

**Biomarkers of airway inflammation:
The use of Exhaled Nitric Oxide
(FeNO) in the management of adult
asthma in UK primary care.**

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Thesis submitted to the University of Nottingham

for the degree of Doctor of Philosophy

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Abstract:

Rationale:

Current asthma guidelines recommend reducing inhaled corticosteroid (ICS) therapy dose by 50% in patients with mild to moderate asthma who have demonstrated three months of good symptom control however there is evidence to suggest that this does not occur.

Objectives:

We tested whether exhaled nitric oxide (FeNO) measurements or other clinical indices could be utilised to predict a safe reduction of ICS dose, without provoking loss of symptom control or exacerbation within 3 months. We also investigated relationships between airway inflammation and asthma symptoms in the mild to moderate asthma cohort.

Methods:

191 patients with stable asthma were recruited from primary care. Patients had their FeNO level measured at baseline and then had their inhaled corticosteroid (ICS) dose reduced by 50%. FeNO measurements were reassessed seven days later. The primary outcomes were whether baseline FeNO or a change in FeNO following ICS dose reduction could predict asthma stability at 3 months.

Results:

128/191 patients (67%) completed the ICS dose reduction successfully at three months. 63/191 patients (33%) suffered from either a loss of control or an exacerbation. Baseline FeNO, or change in FeNO (post step-down minus pre step-down) were not statistically significantly different between the two groups.

Conclusion:

67% of patients with well-controlled asthma can safely reduce their ICS dose by half without suffering from a loss of control or exacerbation within three months; however neither baseline nor change in FeNO measurements or routine clinical indices can be used to predict which patients can or cannot successfully tolerate a reduction in ICS dose.

Publications and abstracts arising from this thesis:

Publications:

DE Shaw, EE Wilson, ID Pavord. Exhaled nitric oxide in asthma. Eur Respir Mon 2010. 49, 32-44

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EE Wilson, TM McKeever, C Lobb, T Sherriff, L Gupta, G Hearson, N Martin, MR Lindley, DE Shaw. Respiratory muscle specific warm-up and elite swimming performance. Submitted to: The European Journal of Applied Physiology.

EE Wilson, T McKeever, B Hargadon, M Shelley, G Hearson, JR Anderson, DB Hodgson, H Bailey, G Meakin, M Thomas, ID Pavord, TW Harrison, DE Shaw. Exhaled nitric oxide and inhaled corticosteroid dose reduction in asthma: a clinical study. Submitted to: The Lancet.

Abstracts:

American Thoracic Society Conference, Denver 2011

Poster session: "The effect of a respiratory muscle specific warm-up on 100m freestyle swimming performance in elite swimmers". EE Wilson, DE Shaw, C Lobb, T Sherrif, L Gupta, N Martin, MR Lindley

American Thoracic Society Conference, San Francisco 2012

Poster discussion session: "Can we identify asthma patients who can safely reduce their inhaled corticosteroid medication without suffering from a loss of control?" EE Wilson, B Hargadon, M Shelley, G Hearson, R Simms, H Bailey, DB Hodgson, J Anderson, T McKeever, M Thomas, ID Pavord, T Harrison, DE Shaw. (*Abstract number: 31012*)

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Poster discussion session: "Does elevated exhaled nitric oxide identify eosinophilic inflammation in mixed granulocytic asthma?" JR Anderson, DB Hodgson, EE Wilson, KM Smith, G Meakin, R Simms, TW Harrison, DE Shaw. (*Abstract number: 31606*)

Poster session: "Neutrophilic airway inflammation and response to prednisolone in patients with refractory eosinophilic asthma." DB Hodgson, JR Anderson, EE Wilson, W Gerrard-Tarpey, H Bailey, G Meakin, DE Shaw, TW Harrison. (*Abstract number: 31084*)

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Declaration of work personally performed:

I personally helped to develop and amend the design of the clinical trial: “Exhaled nitric oxide and inhaled corticosteroid dose reduction in asthma: a clinical study”. I was involved in writing the ethics and R&D approvals for the study and attended the Regional Ethics Committee meeting.

I recruited and obtained informed consent for 200 patients at the Nottingham City Hospital site. I personally performed all of the clinical measurements at this site (amounting to approximately 2200 hours of patient contact time). The research team at Leicester Glenfield Hospital performed consent and clinical tests on the 50 patients recruited into this site.

I was taught how to perform sputum processing and processed approximately 20 initial samples. The remainder of the samples were processed by the laboratory science teams at Nottingham City Hospital and Leicester Glenfield Hospital.

I designed the database and clinical reporting form for the study as well as all study paperwork, advertising and documentation. I entered all of the clinical data into the database and I checked 100% of the data in a complete data audit with the help of the department database manager.

I completed a four week basic statistics course and a 12 week advanced statistics course and personally performed all statistical analysis using STATA 11 with the guidance of a medical statistician.

I was involved in writing an article for the European Respiratory Journal Monograph titled: "Exhaled Nitric Oxide in Asthma". This review article is referred to during this thesis.

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Abbreviations Key:

µg (mcg)	micrograms
ACh	Acetylcholine
ACQ	Juniper Asthma Control Questionnaire
ACT™	Asthma Control Test™
AMP	Adenosine-5'-monophosphate
ANOVA	Analysis of variance
ASTRAL	Asthma Treatment Algorithm Studies
ATS	American Thoracic Society
BAL	Bronchial Alveolar Lavage
BASALT	Best Adjustment Strategy for Asthma in Long Term Trial
BDP	Beclomethasone Dipropionate
BMI	Body Mass Index
BTS	British Thoracic Society
CKD	Chronic Kidney Disease
cNOS	constitutive Nitric Oxide Synthase
COPD	Chronic Obstructive Pulmonary Disease
CRP	C-reactive protein
DDT	Dithiothrietol
DMSO	Dimethyl Sulphoxide
D-PBS	Dulbecco's phosphate buffered saline
ECCS	The European Community of Coal and Steel
ECP	Eosinophilic cationic protein
EDTA	Ethylenediaminetetraacetic acid
eNOS	endothelial Nitric Oxide Synthase

ERS	European Respiratory Society
ESR	Erythrocyte sedimentation rate
FACET	Formoterol and Corticosteroids establishing therapy study
FeNO	Fraction of Exhaled Nitric Oxide
FEV ₁	Forced Expiratory Volume in 1 second
FH	Familial hypercholestromia
FVC	Forced Vital Capacity
GINA	Global Initiative for Asthma
GMP	Guanosine Monophosphate
GMS	General Medical Services
GOAL	Gaining Optimal Asthma Control study
GORD	Gastro-oesophageal reflux disease
HbA1C	Haemoglobin A1C
HEPES	4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid
ICS	Inhaled corticosteroid
IgE	Immunoglobulin E
IL	Interleukin
iNANC	inhibitory non-adrenergic, non-cholinergic
iNOS	inducible Nitric Oxide Synthase
LABA	Long-acting beta ₂ (β ₂) agonist
L-NAME	L-NG-nitro-arginine methyl ester
L-NMMA	L-NG-Monomethylarginine
MDI	Metered Dose Inhaler
mRNA	messenger ribonucleic acid
NF-κB	Nuclear Factor Kappa B

NIHR	National Institute for Health Research
nNOS	neural Nitric Oxide Synthase
NO	Nitric Oxide
NOS	Nitric Oxide Synthase
PBS	Phosphate buffered saline
PC ₂₀	Provocative concentration for 20% fall in FEV ₁
PCR	Polymerase chain reaction
PD ₂₀	Provocative dose for 20% fall in FEV ₁
PEF	Peak flow
QOF	Quality and Outcomes Framework
R&D	Research and Development
REC	Regional Ethics Committee
ROC	Receiver Operator Characteristic
SABA	Short-acting beta ₂ (β ₂) agonist
SIGN	Scottish Intercollegiate Group Network
SMART	Symbicort® Maintenance and Reliever Therapy
SST	Serum separator tube
TIA	Transient ischaemic attack
TNFα	Tumour Necrosis Factor Alpha
VIP	Vasoactive intestinal peptide
VTE	venous thromboembolism

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Chapter 1 - Introduction:

Asthma is a disease defined as “a chronic inflammatory disorder of the airways. The disease is usually “associated with widespread yet variable airway obstruction and an increase in airway sensitivity and response to a variety of stimuli” (1). However, these definitions remain somewhat controversial in clinical practice due to the fact that the presentation of symptoms in asthma are highly variable and the British Thoracic Society state that “the absence of a gold standard definition (of asthma), means that it is not possible to make clear evidence-based recommendations on how to diagnose asthma” (2).

Central to all definitions of asthma is the presence of symptoms and variable airflow obstruction. These symptoms include wheeze, breathlessness, chest tightness and cough. More recent definitions of asthma also include airway hyperresponsiveness and airway inflammation as disease components. How all these features of the disease inter-relate and how they subsequently contribute to the clinical manifestations of asthma (due to the variability of the disease) is questioned (2). Because of these clinical uncertainties, guideline lead definitions of asthma serve to tell us very little about the disease course or the risk and progression of exacerbation. Although quantifiable measures of airway hyperresponsiveness and airway inflammation are becoming more common in secondary care and research environments, time and cost constraints prevent the simple transition of such tests into primary care. In practice, many decisions are made based upon the

presence or absence of symptoms despite the fact that extensive research has shown that symptoms are a poor predictor of outcome (3). Only airflow obstruction is quantified and measured for diagnostic and management purposes in primary care centres. This means that there still remains a need to develop a marker of disease (feasible in primary care) which can be used to inform clinicians about diagnosis, medication response (particularly to oral and inhaled steroids) and exacerbation risk.

The UK has one of the highest incidence rates of asthma of any country in the world. Development of such a marker of disease would be particularly beneficial to clinicians and patients in the UK. There are approximately 5.2 million people with asthma in the UK, and of these, 1.1 million are children. It is estimated that one in five British households are affected by asthma and every year these patients account for 4.1 million GP consultations and one hospital admission every 7.5 minutes (4). Despite the high level of medical intervention dedicated to the care of these patients, asthma is still responsible for 1400 deaths in the UK each year. These facts point to a definite need for more specific diagnostic and management tools in UK asthma care.

Asthma costs the UK over £2.3 billion a year, of this, approximately £889 million is through NHS costs (prescriptions, dispensing, hospital admissions and GP consultations). £1.2 billion is the estimated non-NHS cost of asthma to the UK government. This mainly derives from

loss of productivity through 12.7 million lost work days per year, however, an additional £260 million is spent by the government each year in social benefits to asthma sufferers. The biggest expenditure on asthma for the NHS is medication costs: totalling over £659 million a year (Refer to appendix section 7 for medication costs in England and Wales). In 1969, Ventolin[®] (salbutamol) was introduced into the UK market followed by other short-acting beta₂ (β₂) agonist bronchodilators to reverse asthma induced bronchoconstriction. In 1972, the first inhaled corticosteroid (ICS) medication was prescribed (Becotide[®] beclomethasone dipropionate) as a treatment for airway inflammation and a preventer for asthma exacerbation. In more recent years additional medications including long-acting β₂ agonists, newer inhaled corticosteroid preparations and combined inhalers have been introduced. Monoclonal anti-IgE antibodies have also been marketed in the past five years. These drugs use the anti-IgE antibody to bind IgE molecules in the blood stream allowing the body to remove them.

New treatments for asthma are largely developed to reduce airway inflammation effectively but many clinical decisions are made based upon the presence or absence of asthma symptoms despite the fact that there is poor correlation between symptoms and airway inflammation (3). If a clinical marker could be developed to assess airway inflammation in a safe, cost-effective and simple test; personalised asthma plans and medication regimes could be implemented more readily in UK primary care. The marker would ideally

need to be useful in predicting future exacerbation likelihood, saving money by preventing over-treatment and provide beneficial additional clinical information which is not currently available in primary care.

Unlike the pharmacological progression in asthma medications; the methods used to diagnose, predict and prevent exacerbations, as well as evaluate responses to medication, have not dramatically changed since the introduction of the peak flow meter in 1959 by Wright and McKerrow. Peak flow monitoring is regularly used in primary and secondary care to predict exacerbation, however the use of peak flow monitoring has been questioned in a study by Harrison *et al.* (2004) (5) which concluded that using a combination of a falling peak flow with a doubling dose of inhaled corticosteroid therapy medication cannot prevent an asthma exacerbation (5). Preventing, predicting and treating asthma exacerbation is important as exacerbations cause patients deep concern and distress, cost a disproportionate amount and can lead to sudden death (6-8).

Post-mortem studies show that airway inflammation is prominent in asthma associated deaths (9), and the question is raised as to whether the assessment of airway inflammation using non-invasive techniques may lead to better recognition and management of patients at risk of exacerbation.

The use of invasive techniques to assess airway inflammation such as bronchial lavage are available in secondary care, however, they are time-consuming and expensive procedures requiring theatre staff input

and patient sedation. Less invasive techniques such as sputum analysis are used more extensively now especially in research settings, but they are still expensive and highly involved techniques. It is unlikely that they will ever be feasible and affordable options in primary care. Blood eosinophil count and blood eosinophilic cationic protein are also used as measures of inflammation in asthma, however the evidence is to date inconclusive as to the validity of these measurements in diagnosis and managing asthma, and sputum eosinophil count is the more accurate marker of eosinophilic airway inflammation (10). A non-invasive technique is required for accurate assessment of airway inflammation in primary care. The question as to whether a non-invasive marker is feasible has become particularly pertinent since it has become clearer that the pattern of treatment responsiveness of airway inflammation in asthma is heterogeneous (11).

The relationship between airway inflammation, airway dysfunction and symptoms is weak (12). There have been some recent advances in assessment of airway inflammation using non-invasive techniques. These tools have provided opportunities to try and predict and prevent asthma exacerbations and further explore the heterogeneity of asthma. Such tools of non-invasive assessment of airway inflammation need further testing and clarification of their use and efficacy before they are widely invested in.

This thesis examines exhaled nitric oxide as a non-invasive biomarker of airway inflammation and its potential to be a clinical tool for asthma diagnosis and management in primary care; the heterogeneity and importance of airway inflammation monitoring will also be discussed. The content of the first section will review and explore the concept of exhaled nitric oxide as a biomarker of airway inflammation and the feasibility for use in UK primary care. The second section will concentrate on a pilot study and related sub-group analyses evaluating the use of exhaled nitric oxide as a non-invasive biomarker of airway inflammation in the prediction of over-treatment and exacerbation in primary care patients with well-controlled asthma.

1.1 - Introduction to asthma:

Asthma is a chronic inflammatory condition of the airways. Breathlessness, tightness of the chest and wheezing are characteristics of reduced lung function caused by this obstructive airways disease. Acute exacerbations can be rapid or gradual in onset, and may become life threatening. Microscopic examination reveals extensive inflammatory infiltration of the airways with oedema due to vasodilatation. Biopsies have shown increased numbers of leukocytes, particularly eosinophils, mast cells and T-lymphocytes, in the airways, and increases in the markers of lymphocyte activation. Structural changes resulting from chronic inflammation include bronchial smooth-muscle hypertrophy and hyperplasia. New vessel formation, interstitial collagen deposition resulting in basement membrane thickening, and airway wall remodelling have also been noticed (13, 14).

