)</td>
<td>40(29)*</td>
</tr>
<tr>
<td>VAS (mm)</td>
<td>59(9)</td>
<td>48(5)</td>
</tr>
<tr>
<td>FEV₁% pred</td>
<td>82(5.7)</td>
<td>89(2.4)</td>
</tr>
<tr>
<td>FEV₁/FVC ratio</td>
<td>72(3.2)</td>
<td>74(0.6)</td>
</tr>
<tr>
<td>% atopic</td>
<td>9.1</td>
<td>42.1*</td>
</tr>
<tr>
<td>No of current (ex) smokers</td>
<td>2(5)</td>
<td>3(10)</td>
</tr>
<tr>
<td>PEF A%M</td>
<td>22.8(4)</td>
<td>17.0(1.5)</td>
</tr>
<tr>
<td>Methacholine PC₂₀ (mg/ml)‡</td>
<td>1.0(0.2)</td>
<td>1.3(0.11)</td>
</tr>
<tr>
<td>Sputum eosinophil count (%)†</td>
<td>0.2(0.9)</td>
<td>6.0(7.2)*</td>
</tr>
<tr>
<td>Sputum neutrophil count (%)</td>
<td>78.5(2.6)</td>
<td>49.5(4.1)*</td>
</tr>
</tbody>
</table>

¹Patients with sputum neutrophils >65.3% and eosinophils <1.9%
²Patients with sputum eosinophils >1.9% or neutrophils <65.3%

Mean (s.e.m). † Median (IQR). ‡ Geometric mean (log s.e.m.)
Figure 4.1. Change following 2 months treatment with Budesonide 400µg bd in neutrophilic patients (open bars) and all other patients (closed bars)

Mean (SEM) *p<0.05
Discussion

We have analysed the extent and nature of airway inflammation in induced sputum in normal controls and in a large population of well characterised patients with asthma. Our estimates of normal ranges, whilst derived from small numbers, are very similar to findings in larger populations (Belda et al. 2000; Spanevello et al. 2000). In this large prospective observational study of adults with asthma receiving treatment at BTS stages 1-3 and with relatively normal spirometry we found considerable heterogeneity in induced sputum inflammatory cell counts. Importantly, a number of predominantly female, adult onset, non-atopic subjects had a distinctive sputum inflammatory cell profile consisting of a sputum neutrophilia and a normal sputum eosinophil count. Furthermore, a subgroup of steroid naïve subjects with this isolated neutrophilic inflammation demonstrated an impaired response to treatment with inhaled corticosteroids.

Previous studies have noted sputum evidence of isolated neutrophilic airway inflammation in some patients with severe asthma (Jatakanon et al. 1999c) (Louis et al. 2000b), and in a minority of adults studied during asthma exacerbations (Fahy et al. 1995a). Gibson et al have used induced sputum to assess 56 patients with persistent asthma taking high doses of inhaled corticosteroids, and report that 59% of patients in this group had suppressed sputum eosinophil counts but evidence of neutrophilic inflammation. (Gibson, Simpson, & Saltos 2001) Wenzel et al have used bronchoscopic techniques to characterise the underlying airway immunopathology of a group of patients with severe refractory asthma who had severely impaired lung function and were treated with high dose inhaled steroids and oral prednisolone and have suggested the presence of a subgroup who have a predominant neutrophilic airway inflammation, absence of eosinophils and normal basement membrane thickness (Wenzel et al. 1999). It is not clear whether the findings are peculiar to severe asthma or reflect the effects of treatment with high doses of corticosteroids. Our results provide support for the presence of such a distinct asthma phenotype and for the first time show that it is a relatively common finding in patients with milder asthma and, in some subjects at least, that it is not an artefact due to corticosteroid treatment. The incidence of neutrophilic inflammation was higher.
in the population studied by Gibson et al (Gibson, Simpson, & Saltos 2001) and in the patients with severe asthma studied by Wenzel et al (Wenzel et al. 1999), and it remains possible that this phenotype is particularly associated with more severe disease. We have further extended these earlier findings by showing a significantly impaired response to inhaled corticosteroids in a subgroup of the subjects with an isolated neutrophilia. The poor response to inhaled corticosteroid is not only of obvious clinical significance but it also provides a possible mechanism where subjects might be particularly likely to evolve into more severe, refractory cases.

We do not have a clear explanation for the development of neutrophilic airway inflammation in these patients. All patients had a smoking history of less than 10 pack years, only a small minority were current smokers and the patients with isolated sputum neutrophilia were no more or less likely to have ever smoked than the remaining group. We therefore doubt that current smoking was an important explanation for the unusual inflammatory cell profile. All the patients presented with symptoms consistent with asthma, had normal chest X-rays and no clinical evidence of acute infection, although we cannot exclude the possibility of subtle, subclinical bronchiectasis or lower respiratory tract infections. Idiopathic chronic cough has a similar female predominance and age at onset of symptoms and is associated with a sputum neutrophilia. (Jatakanon et al. 1999b) These similarities suggest there might be parallels between these conditions. Further work is required to define the lower airway immunopathology in more detail and to investigate its aetiology.

This large observational study of adults with asthma provided us with the opportunity to compare sputum markers of airway inflammation in subjects categorised according to atopic status and use of inhaled corticosteroids, variables that have been traditionally used to phenotype asthma. We identified several differences between atopic and non-atopic subjects that have not been reported before. The higher sputum neutrophil count in non-atopic subjects could reflect the higher incidence of neutrophilic asthma in this group. Non-atopic subjects also had less airway hyperresponsiveness and were more likely to have sputum evidence of persistent eosinophilic airway inflammation despite
treatment with inhaled corticosteroids. These differences support suggestions that non-atopic and atopic asthma represent distinct disease phenotypes (Rackemann F.M. 1921). Further work is required to determine whether they are clinically significant. The sputum eosinophil count was significantly lower in atopic subjects treated with inhaled corticosteroids compared to non-atopic subjects so one possibility is that atopic patients might not respond as well to a higher dose of inhaled corticosteroids.

There was no correlation between airway hyperresponsiveness and eosinophilic airway inflammation in the population as a whole, although there was a weak negative correlation when atopic subjects were considered alone. These findings challenge the widely held view, reflected by recent definitions of asthma (Barnes et al. 1997; Global Initiative for Asthma 1995), that there is a simple causal association between eosinophilic airway inflammation and disordered airway function and suggest a more complex relationship. Other studies examining the relationship between the sputum eosinophil count and airway responsiveness have produced mixed results (Crimi et al. 1998; Adelroth et al. 1990; Claman et al. 1994; Lim et al. 1999; Pizzichini et al. 1996a; Rosi et al. 1999) although it is notable that those studies showing a significant correlation have been largely confined to atopic subjects (Claman et al. 1994; Lim et al. 1999; Pizzichini et al. 1996a).

We describe a single observation, and in a disease characterised by variability we cannot be sure that the distinctive phenotype seen in our population of adults with asthma is stable. Our estimates of incidence might also be incorrect, since we have studied subjects referred for secondary care, who might be particularly likely to display unusual features. Longer-term studies of a more typical population of asthmatics are required to estimate the true prevalence of this asthma phenotype and to determine whether it is stable. Placebo controlled longer-term intervention studies with inhaled corticosteroids and other treatments are also required to fully assess the efficacy of these interventions. Our findings raise the possibility of a distinct phenotype of asthma, with active neutrophilic and suppressed eosinophilic airway inflammation, across the range of severity of
asthma that differs in response to treatment and could have important implications for our understanding and treatment of the disease.
4.2 Reduced asthma exacerbations with a management strategy directed at normalising the sputum eosinophil count. A randomised comparison with traditional management.

Abstract

Treatment decisions in asthma are traditionally based on assessments of symptoms and simple measures of lung function. These features do not relate closely to underlying eosinophilic airway inflammation. However airway inflammation has been associated with exacerbations of asthma, suggesting that a management strategy that minimises eosinophilic inflammation may reduce exacerbations. We have tested this hypothesis in a randomised controlled trial. 74 subjects with moderate to severe asthma recruited from hospital clinics were randomised into two groups: one managed by standard British Thoracic Society asthma guidelines (BTS management group) and one managed using an algorithm aimed at normalising the induced sputum eosinophil count as well as minimising symptoms (sputum management group). Patients were seen nine times over 12 months and on each occasion sputum was induced and processed, but the results were not disclosed in the BTS guidelines group. Assessed over 12 months as the area under the curve, the sputum eosinophil count was 63% (95% CI 24-100%) lower in the sputum management group (p=0.002). Patients in the sputum management group experienced significantly fewer severe asthma exacerbations than patients in the BTS management group (35 v 109, p=0.01) and significantly fewer patients were admitted to hospital with asthma (1 v 6, p=0.047). There were no significant differences in the average daily dose of inhaled or oral corticosteroids between the two groups. A treatment strategy directed at normalising the induced sputum eosinophil count reduces asthma exacerbations and hospital admissions without the need for additional anti-inflammatory therapy.
Introduction

Asthma is a disease characterised by variable airflow obstruction, airway hyperresponsiveness and chronic airway inflammation, which is generally eosinophilic (Wardlaw et al. 2000). It is a common disease which can cause considerable morbidity and a significant mortality (Janson et al. 1997) (Campbell et al. 1997). While as required bronchodilators and moderate doses of regular inhaled steroids provide good control in the majority of asthmatics, some patients suffer severe exacerbations that are difficult to prevent and can lead to time off work, hospital admission and life threatening attacks. Management decisions in asthma are traditionally based on assessment of symptoms, airway function and rescue β2-agonist use (BTS 1997). Recent evidence has shown that these features do not relate closely to the degree of underlying eosinophilic airway inflammation (Crimi et al. 1998) and, as a consequence, clinicians have a limited capacity to predict the nature and extent of lower airway inflammation in patients with symptomatic asthma (Parameswaran et al. 2000).

There is strong circumstantial evidence that eosinophils play an important pro-inflammatory role in the pathogenesis of asthma. Eosinophils and their mediators are consistently found in the asthmatic but not normal lung, they are relevant to the asthma process and suppression of eosinophil infiltration in clinical disease by glucocorticoids or in animal models is usually associated with an amelioration of the pathophysiological features of the disease (Wardlaw et al. 2000). Although the use of an anti-IL-5 antibody has not provided support for a role for eosinophils in the development of the early or late response to allergen challenge (Leckie et al. 2000), there is growing evidence that eosinophils are associated with asthma exacerbations (Pizzichini et al. 1999b). Furthermore corticosteroid reduction studies have shown that a sputum eosinophilia develops well before the onset of an exacerbation (Pizzichini et al. 1999b) (Jatakanon, Lim, & Barnes 2000). We therefore hypothesised that a management strategy that aims to control lower airway eosinophilic inflammation as well as symptoms would result in fewer exacerbations than a traditional approach. The development of non-invasive techniques to assess airway inflammation has made it feasible to
investigate such an approach to asthma management (Pavord et al. 1997). We therefore set out to test the hypothesis in a randomised controlled trial.

**Methods**

**Patients**

All eligible patients aged 18-75 with a diagnosis of asthma attending three specialist clinics at Glenfield Hospital between March and October 2000 who were thought to require continued hospital follow-up were invited to participate. Patients were excluded if they were current smokers or had a smoking history of >15 pack years or if they had significant co-morbidity. We also excluded those that were considered by the referring physician to comply poorly with medication, had inadequately controlled aggravating factors such as rhinitis or gastro-oesophageal reflux, or had had a severe exacerbation within 4 weeks of trial entry, to maximise the chance that the current symptoms were due to asthma, that they were stable, and that they would take treatment as instructed during the trial. Severe exacerbations were defined as a decrease in the morning peak expiratory flow to more than 30 percent below the baseline value on two or more consecutive days, or deterioration in symptoms requiring treatment with oral corticosteroids (Pauwels et al. 1997). All patients had symptoms consistent with the diagnosis of asthma and one or more of: a >15% increase in FEV₁ following 200μg inhaled Salbutamol; >20% within day variability in PEF assessed twice daily over 2 weeks; or a concentration of methacholine causing a 20% fall in FEV₁ (methacholine PC₂₀) of <8 mg/ml. The study was approved by the local research ethics committee and all patients gave written informed consent.

**Study design**

After recruitment baseline measurements were recorded and then patients underwent a two week run-in period during which they were maintained on their usual medication. Symptom scores, peak flow measurements and use of rescue
therapy during the final week of the run-in period were used as the baseline measurements for each patient. If during the run-in they had nocturnal wakening, daytime symptoms on four or more days, or if their PEF was less than 80% of predicted on two or more consecutive days, then they had a further two week run-in period during which, in order to maximise their asthma control they received oral prednisolone 30mg daily in addition to their usual medication. In these patients, the second week of this run-in period was then used as baseline. At the end of the run-in period patients were randomised to either a group whose asthma was managed according to a modified version of the British Thoracic Society guidelines (BTS 1997) (BTS management group) or a group whose asthma was managed with reference to the induced sputum eosinophil count (sputum management group). Randomisation was performed by an independent individual (CEB) using the method of minimisation (Treasure & MacRae 1998), stratified by the number of rescue courses of oral corticosteroids required in the previous 12 months, the baseline induced sputum eosinophil count and the baseline methacholine PC_{20}. Patients were followed for 12 months after randomisation with monthly visits for the first four months and bimonthly visits thereafter. Decisions about asthma control were made per protocol by blinded observers (SM,BH) and management decisions were made by an independent observer who was blind to the sputum eosinophil count in the BTS management group (RHG) and to clinical control in the sputum management group (IDP). Patients and their primary care physicians were fully blinded to the management groups and had no knowledge of treatment protocols. All patients underwent identical investigations and were contacted three to five days after the clinic visits with treatment instructions. Patients and their primary care physicians were encouraged to treat asthma exacerbations in the standard way as per the British Thoracic Society asthma guidelines and the patients were issued with individualised self management plans (BTS 1997). At the end of the study patients were asked to record which group they thought they had been randomised into as an assessment of the success of blinding. Follow-up was completed in October 2001.
Management decisions

BTS management group

Treatment decisions in this group were based on traditional assessments of symptoms, PEF, and β2-agonist use. At each visit asthma control was compared to baseline. Asthma control was deemed inadequate and a decision to step-up treatment was made if i) an asthma exacerbation had occurred since the previous visit ii) day time or night time symptom scores were on average 0.5 points greater than baseline, iii) rescue β2 agonist use was >0.5 puffs per day greater than baseline, or iv) if the PEF was <80% of baseline personal best on two or more consecutive days. If asthma control was stable for two months or greater treatment was stepped down. Treatment steps were based on current BTS guidelines modified in the following way. (1): as required β2 agonists only; (2): addition of low dose inhaled corticosteroid (up to 800µg day of Beclomethasone or equivalent); (3): low dose inhaled corticosteroid plus addition of long acting β2 agonist; (4): high dose inhaled corticosteroid (>800µg Beclomethasone or equivalent) plus long acting β2-agonist; (5): high dose inhaled corticosteroid plus additional treatment given in the following sequence: leukotriene antagonist, theophylline, nebulised bronchodilators (6) addition of regular oral prednisolone. Long acting β2 agonists, theophylline and leukotriene antagonists were discontinued if there was no objective evidence of improvement following at least one month of treatment.

Sputum management group

Decisions regarding anti-inflammatory treatment in this group were made according to an algorithm which was based on the principle of maintaining a sputum eosinophil count <3% with a minimum dose of anti-inflammatory treatment. We chose 3% as we have previously shown that this identifies individuals with corticosteroid-responsive asthma (Pavord et al. 1999a). Thus 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 (table 4.4). Decisions about changes in bronchodilator treatment were based on individual patients’ symptoms, PEF readings and use of rescue β2 agonist compared to baseline using the same criteria as in the BTS management group. Management decisions were made by an independent individual (IDP) blinded to the clinical characteristics of the patient, who recorded two separate treatment plans to be followed if the patient was well or poorly controlled. Where sputum induction was not successful, the exhaled nitric oxide concentration was used as a surrogate marker of eosinophilic airway inflammation since we have shown a reasonable correlation between these measures in a similar patient population (Green et al. 2000). Treatment was targeted to achieve an exhaled NO concentration of <8 ppb. We assigned treatments as anti-inflammatory or bronchodilator on the basis of known pharmacological effects and evidence of the effects of treatment on eosinophilic airway inflammation (Pavord et al. 1999a) (Turner et al. 1998) (Louis et al. 2000a) (Pizzichini et al. 1999a). The hierarchy of anti-inflammatory and bronchodilator treatment used in the sputum management group is illustrated in Table 4.5.
Table 4.4. Treatment protocol for sputum management group

<table>
<thead>
<tr>
<th>Sputum eosinophil count</th>
<th>Good</th>
<th>Poor</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;1%</td>
<td>Reduce anti-inflammatory treatment AND Reduce bronchodilator treatment if stable &gt;2 months</td>
<td>Reduce anti-inflammatory treatment AND Increase bronchodilator treatment</td>
</tr>
<tr>
<td>1-3%</td>
<td>No change in anti-inflammatory treatment AND Reduce bronchodilator treatment if stable &gt;2 months</td>
<td>No change in anti-inflammatory treatment AND Increase bronchodilator treatment</td>
</tr>
<tr>
<td>&gt;3%</td>
<td>Increase anti-inflammatory treatment AND Reduce bronchodilator treatment if stable &gt;2 months</td>
<td>Increase anti-inflammatory treatment AND Increase bronchodilator treatment once on maximum anti-inflammatory treatment</td>
</tr>
</tbody>
</table>
Table 4.5 Hierarchy of anti-inflammatory and bronchodilator treatment for sputum management group.

ANTI-INFLAMMATORY TREATMENT:†
1.) Low dose inhaled steroid (100-200μg bd beclomethasone or equivalent)
2.) Moderate dose inhaled steroid (200-400μg bd beclomethasone or equivalent)
3.) High dose inhaled steroid (400-800μg bd beclomethasone or equivalent)
4.) Higher dose inhaled steroid (1600μg bd beclomethasone or equivalent)
5.) Higher dose inhaled steroid (1600μg bd beclomethasone or equivalent) plus leukotriene antagonist
6.) Higher dose inhaled steroid (1600μg bd beclomethasone or equivalent) plus oral Prednisolone 30mg 2/52
7.) Higher dose inhaled steroid (1600μg bd beclomethasone or equivalent) plus oral Prednisolone 30mg 2/52 followed by maintenance oral prednisolone titrated to sputum eosinophil count

† Sputum eosinophils 3-10% start at treatment (1) or (2)
Sputum eosinophils 10-30% start at treatment (3) or (4)
Sputum eosinophils >30% start at treatment (5) or (6)

BRONCHODILATOR TREATMENT:
1.) Long acting β2 agonist
2.) Long acting β2 agonist plus theophylline
3.) Long acting β2 agonist plus theophylline plus nebulised bronchodilator
Clinical Measurements

At entry we performed allergen skin prick tests and recorded details of smoking status, current treatment, duration of asthma, courses of rescue corticosteroids and hospital admissions in the previous year from patients' histories corroborated by clinical records. Patients attended at the same time of day on each occasion, 4-6 hours and 24 hours after the last doses of short and long acting β2-agonist respectively. At each visit we assessed patients by measurement of exhaled nitric oxide concentrations (NO), spirometry before and after 200μg inhaled salbutamol, the asthma quality of life questionnaire (AQLQ) (Juniper et al. 1993), visual analogue scale symptom scores and sputum induction for differential cell count. The order and timing of investigations was standardised. Methacholine PC20 was performed in place of bronchodilator reversibility at the baseline, six and twelve month visits unless patients were seen within 2 weeks of a severe exacerbation or the FEV1 was ≤1 litre.