The mechanism of asthma and allergic response:

In the majority of cases, asthma is an allergic response disorder which is controlled in large-part by IgE mediated mechanisms. Allergens such as dog and cat hair, house-dust mite and aspergillus are common stimulators of the asthmatic allergic response. Once an individual has been exposed to an allergen, there is an uptake of this allergen and presentation by dendritic cells, activation of T-helper lymphocytes, cytokine release from T-helper cells and subsequent secretion of allergen specific IgE antibodies from B-lymphocytes. At this point, the allergen-specific IgE binds to mast cells leading to the release of

inflammatory mediators (14). Exposure to these allergens can also provoke T-cell activation, cytokine and chemokine release, and again, production of inflammatory mediators by IgE independent mechanisms. In short, inflammatory mediators released in either of these ways leads to damage of the epithelium, stimulation of the nerves, swelling, mucus secretion and contraction of the airway smooth muscle. Chronic inflammation, which results from release of inflammatory mediators leads to two principal disorders of lung function in patients with asthma. These are bronchial hyperresponsiveness and limitation of airflow. Patients with asthma have demonstrated increased bronchoconstrictor responses to direct airway smooth muscle stimuli such as histamine and methacholine, and to indirect stimuli including exercise, adenosine monophosphate and cold/dry air. All these stimuli lead to airway narrowing secondary to the initial release of inflammatory mediators. The consequences of this are, increased variability in airway diameter and lung function measurements, including PEF (which can fluctuate more than 20% between morning and evening measurements) (2, 14, 15). Acute bronchoconstriction may result from airway swelling, mucus plugging or airway wall remodelling. Allergen-induced release of inflammatory mediators (such as histamine, prostaglandins and leukotrienes) can lead to acute bronchoconstriction, while swelling of the walls of the airways is caused by oedema, (this can occur with or without bronchoconstriction). Hypersecretion of mucus leading to plugging of the airways and airway remodelling can also be caused by chronic airway inflammation (2, 16). At first exposure to allergen, the

mast cells are primarily involved and produce a response within minutes of allergen detection, and acute symptoms typically peak at around 10-15 minutes post-exposure. It is believed that micro-localisation of mast cells in the smooth muscle and subsequent interactions between the two are responsible for the typical disordered airway function characteristic in asthma. When the mast cell surface-bound IgE is cross-linked by the antigen, the mast cell is activated and degranulation occurs releasing the inflammatory mediators by exocytosis. Bronchospasm and constriction are induced and an early phase response such as this may result in more than a 25% reduction in FEV₁ (13, 14). After 4-6 hours, the allergic response moves into the "late phase" at which point, T-cells, B-cells and eosinophils are recruited. Like mast cells, eosinophils too contain inflammatory mediators which can induce airway damage and contribute to airway hyperresponsiveness. It has also been demonstrated that as well as inflammatory mediators, eosinophils also generate leukotrienes, cytokines, matrix metalloproteinase and reactive oxygen species, which could contribute to the overall symptoms of airway obstruction, injury and damage which are characteristic of asthma. As the late phase of the allergy progresses, nasal congestion and urticaria may also develop. At this point a reduction in FEV₁ by as much as 75% may be seen (13, 14).

1.2 - The diagnosis and management of asthma:

The diagnosis of asthma in primary care (and some secondary care) settings depends upon evaluations of variable airflow obstruction with spirometry (and reversibility), peak flow measurements / variability and assessment of symptom control. In the majority of instances, airway inflammation and hyperresponsiveness are not assessed despite being important characteristic features of asthma (17). The result of this is that many patients often commence treatment for asthma without there being sufficient evidence to justify doing so (18). The implications can be serious, and if misdiagnosed, patients can potentially be taking therapy unnecessarily before a correct alternative diagnosis is made (19). Current research aims to find more definitive methods of diagnosing asthma to avoid these situations arising during the subsequent management of the disease (16).

Asthma management is defined as “accurate initial diagnosis with effective control of the symptoms (including nocturnal and exercise-induced symptoms), prevention of further exacerbation (without inducing too many other side-effects), maintenance of pulmonary function levels (as close to normal as possible), maintenance of “normal” activity levels, prevention of development of irreversible airways damage and prevention of asthma mortality.” (2)

The overall aim of asthma management is to optimise control whilst maintaining the lowest doses of medication possible to achieve this.

The Official American Thoracic Society / European Respiratory Society
Statement: Asthma Control and Exacerbations defines asthma control
as “the extent to which the various manifestations of asthma have been
reduced or removed by treatment. This includes two components:

1. The level of clinical asthma control, which is gauged from features such as symptoms and the extent to which the patient can carry out activities of daily living and achieve optimum quality of life, and
2. The risk of future adverse events including loss of control, exacerbations, accelerated decline in lung function, and side-effects of treatment” (20).

Successful management of asthma comes from a combination of attention to preventative measures, such as allergy avoidance and effective medication use to prevent and /or treat acute attacks. Asthma management depends upon both control of day-to-day symptoms and prevention and treatment of asthma exacerbations. Symptom control depends in the most part on bronchodilation and allergen avoidance as well as use of inhaled corticosteroids (ICS) to reduce airway inflammation. ICS are also used to prevent exacerbations which are dependent on an increase in symptoms not responding to an increase in short-acting β_2 agonist (SABA) use. Most exacerbations are virally induced and once established, an asthma exacerbation is controlled by systemic steroid use. There are still problems which remain in asthma

management which the previous aims attempt to minimise, however, some patients are still either, misdiagnosed and unnecessarily treated with asthma medication or poorly controlled patients, or patients who appear to be well-controlled but could potentially remain well-controlled on lower doses of medication (16).

Another problem faced by patients and clinicians is that some patients who appear to be well-controlled on their medication may still demonstrate evidence of airway inflammation and hyperresponsiveness (21, 22) and therefore may be at risk of developing an exacerbation, therefore just assessing a patient as “well-controlled” on medication may not be sufficient and the exacerbation risk also needs to be quantified (13).

1.2.1 - The management of asthma through medication:

Asthma management medication is typically classified into two groups: controller medications (also known as preventer medications) and reliever medications (also known as rescue medications). Controller medications are those which patients take on a daily basis over the long-term to control asthma symptoms, prevent loss of control and prevent exacerbation. Reliever medications are those which patients take “when required” to relieve bronchoconstriction (23).

Short-acting beta₂ (β₂) agonists (SABAs):

Salbutamol and terbutaline are examples of SABAs. SABAs are used to reduce bronchoconstriction and are the primary choice for treatment of acute exacerbations and exercise-induced asthma. There are other forms of reliever medication, however, SABAs are the best drugs for relieving acute bronchospasm, and increased dependence on SABA is a good indication that a patient is not well-controlled on their preventer medication. However, SABA is not recommended for long-term use as there is evidence that a refractory response may develop and thus the asthma may worsen (23).

Long-acting beta₂ (β₂) agonists (LABAs):

Salmeterol and formoterol are examples of LABAs. LABAs typically act for over 12 hours, by relaxing the smooth muscle in the airway, enhancing mucociliary clearance, and decreasing vascular permeability. It is also believed that LABAs may be involved in moderating

inflammatory mediator release from mast cells and basophils. Treatment with LABA improves lung function and nocturnal asthma, and is shown to particularly benefit those patients who do not obtain adequate symptom control using starting doses of ICS alone. LABAs should not be administered without an ICS in combination (23). Combination inhalers containing LABAs and ICS are now available such as Seretide® and Symbicort® in single devices. These are beneficial as they improve medication compliance in patients.

Inhaled corticosteroids (ICS):

Beclomethasone dipropionate and budesonide are examples of ICS. ICS are currently the most effective anti-inflammatory medications available. They reduce airway inflammation, allowing lung function and asthma symptoms to improve, bronchial hyperresponsiveness to decrease and exacerbation frequency and severity to lessen. ICS affect the signalling pathway for the production of pro-inflammatory molecules. This is mainly achieved through the down-regulation of genes which express inflammatory mediators and the up-regulation of those genes expressing anti-inflammatory mediators. ICS are favoured over oral corticosteroids where possible as there are fewer systemic side effects (23).

Systemic corticosteroids:

Prednisolone is an example of a systemic corticosteroid. It can be administered either orally or parenterally. Short courses of 5-7 days are

often used in patients with uncontrolled asthma, or during periods of exacerbation. It is accepted that long-term oral treatment may be necessary in patients demonstrating severe, persistent asthma with poor control. However it is recommended that specialists alone initiate this form of treatment after all other avenues have been explored (23).

1.3 - Asthma control and severity:

One of the difficulties physicians face when treating asthma and also designing clinical trials, is differentiating between asthma control, severity and exacerbations. These terms are typically used interchangeably but actually mean different things. This difficulty is further worsened by the fact that patients respond in different ways to treatment and asthma symptoms can change over time (20).

Cockcroft and Swystun, 1996 (24) defined good asthma control as “minimal symptoms with minimal use of rescue β_2 agonist with near normal lung function, little resting bronchoconstriction and a small response to bronchodilator” (24). This definition fits broadly with the Global Initiative for Asthma (GINA) guidelines for optimal management of asthma (25) and the ATS/ERS guidelines (20). Asthma severity was defined as the “minimum amount of medication needed to achieve adequate control”, rather than defining it solely on symptoms alone. This means that it would be possible to have severe but well-controlled asthma or poorly controlled mild asthma. The former would be on high doses of inhaled and/or oral corticosteroids and experience few symptoms, whilst the latter would have symptoms but only require low doses of corticosteroids to alleviate them. These differences become important when designing and recruiting into clinical trials, as inclusion criteria may often require the patient to demonstrate particular levels of control, but because asthma is a variable episodic condition, spontaneous improvement or decline is often likely.

Reddel *et al.* (1999) (26) evaluated the differences between asthma control and severity using changes in peak flow variability. The hypothesis that asthma exacerbations had the same degree of peak flow variability as episodes of poor asthma control was tested (26). The results showed that poor asthma control and asthma exacerbations differed in their response to short-acting β_2 agonist use. During periods of poor asthma control (before treatment with inhaled corticosteroids) the average post-bronchodilator peak expiratory flow was 28% higher than the pre-bronchodilator value, whilst during an asthma exacerbation there was no response to bronchodilator and therefore no difference between pre and post bronchodilator peak flow. Most exacerbations were associated with evidence of viral infection, this lead the authors to draw the conclusion that asthma exacerbations were not the same as poor asthma control (26). This study had a high rate of viral exacerbation and did not take into account asthma severity so the view that exacerbations and poor control are linked still persists.

1.4 - Asthma exacerbations:

The incidence of asthma exacerbations in studies varies depending on the definition used and the baseline severity and control of the study population. Criteria used to define an exacerbation in previous studies have included: a drop in peak flow from a pre-determined baseline, need for rescue oral corticosteroids, increase in the use of short-acting β_2 agonist, night time awakening and increased symptom scores. Exacerbations are an important clinical feature of asthma and exacerbation frequency is increasingly measured as an important outcome variable in many clinical trials (24).

In the Formoterol and Corticosteroids Establishing Therapy (FACET) study (17), (which was designed to evaluate the benefits of adding a long-acting β_2 agonist to different doses of inhaled corticosteroid), a severe exacerbation was defined as an episode “requiring treatment with oral corticosteroids”, as judged by the investigator, or a “decrease of more than 30% below baseline value in the morning peak flow on two consecutive days” (the baseline value was established during a run in period) (12). Mild exacerbations were defined as 20% decrease in peak flow from baseline on two consecutive days, nocturnal awakening or three additional inhalations of terbutaline when compared to the study run in period. Approximately 850 patients were entered into the study and were randomised into one of four groups. The total number of severe exacerbations was 425 over a 12-month period, giving an

overall exacerbation rate of 0.5 exacerbations/patient/year. The total number of mild exacerbations was 16,463 (17).

In the Gaining Optimal Asthma Control study (GOAL) (27) the combination of salmeterol/fluticasone (as Seretide®) was compared to fluticasone alone in three different groups of patients over the course of one year; an exacerbation was defined as hospitalisation or as requiring antibiotics or oral corticosteroids. The baseline demographics revealed that 3416 patients experienced 1832 exacerbations, giving an exacerbation rate of 0.54 exacerbations/patient/year prior to the study (27). These figures demonstrate that severe asthma exacerbations are common and that the addition of a long-acting β_2 agonist reduces asthma exacerbations. The FACET study also revealed that higher dose inhaled corticosteroids have a marked beneficial effect of exacerbation frequency but less effect on symptoms and peak expiratory flow, whereas with the addition of long-acting β_2 agonists the opposite was shown to be true (12). This indicates that exacerbation frequency does not relate closely to symptoms and measures of disordered airway function, suggesting that the mechanisms responsible for these particular features of asthma are different (28). This demonstrates that different strategies are needed to reduce asthma exacerbations, as well as optimise asthma control.

Studies have consistently shown that poorly controlled asthma and asthma exacerbations cost a great deal more than well-controlled

asthma; Hoskins *et al.* (2000) (7) found the average cost per patient was 3.5 times higher for a patient having an asthma exacerbation, compared to a patient who did not (7). Similar figures have been published by Van Ganse *et al.* (2002) (8). Barnes *et al.* (1996) (6) suggested that there was significant scope for cost reduction by improving disease control, as one third of the direct cost of asthma was related to accident and emergency admission, hospitalisations and death (6).

Prevention of asthma exacerbations:

The current strategy recommended by the British Thoracic Society / Scottish Intercollegiate Group Network suggests a stepwise approach to the control of asthma symptoms and exacerbations (2). However, patients who appear clinically well-controlled on inhaled corticosteroid can still have evidence of airway inflammation and airway hyperresponsiveness (22) and vulnerable to exacerbations, airway remodelling and possibly fixed airways obstruction (13). These findings have important implications for the management of asthma particularly in primary care. This will be discussed further in Chapter 1.10.

Self management plans advocate doubling the dose of inhaled corticosteroid if the peak flow drops below a pre-determined baseline value. This approach has been questioned, and Harrison *et al.* (2004) (5) found that doubling the dose of inhaled corticosteroid therapy, based on a fall in peak flow of >15% from baseline or an increase in the

asthma symptom score from baseline, did not prevent the need for oral corticosteroids (5). The authors concluded that a higher dose of inhaled corticosteroid medication might be needed in order to prevent asthma exacerbation. Foresi *et al.* (2000) (29) demonstrated that quadrupling the inhaled corticosteroid dose at the onset of an asthma exacerbation had a beneficial clinical effect and reduced the requirement for oral corticosteroids compared to placebo (29). This suggests that once peak flow or symptoms begin to deteriorate an asthma exacerbation may still be prevented. New treatment regimes have been suggested to improve asthma control and prevent asthma exacerbations. O'Byrne *et al.* (2005) (30) evaluated the use of budesonide and formoterol (Symbicort[®]) as both maintenance and reliever medication (SMART[®] plan) (30), and compared it to budesonide/formoterol fixed dose therapy and high dose budesonide in patients with moderate persistent asthma and poor symptom control despite inhaled corticosteroid therapy. Overall SMART[®] reduced the number of exacerbations when compared to fixed dose therapy. However this approach may lead to over or under-treatment of asthma so caution is needed when using SMART[®] outside of the study environment (31). Exacerbations are frightening and debilitating for the sufferer and expensive for the NHS. Preventing exacerbations is one of the ultimate aims of asthma management, however, this proves difficult in many patients with asthma as it is difficult to predict when these exacerbations may occur and why.

1.5 - The heterogeneity of asthma – Introduction:

There are four important features of asthma: airway inflammation, airway hyperresponsiveness, variable airway obstruction and associated symptoms (1). Within asthma these features are often seen to overlap, occur independently or change over time. This can be in response to medication or other factors including infection and allergen exposure. The other common problem, which is seen in clinical practice, is that some of these features also occur in other airway diseases such as eosinophilic bronchitis (32) and COPD (33). This often leads to diagnostic difficulties and treatment uncertainties. The four features mentioned above will be discussed individually to present the importance of each feature in the asthma phenotype.

1.5.1 - Airway inflammation in asthma:

It is widely hypothesised that airway inflammation causes airway hyperresponsiveness which subsequently causes variable airway obstruction and asthma symptoms. If left untreated, airway inflammation may eventually cause airway smooth muscle remodelling. This hypothesis is firmly set in both research and clinical settings and it has even become central to guideline definitions of asthma (2) . However, large cross-sectional and longitudinal studies of airway inflammation using sputum induction (in larger populations with a diverse range of asthma presentations) demonstrate disparity between airway inflammation and other measures of airway function, suggesting that this hypothesis may require modification.

The pathophysiology of airway inflammation in asthma:

The role of eosinophilic airway inflammation in the pathogenesis of asthma has been researched by bronchoscopy studies performed over the last 20 years (34). These studies were largely limited to young volunteers with mild airway inflammation. The development of a non-invasive technique (sputum induction) to measure airway inflammation has made it possible to assess the presence of airway inflammation and relate it to measures of airway dysfunction in larger and more heterogeneous populations than was possible with invasive bronchoscopy studies. In general these studies have contradicted findings in the earlier bronchoscopy studies and they have not found a

correlation between sputum eosinophil levels and various markers of airway dysfunction, such as spirometry (28, 35-37).

Encouragingly the results of the research presented in this thesis show similar correlations between airway inflammation and airway dysfunction. The results of this research can be found in Chapters 4 and 5.

One observation has been that subset of patients with symptomatic asthma do not have sputum evidence of eosinophilic airway inflammation (38-40). Many patients present with sputum neutrophilia. This sputum profile is evident in corticosteroid-naïve (39) as well as corticosteroid treated patients with asthma (37, 41, 42) suggesting this profile is not always related to treatment. It has been noticed that patients with non-eosinophilic asthma respond less well to inhaled corticosteroid therapy than those with a more typically eosinophilic sputum profile (36). Similar sputum findings have been reported in more severe patients with asthma also (37, 38, 41, 42) and have identified a sub-group of patients with refractory asthma who have bronchoscopic evidence of neutrophilic airway inflammation, normal eosinophil counts and a normal basement membrane thickness. These findings suggest the presence of a distinct asthma phenotype characterised by a predominantly neutrophilic airway inflammatory response and relative corticosteroid resistance. However, these findings are based on single observations, and in a disease as variable as asthma there is a distinct need to establish a clearer understanding of whether a neutrophilic

asthma phenotype is persistent and whether relative corticosteroid resistance of these individuals is long-term. If this is the case, there is little point in exposing these patients to corticosteroid medication. As such the evidence now suggests that airway dysfunction and eosinophilic airway inflammation appear to be independent of one another. Further detail on the importance of sputum phenotypes and treatment decisions can be found in Chapter 5.4.

There is however a relationship between the change in airway function and eosinophilic airway inflammation following intervention with allergen challenge (43) or corticosteroid treatment (40) suggesting that there may be a within patient association with changes in these markers rather than a between patient association. The question as to whether changes in eosinophilic airway inflammation are linked to changes in airway function has been questioned by Leckie *et al.* (2000) (44). One study has shown that the humanised monoclonal antibodies to IL-5 cause a profound and long lasting reduction in blood and sputum eosinophilia, but they had no effect on airway responsiveness, lung function or symptoms pre and post allergen challenge (45). In another study there was no evidence of improvement in traditional markers of asthma control in a cohort of patients with more severe asthma who were symptomatic and had disordered airway function despite treatment with high doses of inhaled corticosteroids (46). One problem in interpreting these studies is that the anti-IL-5 antibody only partially reduced the tissue eosinophilia (45) although the effects seen were

significant. One view is that the findings of anti-IL-5 monoclonal antibodies suggest that changes in airway function and eosinophilic airway inflammation are independent and that the abnormalities of airway function seen in asthma are causally linked to other aspects of the inflammatory response which, although closely linked to airway inflammation, can be disassociated from it (47).

Both eosinophilic bronchitis and asthma are associated with cough and it is possible that eosinophilic airway inflammation is directly responsible for this aspect of the asthmatic process. The previous demonstration of a significant correlation between the improvement in cough reflex sensitivity and fall in induced sputum eosinophil count following treatment of subjects with eosinophilic bronchitis with inhaled corticosteroids would be consistent with a causal association (48). Other reports suggest an increased rate of decline in FEV₁ with the development of fixed airflow obstruction in eosinophilic bronchitis (49); it is possible that this complication of chronic asthma is also related to eosinophilic airway inflammation. This section highlights the importance in understanding the overlap of clinical features between asthma and other respiratory diseases. The diagnosis and management of asthma can therefore be exceptionally difficult especially in centres where only tests of airflow obstruction are available. The problem this poses to the diagnosis and management of asthma in primary care will be discussed in further detail in Chapter 1.10.

Inflammatory phenotypes and the stability of airway inflammation in asthma:

Asthma has been recognised as a heterogeneous disease for many years. However, in recent years there has been interest surrounding the identification of asthma phenotypes based upon the highly variable patterns of airway inflammation in asthma. Asthma airway inflammation is not a stable entity and the levels of airway inflammation fluctuate over time. The need to assess asthma as a continuum of variable symptoms over time, rather than at one fixed time point has become more apparent in recent years.

The introduction of induced sputum as a non-invasive “measure” of airway inflammation has facilitated knowledge of asthma phenotypes: eosinophilic and non-eosinophilic. It has become popular to characterise patients with asthma in this way as these two particular phenotypes appear to determine the likelihood of response to treatment, particularly with inhaled corticosteroids, which has been shown to differ according to the pattern and extent of airway inflammation. The concept of differing phenotypes has lead to researchers considering the possibility of individualised asthma treatment and management based upon the phenotype of airway inflammation which patients personally express.

Numerous studies have been undertaken to attempt to refine and explain the phenotypic diversity which is seen in asthma. Adult studies which have employed induced sputum, have consistently identified

distinct eosinophilic and non-eosinophilic subgroups of asthma. In addition inhaled corticosteroids, which are used to suppress airway inflammation and sputum eosinophilia, are known to be a significant cofounder in such studies. Normal sputum eosinophil counts have been reported in up to 25% of adult patients with untreated symptomatic asthma (36) and for over 50% of adult patients treated with high dose inhaled corticosteroids (38).

In a study by Simpson *et al.* (2006) (50) it was suggested that airway inflammation in adult asthma could be categorised into four inflammatory subtypes based upon the sputum profile: Neutrophilic asthma (Neutrophils >61%), eosinophilic asthma (eosinophils >3%), paucigranulocytic asthma (neutrophils <61% and eosinophils <3%) and mixed granulocytic asthma (neutrophils >61% and eosinophils >3%) (50). In stable adult asthma, where inhaled corticosteroids are the most prevalent treatment, paucigranulocytic asthma is seen to be the most common inflammatory phenotype followed by neutrophilic asthma (51, 52). In a study which examined adults during the stable phase of asthma, many clinical features were consistent across the four inflammatory phenotypes. Although the presence of the inflammatory phenotype appeared to predict a greater likelihood of future exacerbation (39) and non-eosinophilic patients were more likely to be female subjects and non-atopic than the remaining population (36).

Adults with an acute severe exacerbation of asthma are more likely to present with neutrophilic or paucigranulocytic sputum (51). The evidence is mounting to suggest that a “one size fits all” approach to asthma management is neither effective nor acceptable. Researchers are now searching for individualised phenotype-specific targeted treatment regimes. These different inflammatory profiles may also dictate treatment response, and as such, more accurate initial investigation of the inflammatory phenotype in individuals may prevent incorrect and “trial and error” treatment protocols.

However, a continuous problem which arises in most studies which use sputum profiles to define asthma phenotypes, guide treatment regimes or predict exacerbation, is that they confine their analyses to cross-sectional data measured at a single time point thus assuming temporal phenotypic stability. This is potentially a significant limitation of these studies given that asthma by definition is a variable disease (2). Simpson *et al.* (2010) (53) showed that the absence of a sputum eosinophilia was a consistent finding four weeks and five months after it was first demonstrated (53) and identified a subgroup of patients with predominantly non-eosinophilic sputum on repeated observations made over 12 months (39).

In a study by Jayaram *et al.* (2006) (54) the pattern of sputum inflammation was similar at baseline and during exacerbation in adults with asthma studied longitudinally over two years, indicating that patients with non-eosinophilic asthma were far less likely to have

eosinophilic exacerbations (54). In a prospective, double-blind, placebo-controlled trial of inhaled corticosteroids in non-eosinophilic patients with asthma a bronchoscopy at baseline was performed and then patients underwent repeated induced sputum six times over six months. None of the eleven patients studied demonstrated an airway eosinophilia at any point and at bronchoscopy all had normal basement membrane thickness (55). This supports the hypothesis that the non-eosinophilic phenotype is stable in adults since increased basement membrane thickness has been shown to be a long-term marker of eosinophilic airway inflammation (56).

The fact that inflammometry using induced sputum has been shown to be a successful strategy to prevent asthma exacerbations in adults (39, 54) may also support the theory that the stability or significance of inflammatory markers change over time. Phenotypic analysis using induced sputum does appear to have clinical value, not least as an inflammometer to guide inhaled corticosteroid treatment in adults with refractory disease, but uncertainties remain. It is now believed that variability over time is important in asthma management and that simple classifications of asthma based upon characteristics such as airway inflammation at a single time point represents over-simplification. Even studies which have included multiple factors and mathematical modelling techniques (57, 58) have not yet included variability over time. In a study by Anderson *et al.* (2008) (59) it was suggested that research should focus on searching for stable subgroups defined by unique and

specific genetic and molecular characteristics rather than “phenotypes” which can lead to uncertainty. However the other alternative is to develop a biomarker of inflammation which can distinguish between different inflammatory phenotypes, but which is safe, cost-effective and simple enough to be performed on a regular basis to assess changes in inflammation accurately and easily over time.

Sputum induction is not a feasible option as a long-term regular biomarker in primary care and patient’s homes, it is expensive, time-consuming and a highly involved technique requiring specialist input. Exhaled nitric oxide using the Aerocrine™ NIOX MINO® portable analyser, is a quick and cost-effective technique. It is similar to an alcohol breathalyser in that the test only requires the patient to be able to perform one breath, unlike sputum analysis. Exhaled nitric oxide has been shown to correlate well with sputum eosinophilia in moderate to severe patients with asthma, and can distinguish between eosinophilic sputum and non-eosinophilic sputum in this selected population. Encouragingly the research performed in this thesis yielded similar results showing that FeNO can differentiate eosinophilic inflammation from both neutrophilic and paucigranulocytic asthma. The results can be found in Chapter 5.4.

1.5.2 - Airway hyperresponsiveness in asthma:

Airway hyperresponsiveness is considered to be one of the characteristic clinical features of asthma (60). Airway hyperresponsiveness is defined as an “increased sensitivity to an inhaled constrictor agonist and a steeper slope of the dose response curve”. Two main forms of bronchoconstrictor stimuli exist; direct stimuli and indirect stimuli. Direct bronchoconstrictors consist of agents such as histamine and methacholine. These stimulate receptors on the airway smooth muscle and as such cause direct bronchoconstriction. Indirect bronchoconstrictors cause bronchoconstriction via the release of secondary bronchoconstrictor mediators from mast cells. For example; inhaled adenosine monophosphate or inhaled sodium metabisulphate.

Airway responsiveness is usually measured as the provocation dose / concentration of methacholine causing a 20% fall in FEV_1 by linear interpolation of the log dose / concentration response curve (PD_{20}/PC_{20}). In the general population the distribution of airway hyperresponsiveness follows a continuous unimodal log-normal distribution, with asthma sufferers representing the hyperresponsive part of the log-normal distribution curve. A PC_{20} is not usually measurable in normal healthy individual, this suggests that there is a large difference in the airway responsiveness between normal healthy individuals and patients with asthma. The cut-off used to identify asthma is normally taken to be a methacholine concentration of $<8\text{mg/ml}$. This value had a sensitivity of

100%, a specificity of 93% and a negative predictive value of 100% in a study on a population of 500 college students with a diagnosis of current and symptomatic asthma. Cockcroft *et al.* (1992) (61) concluded that a PC₂₀ of greater than 8mg/ml ruled out presence of current asthma and that a PC₂₀ value of less than 1mg/ml was almost certainly a positive diagnosis of current asthma. The values of responsiveness between 1mg/ml and 8mg/ml were regarded as intermediate (61). In patients with asthma the presence of airway hyperresponsiveness is highly variable. In many patients airway responsiveness remains stable over long periods of time but can increase during exacerbations of allergen exposure (60). Airway responsiveness may occasionally normalise after withdrawal from the allergen exposure or after corticosteroid therapy, but is seen to persist in the majority of patients even after appropriate treatment (62).

The use of methacholine PC₂₀ to diagnose asthma has been evaluated: Hunter *et al.* (2002) (18) demonstrated that when asthma was defined as “consistent symptoms with objective evidence of abnormal variable airflow obstruction”. A positive methacholine challenge was more sensitive than peak flow percentage mean and acute bronchodilator response (reversibility) in diagnosis (18).

The pathophysiology of airway hyperresponsiveness in asthma:

Various mechanisms are involved in development of airway hyperresponsiveness in asthma. Airway wall thickening is implicated: patients with asthma demonstrate subepithelial thickening (63) and exudation of plasma (64). This leads onto airway wall thickening, as the airway luminal resistance induced by a certain degree of airway smooth muscle shortening is then enhanced (65). Secondly, epithelial damage may allow greater exposure of bronchial smooth muscle to bronchoconstrictor mediators and decrease the amount of bronchodilating mediators released (66). Other possible mechanisms include both loss of sympathetic innervation to the lung (67), and loss of the bronchoprotective effect of deep inspiration in asthma (68).

Airway hyperresponsiveness and eosinophilic airway inflammation in asthma:

The relationship between eosinophilic airway inflammation and airway hyperresponsiveness is complex. Crimi *et al.* (1998) (35) performed measurements of airway hyperresponsiveness and eosinophilic airway inflammation (using bronchial wash, bronchoalveolar lavage and induced sputum) in 71 subjects. They found no significant correlations between eosinophilic airway inflammation and airway hyperresponsiveness (35). Some studies investigating patients with atopic asthma have found weak correlations between eosinophilic airway inflammation and airway hyperresponsiveness. There was a weak inverse correlation ($r=-0.4$) in a study involving 35 patients with

mild asthma (69). Similar relationships have been described in patients receiving inhaled corticosteroid medication (70, 71). The current view is that although interrelated, eosinophilic airway inflammation and airway hyperresponsiveness are, to a large degree, independently regulated. Support for this view comes from a factor analysis of 99 patients with mild asthma (28), and from the recognition that eosinophilic airway inflammation can occur without airway hyperresponsiveness in patients without functional airway abnormalities seen in asthma, but with a corticosteroid responsive cough and associated eosinophilic airway inflammation (32).

1.5.3 - Variable airflow obstruction in asthma:

Variable airflow obstruction has long since been considered the hallmark of asthma (72, 73). Airflow obstruction and its reversibility to treatment, or variability in response to stimuli, are incorporated in all the asthma guidelines (1, 2, 74). Although variable airflow obstruction forms only part of the asthma phenotype, due to its ease of measurement it predominates in the diagnosis and assessment of asthma especially in primary care settings. Both peak flow and spirometry accurately reflect changes in large airway calibre and can be easily measured. Variable airflow obstruction, defined as a 200ml or 12% improvement in FEV₁ either spontaneously, or following administration of a bronchodilator or glucocorticoid (reversibility), is considered diagnostic of asthma and is currently the most commonly used diagnostic test (75).

The pathophysiology of variable airflow obstruction in asthma:

Airflow obstruction is normally induced by a variety of stimuli including allergen exposure, exercise, cold air and dust. Four mechanisms which are believed to relate to underlying airway inflammation have been implicated. Firstly, allergen exposure leads to an IgE-dependent release of mediators from airway mast cells, including histamine and prostaglandins, leading to the early phase asthmatic response. Other stimuli cause combinations of direct contraction of smooth muscle, mediator release from cytokine “primed” inflammatory cells, and stimulation of local and central neural reflexes. All of these responses

lead to contraction of the airway wall smooth muscle and consequently airflow obstruction (76).