Allergen skin prick tests were performed to *Dermatophagoides pteronyssinus*, cat fur, grass pollen and *Aspergillus fumigatus* solutions with normal saline and histamine controls (Alk-Abelló, Berkshire, UK). End-exhaled NO was measured with a chemiluminescence analyser (Logan Research, Rochester, UK) with subjects exhaling at a flow rate of 250ml/second from total lung capacity. NO was sampled from a sidearm attached to the mouthpiece and the mean NO value was taken from the point corresponding to the plateau of the end-exhaled CO2 reading (Kharitonov, Alving, & Barnes 1997). Methacholine challenge testing was performed using the tidal breathing method with doubling concentrations of methacholine (0.03 to 16 mg/ml) nebulised via a Wright nebuliser (Juniper, Cockcroft, & Hargreave 1994). Symptoms were recorded on 100mm visual analogue scales (VAS) from no symptom (0mm) to the worst ever symptom (100mm) for breathlessness, wheeze and cough and a total VAS score (from 0-300mm) was calculated as the sum of the three individual scores. Sputum was induced and processed as previously described (Pavord et al. 1997). An experienced observer (DP) blinded to the clinical characteristics of the subjects
performed the cell counts within 4 days of the clinic visits. Patients completed daily diary cards throughout the study recording daytime and night-time symptoms, twice daily pre bronchodilator peak expiratory flow (PEF), medication use including rescue $\beta_2$ agonist and oral prednisolone, days off work and emergency care visits. PEF was recorded as the best of three successive readings using a Mini-Wright peak flow meter (Clement Clarke International Ltd., Harlow, UK). Symptom scores ranged from 0 to 3 (for day time symptoms: 0=none, 1=occasional symptoms, 2=symptoms most of the day, 3=asthma very bad, unable to do 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). In both groups compliance was assessed using clinical impression with tablet counting, prescription checking, weighing of inhalers and monitoring of prednisolone or theophylline levels where there was any doubt. (Methods 3.1.1-3.1.9, 3.2.1).

Statistical Analysis

A power calculation, based on our own observations of the frequency of exacerbations amongst a similar group of patients with asthma attending our clinics (mean (SD) exacerbations 3.2(2.1) per patient per year), showed that 30 patients were needed in each group to demonstrate a 50% reduction in severe exacerbations ($\alpha=0.05$, $\beta=0.02$). The primary outcome variables were the number of severe asthma exacerbations and control of eosinophilic airway inflammation measured by the induced sputum eosinophil count. Secondary outcome variables were exhaled nitric oxide concentrations, symptom scores, total AQLQ scores, peak flow amplitude % mean (PEF A%M), FEV$_1$, change from baseline of methacholine PC$_{20}$, medication use and hospital admissions due to asthma. If patients withdrew due to poor asthma control their data was analysed on an intention to treat basis and extrapolated for the 12 month follow-up period; data from patients who withdrew for other reasons was included in the analysis until the time of withdrawal. Induced sputum eosinophil counts exhaled nitric oxide concentrations and methacholine PC$_{20}$ were log normally distributed and were expressed as the geometric mean (log standard error). Age and age at
onset were expressed as median (interquartile range). All other baseline variables were expressed as mean (SEM). Differences in severe asthma exacerbations were compared by the Mann-Whitney U test. The daily doses of inhaled and oral corticosteroids were compared between groups by an independent t-test. Additional treatments were not used universally by all patients, but where they were used the dose was constant. Therefore, proportions of patients receiving each type of additional medication were compared by the chi-squared test, as was the proportion of patients having one or more exacerbations and the proportion having a hospital admission due to asthma. Change from baseline in methacholine \( \text{PC}_{20} \) was compared by an independent t-test. For all other outcome variables, the area under the curve (AUC) over the twelve month period was calculated for each patient and compared between groups by an independent t-test. Post-hoc analyses of the between group differences in the change from baseline in inhaled and oral corticosteroid doses in non-eosinophilic and eosinophilic subgroups was also done by an independent t-test. Doses of inhaled corticosteroid have been expressed as Beclomethasone dose equivalents (with fluticasone considered to be twice as potent and budesonide equipotent.) The proportions of severe exacerbations occurring in males and females were compared using the chi square test. Independent risk factors for severe asthma exacerbations were assessed using a multiple linear regression model including the following in the model: age, gender, atopy, symptom scores, rescue \( \beta_2 \)-agonist use, inhaled steroid dose and PEF A%M during the run-in, pack years smoked, the sputum eosinophil AUC and the geometric mean methacholine \( \text{PC}_{20} \). Longitudinal data were calculated as fold change in sputum eosinophil count and fold change in exhaled nitric oxide level and the correlation between these variables was calculated using Spearman Rank method. The overall cost of each management strategy was calculated using our estimates of the cost of sputum induction and processing (£15 per sample), the 2001 Unit Costs of Health and Social Care (www.ukc.ac.uk/PSSRU/), the Department of Health 2001 reference costs (www.doh.gov.uk/nhsexec/refcosts.htm) and the British National Formulary, (September 2001). Total costs for each patient were calculated as the sum of the costs of hospital out-patient appointments, primary care visits, hospital admissions and medication use throughout the 12 months, with the
addition of the costs of sputum induction and processing for patients in the sputum management group only. The mean costs were then compared using an independent t-test (Thompson & Barber 2000). p values of less than 0.05 were considered significant. All data was analysed with SPSS for Windows (version 10.0, SPSS Inc, Chicago, USA).

Results

82 patients were recruited, of which 74 were randomised. Eight patients withdrew during the run-in period and a further six patients withdrew during follow-up, leaving 34 patients in each group who completed the study (Figure 4.2). No patients withdrew due to poor asthma control. The two treatment arms were matched at baseline for demographic and clinical features and similar numbers of patients in each group received an oral corticosteroid trial (Table 4.6).
Figure 4.2. Trial profile

108 patients approached

22 patients declined

86 agreed to participate

4 patients had no objective evidence of asthma

82 patients recruited

8 patients withdrew during run-in:
1 asthma too unstable
2 changed their minds
2 poor compliance
1 steroid induced psychiatric illness
2 developed other medical problems

74 patients randomised

37 Sputum management group
3 withdrew during follow-up:
1 changed mind
1 moved away
1 acute myocardial infarction
34 completed 12 month follow-up

37 BTS management group
3 withdrew during follow-up:
3 changed minds
34 completed 12 month follow-up
Table 4.6. Baseline characteristics

<table>
<thead>
<tr>
<th>Group</th>
<th>Sputum management</th>
<th>BTS management</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>37</td>
<td>37</td>
</tr>
<tr>
<td>Male (n)</td>
<td>19</td>
<td>21</td>
</tr>
<tr>
<td>Age (yrs)†</td>
<td>50(19-73)</td>
<td>47(20-75)</td>
</tr>
<tr>
<td>age onset (yrs)†</td>
<td>36(1-67)</td>
<td>32(1-73)</td>
</tr>
<tr>
<td>Atopic (n)</td>
<td>22</td>
<td>20</td>
</tr>
<tr>
<td>FEV₁%pred (%)</td>
<td>73.4(21.3)</td>
<td>75.9(26.1)</td>
</tr>
<tr>
<td>FEV₁/FVC ratio (%)</td>
<td>63.2(10.9)</td>
<td>64.5(12.8)</td>
</tr>
<tr>
<td>methacholine PC_{20} (mg/ml)‡</td>
<td>0.8(0.7)</td>
<td>0.7(0.8)</td>
</tr>
<tr>
<td>sputum eosinophil count (%)‡</td>
<td>2.4(0.7)</td>
<td>2.0(1.0)</td>
</tr>
<tr>
<td>sputum neutrophil count</td>
<td>49.2(31.6)</td>
<td>44.8(28.6)</td>
</tr>
<tr>
<td>exhaled NO (ppb)‡</td>
<td>4.4(0.6)</td>
<td>3.7(0.6)</td>
</tr>
<tr>
<td>Total VAS score (mm 0-300)</td>
<td>97(67)</td>
<td>85(67)</td>
</tr>
<tr>
<td>Total AQLQ score (1-7)</td>
<td>5.1(1.2)</td>
<td>5.2(1.2)</td>
</tr>
<tr>
<td>rescue corticosteroid courses previous year</td>
<td>2.1(2.4)</td>
<td>2.0(3.0)</td>
</tr>
<tr>
<td>asthma admissions previous year (group total)</td>
<td>10</td>
<td>11</td>
</tr>
<tr>
<td>BTS treatment stage†</td>
<td>4(1-5)</td>
<td>4(1-5)</td>
</tr>
<tr>
<td>mean dose inhaled steroid (mcg/pt/day)</td>
<td>1930(1338)</td>
<td>1680(1216)</td>
</tr>
<tr>
<td>refractory asthma (n)</td>
<td>28</td>
<td>27</td>
</tr>
<tr>
<td>oral corticosteroid trial during run-in (n)</td>
<td>12</td>
<td>13</td>
</tr>
<tr>
<td>optimal control not achieved despite steroid trial (n)</td>
<td>5</td>
<td>4</td>
</tr>
</tbody>
</table>

Mean (s.d.) † Median (range) ‡ Geometric mean (log s.e.m.)
Control of airway inflammation and hyperresponsiveness

Sputum induction was successful in 87.3% of attempts. Assessed over 12 months as the AUC, the sputum eosinophil count was 63% lower in the sputum management group (95% CI 24% to 100%; p=0.002). Similarly, there was a 48% (95% CI 12% to 85%; p=0.01) reduction in the area under the NO curve in the sputum management group. 25 patients in the BTS management group and 28 patients in the sputum management group had measurements of methacholine PC_{20} at baseline, 6 and 12 months. The change in methacholine PC_{20} was significantly better in the sputum management group at 6 months (+1.0 v -0.7 doubling doses, mean difference 1.6, 95% CI 0.2 to 3.1; p=0.03) and 12 months (+0.2 v –1.3 doubling doses, mean difference 1.5, 95% CI 0.3 to 2.6; p=0.015) (Figure 4.3).

Exacerbations and hospital admissions

There were significantly fewer severe exacerbations in the sputum management group than the BTS management group (35 v 109 total exacerbations, p=0.01, figure 4.4) and fewer rescue courses of oral corticosteroids (24 v 73, p=0.008), 80% of which (19/24 v 59/73) were commenced by the patient or the primary care physician. Significantly fewer rescue courses of oral corticosteroids to that seen in the year preceding the study were seen in the sputum management group (24 v 70, p=0.004) but not in the BTS group (73 v 74, p=0.97). The total number of patients with one or more exacerbation was greater in the BTS management group than the sputum management group (26/37 patients v 18/37 patients, p=0.058). Significantly fewer patients in the sputum management group were admitted to hospital due to exacerbations of asthma (1 v 6, p=0.047).

Symptoms, quality of life and lung function.

There were no significant differences in VAS symptom scores, total AQLQ scores, PEF A%M, post bronchodilator FEV₁ or rescue β₂ agonist use between the two groups (Figure 4.5).
Figure 4.3. Changes in the induced sputum eosinophil count, exhaled nitric oxide and provocation concentration of methacholine causing a 20% fall in FEV$_1$ (methacholine PC$_{20}$) over time, in the BTS management group (open symbols) and the sputum management group (closed symbols).

Points represent geometric mean and log (s.e.m.)
Figure 4.4. Cumulative asthma exacerbations in the BTS management group (dashed line) and the sputum management group (solid line).
Figure 4.5. Changes in visual analogue symptom scores (VAS), the asthma quality of life questionnaire (AQLQ), rescue β₂-agonist use and lung function over time in the BTS management group (open symbols) and the sputum management group (closed symbols).

Points represent mean and standard error of the mean.
Medication usage

Significantly fewer patients in the sputum management group received nebulised bronchodilators than in the BTS management group (4 v 11, p=0.043). Similar numbers of patients in both groups were treated with long acting \( \beta_2 \) agonists (12 v 12, \( p =1.0 \)), leukotriene antagonists (13 v 15, \( p=0.63 \)) and theophylline (12 v 12, \( p=1.0 \)). There were no between group differences in the daily dose of inhaled corticosteroids (1660(215) v 1705 (189) mcg per patient per day, \( p=0.88 \)) or oral prednisolone (2.6(0.6) v 3.0(0.8) mg per patient per day, \( p=0.69 \)). 13 patients in the sputum management group and 11 in the BTS management group had a geometric mean sputum eosinophil count across the twelve months within our normal range (<1.9%). Confining the analysis to these “non-eosinophilic” subgroups, management by sputum guidelines resulted in a 961 mcg per patient per day reduction in the inhaled corticosteroid dose at the end of the study compared to baseline whereas in the BTS management group inhaled corticosteroids were increased by 464mcg per patient per day (mean difference 1425 (95% CI 529 to 2329) mcg per patient per day, \( p=0.001 \)). There were no significant differences in exacerbation numbers or change in PC\textsubscript{20} in these subgroups (table 4.7).
Assessment of Blinding

When asked to document which randomisation group they thought they had been assigned to, 54% of patients recorded "don't know", 28% of patients selected the correct group, and 18% were incorrect. The proportion of responses was similar between the two groups.

Risk Factors for Severe Exacerbations

Females had significantly more severe exacerbations than males (101 v 43 total exacerbations, p=0.017). In the multiple linear regression analysis the only independent predictors of exacerbation frequency were night-time symptoms scores during the run-in (β=0.40, p=0.01) and the sputum eosinophil AUC (β=0.24, p=0.04) (R=0.69, R²=0.48, p<0.0001).

Sputum eosinophil counts preceding asthma exacerbations

The geometric mean (SEM) eosinophil count at study visits immediately preceding an exacerbation was significantly higher in the BTS group compared to the sputum management group (7.0(0.2) v 1.1(0.2), p=0.002.) In addition, the eosinophil count at study visits immediately preceding an exacerbation was significantly greater than that at visits not followed by an exacerbation in the BTS group (geometric mean (SEM) eosinophil count 7.0(0.2) v 3.1(0.1), p=0.006) but not the sputum management group (geometric mean (SEM) eosinophil count 1.2 (0.2) v 1.1(0.1), p=0.66).

Relationship between serial sputum eosinophil count and exhaled NO measurements

There was a significant but weak positive correlation between fold change in exhaled nitric oxide and fold change in induced sputum eosinophil count (r=0.262, p<0.001). The correlation was closer in males (r=0.303, p<0.001) than in females (r=0.183, p=0.016).
Economic Evaluation

The estimated annual total mean (SEM) cost per patient was £1,954 (164) for the BTS management group and £1,755 (119) for the sputum management group (p = 0.30)

Discussion

Severe exacerbations of asthma requiring courses of oral corticosteroids or hospital admission are the most serious manifestation of this disease. They lead to asthma deaths, considerable patient morbidity and a high cost to the health service in terms of doctor consultations, medication use and hospital beds (Hoskins et al. 2000). In this study we show for the first time that a strategy directed at maintaining a normal airway eosinophilic count caused a dramatic reduction in the number of severe exacerbations in a group of moderate to severe asthmatics attending our outpatient clinic. We believe this has profound implications for the management of asthma in that it strongly supports the view that regular monitoring of airway inflammation is required for the optimal treatment of this group of difficult patients.

A second important message of this study is that it provides further support for a central role for eosinophils in the pathogenesis of asthma. The idea that eosinophils are important pro-inflammatory cells in asthma has been undermined recently by a study in which a humanised anti-IL5 monoclonal antibody was able to reduce the sputum eosinophilia after allergen challenge, but had no effect on the early or late fall in FEV₁, or on airway responsiveness (Leckie et al. 2000). In addition it has become clear that an airway eosinophilia can be dissociated from airway responsiveness and variable airflow obstruction in asthma (Crimi et al. 1998). We have recently demonstrated in a study comparing the immunopathology of asthma and eosinophilic bronchitis that airway hyperresponsiveness is more closely associated with mast cell infiltration of the airway smooth muscle than with the presence of eosinophils, basement membrane thickening, and activated Th2 cells in the airway submucosa (Brightling et al. 2002). However the pathophysiology of severe asthma
exacerbations is complex and involves mucosal oedema, mucus hypersecretion and impaction of the lumen of the bronchi with cellular debris as much as smooth muscle contraction (Carroll et al. 1996). As one of the few longitudinal studies of airway inflammation undertaken in asthma our study supports a crucial role for eosinophils in this process by showing that eosinophilic airway inflammation was an independent risk factor for exacerbations and that a reduction in eosinophils brought about by glucocorticoids prevented exacerbations. Although this doesn’t show causation, and it remains possible that the effect is mediated via another corticosteroid sensitive mechanism, at the very least the clear implication is that eosinophilic airway inflammation is a more valid surrogate marker of exacerbation frequency than the other outcome measures assessed in this study. A key question is whether anti-IL5, which has a more specific effect on eosinophilic inflammation, is able to reduce the number of severe exacerbations.

Eosinophilic airway inflammation and airway responsiveness tended to increase in the traditional management group and post bronchodilator FEV₁ declined in both groups, presumably reflecting regression towards the mean and the effects of the oral corticosteroid trial during the run-in period on early measurements. Interestingly, this effect was not seen with symptoms, β₂ agonist use and peak flow variability. Moreover, the improvement in eosinophilic airway inflammation and exacerbation frequency in the sputum management group was not associated with improvements in these traditional markers of asthma control. These observations further support the idea that the mechanisms that cause exacerbations and eosinophilic airway inflammation can be dissociated from those that underlie symptoms and variable airflow obstruction. The fact that higher doses of inhaled steroids are more effective in controlling exacerbations but less effective at controlling symptoms and peak flow variability compared to the addition of long acting β₂ agonists (Pauwels et al. 1997) is consistent with this view. This apparent disassociation between control of inflammation/exacerbations and symptoms/variable airflow obstruction has important implications for the management of asthma suggesting, for example, that a traditional approach where treatment is stepped down or discontinued if
there is no evidence of symptomatic benefit or objective improvement in peak expiratory flow variability might need to be re-examined.

We chose our definition of an exacerbation to be consistent with the definition of severe exacerbations used in the FACET study (Pauwels et al. 1997). We noted more exacerbations, presumably because we studied a more severe population, and unlike in the FACET study, we did not exclude patients who exacerbated frequently. The increased exacerbation frequency may also be due to misclassification of periods of poor asthma control. Reddel et al have suggested that these can be distinguished from exacerbations as they are associated with increased symptoms, increased PEF variability and increased response to $\beta_2$-agonists (Reddel et al. 1999). We doubt that the improved outcome seen in the sputum management group reflects reductions in periods of poor asthma control rather than exacerbations since control of symptoms and PEF variability was similar in the two groups.

At first sight it may seem surprising that greater control of eosinophilic airway inflammation and exacerbations was achieved with the sputum management protocol despite no increase in overall treatment. This may be partly because step down in treatment occurred more quickly. However in an exploratory post hoc analysis we noted a number of subjects whose sputum eosinophil count was predominantly within the normal range throughout the study period. In these subjects the dose of corticosteroids was markedly reduced in the sputum management group without evidence of deterioration in control. In contrast, amongst the eosinophilic patients, the doses of inhaled and oral corticosteroids were increased in both treatment groups, although this presumably occurred before the onset of an exacerbation in the sputum management group and in response to exacerbations in the BTS management group. Thus there was evidence that monitoring of airway inflammation allowed treatment to be targeted and used more efficiently. We have previously identified a group of non-eosinophilic patients with symptomatic asthma and have associated the absence of sputum eosinophils with a poor response to short-term treatment with inhaled corticosteroids (Pavord et al. 1999a). The current study provides some support for the presence of a non-eosinophilic, corticosteroid resistant asthma
phenotype. It extends our previous study by providing evidence that the phenotype is relatively stable over the longer-term and emphasises that the longer term response to corticosteroids is also related to the presence of airway eosinophils.

The sputum management protocol resulted in improved airway responsiveness at 6 and 12 months. A recent study has shown that an asthma management strategy targeted to improving airway responsiveness resulted in reduced asthma exacerbations, when compared to traditional management (Sont et al. 1999). The improved outcome seen in this study may have been primarily due to improvement in eosinophilic airway inflammation since the intervention population received a significantly higher dose of inhaled corticosteroids and had improvements in bronchoscopic measures of airway inflammation at the end of the study. However it is possible that a treatment strategy that targets both airway responsiveness and eosinophilic inflammation may achieve even better results and further studies are required to investigate this possibility.