Secondly, airway wall oedema can independently cause airflow obstruction. An increase in microvascular permeability and leakage leads to mucosal thickening and swelling of the airway wall outside the smooth muscle. This swelling of the airway wall and loss of elastic recoil pressure contribute to an increased resistance to airflow within the airway. This component of asthma is referred to as the late phase asthmatic response and is similar to the reduction in airway calibre that is characteristically seen 6 to 24 hours post allergen challenge (76). Thirdly, increased exudation of serum protein and cell debris combined with mucus production lead to the plugging of the small bronchi with a subsequent increase in airflow obstruction (77). Lastly, chronic inflammation can cause long-term structural changes if left untreated in the airway wall matrix. This is known as airway wall remodelling (78, 79).

Variable airflow obstruction and eosinophilic airway inflammation in asthma:

A relationship between FEV₁ and sputum eosinophils has been demonstrated: Woodruff *et al.* (2001) (80) used multivariate analysis of data collected during screening and enrolment of 205 adults with asthma (80). After controlling for confounding factors, their analysis demonstrated that the induced sputum differential eosinophil count was independently associated with a lower FEV₁ and a lower PC₂₀; an

increased sputum neutrophil percentage was independently associated with lower FEV₁ but not with the PC₂₀. These results suggest that both eosinophilic inflammation and neutrophilic inflammation independently contribute to abnormalities in FEV₁ in asthma. Ten Brinke *et al.* (2001) (81) found that the only independent factor associated with persistent airflow limitation was a sputum eosinophilia. Other factors examined included; age at onset, smoking history, atopic status, bronchodilator reversibility, PC₂₀ with histamine, exhaled nitric oxide, blood eosinophils and blood IgE. This was a smaller homogeneous population with a more limited analysis of dichotomous variable; 132 non-smoking patients with asthma receiving high dose inhaled corticosteroids were studied and persistent airflow limitation was defined as a post bronchodilator FEV₁ or FEV₁/FVC ratio of less than 75% predicted. The association was not apparent in the sub-group receiving oral corticosteroid therapy (82). Balzano *et al.* (1999) (83) had previously examined 46 patients who were diagnosed with a mixture of different airways diseases; there was a significant inverse correlation between both FEV₁ and sputum neutrophils, eosinophils and eosinophilic cationic protein. There was also a significant correlation between FEV₁/FVC and the same sputum markers of airway inflammation (83).

1.5.4 - Asthma symptoms:

One of the biggest difficulties faced by physicians diagnosing asthma is the variability of symptoms. Asthma control and severity is covered in detail in Chapter 1.3. This section focuses on the disassociation between asthma symptoms and airway inflammation. A correct diagnosis of asthma is essential if appropriate drug therapies are to be administered. Asthma symptoms may be intermittent and their significance may be overlooked by patients and physicians, or, because symptoms are regularly “non-specific” may result in misdiagnosis (e.g. wheezy bronchitis and COPD which present with similar symptoms) (25).

A clinical diagnosis of asthma is often prompted by symptoms such as episodic breathlessness, wheezing, cough and chest tightness (25).

Episodic symptoms after allergen exposure, seasonal variability of symptoms, a family history of asthma and other atopic disease are also helpful in diagnosis. Asthma associated with rhinitis may occur intermittently, with patients being entirely asymptomatic between seasons or it may involve some seasonal worsening of asthma symptoms or a background of persistent asthma (25). The effective management of asthma relies on monitoring lung function and symptoms. There are a number of asthma symptom questionnaires which have been validated to assess the presence and severity of asthma symptoms in patients (e.g. Juniper Asthma Control Questionnaire (84)).

The Juniper Asthma Control Questionnaire (ACQ) (84) was developed by Juniper and coworkers for assessing asthma control in clinical trials and clinical practice. Questions are based on recalling asthma symptoms from the previous 7 days and comprise breathlessness, nocturnal waking, symptoms on waking, activity limitation, wheeze, frequency of short-acting β_2 agonist use, and pre-bronchodilator FEV₁% predicted. All seven items are scored on a 7-point scale without weighting (0 = good control, 6 = poor control) and the overall score (range, 0–6) is the mean of the responses (20, 84).

The ACQ has been validated against quality of life and physician assessment (84-88) and the minimum clinically important difference is 0.5 (86). The optimal cut-point for “well-controlled” using the Gaining Optimal Asthma Control (GOAL) study classification was less than or equal to 0.75, and a value of greater than or equal to 1.50 confirms “not well-controlled” asthma (88). Shortened versions, with omission of short-acting β_2 agonist use and/or FEV₁, perform almost as well as the 7-item version (86, 88) and are likely to be suitable for completion in primary care (89). Wording of the validated ACQ is slightly different from the originally published version (20, 84).

A shortcoming of the ACQ is the observation that most patients' scores are less than or equal to 2.5, with scores of greater than or equal to 4 only occurring with severe exacerbations. This suggests that the range and intervals for individual item scores could be improved. Also, the response scales may be more complex and time-consuming than is

necessary, and its acceptance for use in primary care needs to be demonstrated. Although ACQ includes pre-bronchodilator FEV₁ (a predictor of risk of exacerbations), change in this component may be outweighed by the remaining six symptom/reliever components, as was seen in a study by Jenkins *et al.* (2005) (90) of long-acting β_2 agonist monotherapy (90).

(A copy of the Juniper Asthma Control Questionnaire can be found in appendix section 3).

Questionnaires which assess symptoms are subjective and many patients with asthma suffer from symptoms but have normal lung function and no evidence of airway inflammation (21), conversely there are also patients with asthma who have very low symptom scores but suffer with airway inflammation (69). Clinical decisions are typically based upon the presence or absence of symptoms despite the fact that inhaled corticosteroid medications are used to reduce airway inflammation (69).

1.5.5 - Summary of asthma heterogeneity:

The clinical evidence today suggests that asthma is not a single disease, rather it is a collection of related diseases with different phenotypic characteristics. This makes assessment of asthma-related symptoms difficult. Currently there are no diagnostic tests which are sufficiently sensitive to rule out asthma completely (18). Whilst all the tests mentioned in this chapter contribute to providing additional valuable clinical information, none of them provide the “gold standard” in asthma diagnosis. Despite the fact that these tests cannot give a conclusive diagnosis of asthma, in combination, they provide evidence to suggest a diagnosis of asthma. However, these tests are not readily available in all settings and this has serious implications for the diagnosis and management of asthma in primary care.

1.6 - Exhaled nitric oxide:

Nitric oxide (NO) is an important signalling molecule involved in many physiological and pathological processes and its presence can be both beneficial and detrimental (2). NO is required in appropriate levels for the protection of organs such as the liver from ischaemic damage, However increased levels can cause vascular collapse associated with septic shock and chronic production has been linked with various carcinomas and inflammatory conditions, including asthma (16). NO is a highly reactive molecule which displays important functions in the respiratory system (91-96). It was previously known as "endothelial derived relaxing factor" (15, 97) and promotes vascular and bronchial dilatation in the lungs (91-96).

1.6.1 - Formation of nitric oxide:

The synthesis of NO is mediated by NO synthases (NOS). Two forms exist: constitutive (cNOS) and inducible (iNOS). The constitutive forms exist as endothelial (eNOS) and neural (nNOS). iNOS has been shown in bronchial epithelial cells, alveolar macrophages (98-101), nasal vascular endothelial cells (102) and nasal ciliated epithelial cells (102, 103). iNOS can produce much greater amounts of NO than cNOS (nanomolar concentrations) and increased iNOS is found in airway epithelial cells and in asthma. Guo *et al.* (2000) (104) demonstrated that patients with asthma exhibit increased expression of iNOS mRNA in the airways compared with healthy controls (104), and that patients using ICS had decreased expression of iNOS protein and mRNA compared with those not receiving ICS.

Each of the three NOS isoforms is present in the airways and can contribute to the formation of NO. In a study on healthy, atopic and children with asthma NOS2 (iNOS) mRNA was detected in bronchial epithelial cells from all groups, whereas NOS1 (nNOS) mRNA was not detectable and NOS3 (eNOS) mRNA was found in only 36 of the 43 samples obtained. The levels of iNOS correlated with the fractional concentration of NO in exhaled breath (FeNO) measured at a flow of 200mls/sec, adding evidence that the raised NO in the airways is due to iNOS production (105).

Other factors may be important in the formation of NO in the airways. At least two other mechanisms for the formation of NO have been postulated, including the release of NO from S-nitrosothiols, (this may account for approximately 80% of NO release) (106) and nitrite protonation to form nitrous acid which releases NO gas with acidification (107).

Genetic factors are known to affect the production of NO. van's Gravesande *et al.* (2003) (108) demonstrated a strong relationship between a known functional NOS3 mis-sense sequence variant in the endothelial NO gene (G894T) and NO level in a cohort of subjects with asthma (108). NOS1 polymorphisms also appear to be associated with asthma symptoms and IgE levels (109); further work has shown the number of AAT repeats in intron 20 of this gene correlate with NO levels, with a higher number of repeats correlating with lower NO levels (110).

In general, it has been shown that eNOS is predominantly found in endothelial cells within the bronchial circulation. However, there is some evidence to suggest that eNOS is expressed in the epithelial cells also (111). Similarly, nNOS has been reported in epithelial cells (112) even though it is generally localised to the cholinergic airway nerves (113).

1.6.2 - Function of nitric oxide:

NO is a free radical with one unpaired electron and a short half life of 1-5 seconds. NO is also a ubiquitous messenger molecule that is involved in the homeostasis of multiple biological functions. As well as these functions, NO also serves as a pro-inflammatory molecule in the lung. It is produced by alveolar macrophages in response to stimulation by endotoxins and cytokines (114, 115). NO also has a toxic effect in the lung where it is oxidised to peroxynitrite, a potent epithelial toxin found in asthmatic airways after allergen exposure. NO has other important functions in the respiratory system, including promoting vascular and bronchial dilatation, mediating ciliary beat frequency, promoting mucus secretion and acting as a neurotransmitter for non-adrenergic, non-cholinergic neurons (91, 92, 94-96, 116). Other roles for NO include promoting Th2 lymphocyte proliferation (117) and acting as a potent mediator of neurogenic oedema in animal models (92, 118). The balance of NO activity is controlled by uptake by antioxidant molecules such as haemoglobin and glutathione.

It is apparent that NO has many effects on airway function, however, the effects of endogenous NO are strongly dependent upon the site of NO production and the amount which is being produced (119). NO and NO-donor compounds relax the smooth muscle of human airways in vitro by activating guanylyl cyclase and increasing cyclic GMP (120, 121). In guinea pigs it has been shown that inhaling high concentrations of NO causes bronchodilation and protects against cholinergic

bronchoconstriction (122). In human subjects however, inhalation of high concentrations of NO (above 80ppb) has been shown to have no effect on lung function in normal participants and causes only weak and varied bronchodilation in participants with asthma (119, 123, 124). It may be the case that NO is the major neurotransmitter of bronchodilator nerves in the human airways. In the proximal human airways there is a prominent inhibitory non-adrenergic, non-cholinergic (iNANC) bronchodilator neural mechanism, which has particular functional importance as it is the only endogenous bronchodilator pathway present in the human airways (96). It has been determined that NO is the neurotransmitter of the iNANC pathway in human airways as studies have shown that NOS inhibitors almost completely abolish the neural response (96, 125, 126). In addition to this, iNANC stimulation of human airways causes an increase in cyclic GMP without any increase in cyclic AMP occurring (120).

In terms of vascular effects, NO is a potent vasodilator in the bronchial circulation and is also thought to have an important role in airway blood flow regulation (127-130). It is believed that endogenous NO may increase plasma exudation by increasing the blood flow to leaky post-capillary venules. This subsequently causes an increase in airway oedema (118). However, in a study by Erjefalt *et al.* (1994) (131) it was shown that when iNOS inhibitors were applied to the surface of the airways, there was an increase in plasma exudation. This suggests that the basal release of NO has an inhibitory effect on microvascular

leakage (131). In relation to airway secretions, L-NAME has been shown to increase baseline airway mucus secretions. This suggests that eNOS derived NO typically inhibits mucus secretion (132). Conversely, NO donors have been shown to increase mucus secretion in human airways in vitro (133).

In terms of the inflammatory effects of NO, there is evidence to suggest that high concentrations of NO may have effects on the immune system and the subsequent inflammatory response. With regard to the production of NO in asthmatic airways, previously it has been demonstrated that there is evidence for an increase in the expression of iNOS in these subjects (primarily from epithelial cells and macrophages) (119, 134). It is believed that this trend arises due to pro-inflammatory cytokines, oxidants and various other inflammatory mediators. Since NO is a gas, it diffuses readily into the airway lumen and thus is detectable in exhaled air (135). There is an increase in the level of FeNO in asthmatic patients (136, 137), which is understood to be, in the most part, derived from the lower airways (138, 139). This increase in FeNO in subjects with asthma is correlated to airway inflammation (140), is increased during the late phase response to allergen (141), during asthma exacerbation (142) and has also been shown to be subsequently reduced after treatment with ICS (143).

1.6.3 - Measurement of exhaled nitric oxide:

The fraction of FeNO present in exhaled breath (FeNO) can be measured by chemiluminescence. This is the most sensitive method and uses ozone to react with NO and produce nitrogen dioxide. This reaction emits photons in a stoichiometric relationship correlating with the amount of NO present. This allows measurements down to 1 part per billion (ppb) (144). FeNO can be measured either “offline” or “online”. (Offline measurements are rarely used now). Online measurement involves the inhalation of NO free air immediately followed by exhalation at a steady flow directly into the measuring apparatus. FeNO measurements are influenced by a number of variables. The most crucial is the exhaled flow. As NO is produced continuously in the airways, the concentration of NO measured at the mouth will vary with the flow of exhaled air. Various models describing the relationship between flow and NO concentration have been proposed. A trumpet shaped model of NO production has now replaced a two-compartmental model (145, 146). These studies incorporate axial diffusion into a one dimensional model of NO gas exchange in the lungs and predict a significant back diffusion of NO from the airways into the alveolar region, resulting in loss of NO that would therefore not appear in exhaled breath; this may cause an underestimation of both the maximum airway flux and the airway diffusing capacity for NO. This outcome depends upon on a significant proportion of NO being produced by the small airways (147).

The joint American Thoracic Society/ European Respiratory Journal guidelines 2005 recommend measuring FeNO at a flow of 50ml/sec (148). Prior to this joint statement, the two organisations recommended different flows, making study comparisons difficult (148); some of the larger original studies comparing differential sputum eosinophil counts and FeNO measurements were performed at a flow of 250ml/sec. Comparison between the two flows and different models have been performed with varying results (149, 150). In general the values obtained correlate, but the relationship is dependent upon both exhalation pressure and flow.

The technique for measuring FeNO is simple and well within the scope of most patients in primary care, including children as young as five years of age. FeNO is best measured before other spirometric manoeuvres and nasal clips should not be worn. In summary, a patient inhales NO free air to total lung capacity and then exhales at a rate of 50 ml/sec maintained within +/- 10% for > six seconds, and with an oral pressure of 5–20cmH₂O to ensure velum closure. A disposable filter is required, and normally the mean of three measurements is used. The test is quick and results instantaneous; three measurements can be completed in approximately three minutes.

1.6.4 - Reference values for exhaled nitric oxide:

Reference values for FeNO have been determined from small adult and paediatric populations. Values for normal healthy adults range between 5ppb and 35ppb and between 5ppb and 25ppb in children. 97% of healthy individuals have levels of <35ppb; this drops to <22.4ppb if outliers and subjects with evidence of atopy are removed (151).