Our finding of a significantly greater rate of exacerbations in females is in keeping with previous studies which have shown that females are more likely to have severe asthma exacerbations (Tattersfield et al. 1999), more hospital admissions due to asthma (Osborne et al. 1998; Trawick, Holm, & Wirth 2001) and greater impairments in quality of life (Osborne et al. 1998) than men with similar lung function, despite a similar incidence and prevalence of the disease (Prescott, Lange, & Vestbo 1997). The reason for these differences is unclear. There have been suggestions that the use of exogenous oestrogens is associated with an increased risk of developing asthma (Troisi et al. 1995) and that there are abnormalities of regulation of beta2-adrenoceptors by female sex hormones in women with asthma (Tan, McFarlane, & Lipworth 1997). In our study, female gender was not an independent risk factor for exacerbations after correcting for confounding factors suggesting that there may be important differences in disease severity between men and women. The role of female sex hormones on airway inflammation and asthma severity warrants further investigation. Nocturnal wakening during the stable run-in period proved to be the other independent risk factor for subsequent asthma exacerbations in our study. Whilst
this may reflect the identification of patients with persistently poor asthma control, there is some evidence to suggest that nocturnal asthma is associated with increased alveolar inflammation (Kraft et al. 1996; Kraft et al. 1999). It is possible that assessments of alveolar airway inflammation, for example by measuring exhaled NO at differing flow rates and calculating NO flux (Tsoukias & George 1998), might also be useful in guiding asthma therapy and preventing asthma exacerbations.

The association between the change in induced sputum eosinophil count and the change in exhaled NO was weak, suggesting that the two variables identify a common airway abnormality but are regulated differently by factors that alter airway inflammation. One difficulty with interpreting this relationship is that the patients underwent multiple interventions which may have a different effect on sputum eosinophil count and exhaled nitric oxide concentration. Jatakanon showed that addition of inhaled steroid in subjects with symptomatic asthma had a more complete suppression effect on exhaled NO concentration than sputum eosinophilia (Jatakanon et al. 1999b). The different relationship between exhaled NO concentration and sputum eosinophil count between males and females was unexpected. A possible explanation is the effect of female sex hormones on expression and activity of nitric oxide synthase in the airway epithelium. Oestrogen is known to increase nitric oxide concentration in myometrial cells (Zervou, Klentzeris, & Old 1999) and there is evidence that it does the same in airway epithelial cells (Kirsch et al. 1999). Normal females have been demonstrated to have significant variability of exhaled nitric oxide concentration throughout their menstrual cycle with peak values at day 14 (Kharitonov et al. 1994a), in keeping with an important role of female sex hormones in determining exhaled NO concentration in vivo. Another contributing factor to this difference is suggested by the findings of Grasemann et al, who have recently described gender differences in exhaled NO concentration and have that the number of intronic AATn repeats in NOS1 has a significant effect on exhaled NO concentration in women but not in men (Grasemann et al. 2003). Whilst measuring exhaled NO can be carried out quickly and provide an immediate result our data suggest that the relationship between the two measurements in adults is complex and that it might be unwise to extrapolate findings with a
management strategy that targets sputum eosinophil count to one using exhaled nitric oxide concentration.

It is important to recognise some limitations of our study, some of which are common to studies comparing management approaches rather than specific treatments. Firstly, it was not possible to perform this study in a true double-blind fashion. However management decisions were strictly protocol driven and were made blinded to the sputum eosinophil count in the BTS management group and to clinical control in the sputum management group. Furthermore, courses of rescue corticosteroids (representing over two thirds of the primary outcome variable of asthma exacerbations) were almost always initiated by the patients or their primary care physician who were blinded so we doubt that bias due to unblinding had an important effect on the study. Secondly, the sputum management protocol may have been biased to achieving more rapid control of airway inflammation and the improved outcomes in this group may reflect this. However there was no evidence that the differences in exacerbation frequency lessened with time. Nevertheless it remains possible that this would be seen with a longer study. Thirdly, our criteria for the assessment of clinical control were arbitrary, as in previous studies (Sont et al. 1999), and although equivalent and effective symptom control was achieved, we cannot exclude the possibility that tighter control would have reduced the difference seen. Finally, our study was confined to patients attending a specialist hospital clinic who were thought to be compliant a high proportion of whom had refractory asthma (American Thoracic Society 2000). These patients may be particularly likely to benefit from the sputum management strategy since there is particularly strong evidence of heterogeneity of lower airway inflammatory responses in this group of patients (Wenzel et al. 1999) (Gibson, Simpson, & Saltos 2001). In addition the presence of severe eosinophilic airway inflammation has been shown to be associated with reduced perception of bronchoconstriction in similar populations of patients (Veen et al. 1998). Thus although our management strategy is feasible, cost effective and efficacious in secondary care, we would be cautious in extrapolating our findings to patients with milder disease managed in primary care. We would also have reservations about the feasibility of performing sputum induction in a primary care setting although exhaled nitric oxide would be more
suitable and further studies are required to prospectively assess its use in the management of asthma.

In conclusion we have shown that when compared to current clinically based management guidelines, a treatment strategy directed at normalising the induced sputum eosinophil count reduces asthma exacerbations and hospital admissions without the need for additional anti-inflammatory therapy. This supports the need for regular monitoring of airway inflammation in patients with moderate to severe asthma.
4.3 A placebo controlled comparison of formoterol, Montelukast or higher dose of inhaled corticosteroids in subjects with symptomatic asthma despite treatment with low dose inhaled corticosteroids.

Abstract

Few placebo controlled studies have directly compared the different treatment options for patients with asthma who remain symptomatic despite inhaled steroids. We performed a double blind 4 way cross-over study comparing 1 month’s treatment with budesonide 400mcg bd, additional formoterol, additional montelukast and placebo in 49 patients with uncontrolled asthma despite budesonide 100mcg bd. Each treatment was separated by a 4 week washout period. We measured exhaled nitric oxide (NO), FEV$_1$, methacholine PC$_{20}$, visual analogue symptom scores (VAS) and induced sputum before and 12 hours after each treatment and peak expiratory flow (PEF) was recorded twice daily. Overall the improvements seen with each treatment were small. Compared to placebo, high dose budesonide resulted in significant improvements in VAS (-21.3 mm; 95% CI -40.4,-2.3) morning PEF (16.5 l/min; 95% CI 2.3, 30.7), FEV$_1$ (0.14 l; 95% CI 0.0,0.28) and NO (fold reduction 1.9; 95% CI 1.1, 3.1). Formoterol caused similar improvements in morning PEF (17.5 l/min, 95% CI 4.0, 31.0). However the change in sputum eosinophil count with formoterol (2.4% to 3.8%; fold reduction 0.6, 95% CI 0.5,0.9) differed significantly from placebo (2.8% to 2.5%; fold reduction 1.1, 95% CI 0.7,1.6; p=0.03) and high dose budesonide (2.7% to 1.6%; fold reduction 1.6, 95% CI 1.2,2.2; p<0.001). Montelukast did not result in any greater improvements than placebo. We conclude that treatment given in addition to low dose inhaled corticosteroids results in modest benefits. Despite similar effects on PEF, formoterol and high dose budesonide have contrasting effects on eosinophilic airway inflammation.
Introduction

A considerable number of patients with asthma remain symptomatic despite treatment with low dose inhaled corticosteroids: a large primary care based audit showed that almost half of all patients were taking low dose inhaled corticosteroids along with as required $\beta_2$ agonists and that 54% of these were taking more than two puffs of beta-agonist/day suggesting the need for treatment step up (Neville et al. 1999). The clinician is faced with an increasing number of treatment options for this important group of patients but relatively little data from placebo-controlled studies to guide treatment decisions.

Interpretation of the available studies is not always straightforward since the findings often differ with different outcome variables. In the FACET study for example, increasing the dose of budesonide resulted in a greater reduction in the rate of severe asthma exacerbations but was less effective in improving symptom control than the addition of the long acting bronchodilator formoterol (Pauwels et al. 1997). Finally there are important concerns about the ability to generalise the results of clinical trials in this area to everyday practice. Almost all of the previously published clinical studies in patients with asthma who remain symptomatic despite low dose inhaled corticosteroids only recruited patients who demonstrated marked acute improvements in FEV$_1$ following inhaled bronchodilators, typically of at least 15%. Such degrees of bronchodilator reversibility are distinctly unusual in clinical practice. For example, only 5-10% of the patients seen in our clinic with asthma have a 15% or greater improvement in FEV$_1$ after 200\mu g inhaled salbutamol (Hunter et al. 2002) and 28% of patients with asthma in general practice demonstrate a bronchodilator response of this magnitude following 2.5mg nebulised salbutamol (Jamison & McKinley 1993).

We set out to directly compare the effects of higher dose budesonide, low dose budesonide plus formoterol and low dose budesonide plus montelukast on a range of outcome variables in a prospective, placebo controlled four way cross over study of an unselected population of patients with symptomatic asthma.
Methods

Subjects

Volunteers aged 18-75 with a diagnosis of asthma treated with the equivalent of 400 μg/day Beclomethasone Dipropionate or less were invited to participate following advertisements in the local media. Subjects were excluded if they were current smokers or had a smoking history of >10 pack years, had significant comorbidity, were receiving oral corticosteroids, long acting B2-agonists, leukotriene antagonists or theophylline or had had an asthma exacerbation or lower respiratory tract infection within 4 weeks of trial entry. All patients had symptoms consistent with the diagnosis of asthma and one or more of: a >15% increase in FEV₁ following 200μg inhaled Salbutamol; >20% within day variability in PEF assessed twice daily over 2 weeks; or a concentration of methacholine causing a 20% fall in FEV₁ (methacholine PC₂₀) of <8 mg/ml. Subjects were established on a standard dose of budesonide (100 μg twice daily via a turbohaler) for four weeks before entry and were eligible to participate in the study if they had asthma symptoms on at least four days in the third or fourth baseline week. The study was approved by the local research ethics committee and all patients gave written informed consent.

Study design

We performed a randomised, double blind, placebo controlled four way cross-over study. After recruitment baseline measurements were recorded and then patients underwent a one month run-in period during which they took budesonide 100μg twice daily via a turbohaler along with salbutamol as required for symptom relief. They then attended for measurement of exhaled nitric oxide, spirometry, methacholine PC₂₀, Juniper Asthma Quality of Life Questionnaire (AQLQ) (Juniper et al. 1992), Visual Analogue Symptom scores (VAS) and sputum induction. Suitable subjects were randomised to receive one of the following for 1 month each: 1) Budesonide 100 μg twice daily alone; 2) Budesonide 400 μg twice daily; 3) Budesonide 100 μg twice daily and oral
montelukast 10mg daily; 4) Budesonide 100 μg twice daily and inhaled formoterol 12 μg twice daily. All inhaled study medication was delivered via a turbohaler. Patients then completed a one month “washout” period during which they were maintained on budesonide 100 μg twice daily and as required salbutamol only. At the end of this time clinical measurements were repeated and patients crossed-over to a second of the above four treatments which they received for a further one month. The process was then repeated until the subjects had received each of the four treatments, with a four week washout period between each. Clinical measurements were taken before and 12 hours after each treatment. The order of treatments was randomly allocated to each patient. Blinding was maintained by issuing identical active and placebo turbohalers (Astra Zeneca, Lund, Sweden) and montelukast and placebo tablets which were each prepared in a single white capsule (Royal Hallamshire Hospital Pharmacy Department, Sheffield, UK). Randomisation was performed by the Glenfield Hospital Pharmacy Department.

**Asthma exacerbations**

Severe asthma exacerbations were defined as a decrease in the morning peak expiratory flow to more than 30 percent below the baseline value on two or more consecutive days, or deterioration in symptoms requiring treatment with oral corticosteroids (Pauwels et al. 1997). If a severe exacerbation occurred this was treated with prednisolone 30mg daily for 2 weeks followed by a six week washout period of budesonide 100μg twice daily and as required salbutamol only. Patients who experienced two severe exacerbations were withdrawn from the study.

**Measurements**

At entry we performed allergen skin prick tests and recorded details of smoking status. Patients attended at the same time of day on each occasion, 12 hours after the last dose of study medication or budesonide wash-out and > 6 hours after the last dose of rescue Salbutamol. At each visit we assessed patients by
measurement of exhaled nitric oxide concentrations (NO), spirometry, methacholine PC<sub>20</sub>, the asthma quality of life questionnaire (AQLQ), visual analogue scale symptom scores and sputum induction for differential cell count and cysteinyl leukotriene assay. The order and timing of investigations was standardised. The methacholine PC<sub>20</sub> was omitted if patients were seen within 2 weeks of a severe exacerbation or the FEV<sub>1</sub> was ≤ 1 litre. Patients completed daily diary cards throughout the study recording daytime and night-time symptoms, twice daily peak expiratory flow (PEF) and rescue β<sub>2</sub> agonist use. Compliance was assessed by tablet counting and was defined as acceptable if the subjects used >75% of the recommended dose of montelukast or placebo. Following sputum induction the cell free sputum supernatant was removed and stored at -80°C until analysis. The cysteinyl leukotrienes LTC<sub>4</sub>/LTD<sub>4</sub>/LTE<sub>4</sub> were measured using a commercial ELISA (Cayman Chemical, Ann Arbor, MI) with the standard curve spiked with DTT at the same concentration as the unknown. The intra- and interassay coefficients of variability were <10% and the lower limit of detection was 13 pg/ml of sample. (Methods 3.1.1-3.1.9, 3.2.1, 3.2.2)

**Statistical Analysis**

The primary outcome variables were the change from the pre-treatment baseline in the methacholine PC<sub>20</sub> (in doubling doses), fold change in sputum eosinophil count and the change in global VAS symptom score. Secondary outcome measures were the change from the pre-treatment baseline in FEV<sub>1</sub>, total AQLQ score, fold change in exhaled NO and the change in morning PEF calculated as the mean change between the final week of the washout period and the final week of treatment. Based on our own estimates of the within subject repeatability of the primary outcome variables, (Pavord et al. 1999a) the study had an 80% chance of detecting a one doubling dose difference in change in methacholine PC<sub>20</sub>, a 2 fold difference in sputum eosinophil count between treatment and a 20 mm difference in VAS symptom scores at the 5% level.
Patients who had a severe exacerbation during a treatment month were assigned the lowest observed value for each outcome measure for that treatment. If a treatment was stopped early due to adverse effects measurements were taken within 12 hours of the last dose of treatment and the results were included on an intention to treat basis. Patients who withdrew from the study for reasons other than a severe exacerbation were assigned the mean value for each outcome variable.

Induced sputum eosinophil counts, exhaled nitric oxide concentrations and methacholine PC$_{20}$ were log normally distributed and were expressed as the geometric mean (log standard error). Age and age at onset were expressed as median (interquartile range). All other baseline variables were expressed as mean (SEM). Primary and secondary outcome measures were compared between groups using a two way ANOVA. The chi squared test was used to compare the numbers of patients having severe asthma exacerbations during each of the treatment periods.

**Results**

65 patients were recruited, of which 49 were randomised. Ten patients withdrew early and therefore failed to complete one or more of the study treatments (figure 4.6.) Baseline demographic data and lung function are given in table 4.8. There were no period or order effects and pre-treatment values were well matched for each outcome variable (table 4.9). 18 severe exacerbations occurred during the following treatment periods: washout periods 7; high dose budesonide 1; formoterol 2; montelukast 4; placebo 4. The difference in exacerbation frequency between treatments was not significant (p=0.46).
Table 4.8. Baseline characteristics

<table>
<thead>
<tr>
<th>Baseline characteristics</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>49</td>
</tr>
<tr>
<td>male (n)</td>
<td>25</td>
</tr>
<tr>
<td>age (yrs)†</td>
<td>42(19-73)</td>
</tr>
<tr>
<td>age onset (yrs)†</td>
<td>13.5(1-67)</td>
</tr>
<tr>
<td>atopic (n)</td>
<td>37</td>
</tr>
<tr>
<td>FEV₁%pred (%)</td>
<td>74.8(3.1)</td>
</tr>
<tr>
<td>FEV₁/FVC ratio (%)</td>
<td>67.6(2.2)</td>
</tr>
<tr>
<td>% increase in FEV₁ post bronchodilator</td>
<td>13.2(1.5)</td>
</tr>
<tr>
<td>&gt;15% increase in FEV₁ post bronchodilator (n)</td>
<td>15</td>
</tr>
<tr>
<td>PEF A%M</td>
<td>13.1(1.4)</td>
</tr>
</tbody>
</table>

Mean (s.e.m.) † Median (range)
Figure 4.6. Trial profile

66 subjects recruited

49 subjects randomised

11 had no objective evidence of asthma
5 had no symptoms during run-in
1 developed other illness

4-way cross-over study

10 subjects withdrew:
5 had 2 severe exacerbations
4 changed their minds
1 developed other illness

39 completed all 4 treatments

117
Table 4.9 Pre-treatment results

<table>
<thead>
<tr>
<th></th>
<th>Budesonide</th>
<th>Formoterol</th>
<th>Montelukast</th>
<th>Placebo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Morning PEF</td>
<td>417(14)</td>
<td>423(12)</td>
<td>418(13)</td>
<td>418(14)</td>
</tr>
<tr>
<td>FEV₁(I)</td>
<td>2.52(0.1)</td>
<td>2.51(0.1)</td>
<td>2.54(0.1)</td>
<td>2.51(0.1)</td>
</tr>
<tr>
<td>Methacholine PC₂₀ (mg/ml) †</td>
<td>0.39(0.1)</td>
<td>0.37(0.1)</td>
<td>0.28(0.1)</td>
<td>0.29(0.1)</td>
</tr>
<tr>
<td>sputum eosinophil count (%) †</td>
<td>2.6(0.1)</td>
<td>2.2(0.1)</td>
<td>2.0(0.1)</td>
<td>2.8(0.1)</td>
</tr>
<tr>
<td>sputum neutrophil count</td>
<td>52.3(3.9)</td>
<td>54.3(3.7)</td>
<td>60.4(3.6)</td>
<td>53.8(3.2)</td>
</tr>
<tr>
<td>Exhaled NO (ppb) †</td>
<td>6.2(0.1)</td>
<td>5.4(0.1)</td>
<td>6.5(0.1)</td>
<td>6.2(0.1)</td>
</tr>
<tr>
<td>Sputum LTC₄/D₄/E₄ (ng/ml) †</td>
<td>4.6(0.1)</td>
<td>4.9(0.1)</td>
<td>6.6(0.1)</td>
<td>5.8(0.1)</td>
</tr>
<tr>
<td>Total VAS score (mm 0-300)</td>
<td>72.8(8.5)</td>
<td>75.6(9.2)</td>
<td>79.6(9.5)</td>
<td>73.8(7.9)</td>
</tr>
<tr>
<td>Total AQLQ score (1-7)</td>
<td>5.6(0.1)</td>
<td>5.5(0.1)</td>
<td>5.5(0.1)</td>
<td>5.5(0.1)</td>
</tr>
</tbody>
</table>

Mean (s.e.m.) † Geometric mean (log s.e.m.)
Primary Outcome Variables

Methacholine $PC_{20}$
There were no significant differences in the doubling dose change in methacholine $PC_{20}$ between any of the treatment groups and placebo nor across the individual treatments (table 4.10, figure 4.7).

VAS symptom scores
Higher dose budesonide resulted in a significant improvement in the global VAS symptom score compared to low dose budesonide plus placebo (mean difference 21.3mm, 95% C.I. -39.8, -2.9; $p=0.023$). Additional montelukast and additional formoterol did not result in significant improvements in VAS symptom scores compared to placebo and the difference across the 4 treatment arms did not reach statistical significance ($p=0.054$) (table 4.10, figure 4.7.)

Sputum eosinophils
There was a significant difference in the fold change in the sputum eosinophil count across the groups ($p=0.005$) (table 4.10, figure 4.7.) Formoterol resulted in a significant increase in the sputum eosinophil count compared to placebo (mean (95% C.I.) difference 1.75 (1.03, 2.92) fold; $p=0.03$) and high dose budesonide mean (95% C.I.) difference 2.54(1.51, 4.27) fold; $p=0.003$).