The between subject standard deviation is 25ppb in asthma and 8ppb in normal healthy controls (152)

Table 1.1:

Distribution of normal FeNO values (153).

Exhaled Nitric Oxide according to classes of height and age among 1131 healthy never-smoking subjects					
Height (cm)	Age (years)				
	25-34	35-44	45-54	55-64	65-75
<160	24.0	24.5	28.8	31.5	34.1
160-169	27.4	29.7	32.8	35.9	38.9
170-179	31.2	34.1	37.3	40.9	44.3
180-189	35.5	38.9	42.5	46.5	50.4
>190	40.4	44.3	48.4	53.0	57.4
Data are presented as the 95% upper limit of FeNO (in ppb) calculated as mid-class values.					

Table is adapted from research published by Olin et al. (2007) (153).

There is still controversy over FeNO reference values. Current research aims to refine these reference values and produce clearer baseline figures and guidelines. However, previous research has produced reference values which are currently being used in the clinical setting to assess airway inflammation. NIOX FlexFlow[®] (Aerocrine[™], Sweden) is

one such device which measures the level of FeNO in the breath. It takes measurements at five different flow rates (10ml/sec, 30ml/sec, 50ml/sec, 100ml/sec and 200ml/sec) it then uses the slope and intercept of the line-graph produced from the five values to ascertain the alveolar and bronchial NO levels respectively. Currently it is the 50ml/sec measurement which is deemed to be the most important value (single flow NIOX MINO[®] machines only measure at 50ml/sec). Figure 11.1 shows the Aerocrine™ FeNO reference values for “normal” patients based on their sex, height, smoking status, allergy status and infection status. Normal values for individuals range from 9ppb (50ml/sec) to 50ppb (50ml/sec), therefore it is important that these normal values are taken into account when an individual is performing the test. It is generally accepted that a mean cut-off value for “normality” is around 25-27ppb (50ml/sec), but it should not automatically be assumed that all subjects with higher NO levels than this have asthma. Their demographics, atopic status and other confounding factors may account for the higher measurements. See chapter 1.6.5 for further information.

Figure 1.1:

Guideline values for FeNO using the Aerocrine™ NIOX FlexFlow® system (154).

NIOX® reference values

Use this guide to find out the expected normal value in your patient. It is dependant on – if the patient is a smoker, has a respiratory infection or has an allergy. Your patient's height and sex also do influence the expected normal value.

Find out the expected normal value (ppb) in 4 steps:

1. Regular smoking in the last 4 weeks?	Non-Smoker						Smoker										
2. Respiratory infection in the last 4 weeks?	No Infection			With Infection			No Infection			With Infection							
3. Known allergic rhinitis?	No Allergy		With Allergy	No Allergy		With Allergy	No Allergy		With Allergy	No Allergy		With Allergy					
4. Height	155 cm	15	22		18	28		9	14		12	17					
WOMAN	160 cm	16	18	24	28	19	23	29	34	10	12	15	17	12	14	18	21
	165 cm	17	20	25	29	21	24	31	36	10	12	16	18	13	15	19	23
	170 cm	18	21	26	31	22	25	32	38	11	13	16	19	14	16	20	24
	175 cm	19	22	28	32	23	27	34	40	12	14	17	20	14	17	21	25
	180 cm	20	23	29	34	24		36	42	12	14	18	21	15	18	23	26
MAN	185 cm	21	24	31	36	25	30	38	45	13	15	19	23	16	19	24	28
	190 cm	22	25	32	38	27	31	40	47	14	16	20	24	17	20	25	29
	195 cm		27	40		33		50		17		25		21		31	

Infection = respiratory tract infections
Allergy = respiratory allergies, allergic rhinitis
Values are given as geometric mean. The Data is based on 897 subjects, primarily Caucasian, aged 14±13 years

Adapted from Dressel H, de la Motte D, Reichert J, Ochmann U, Petro R, Angerer P, Holz O, Nowak D, Jones RA. Exhaled nitric oxide. Independent effects of atopy, smoking, respiratory tract infection, gender and height. Respir Med. 2008 Apr 4.

Figure is taken from an educational brochure produced by Aerocrine™ for clinicians and researchers using exhaled nitric oxide (154). The original research was performed by Dressel et al. (2008). This figure is adapted from the original work.

1.6.5 - Confounding factors in exhaled nitric oxide reference values:

The effect of atopy on FeNO levels is controversial. Some authors argue that there is no influence; Gratziou *et al.* (1999) (155) found no difference in FeNO levels between atopic and non-atopic individuals (155). This study was corroborated by a further study from Berlyne *et al.* (2000) (156). However there is a large body of evidence that atopy is associated with high FeNO levels, and that the relationship is such that in trials using FeNO as a marker of airway inflammation in asthma, the influence of atopy can be difficult to exclude (157). Various studies have shown that patients with atopic asthma have FeNO levels which are higher than matched controls with non-atopic asthma (158-161). There is also evidence that atopic individuals without asthma have higher FeNO levels than non-atopic individuals without asthma (162); this also applies in children. FeNO levels are also higher in children sensitised to various aeroallergens, including house dust mite (161, 163) and grass pollen. The relationship between atopy and FeNO may just be due to exposure; Olin *et al.* (2004), (2007) (153, 164) found that FeNO levels rose in atopic patients after exposure to the relevant antigen, and that atopic individuals who had never been exposed to the relevant antigen or who had never experienced asthma had normal FeNO levels (153, 164). This issue is complicated by the influence of rhinitis with both asthma and atopy. Levels of FeNO generated in the nose are much greater than those generated in the lower airway (165), and nasal contamination could theoretically cause difficulties with measurement.

In general FeNO is thought to reflect lower airway inflammation rather than upper airway inflammation (caused by atopy or allergic rhinitis) as the flow used when performing FeNO measurements generates an excess pressure in the oral cavity; this is assumed to close the velum and prevent contamination from NO produced in the para-nasal sinuses (166, 167).

Other factors are known to affect FeNO readings. Causes of lung inflammation which increase FeNO levels include bronchiectasis, viral infection, fibrosing alveolitis, allergic rhinitis, pulmonary tuberculosis, COPD and pulmonary sarcoidosis. However pneumonia (167) and cystic fibrosis (168) have both been shown to reduce FeNO levels. Caffeine ingestion and smoking (169, 170) both reduce FeNO levels, and a nitrate- rich diet increases them (171). Interestingly, Horvath *et al.* (2003) (162) demonstrated that FeNO levels were higher in smokers with asthma than in smokers with no asthma (162), however Shaw *et al.* (2007) (172) found that the relationship between FeNO and sputum eosinophil counts was much weaker in smokers with asthma (172). There has also been debate about diurnal variation in FeNO levels, but this aspect is now thought to be minimal (173). More importantly measures of lung function and airway hyperresponsiveness have both been shown to reduce FeNO levels (174, 175). This has practical implications; and as mentioned previously, measurements of FeNO should always be performed before any other airway manoeuvre.

Virally-induced upper respiratory tract infections have been shown to cause a rise in FeNO levels, it has been suggested that this may be as a result of the expression of iNOS in response to NF- κ B activation by the rhinovirus (176). In bronchiectasis there may also be elevated FeNO and in the first instance it is believed to be related to the extent of lung involvement (173). Conversely, FeNO is reduced in cystic fibrosis (177) and the theory is that this is due to the level of intense neutrophilic inflammation and the NO is converted into peroxynitrite by the superoxide anions. Similarly, FeNO is much lower in COPD also, despite the fact that there is active inflammation present (178). A loose prediction is that this may be related to the effects of smoking, which lowers FeNO levels however, this is not definitive (179), alternatively it may be due to neutrophilic inflammation. In lung parenchymal inflammation FeNO is seen to be elevated as well as in patients with active fibrosing alveolitis, however, trends suggest that when fibrosis intervenes FeNO levels may begin to fall (180), in this instance FeNO could therefore be used as a marker of disease progression and activity. This is consistent with histological studies which have demonstrated an increase in iNOS expression during the active inflammatory phase of the disease, with no iNOS expression in the areas of fibrosis (140). FeNO values should always be interpreted with caution paying specific attention to the patient's history and confounding factors.

1.6.6 - Relationship between exhaled nitric oxide and eosinophilic airway inflammation in asthma:

When studying the relationship between eosinophilia and airway inflammation it becomes apparent that variations exist depending upon the populations being investigated. The use of FeNO tests have been conducted most extensively within populations of children as the test serves as a non-invasive alternative to traditional airways tests, such as the methacholine challenge, which would be likely to cause distress in young children. Payne *et al.* (2001) (177) studied the possible correlation between eosinophilia and FeNO levels in endobronchial biopsies. The study included 31 children with difficult-to-control asthma and 7 children acting as controls. FeNO and eosinophilia were measured before and after a two week course of oral prednisolone. From the study it was demonstrated that, in children, FeNO levels correlate with eosinophilia score (177). This relationship between eosinophilia and FeNO level has also been demonstrated in bronchoalveolar lavage fluid (178) and induced sputum in children with asthma (179, 180). In adults with mild asthma it has been demonstrated that sputum eosinophil counts also correlate with FeNO levels (140). There was no evidence that this was influenced by gender, age, atopic status or lung function. However, smoking status did affect the relationship (as mentioned previously smokers tend to have a low FeNO typically 2-10ppb despite inflammation possibly being present). A significant but weak correlation between FeNO levels and eosinophil differential counts was demonstrated by Berlyne *et al.* (2000) (156) in

subjects with steroid-naïve asthma, in subjects with eosinophilic bronchitis without asthma and also in healthy atopic subjects. In subjects who were taking steroids, FeNO levels were seen to be significantly lower than the levels in those participants who were not. This trend was seen despite there being no difference in the sputum cell counts between the two groups of participants (156). The conclusion drawn was that FeNO measurements have limited utility as a surrogate biomarker of inflammation in the airways except for in those participants which are steroid-naïve (156, 181).

A study by Silkoff *et al.* (2005) (182) which was developed using FeNO to predict persistent eosinophilia in severe refractory asthma demonstrated that FeNO levels which exceed 72.9ppb were associated with a sensitivity of 0.56 and specificity of 1.0 for identifying persistent eosinophilia, as measured by endobronchial biopsy or bronchoalveolar lavage. It was also determined that FeNO levels were correlated with tissue eosinophil level (182). Despite these studies, there have been complications in the relationship between FeNO and airway eosinophils in bronchial biopsies as two trials discovered there to be no significant correlation between the two (183, 184). A further complication arose in a study examining the potential use of interleukin-5 in subjects with asthma. Mepolizumab (anti-IL5) was trialled on 29 patients with a diagnosis of refractory asthma. The results showed that exacerbation frequency and differential sputum eosinophil counts were reduced in those subjects taking the active mepolizumab when compared to

placebo. However, there was no significant decrease in the FeNO levels in subjects taking the active medication compared to those taking the placebo medication. This trend in FeNO levels was seen despite the fact that sputum eosinophil count in subjects taking Mepolizumab had been reduced to <1% (185). These results question the biological associations thought to be active between FeNO and sputum eosinophils. However, predictions have been made which may make it plausible that FeNO may in fact be involved in an alternative inflammatory mechanism possibly associated with eosinophil production or recruitment.

1.6.7 - Relationship between exhaled nitric oxide and asthma diagnosis:

As previously mentioned, FeNO has been, and continues to be, used in research to determine its efficacy as a surrogate biomarker of airway inflammation in asthma. However, despite its prevalence in the research and its potential as a useful clinical diagnostic tool, no studies to date have conclusively proved that FeNO can be used as a clinical diagnostic test. In a study by Smith *et al.* (2004) (186) it was determined that a FeNO level of 20ppb (at the 50ml/sec flow rate) had 88% sensitivity for the diagnosis of asthma in steroid-naïve subjects with symptoms suggestive of asthma (186). The results obtained from the study showed that there was a strong positive correlation between FeNO and sputum eosinophils ($r=0.67$, $p<0.001$) and a strong negative association between FeNO and airway hyperresponsiveness ($r=0.56$, $p<0.001$). In a separate study by Dupont *et al.* (2003) (187), it was found that a FeNO level greater than 16ppb at the 50ml/sec flow rate had a sensitivity of 90% and a specificity of >90% with regard to diagnosing asthma (187). Henriksen *et al.* (2000) (159) performed a large study with 8571 adolescents and combined FeNO level results with the results of methacholine challenge tests. In 75% of the suspected patients with asthma, airway hyperresponsiveness was confirmed versus 25% of the control subjects. Whereas 52% versus 20% respectively had elevated levels of FeNO (>8ppb at 250ml/sec flow rate); the combination of airway hyperresponsiveness and elevated

FeNO levels appeared to be a specific finding for allergic asthma in this population survey (159).

Measuring FeNO levels in conjunction with traditional airways tests such as spirometry and methacholine challenge test can be used by clinicians to aid in the differential diagnosis of eosinophilic bronchial asthma, thus reducing patient exposure to inappropriate or ineffective medications. It was concluded by Smith *et al.* (2004) (186) that measuring FeNO levels offers the clinician “correct asthma diagnosis in over 80% of patients aged 8-75 years using a cut-off score of 20ppb at the 50ml/sec flow rate (186)”. “FeNO offers a higher degree of diagnostic accuracy for asthma (sensitivity 88% at a cut-off of 20ppb) than tests based on lung function. The diagnosis of asthma was ascertained by a positive response to bronchodilator and/or positive bronchial hyperresponsiveness in accordance with ATS guidelines” (186). In a study by Malmberg *et al.* (2003) (188) concluded that “correct asthma diagnosis in nearly 80% of children aged 4-8 years using a cut-off score of 10ppb at the 50ml/sec flow rate (188).”

Another benefit of FeNO measurement is that it offers rapid identification of non-compliant patients (189) and is therefore a good non-invasive tool for monitoring adherence to steroid treatment (190). If FeNO levels remain elevated in patients taking maintenance doses of steroids it essentially means one of two things clinically. Either the patient is not being prescribed enough anti-inflammatory therapy or the

patient is not adhering to the medication which has been prescribed to them. In most instances, FeNO levels fall in patients receiving anti-inflammatory treatment. However, some patients display elevated FeNO levels despite taking anti-inflammatory steroid medication. The most common cause of such an occurrence is non-compliance on the part of the patient. However, it may also arise from poor inhaler technique, inadequate steroid dose, chronic exposure to allergen or non-eosinophilic airway inflammation as the cause of the asthma (191). Only in very few cases will a patient with asthma be totally steroid-resistant (192).

1.6.8 - Relationship between exhaled nitric oxide and asthma treatment:

Despite its prevalence in research, as yet only two studies have been undertaken which have successfully used FeNO measurements to titrate inhaled corticosteroid dosage in subjects with asthma (193, 194). The first of these studies was performed by Smith *et al.* (2005) (252) and used FeNO measurements (at the 250ml/sec flow rate) to down-titrate ICS in 92 subjects with asthma (193, 194). The trial was a single-blind study and the participants involved were either treated according to their FeNO results or according to current guidelines. All participants were subject to a run-in period (ranging between 3 and 12 months). During this time all subjects were prescribed 750 micrograms of inhaled corticosteroid, after the run-in period the steroid dose was reduced in the FeNO group if the FeNO level measured <15ppb (193).

Steroid reduction in the control group was based on current asthma management guidelines and only occurred when, over the course of the previous week, subjects achieved the following:

- Less than 2 night-time awakenings due to asthma.
- A mean peak-flow amplitude of <20%.
- Bronchodilator use <4 times on 1 or 2 days.
- Minimal asthma symptoms.
- FEV₁ >90% predicted.