Secondary outcome variables

Morning PEF
Significant differences in the change in mean morning PEF over the final week of treatment were observed across the four treatments ($p=0.019$). A significant improvement in mean morning PEF was seen with both high dose budesonide (16.3 l/min; 95% C.I. 3.8, 28.8; $p=0.01$) and additional formoterol (17.2 l/min, 95% C.I. 4.7, 29.7; $p=0.007$) compared to placebo (table 4.10).

Fold change in eNO
There was a significant difference in the fold change in exhaled nitric oxide across the groups ($p=0.011$) The change in eNO with high dose budesonide differed significantly from the change seen with placebo (mean (95% C.I) difference 1.86 (1.21, 2.86) fold; $p=0.005$) and formoterol (mean (95% CI) difference 1.95 (1.27,3.00) fold; $p=0.003$) (table 4.10).
Pre bronchodilator FEV₁

High dose budesonide resulted in a significantly greater improvement in pre bronchodilator FEV₁ than low dose budesonide plus placebo (mean difference 0.14l, 95% C.I. 0.02,0.26; p=0.009). Additional montelukast and additional formoterol did not result in significant improvements in FEV₁ compared to placebo and the difference across the 4 treatment arms did not reach statistical significance (p=0.133) (table 4.10).

Change in Total AQLQ score

There were no significant differences in the change in the total AQLQ score across the four treatments (p=0.81) and none of the treatments resulted in a greater improvement in AQLQ compared to placebo (figure 4.9).

Subgroup analyses

The response to any of the treatments studied was not influenced by atopic status, degree of acute bronchodilator reversibility, pre-treatment sputum eosinophil counts or pre-treatment sputum cysteinyl-leukotriene concentrations.
Figure 4.7. Change in primary outcome variables compared to placebo.
Table 4.10  Change from baseline in primary and secondary outcome variables.

Mean (95% confidence intervals) * p<0.05 ANOVA

<table>
<thead>
<tr>
<th></th>
<th>Budesonide</th>
<th>Formoterol</th>
<th>Montelukast</th>
<th>Placebo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients completing treatment (n)</td>
<td>42</td>
<td>40</td>
<td>43</td>
<td>41</td>
</tr>
<tr>
<td>Methacholine PC_{20} (doubling dose)</td>
<td>0.4(-0.2 to 1.0)</td>
<td>0.2(-0.5 to 1.0)</td>
<td>0.4(-0.3 to 1.0)</td>
<td>0.1(-0.4 to 0.6)</td>
</tr>
<tr>
<td>Sputum eosinophils (fold reduction)</td>
<td>1.6(1.1 to 2.2)</td>
<td>0.6(0.5 to 0.9)*</td>
<td>0.8(0.5 to 1.3)</td>
<td>1.1(0.7 to 1.6)</td>
</tr>
<tr>
<td>VAS symptom score (mm)</td>
<td>-12.6(-22.7 to -2.6)*</td>
<td>-3.5(-15.2 to 8.2)</td>
<td>+10.1(-9.3 to +29.5)</td>
<td>+5.5 (-10.0 to 21.1)</td>
</tr>
<tr>
<td>Morning PEF</td>
<td>15.2(6.4 to 24.0)*</td>
<td>16.4(8.3 to 24.6)*</td>
<td>4.9(-5.1 to 14.9)</td>
<td>-1.3(-12.2 to 9.5)</td>
</tr>
<tr>
<td>FEV\textsubscript{1}(l)</td>
<td>0.07(0.01 to 0.12)*</td>
<td>-0.05(-0.15 to 0.06)</td>
<td>-0.05(-0.13 to 0.03)</td>
<td>-0.07(-0.20 to 0.05)</td>
</tr>
<tr>
<td>Total AQLQ</td>
<td>0.2(0.0 to 0.4)</td>
<td>0.1(-0.2 to 0.4)</td>
<td>0.2(0.0 to 0.4)</td>
<td>0.0(-0.2 to 0.3)</td>
</tr>
<tr>
<td>Exhaled NO (fold reduction)</td>
<td>1.3(0.9 to 1.8)*</td>
<td>0.7(0.5 to 0.9)</td>
<td>0.9(0.7 to 1.2)</td>
<td>0.7(0.5 to 1.0)</td>
</tr>
</tbody>
</table>
Discussion

We have compared the effect of high dose inhaled budesonide, additional formoterol and additional montelukast on a range of outcome measures in patients with mild to moderate asthma who remain symptomatic despite low dose inhaled budesonide. High dose budesonide was the most efficacious treatment resulting in significant improvements in global VAS, morning PEF, and exhaled NO compared to placebo. Formoterol was the next most efficacious treatment resulting in significant improvements in morning PEF. There was no evidence that the response to Montelukast differed from placebo. This is the first placebo controlled comparison of the treatment options for patients with symptomatic asthma despite low dose inhaled corticosteroids and the first to study an unselected population. We provide an estimate of treatment response on a wide range of outcome measures and have shown evidence of important heterogeneity of treatment effects.

Overall the benefit provided by each of the additional treatments in this group of patients was minor. We chose our primary outcome variables as they had been shown to be the most responsive to change in an earlier study (Pavord et al. 1999a) and to provide estimates of the effects of treatment on different aspects of the asthmatic state. In corticosteroid naïve asthma, inhaled corticosteroids result in 1-4 doubling dose improvements in airway responsiveness (Pavord et al. 1999a;Vathenen et al. 1991) and 6-10 fold reductions in the sputum eosinophil count (Jatakanon et al. 1998a;Pavord et al. 1999a). The much smaller effects seen in this study indicate that most patients taking low dose inhaled corticosteroids are near the top of the dose response curve for these variables. Symptom scores and morning PEF were more responsive in the current study emphasising the complex relationship between airway inflammation, airway responsiveness, simple tests of airway calibre and clinical expression of the disease.

Previous studies of long acting β₂ agonists (Green et al. 2002a;Greening et al. 1994;Pauwels et al. 1997) or montelukast (Laviolette et al. 1999;Wilson et al. 124
2001) given to patients who remain symptomatic despite low dose inhaled corticosteroids have reported greater improvements in morning PEF than those seen in our study. In contrast, we have demonstrated rather greater clinical benefits from a high dose of inhaled corticosteroids than has previously been reported (Green et al. 2002a; Greening et al. 1994; Pauwels et al. 1997). We do not feel that the differences are due to the small size of our study since we had sufficient power to detect changes in PEF of the magnitude seen in the earlier trials. One reason for these apparent discrepancies may be the patient population studied. Unlike the previous studies we did not confine our recruitment to subjects who demonstrated a marked acute bronchodilator response, a population who might be particularly likely to respond to a long acting β2 agonist. The fact that the only other study which has not had an entry criterion of a large bronchodilator response showed no significant improvement in FEV1, PD20 methacholine, symptom scores or exacerbation rates when salmeterol was added to Beclomethasone would be consistent with this view (Verberne et al. 1998).

We did not find a relationship between bronchodilator response and the effect of long acting β2 agonists, although this was a relatively small study and our power to demonstrate such a relationship was low and the treatment effects observed were small.

The relative efficacy of the treatments differed considerably with the different outcome variables studied. The most striking example of this can be seen with the contrasting effects of formoterol and high dose budesonide on markers of eosinophilic airway inflammation. The addition of formoterol led to a significant increase in the sputum eosinophil count and exhaled NO compared to placebo, despite resulting in similar improvements in morning PEF as high dose inhaled budesonide. This finding of an apparent worsening in eosinophilic airway inflammation following the addition of formoterol despite improvements in PEF is consistent with the increasing evidence of a dissociation between eosinophilic airway inflammation and day to day symptom control/ airflow obstruction in asthma (Brightling et al. 2002; Green et al. 2002a). In contrast, eosinophilic airway inflammation is strongly implicated in the pathophysiology of severe asthma exacerbations (Green et al. 2002a; Pizzichini et al. 1999b). The fact that
higher dose budesonide resulted in greater reductions in severe asthma exacerbations than low dose inhaled budesonide plus formoterol despite less improvement in PEF in the FACET study is in keeping with this (Pauwels et al. 1997). We did not demonstrate significant differences in exacerbation frequency between the treatment arms in our study but our study was not powered to show this. It is possible that a larger or longer study may have done so.

If eosinophilic airway inflammation is linked to exacerbations and formoterol increased the sputum eosinophil count then why have studies not shown an increase in exacerbation frequency with formoterol and other long acting β2-agonists? (Pauwels et al. 1997; Shrewsbury, Pyke, & Britton 2000). This may be a function of the selection of a population who respond particularly well to β2-agonists as well as the definition of exacerbations which depends on a >30% fall in PEF from pre-treatment baseline. Since patients treated with formoterol had a marked improvement in PEF from baseline, subsequent falls in PEF would have to be much greater to reach this threshold, thus potentially introducing bias in favour of the long acting β2-agonist. It remains possible that long acting β2-agonists are associated with an increase in exacerbation frequency in a more representative population. Such an effect has been seen with the regular use of short acting β2-agonists (Taylor et al. 1993) and this treatment has been associated with a significant increase in sputum eosinophil counts (Aldridge et al. 2000).

It is also possible that long acting β2-agonists might prevent exacerbations by increasing the threshold at which increased airway inflammation results in an asthma exacerbation. This concept is supported by the findings of McIvor et al who showed that subjects with moderate asthma treated with salmeterol developed an exacerbation later and at a higher sputum eosinophil count than those treated with placebo. However the clinical relevance of any such effect is unclear. We have considered whether the increase in sputum eosinophil count seen in our study with formoterol treatment occurred because patients reduced their inhaled corticosteroid dose because of better control of symptoms. However
we think that this is unlikely since compliance was carefully assessed throughout the study.

Our findings contrast with those of Kips et al who studied the effects of low dose budesonide plus formoterol versus high dose budesonide on sputum inflammatory cells in a subset of the participants of the FACET study (Kips et al. 2000). They found a slight increase in the sputum eosinophil count in the budesonide plus formoterol group compared to the group treated with higher dose budesonide. This increase did not reach statistical significance, although there were clinically important differences in the two groups at baseline which complicate the interpretation of this data. Bronchial biopsy studies of the effects of long acting β2-agonists on airway inflammation in asthma have neither shown an increase in mucosal eosinophil numbers (Li et al. 1999; Wallin et al. 1999) nor consistent evidence of anti-inflammatory effects (Green et al. 2002a; Li et al. 1999; Roberts et al. 1999; Wallin et al. 1999). It is possible that the increase in sputum eosinophils following formoterol treatment seen in our study occurred as a result of increased trafficking of eosinophils away from the airway mucosa rather than an increase in eosinophilic airway inflammation per se. Further studies are required to clarify this.

We conclude that in an unselected population treatment given in addition to low dose inhaled corticosteroids results in modest benefits. Furthermore, the response to individual treatments appears to differ with the outcome variable selected. Further studies addressing the effects of treatment on a range of outcome variables in large numbers of unselected patients are required.
5. Conclusions

5.1 Summary of results

This thesis has explored the use of induced sputum in the clinical assessment and management of adults with asthma and has demonstrated for the first time that measuring airway inflammation in this way leads to improved patient outcomes.

We have used the technique of induced sputum to assess the heterogeneity of airway inflammation in a relatively large population of patients with mild to moderate symptomatic asthma and have demonstrated the presence of a distinct, relatively common asthma phenotype with predominantly neutrophilic inflammation and normal sputum eosinophil counts even in patients with mild, corticosteroid naïve disease (Green et al. 2002b). We have provided some evidence that this non-eosinophilic phenotype is resistant to treatment with inhaled corticosteroids (Green et al. 2002b) and is stable in the longer term (Green et al. 2002a).

We have shown that a management strategy directed at normalising the sputum eosinophil count, as well as controlling symptoms and peak flow readings, leads to a dramatic reduction in severe asthma exacerbations and prevents hospital admissions compared to a traditional clinical approach. In patients with moderate to severe asthma requiring hospital management, we have shown that such an approach is not only beneficial but is feasible and cost effective. Sputum eosinophilia was found to be independently associated with exacerbation frequency but control of sputum eosinophils did not improve symptoms or peak flow variability demonstrating that eosinophilic airway inflammation, exacerbations, symptoms and variable airflow obstruction are likely to reflect different facets of the disease (Green et al. 2002a).

Amongst patients with asthma who remain symptomatic despite low dose inhaled corticosteroids, we found that the overall response to additional treatment was
modest and varied between outcome measures. Despite similar improvements in lung function, high dose inhaled corticosteroids and long acting β2-agonists had contrasting effects on the sputum eosinophil count, providing further evidence for a dissociation between eosinophilic airway inflammation, day-to-day symptoms and variable airflow obstruction in asthma.

5.2 Limitations and areas for future research

Whilst asthma is a disease characterised by variability the study identifying the subgroup of patients with neutrophilic inflammation (chapter 3.1) had a cross-sectional design providing only isolated information about the nature of airway inflammation in these patients. The randomised controlled trial of asthma management described in chapter 3.2 provided some evidence that a non-eosinophilic phenotype is stable in the longer term but prospective longitudinal studies of large numbers of patients with heterogeneous asthma would be required to fully explore this and to further evaluate the relationships between airway inflammation, symptoms and disordered airway physiology. Furthermore the observed differences in induced sputum may reflect differences in the localisation of the inflammatory process within the airway rather than true differences in the cellular nature of the inflammation. A biopsy study comparing the airway immunopathology of non-eosinophilic asthma with eosinophilic asthma would be required to address this. Although we have shown evidence that a subgroup of patients with isolated neutrophilic airway inflammation have an impaired response to corticosteroids, this study was not performed in a double-blind placebo controlled manner and may therefore be open to bias. Additionally, we have speculated that a poor response to corticosteroids in the short term may be associated with a poor long-term outcome but there is as yet no evidence from prospective studies to support this. These are important areas for future study.

Several limitations of the randomised controlled trial of asthma management based on maintaining a normal sputum eosinophil count have already been discussed in chapter 3.2. Whilst we have demonstrated the feasibility of this
approach for patients with difficult to manage asthma requiring hospital follow-up we accept that similar benefits may not be gained amongst wider patient groups and that there are important limitations to the widespread adoption of this technique. Exhaled nitric oxide would be a suitable alternative marker of airway inflammation and prospective randomised controlled trials accessing its application in the management of asthma, including patients with milder disease, are required. Finally we limited our patient follow-up to a year. Longer studies are required to establish whether patients continue to benefit from this approach in the longer term and in particular whether treatment directed at normalising the induced sputum eosinophil count prevents airway remodelling and protects against accelerated declines in lung function.

The variable nature of asthma may also have implications for the interpretation of the cross-over study described in chapter 3.3, since natural variations in disease may mask treatment responses. However, significant pre-treatment differences were not seen and the effects of each treatment was compared to placebo, so we doubt that this had a major influence on the outcomes of the study. The results from this study contrast with much of the published literature in this area. However, our study is the first to include an unselected population of patients with symptomatic asthma. The benefits demonstrated in our study following additional treatment were minor. It is possible that the patients we recruited were only mildly symptomatic and that greater improvements and more clear between treatment differences would have been seen in patients with more poorly controlled disease. Relatively high patient withdrawal rates occurred during this study, largely as a result of severe exacerbations occurring during the low dose inhaled corticosteroid washout months. A shorter washout period may have resulted in fewer withdrawals but at the risk of carry-over effects. However, despite these withdrawals the numbers of patients completing each treatment arm remained within our power calculations. Additionally we extrapolated our data to include all recruited subjects in the analyses on an intention to treat basis wherever possible. Nevertheless, our study may not have been adequately powered to identify important predictors of treatment response and larger studies of similar patient populations are required.
Whilst we included a wide range of outcome variables in this study we did not set out to assess the effects of treatment on exacerbation rates. Again, this would require a considerably larger and longer study. The demonstration of an increase in the sputum eosinophil count following treatment with formoterol in this patient group, along with our previous finding of an important association between sputum eosinophilia and severe asthma exacerbations, however, raise important questions about the effects of long acting β2-agonists. As yet there are no published studies assessing the effects of these drugs on severe exacerbation rates in unselected patient groups. The observation that formoterol resulted in an increase in the sputum eosinophil count does not provide clear evidence of a pro-inflammatory effect and biopsy studies are required to assess the effects on mucosal inflammation.

Our results lend support to the large body of work supporting a role for eosinophils as important effector cells in asthma but do not provide direct evidence that they are involved in its pathogenesis or are a causal factor in the development of severe exacerbations. Corticosteroids clearly have a profound effect on eosinophils and their beneficial effects in asthma appear to go hand-in-hand with their inhibition of tissue eosinophilia, but they have wide ranging effects on many of the other components of the inflammatory pathway in asthma. Whilst our results suggest that eosinophils are extremely useful markers of response to treatment, it is possible that they are innocent bystanders acting as a surrogate of some other corticosteroid responsive abnormality. The evidence would also be consistent with eosinophils being important effector cells. A further possibility is that eosinophils are part of a complex inflammatory process in which they favour one aspect of the pathophysiology such as severe exacerbations. If this is the case, then long-term longitudinal studies using more specific anti-eosinophilic drugs addressing a range of outcome variables including exacerbation frequency will be needed.

It is hoped that the results of future studies addressing these outstanding issues, together with the findings presented in this thesis, will lead to significant advances in the management of patients with asthma.
Appendix I Example of Visual Analogue Symptom scales

Please mark a cross along the scale to show how severe your symptoms are.
Mark 3 separate crosses: 1 for wheeze, 1 for cough and a third for breathlessness
# Appendix II  Example of asthma diary card

<table>
<thead>
<tr>
<th>Name:</th>
<th><strong>DAYTIME ASTHMA</strong></th>
<th><strong>NIGHT TIME WAKENING</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0: Normal</td>
<td>0: None</td>
</tr>
<tr>
<td></td>
<td>1: Occasional wheeze or breathlessness</td>
<td>1: Awoke once due to asthma</td>
</tr>
<tr>
<td></td>
<td>2: Wheeze/ short of breath most of the day</td>
<td>2: Awoke 2-3 times due to asthma</td>
</tr>
<tr>
<td></td>
<td>3: Asthma very bad: unable to do normal activities at all</td>
<td>3: Awake most of the night due to asthma</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th><strong>Week 1</strong></th>
<th><strong>DATE:</strong></th>
<th><strong>Daytime Asthma:</strong></th>
<th></th>
<th><strong>Night-time Wakening:</strong></th>
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<td><strong>Week 2</strong></td>
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<td><strong>Daytime Asthma:</strong></td>
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<td><strong>Number of puffs of</strong></td>
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<td><strong>Number of puffs of</strong></td>
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<td></td>
<td></td>
<td></td>
<td><strong>Ventolin/Briacynl per 24 hours</strong></td>
<td></td>
<td><strong>Ventolin/Briacynl per 24 hours</strong></td>
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<th><strong>Week 1</strong></th>
<th><strong>DATE:</strong></th>
<th><strong>Daytime Asthma:</strong></th>
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<th><strong>Night-time Wakening:</strong></th>
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<td><strong>Week 2</strong></td>
<td><strong>DATE:</strong></td>
<td><strong>Daytime Asthma:</strong></td>
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<td><strong>Ventolin/Briacynl per 24 hours</strong></td>
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<td><strong>Ventolin/Briacynl per 24 hours</strong></td>
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Appendix III  Intra-observer repeatability of sputum differential cell counts

<table>
<thead>
<tr>
<th>Cell type</th>
<th>Intraclass correlation coefficient</th>
</tr>
</thead>
<tbody>
<tr>
<td>eosinophil</td>
<td>0.99 (0.99-1.00)</td>
</tr>
<tr>
<td>neutrophil</td>
<td>0.96 (0.89-0.99)</td>
</tr>
<tr>
<td>macrophage</td>
<td>0.99 (0.96-1.00)</td>
</tr>
<tr>
<td>lymphocyte</td>
<td>0.31 (0.22-0.70)</td>
</tr>
<tr>
<td>epithelial cell</td>
<td>0.90 (0.73-0.97)</td>
</tr>
</tbody>
</table>

Values are mean (95% C.I.)
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Ref Type: Abstract


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