If a patient did not fulfil all these criteria, an episode of loss of control was counted. Within the FeNO group, the optimal dose was one dose above the dose at which the subjects FeNO level was >15ppb (193). In

the control group the optimal dose was one dose above the dose at which a loss of control had occurred. These figures subsequently became the optimal doses at which subjects entered the final year-long phase of the study. During this stage, the steroid dose was increased in the FeNO group if the measured FeNO level exceeded 15ppb. Within the control group the optimal dose was increased if a loss of control (defined as for the dose optimisation period) occurred. The steroid dose was reduced on predetermined criteria in both groups, but never below the optimal starting dose (193). Although it was noted that there were fewer exacerbations in the FeNO group, it was not great enough to demonstrate significance statistically. However, it was determined over the course of the study that participants in the FeNO group used 45% less inhaled corticosteroid when compared to the control group. There was a significant difference in the distribution of doses of inhaled fluticasone at the end of the study. The mean dose was 370 micrograms per day in the FeNO group and 641 micrograms per day in the control group. At the end of the study, the control of asthma in the FeNO group was not significantly better (193). The results of the FeNO differences looked impressive, however, it must be noted that the dosage difference was likely to be accounted for by the fact that there was a dose increase of steroids in the control group of participants (and some participants were on LABA medication also) (195). The study does however, confirm that a management plan based on tracking changes in FeNO level offers a safe and practical method of asthma management and may allow steroid reduction without a concomitant

increase in asthma exacerbations. This outcome was also demonstrated by Green *et al.* (2002) (39) who showed that “titrating steroid dose to match the severity of airway inflammation results in a healthier patient with fewer emergency room visits and significantly fewer exacerbations (39).” This not only reduces the amount of treatment a patient is exposed to, but also reduces cost to the individual and the NHS.

In a second study Pijnenburg *et al.* (2005) (194) compared a FeNO algorithm to a symptom based algorithm to titrate inhaled corticosteroid doses in 39 and 45 children respectively. Subsequently, at a one year follow up there was no difference in the symptom scores, lung function, inhaled corticosteroid doses or exacerbation rates between the two groups, but there was a significant improvement in the methacholine PD₂₀ in the FeNO group compared with the control group (194). Aerocrine™ used the results of these three studies to confirm that “routine monitoring of the FeNO level as a marker for inflammation makes it possible to titrate the steroid dose according to the patient’s specific needs (193, 194).”

In summary, research has determined that the use of FeNO measurements can significantly reduce maintenance doses of inhaled steroids without compromising asthma control (193). In those patients who already take steroids, the dose can be gradually reduced to the point at which the FeNO level starts to increase again. At this point the

steroid dose should be raised, but only by the minimum amount required to maintain the FeNO measurement at a stable level. This method may, in the future, allow personalised asthma treatment plans to be initiated, through FeNO level measurement tracking. This method, compared to conventional treatment guidelines improves asthma prognosis (as measured by improving hyperresponsiveness and reduced inflammation) (194).

1.6.9 - Using exhaled nitric oxide to predict treatment response in asthma:

As previously mentioned, some studies have been using FeNO measurements to track changes in the inflammation levels and increase or decrease inhaled corticosteroid treatment accordingly. Other studies have used FeNO measurements as a baseline for predicting treatment response.

Wilson *et al.* (2006) (196) studied the effect of ciclesonide in low doses in mild to moderate patients with asthma to determine whether it exhibited significant anti-inflammatory effects after 4 weeks of 160 micrograms per day of treatment. The FeNO level (along with other lung function tests) was measured after the 4 weeks of ciclesonide treatment and again after a subsequent drug wash-out period. It was established that there was no significant difference between the run-in period, wash-out period or placebo for any of the end points that were measured (196). However, the difference between ciclesonide and placebo was significant for the FeNO results [47(95% CI: 15-81) ppb]. The authors acknowledged that FeNO is particularly sensitive to the effects of inhaled corticosteroid (197) and other studies have shown significant suppression of inflammation at low to moderate doses. Lee *et al.* (2005) (198) demonstrated significant suppression of inflammation using high-doses of ciclesonide (1280 micrograms per day) (198), but not using moderate doses (320 micrograms per day) (199). Conversely, Kanniess *et al.* (2001) (200) showed significant suppression of airways

inflammation with 320 micrograms per day. The evidence either way is inconclusive as different studies obtained opposing results.

In a second study using FeNO to predict treatment response, Jones *et al.* (2002) (201) performed a trial to evaluate the usefulness of FeNO in investigating the dose response relationship for inhaled beclomethasone dipropionate (BDP), and to compare FeNO levels with other markers of airways inflammation (201). 65 participants were withdrawn from their inhaled corticosteroid therapy and were entered into a double-blind, parallel-group, placebo-controlled trial involving 50, 100, 200 micrograms BDP per day for 8 weeks. The relationship between the dose of inhaled corticosteroid and change in FeNO showed a dose-response curve which appears to be linear, at least up to a dose of 500 micrograms BDP equivalent (201). In this study Jones *et al.* (2002) (201) have shown that FeNO measurements have been used to predict deteriorating asthma conditions and control, and potentially may reflect underlying changes in the inflammation of the airways (201). The findings from the Jones' study along with results from similar trials, offer further evidence that repeating FeNO measurements may serve as a useful guide for the adjustment of inhaled corticosteroid dosage in patients with persistent asthma (201). It was established by Kharitonov *et al.* (2002) (202), that the recovery of FeNO back to baseline following withdrawal of inhaled corticosteroid therapy is rapid but also dose-dependent (202).

In a similar study, Silkoff *et al.* (2001) (203) demonstrated the dose response and the reproducibility of the FeNO measurements fell following inhaled BDP therapy in non steroid-treated patients. From the results of the study the general model showed that the test for treatment differences in FeNO was highly significant. There was no significant difference between FeNO at baseline and after one week of placebo inhaler treatment. A progressive fall in FeNO was seen as BDP dose was increased, and all doses of BDP were associated with significant change in the FeNO levels from baseline and placebo values. The level of FeNO in subjects taking 100 micrograms BDP was significantly different from those taking 800 micrograms BDP. However, there was no significant difference between the 100 micrograms and 400 micrograms doses or between the 400 micrograms and 800 micrograms doses (203).

In summary, the authors concluded that FeNO was “superior to both FEV₁ and PC₂₀ in establishing a dose response for 100 micrograms per day and 800 micrograms per day BDP, but was unable to distinguish between the interim doses.” It was again suggested that FeNO monitoring may be useful in determining minimal effective doses of ICS. However, they believed it may only be useful in patients with raised baseline FeNO values which could be used to compare the potencies of anti-inflammatory medications (203).

Other studies which have used FeNO to predict treatment response have focussed on the time it takes for FeNO levels to rise or fall before

or after inhaled corticosteroid therapy is administered respectively. In one such study investigations into whether systemic glucocorticoid therapy (like inhaled corticosteroid therapy) could reduce FeNO levels in a given individual. All participants who underwent emergency treatment for asthma with systemic corticosteroids had their FeNO levels monitored repeatedly. FeNO levels were shown to fall as the time since treatment increased. At the same time, relief from airway obstruction was also shown to improve (142). The decrease in FeNO levels was evident from around 48 hours post-therapy. This study provides further evidence that using FeNO levels to track changes in patient airway inflammation can aid recovery through the use of patient-specific treatment plans (142).

The fall in FeNO levels was much more rapid in treatment of acute asthma in children with a single dose of nebulised budesonide. Tracking the FeNO changes showed a decrease in 6 hours, as FeNO decreased this was correlated with an increase in peak expiratory flow rate (204). These studies not only offer evidence into the efficacy of FeNO as a traceable biomarker, but also offer insight into the most effective medication types available. In summary, using FeNO measurements to predict treatment response firstly offers insight into systemic steroid and ICS efficacy. FeNO responds faster than any other marker to changes in steroid intake (142, 204). There is a clear dose-dependent relationship between the inhaled steroid dose and the fall in FeNO level (203). A reduction in FeNO of at least 20% in unstable patients

indicates efficacy of the anti-inflammatory treatment (205). The implications for clinical practice are that monitoring a patient's FeNO levels before and during anti-inflammatory therapy is now "a simple, quick and patient-friendly way of checking that steroid therapy is having the desired effect on airway inflammation levels" (203, 205).

Secondly, FeNO measurement offers prediction of steroid response. In patients with non-specific symptoms, a FeNO value of >47ppb is highly indicative of a subsequent corticosteroid response (206). FeNO measurement correctly identifies the responders better than spirometry or PEF variability. In this group of patients with non-specific respiratory symptoms, FeNO measurement was significantly better than FEV₁ bronchodilator response in correctly identifying those who will respond to inhaled fluticasone (206). FeNO as a predictor of response might help to identify individual children who achieve a greater improvement in asthma control days with an ICS compared with a leukotriene receptor antagonist (207). Patients with normal FeNO levels who do not show any symptomatic response to anti-inflammatory treatment may have little or no underlying inflammation, in which case other forms of treatment should be considered, and discontinuation of ineffective anti-inflammatory treatment is likely to be appropriate (207).

1.6.10 - Corticosteroid treatment and exhaled nitric oxide in asthma:

Inhaled and oral corticosteroids are used to treat airway inflammation in asthma. Exhaled nitric oxide measurements are shown to fluctuate in response to changes in corticosteroid medication use.

In a study performed by Zanconato *et al.* (2002) (232), 30 children with asthma exacerbation were treated with oral prednisolone (systemic steroids) for 5 days. FeNO levels were recorded before and after the oral corticosteroid therapy. A control group, consisting of healthy, non-atopic children also had their FeNO levels measured. The results determined that FeNO values were higher in children with asthma (FeNO online, 74.9 ± 0.4 ppb; FeNO offline, $20.2 \text{ppb} \pm 1.4 \text{ppb}$) than in the control group (FeNO online, 10.1 ± 0.8 [p<0.0001]; FeNO offline, 5.9 ± 0.4 ppb [p<0.0001]) (176). The conclusions drawn were that there was a significant decrease in FeNO levels in children with asthma exacerbation after a course of oral prednisolone therapy, and that the effect of oral corticosteroids is similar to those of ICS but ICS onset is much slower (142, 176).

In a further study by Slats *et al.* (2006) (208) the effect of combining oral corticosteroid therapy with ICS on FeNO levels was investigated. It was hypothesised that airway inflammation can persist despite ICS treatment and it may be possible to further reduce inflammation by prescribing an oral corticosteroid therapy in combination with ICS.

FeNO was used to measure the level of airways inflammation. From the results obtained, two weeks of treatment “did not significantly change the level of FeNO either within the prednisolone group (mean change, -14.0[ppb; SD 33.4ppb) or within the placebo-treated group (mean change, 9.7ppb ; SD 12.8ppb)” (208).

A common theme for research has been using FeNO to assess the response of an individual to ICS therapy. One such study was performed by Smith *et al.* (2005) (206) and assessed the predictive accuracy of FeNO to identify steroid response in 52 patients presenting with undiagnosed respiratory symptoms in a single-blind, fixed-sequence, placebo-controlled trial of inhaled fluticasone for four weeks (206). In this study, steroid response was defined as a “change in symptoms, peak flows, spirometry or airway hyperresponsiveness to adenosine, based on guidelines and recommendations” (206). The results showed that response to steroid treatment was significantly higher in the highest FeNO group (>47ppb) for each endpoint. The interesting factor is that these results held true irrespective of the diagnostic label. In all instances the predictive values for FeNO were significantly greater for almost all other baseline predictors, with an optimum cut-point of 47ppb (206).

1.7 - Asthma control and exhaled nitric oxide:

FeNO measurement offers both the individuals and the clinicians' notification of loss of control. The general understanding is that, if a patient's FeNO level increases by more than 60% from one visit to the next, even in the absence of asthma symptoms, this increase has a positive predictive value of over 80% of an imminent deterioration in asthma control (209). Loss of control is different to exacerbation. Loss of control can be reversed by increasing the ICS and SABA dose, however, exacerbation requires systemic steroids to reduce the inflammation. FeNO measurement also offers prediction of asthma relapse. In a study by Pijnenburg *et al.* (2005) (210) when asymptomatic children in clinical remission stopped taking steroids, a FeNO level of more than 49ppb 2-4weeks later was an effective predictor of asthma relapse (210).

In a study by Szeffler *et al.* (2005) (211) it was investigated whether the equivocal role of FeNO in clinical asthma management could be made more certain as a reliable biomarker of airway inflammation. The study consisted of a randomised, double-blind, parallel-group trial at 10 centres in the USA. 780 inner-city adolescents with persistent asthma were screened. All participants were entered into a three week run-in period based on a standard treatment regime. After this time, 546 participants who demonstrated adherence to the run-in phase of treatment were randomly assigned to a programme of either 45 weeks of standard treatment on the basis of measurements of FeNO or control

group based on conventional asthma management plans. For this study, the primary outcome measure was the number of days a participant presented with asthma symptoms (211). The results of the study showed that the mean number of days with asthma symptoms did not differ between the treatment groups in the FeNO monitoring group versus the control group. All other measurements including, lung function and asthma exacerbation did not differ between the two groups (211).

The conclusions drawn from the study were that conventional asthma management plans resulted in good control of symptoms in most participants. Using FeNO as an additional control measure of asthma resulted in higher doses of ICS, without clinically important improvements in symptomatic asthma control (211). This is an important result as at times research produces results which look impressive in terms of statistical significance, however in relation to clinical improvement they are insignificant and little or no improvement is seen. With regard to the patients in the clinical setting, it is a significant improvement in symptoms which is ultimately most important.

Following the results from the Szeftler *et al.* (2008) (211) study, a similar study by de Jongste *et al.* (2009) (212) based on telemonitoring of FeNO levels in 151 children produced similar results. Participants were randomly assigned to one of two groups: FeNO plus symptom monitoring, or monitoring of symptoms only. All participants were asked

to score their asthma symptoms in an electronic diary over 30 weeks; of these 77 were given a portable FeNO analyser. Data were transmitted daily to the coordinating centre. Every three weeks, participants were telephoned and steroid dose was adapted according to FeNO and symptoms, or according to symptoms. The primary endpoint was the proportion of symptom-free days in the last 12 weeks of the study.

The results demonstrated that telemonitoring was feasible with reliable FeNO data for 86% of days, and valid diary entries for 79% of days (212). Both groups showed an increase in symptom-free days, improvement of FEV₁ and quality of life, and a reduction in steroid dose. None of the changes from baseline differed between groups. The difference in symptom-free days over the last 12 weeks was 0.3%. There was a trend for fewer exacerbations in the FeNO group. These trends may purely be representative of patients becoming more compliant to their medication regimes due to the fact that they knew they were in a clinical trial (212). The conclusions drawn from the study were that 30 weeks of daily FeNO monitoring and symptom telemonitoring was associated with improved asthma control and a lower steroid dose. There was no added value of daily FeNO monitoring compared with daily symptom monitoring alone (212). Both of these studies indicate that there is no added benefit to using FeNO measurements to evaluate asthma control.

A study by Van Den Toorn *et al.* (2001) (213) looked at asthma control in a different way. It is clear trend that symptoms of atopic asthma often

disappear during puberty (213). Significant airway remodelling was found in subjects in clinical remission. This study has shown that there is ongoing airway inflammation and airway remodelling in adolescents in clinical remission of atopic asthma. Subclinical airway inflammation may well determine the risk of an asthma relapse later in life. In conclusion, FeNO levels may reflect airway inflammation and remodelling in asymptomatic individuals and this may be able to be used on a daily basis to predict potential relapse in the future (213).

It is clear to see that the evidence from different studies provides different conclusions regarding the efficacy of using FeNO as a predictor of asthma relapse / loss of control. More studies are needed before a conclusive answer can be determined. The answer may be that FeNO is best used to personalise individual treatment regimes and management plans based on individual changes from baseline over time. Large population studies are needed to assess the efficacy of FeNO as a management tool in patients who present with widely variable asthma phenotype profiles.

1.8 - Asthma exacerbation and exhaled nitric oxide:

In terms of asthma research, an area which shows a distinct lack in the number of studies, is looking at the relationship between FeNO and asthma exacerbation prediction in adults. Leuppi *et al.* (2001) (214) addressed the issue that it would be helpful to have predictors for failure or success of a planned ICS reduction. 50 participants with well-controlled asthma, taking a median dose of 1000 micrograms per day had their ICS dose halved every 8 weeks. Measurements of hyperresponsiveness to bronchial challenge test with histamine were measured at baseline. Airway hyperresponsiveness to bronchial challenge test with mannitol, spirometry, FeNO, and, in 31 subjects, sputum inflammatory cells were recorded at baseline and at subsequent monthly intervals (214). The results were as follows: 39 subjects suffered an asthma exacerbation and 7 subjects were completely weaned off ICS without any indication of loss-of control or exacerbation. Analysis of the Kaplan-Meier survival method indicated that the significant predictors of a failure of ICS reduction were being hyperresponsive to both histamine and mannitol at baseline ($p=0.039$), and being hyperresponsive to mannitol during the dose-reduction phase of the study ($p=0.02$). It was also indicated that subjects who were older than 40 years tended to be at greater risk of ICS reduction failure than those under 40 years ($p=0.059$) (214). It was observed that subjects over 40 years of age in the study population had a significantly lower mean FEV₁ percentage predicted and longer duration of asthma than those less than 40 years of age. It is understood that there is some

evidence that as an inflammatory state persists overtime, anatomical and functional airway changes are more likely to occur (215). Response to mannitol and percentage sputum eosinophils were significantly greater before a failed ICS reduction, whereas there were no significant differences in symptoms, spirometry or FeNO. There was also a correlation demonstrating that an increase in sputum eosinophilia over the course of step-down (but not high levels at baseline) was also a significant predictor of failure of ICS reduction. The final set of results indicated that lung function and FeNO measurements did not have any significant value in predicting exacerbation following ICS reduction at any time period. The conclusion drawn in relation to FeNO was that “ICS treatment leads to sustained inhibition of inducible NO synthase (214), so that FeNO does not necessarily increase during exacerbation if ICS medications have been taken recently” (214). However, this theory has not been conclusively studied to date and therefore in this study the data indicated that FeNO is not a significant predictor of exacerbation or ICS reduction failure (214).

A similar study by Deykin *et al.* (2005) (216) investigated the feasibility of using sputum eosinophils, airway hyperresponsiveness and FeNO in predicting an asthma exacerbation following substitution of ICS for either salmeterol or placebo (216). The trial consisted of 164 subjects with mild-to-moderate asthma assessed over 16 weeks. As would be expected, in comparison with continued ICS use, a switch to salmeterol or placebo was associated with increased rates of asthma deterioration

over the 16 week period (9.3% versus 24.1% and 37.5% respectively; $p=0.04$ and $p<0.001$ respectively) (216). The results obtained for FeNO were similar to those gained in the study by Leuppi *et al.* (2001) (214). FeNO was not a significant predictor of subsequent asthma control in subjects who discontinued ICS (216). However, unlike Leuppi *et al.* (2001) (214) Deykin *et al.* (2005) (216) also found that bronchial challenge test with methacholine PC₂₀ was not a significant predictor either (216). Like Leuppi *et al.* (2001) (214), however, both induced sputum counts measured at 2 weeks after a switch from ICS to placebo and changes in sputum eosinophil counts from before ICS cessation to after a switch to placebo predicted subsequent asthma deterioration (area under the ROC curve 0.771 [$p<0.001$] and 0.825 [$p<0.001$], respectively (216). The conclusion in relation to FeNO is that it demonstrates limited utility as a predictive marker of exacerbation. Yet on the basis of a model treatment strategy, the study estimates that allocating patients to ICS therapy on the basis of changes in sputum eosinophil counts after a trial discontinuation could allow 48% of subjects with mild-to-moderate asthma to discontinue ICS therapy without an increased risk of asthma deterioration over a period of at least 14 weeks (216).

With two studies demonstrating the significant efficacy of sputum eosinophil cell counts as a predictive marker of ability to stop ICS therapy and future exacerbation, the discussion is underway as to whether sputum analysis should become routine in all asthma testing. It

is not currently included in the guidelines for routine use within clinical practice, however, the evidence suggests that it may in fact be a valid and useful test (216) and could provide a rationale for more widespread evaluation of sputum eosinophil counts for the optimal management of asthma (216).

In another study by Shaw *et al.* (2007) (172) FeNO was investigated for its utility as a predictive measure of asthma exacerbation, in a similar manner to the two studies mentioned previously. The aim of this study was to assess whether titrating ICS dose using FeNO results in a reduction in the number of asthma exacerbations and thus whether FeNO measurements could result in more efficient use of ICS in comparison to traditional asthma management regimes (172). 118 participants who were previously diagnosed with asthma in the primary care setting (i.e. by traditional spirometry diagnostic methods) were randomised to a single-blind trial of ICS therapy based on either FeNO measurements (n=58) or BTS guidelines (n=60). All participants were assessed monthly over a 4 month period and subsequently every 2 months for a further 8 months. The primary outcome measure was defined as the number of severe asthma exacerbations (172). The results of this study were as follows: the estimated mean exacerbation frequency was 0.33 (S.D 0.69) per patient per year in the FeNO group and 0.42 (S.D 0.79) in the control group treated in line with BTS guidelines. Overall, the participants being treated in the FeNO group used 11% more ICS (95% CI, -17 to 42%; p=0.40), although the final

daily dose of ICS was lower in the FeNO group (557 versus 895 micrograms; mean difference, 338 micrograms; 95% CI, -640 to -37; $p=0.028$) (172). The use of FeNO measurements to determine treatment decisions in asthma did not result in lower exacerbation frequency or in a lower maintenance dose of ICS when compared with traditional BTS guidelines asthma management. However, participants in the FeNO group completed the study on lower ICS doses than the control group, but the use of ICS over the entire 12 months of the study did not differ between either group (172). Both groups experienced fewer exacerbations overall when compared to the previous year, however, this may attributed to more intensive monitoring in the study setting and increase in patient compliance due to being in a trial. These results are consistent with findings from the two studies mentioned previously by Leuppi *et al.* (2001) (273) and Deykin *et al.* (2005) (214, 216).

The three previous studies (Leuppi *et al.* (2001) (273); Deykin *et al.* (2005) (214, 216) and Shaw *et al.* (2007) (172)) have all looked at absolute FeNO values and cut-off figures. There is no significant evidence to state that a specific FeNO cut-off value at a specific flow rate should be used alone as a determining measure of airway inflammation and future exacerbation. There is some evidence to suggest that percentage changes in FeNO and individually tailored FeNO values may provide more useful information on underlying changes in airway inflammation in comparison to set cut-off values

based on demographic data. For example, a patient may have low FeNO values and still suffer an asthma exacerbation. The individual change in FeNO may be an important factor as opposed to absolute figures.

A study by Michils *et al.* (2008) (217) examined FeNO measurements using this change in FeNO approach. ACQ and FeNO values were recorded in 341 unselected patients with asthma. The whole population and subgroups were considered, i.e. both ICS-naïve and low or high-to-medium (\leq or $>$ 500 micrograms BDP equivalents per day) ICS groups. The study concluded that a fall in FeNO by more than 40% from baseline had a positive predictive value of 83% for predicting optimal asthma control (217). The authors concluded that measuring changes in FeNO overtime in individuals is more useful than using single cut-off points in predicting and diagnosing loss of asthma control (217).

In conclusion, there have been a few studies which have investigated the use of FeNO as a predictor of exacerbation and the results are mainly insignificant. However, looking at changes in FeNO overtime as opposed to whole-population cut-off figures has yielded better results.

1.9 - Summary:

Exhaled nitric oxide as a biomarker:

A biomarker is a surrogate marker which can be utilised clinically to understand, characterise and quantify an underlying disease process (218, 219). In many respiratory diseases confirmation of the pathology can be sought using tissue biopsy, however the cost to benefit ratio is too high to make biopsy a routine monitoring tool. Imaging is also available to assess anatomical physiology and respiratory measurements can identify some changes in lung function (e.g. spirometry). However, both imaging and lung function testing are limited. They do not easily track the variability of asthma, they are costly, time consuming and imaging services are largely unavailable in primary care (220).

Exhaled nitric oxide is a non-invasive biomarker of airway inflammation. It is produced by airway wall cells (including epithelial cells), and levels are increased in patients with atopic asthma and rhinitis. Exhaled nitric oxide has been used in studies to predict ICS response and track deterioration on discontinuation of therapy (221).

The performance of exhaled nitric oxide as a biomarker:

The success with which a biomarker operates in diagnostic decision making ultimately depends upon its performance. A sensitive test identifies most patients with a specific condition or disease but can also pick up some patients without the disease. A sensitive test can also rule

out a condition when the result is strongly negative. It is said to have a high negative predictive value or a low negative likelihood ratio. A specific test is a test which identifies people who have a positive test as having a condition or a disease, but some affected patients may be missed. A specific test can rule a condition in when the result is positive. It is said to have a high positive predictive value or a strong likelihood ratio. The severity and presentation of the disease or condition being tested by the biomarker would determine whether it would be best to use a highly sensitive or highly specific diagnostic tool.

An example would be a D-Dimer assay. A normal D-Dimer has a high negative predictive value for venous thromboembolism (VTE >95%) and helps to accurately rule out VTE. However, D-Dimer is associated with only modest positive predictive values and cannot be used therefore as a positive predictor clinical tool (it is not a specific test). Therefore when a D-Dimer is normal clinicians know there is a less than 5% chance that the patient has a VTE. This means that D-Dimer is a sensitive test, this is important because in VTE it is important not to miss patients. When D-Dimer is raised, the clinicians cannot associate the raised value with a definitive diagnosis of VTE and therefore further tests are employed to confirm or deny the diagnosis (222, 223).

The ideas for FeNO use may be similar to that of D-Dimer.

In a study by Dupont *et al.* (2003) (224) the pre-test probability of diagnosis asthma was 67%. The positive predictive value of exhaled

nitric oxide for diagnosing asthma was 90% and the negative predictive value was 90%. In a study by Smith *et al.* (2004) (225) the pre-test probability was lower than Dupont *et al.* (2003) (224) at 36%; the positive predictive value for diagnosing asthma with exhaled nitric oxide was 70% but the negative predictive value was higher at 92%. These and other data by Pedrosa *et al.* (2010) (226) indicate that exhaled nitric oxide values (like D-Dimer), provide more reliable clinical information when the value is low. This however, does not preclude using high values in clinical decision making, but the weight of a high value needs to be appropriately modified to reflect the differing performance characteristics of exhaled nitric oxide as a biomarker (221).

Clinical decisions are generally based on whether an individual result is greater or less than a chosen cut-point for the biomarker. Low/normal values generally indicate the absence of underlying disease activity, and high/abnormal values indicate its presence (227). However, cut-points tend to differ between studies depending on the nature of the trial. For example different studies investigating exhaled nitric oxide may use one, two or even three different cut-points.

The table below examines three different studies which employ FeNO as a tool for monitoring ICS treatment and the cut-points associated with the different trial designs.

Table 1.2:

FeNO cut-off values chosen for different FeNO based intervention studies.

Summary of studies investigating exhaled nitric oxide as a tool for monitoring ICS treatment in asthma			
	Smith (228)	Pijnenburg (194)	Shaw (172)
Design	Single blind Parallel group	Single blind Parallel group	Single blind Parallel group
Population	97 adults Mild-moderate asthma	85 children Atopic asthma	118 adults Primary care diagnosis of asthma
Duration	12 months	12 months	12 months
Intervention	Control: Conventional measures of asthma control Intervention: FeNO <15ppb (250mL/s flow rate)	Control: Control of symptoms Intervention: Control of symptoms and FeNO <30ppb	Control: Control of symptoms Intervention: FeNO <26ppb
Symptoms	Symptoms ≤ 2days/week Night symptoms ≤ 1/week β ₂ ≤ four times/week and ≤ 2days/week PEF variation ≤ 20% FEV ₁ ≥ 90% of baseline	Symptom score >14 (over two weeks) Symptoms ≤ for the first time Symptom score ≤ for the second time	ACQ > 1.57 ACQ <1.57 x 2 visits
FeNO and treatment steps	Controlled if FeNO < 15ppb 250mL/S (35ppb at 50mL/s) = decrease one dose step if controlled Uncontrolled ≥ 15ppb	FeNO >30ppb = increase ICS FeNO ≤ 30ppb and symptom >14 = ICS no change FeNO ≤ 30ppb and symptom ≤ 14 = decrease ICS	FeNO > 26ppb = Double ICS FeNO < 16ppb = Halve ICS FeNO < 16ppb + ACQ >1.57 = +LABA
Outcomes	Decreased exacerbations Decreased ICS dose	Improved AHR and AI Non significant change in ICS dose or exacerbations	Decreased exacerbations Increased ICS dose

In these studies the FeNO cut-points were chosen based upon the upper limits of normal exhaled nitric oxide values derived from a healthy control population (212, 229, 230) or from patients with stable asthma (228). In a study by Shaw *et al.* (2007) (172), a low cut-point, validated against corresponding sputum eosinophil counts, was used to determine the likely absence of underlying disease activity (low exhaled nitric oxide and high negative predictive value for inflammation, and therefore the ICS was reduced). In contrast Hewitt *et al.* (2009) (231)

used a high cut-point related to likely steroid responsiveness (high exhaled nitric oxide and moderate positive predictive value for steroid response, and therefore the ICS dose was increased). The differences between these two studies highlight the difficulties in standardising exhaled nitric oxide as a clinical biomarker.

Exhaled nitric oxide and improving clinical outcomes:

Current asthma guidelines (2, 25) recommend treatment decisions are made based upon the assessment of asthma control using a combination of symptoms and lung function (2, 25).

However, studies which employ biomarkers differ in that they seek improved outcomes by basing treatment decisions upon the biomarkers in question (e.g. airway hyperresponsiveness (232), sputum eosinophils (39, 233), eosinophilic cationic protein (ECP) (234) and exhaled nitric oxide (172, 228-230, 235, 236). All such studies use calculations which provide recommendations for up and down-titration of asthma treatment based upon inflammatory biomarkers. These types of studies are important for improving patient care as research indicates that asthma control remains suboptimal despite the extensive clinical guidelines and effective asthma treatments (237). There is also evidence to suggest that over-treatment of asthma with inhaled corticosteroids is also occurring due to poor compliance with step-down guidelines (238), and further evidence that a different subset of patients remain under-treated (239, 240) and that medication compliance needs to improve (241, 242). Simple biomarker tests may improve the treatment and compliance

regimes in asthma management. Biomarker studies have the potential to improve asthma pharmacotherapy by individualising treatments and adjusting doses based upon personal response, as well as increasing patient participation in pharmacotherapy, thus enhancing compliance and adherence and improving monitoring and subsequent control of asthma. The use of clinical biomarkers can lead to improved asthma outcomes at a similar or lower cost.

The use of sputum eosinophils as a biomarker to guide asthma therapy has reported a high significant reduction in the likelihood of asthma exacerbation by 50% (233). Such studies could be considered the closest studies to a “gold standard” in the use of biomarkers to determine inhaled corticosteroid dose in asthma. The problem with sputum induction is that it is not available in primary care and requires specialist equipment and staff to process the samples. Additionally, although the test is considered relatively safe, it is not feasible to use it as a regular test and many patients with mild to moderate asthma are unable to produce sputum.

Exhaled nitric oxide, which is more accessible and simpler to measure has been used as a surrogate biomarker for sputum eosinophils in five recent studies (172, 194, 228, 229, 236) However, unlike sputum eosinophil studies, exhaled nitric oxide based studies are less successful (172, 194, 228, 229, 235, 236), and unlike sputum

eosinophils, exhaled nitric oxide shows now significant reduction in asthma exacerbation rates to date.

The results of these studies are discordant and only one study by Smith *et al.* (2005) (228) has shown a positive effect of reduced maintenance inhaled corticosteroid dose with exhaled nitric oxide guided therapy. Key points in exhaled nitric oxide studies are the assessment of improved selection of active treatments based upon individual response, and improved titration of treatment using exhaled nitric oxide related treatment outcomes. In asthma, inhaled corticosteroids are the treatment of choice to reduce airway inflammation; but guidelines recommend that treatment decisions are based upon a combination of symptoms and spirometry (2, 25) despite the fact that these variables demonstrate poor correlation with airway inflammation (243). Consequently, these studies have been performed to seek and evaluate the efficacy of treatment and dose adjustment using exhaled nitric oxide as a marker of eosinophilic airway inflammation.

Exhaled nitric oxide responsiveness:

A clinical biomarker needs to be responsive to changes in treatment, responsive to changes in the clinical condition, and sensitive to detect changes early enough. Exhaled nitric oxide has been confirmed as highly responsive to inhaled corticosteroid dosing changes. A strong linear dose-relationship is seen for sputum eosinophils and changes in exhaled nitric oxide also correlate significantly with changes in sputum

eosinophilia (244). As part of this thesis additional data analysis examined the same relationship between exhaled nitric oxide and sputum eosinophil count, the results of this analysis can be found in Chapter 5.2. Encouragingly the results obtained in the studies in this thesis correlated well with the findings of previous studies.

In a study by Silkoff *et al.* (2001) (245) exhaled nitric oxide was shown to be able to detect a dose effect between 100 and 800 micrograms bdp, whereas FEV₁ was not able to detect the same changes (245).

FeNO is considered to be a responsive biomarker, however, the best use for this simple clinical tool needs to be confirmed through further research.

Exhaled nitric oxide reproducibility:

A biomarker needs to be highly reproducible in order to produce accurate and clinically relevant results. Measuring exhaled nitric oxide using chemoluminescence has been shown to be highly reproducible by Taylor *et al.* (2006) (244) and Gabbay *et al.* (1998) (246).

There are some issues with confounding factors when measuring exhaled nitric oxide. While it have been shown that exhaled nitric oxide values are reduced when inhaled corticosteroid is started for treatment of eosinophilic airway inflammation, exhaled nitric oxide is also influenced by factors other than eosinophilic airway inflammation. These include age, atopy, gender, smoking, BMI and genetic variation. This can have the effect of leading to a false negative or false positive

exhaled nitric oxide result when compared to the “gold standard”. In a study by Shaw *et al.* (2007) (172), the relationship between exhaled nitric oxide and sputum eosinophilia was imperfect, resulting in many false-positives when an exhaled nitric oxide cut-point of 26ppb was used.

This limitation surrounding exhaled nitric oxide cut-points can be partially improved by using exhaled nitric oxide in combination with other tests to produce composite markers. Using exhaled nitric oxide in combination with spirometry (247) or airway hyperresponsiveness (248) may improve the prediction of future exacerbation risk. In a different study by Gelb *et al.* (2006) (247), using FEV₁ in combination with exhaled nitric oxide improved the predictive value for asthma exacerbations. A raised exhaled nitric oxide in combination with a reduced FEV₁ percent predicted had a likelihood ratio of 5.94 for predicting subsequent asthma exacerbation.

Biomarker studies are also concerned with determining methods to individualise the marker by investigating individual change from baseline. In a study by Michils *et al.* (2008) (217) change in individual exhaled nitric oxide from baseline had a better predictive value for future loss of control than using “whole-population” fixed cut-points.

The measurement interval of exhaled nitric oxide:

Some clinical markers such as blood glucose level change rapidly over a few hours, whereas others for example HbA1C change more slowly

over a period of weeks or months (249). Exhaled nitric oxide has been shown to rapidly reduce in response to dose-dependent inhaled corticosteroid therapy in asthma. This reduction occurs in the short-term (3-5 days post treatment commencement) (244, 245). Similarly the opposite effect is also true and exhaled nitric oxide levels rise rapidly during the first 3-5 days post cessation of inhaled corticosteroid therapy. There is however, a limiting factor with exhaled nitric oxide measurement and that is the presence of patients who present with a persistently high exhaled nitric oxide which is not reduced by inhaled corticosteroid medication but may still have elevated sputum eosinophils. This phenomenon has been identified and studied by Pijnenburg *et al.* (2005) (250) and seems to affect a small percentage of the asthmatic and non-asthmatic population. Shaw *et al.* (2007) (172) also addressed this occurrence (which was seen in approximately 15% of study subjects), by using sputum eosinophils in combination with exhaled nitric oxide to identify these individuals.

A more recent trial involving exhaled nitric oxide, referred to as the BASALT trial (3), compared “as-needed, symptom-based inhaled corticosteroid use”, as well as exhaled nitric oxide based dosing, with traditional daily inhaled corticosteroid use with dosing determined by a physician. The trial addresses the potential utility of measuring exhaled nitric oxide as a biomarker to guide adjustment of asthma medications (3). Although exhaled nitric oxide is associated with allergic inflammation and the risk of asthma exacerbation, prior studies have

not provided sufficient evidence for the justification of monitoring exhaled nitric oxide as part of routine asthma management (211, 251). In the BASALT trial, dose adjustment of inhaled corticosteroid therapy in response to exhaled nitric oxide monitoring was not shown to be better than the physician lead and symptom lead dosing. The results are consistent with other studies which do not provide conclusive justification for the use of routine exhaled nitric oxide monitoring in asthma management. A recent ATS practice guideline (252) recommends the use of exhaled nitric oxide measurement in monitoring airway inflammation in patients with asthma. However, the results of the BASALT study indicate that it is difficult to justify additional healthcare expenditure for routine exhaled nitric oxide monitoring in mild to moderate adult asthma.

Exhaled nitric oxide has desirable properties as a biomarker to assess airway inflammation and adjust inhaled corticosteroid dosing but requires more specific studies to assess its place in clinical practice.

1.10 - The diagnosis and management of asthma in primary care:

In the UK, approximately 9% of the population have a diagnosis of asthma. Approximately 80% of all patients with asthma are managed solely in primary care (2). The majority of mild to moderate well-controlled patients with asthma will only visit their GP or practice nurse once a year for an annual asthma review consisting of a peak flow measurement. For this reason it is important that the diagnosis and management of asthma in primary care is adequate and carefully monitored to ensure patients receive the appropriate medical care.

It is important to mention that the definitions, goals and guidelines for asthma management and diagnosis are the same for primary care and secondary care, however there are often disparities between the services due to time and equipment constraints.

In 2004 The GMS (General Medical Services) Contract introduced a “pay for performance” scheme called The Quality and Outcomes Framework (QOF). This framework financially rewards GPs who achieve targets set over a wide range of clinical indicators. This is to encourage accurate diagnosis, maintenance of disease registers and proactive care of people who require medical attention for long-term conditions but who do not require hospitalisation and secondary care (253). Of the 1050 QOF points available, 35 of them are allocated to asthma indicators and 30 to COPD indicators with additional points

assigned to generic smoking cessation advice. Approximately 20% of GP income is related to the achievement of the QOF targets (254).

(A copy of the QOF indicators for respiratory diseases can be found in the appendix section 8).

Whilst the QOF system makes certain aspects of asthma management compulsory, it does not address the issue of diagnosis of asthma in primary care.

The lack of a “gold-standard” test means that conclusive diagnostic accuracy cannot always be obtained. In secondary care and research environments there is a greater provision of tests to assess lung function and airway inflammation but even so these tests do not provide definitive diagnoses of asthma. Rather they serve to build up a bigger range of valuable clinical data to support a diagnosis of asthma. However, these tests are not routinely available in primary care settings. This is usually because they are too costly, too time consuming and too complex (often requiring specialist input into the processing and interpretation of results). As a result asthma diagnosis in primary care can be difficult. Before an asthma management plan can be implemented, patients firstly need to be accurately diagnosed. Guidelines from GINA, ATS, BTS and SIGN recommend the assessment of symptoms and measurement of lung function in primary care (2, 25). However, there is a poor relationship between symptoms and lung function and as such asthma is often misdiagnosed. Thus,

whilst lung function testing and symptom assessment is of great value, over-reliance on these techniques (especially in primary care) can be misleading. This has led to increasing evidence that there is a lack of diagnostic rigour in primary care.

A study by Dennis *et al.* (2002) (255) in GPs in the UK reported that 56% of children and 45% of adults received some form of asthma treatment before a diagnosis was established (255).

In addition, lung function results were recorded for <20% of people diagnosed with asthma in primary care. The proportion was even lower in patients who were immediately prescribed anti-asthma medications. The final issue noticed in primary care diagnosis of asthma is the time it takes to establish a firm diagnosis. In the majority of patients and children it is estimated to be around 18 months. But diagnostic delays of up to five years have been reported in some GP surgeries in the UK (256). The problem is not restricted to the UK, across Europe and The USA studies have identified the same trends (257).

Once a diagnosis of asthma has been decided upon and an anti-asthma medication has been prescribed, a future management plan for the patient should be implemented. The management of asthma in both primary and secondary care should follow the same stepwise approach (Figure 1.2). The QOF system ensures that all patients receive an asthma review each year, however the subsequent management of

patients varies from practice to practice, however there are no QOF targets addressing these management issues.

Figure 1.2:

Summary of stepwise management of asthma in adults from the British Thoracic Society guidelines (2).

The stepwise approach diagram below was designed by the British Thoracic Society, however the initial research into step-down was performed by Hawkins et al. (2003) (258).

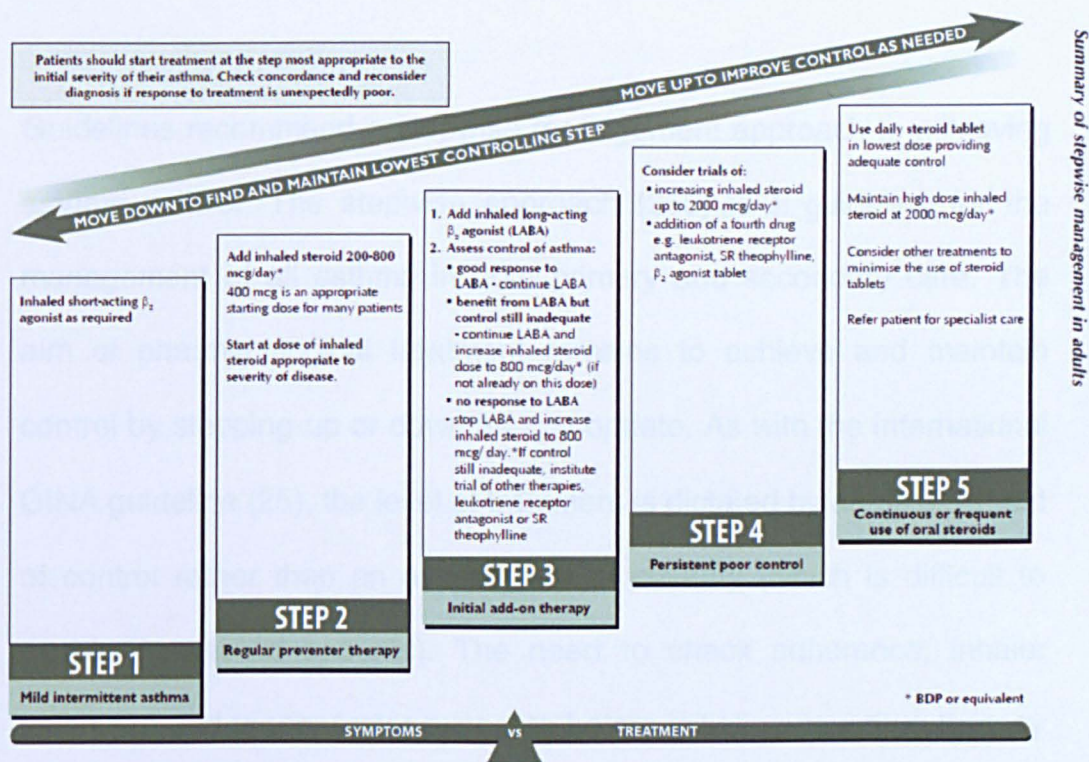


Figure reproduced from the British Thoracic Society Guidelines on the Management of asthma (2).

The BTS guidelines now include a monitoring and management statement within the diagnostic section (2). Effective management

serves to facilitate the diagnostic process by recording and monitoring patient response to treatment thus reinforcing or reducing the probability of asthma whilst also providing clinicians and patients with information to underpin treatment and or referral decisions.

Various tools including composite symptom and quality of life scores, lung function tests, exacerbations, inhaler technique and compliance assessment systems are available for clinicians to use in day to day practice (2). Questionnaires such as the Asthma Control Test™ (ACT™) and Asthma Control Questionnaire (ACQ) are particularly useful validated questionnaires in primary care (259, 260).

Guidelines recommend a stepwise management approach to achieving asthma control. The stepwise approach (258) is a guideline for the management of all asthma in both primary and secondary care. The aim of pharmacological treatment remains to achieve and maintain control by stepping-up or down as appropriate. As with the international GINA guideline (25), the level of treatment is dictated by an assessment of control rather than an assessment of severity (which is difficult to assess in treated patients). The need to check adherence, inhaler technique and trigger factor exposure before initiating new drug therapy is stressed as “vital” to primary care GPs.

1.10.1 - Over-treatment with inhaled corticosteroids:

Under-diagnosis and under-treatment are considered to be a major problem in the management of asthma and COPD in primary care and worries exist about the consequences of under-treatment (261). It is stressed to GPs that symptoms indicating asthma have to be interpreted more carefully as indicators of disease (262). According to international guidelines, a diagnosis should be confirmed by GPs using spirometry (263) and consequently correct levels of medication can be prescribed, and thus the problem of under-treatment is beginning to diminish in primary care. Combined with the growing awareness of asthma in the general population this practice has begun to lead to over-treatment with inhaled corticosteroids (ICS). When the symptom complaints disappear, a healthy person using ICS may be mistaken for being well-controlled and thus be advised to continue treatment with ICS. This may particularly be the case in patients who may have had viral infections. The recent development of diagnostic support services in European primary care (264-266) has triggered awareness that over-treatment is an increasing problem. These services offer GPs the possibility to refer all their patients with respiratory problems for diagnosis and advice. These services are not yet available in the UK and they still do not overcome the problem of over-treatment.

In a study by Lucas *et al.* (2008) (238) the primary care services and the diagnostic support services offered to patients with asthma were assessed through the use of questionnaires and analysis of patient

records. Of the 2271 patients who had their diagnosis reassessed, 36% were diagnosed as asthmatic, 19% as COPD, 6% as asthma and COPD, 20% as neither asthma nor COPD and 19% as unclear diagnosis. Of 1177 taking an ICS medication in 505 patients the use of ICS did not match the results of the spirometry or the medical history. Of these 505 patients, 133 were able to completely stop their ICS medication with no worsening of their symptoms at three months (238). This study only looks at the outcome of patients who had their ICS medication stopped completely, but in reality the BTS guidelines suggest a stepwise reduction (2). In a study by Hawkins *et al.* (2003) (258) stepping-down of ICS medication was evaluated in a randomised controlled trial. The results showed that 49% of participants in the step-down group completed the study taking a 50% reduced dose of the ICS with good control (258). The study was based on existing evidence which states that a stepwise approach to ICS reduction should be implemented, evidence shows that a reduction can be achieved in patients with mild disease and the clinical implications of stepping-down ICS in moderate to severe disease are not conclusive. The results of the study (258) show that adopting a step-down approach to the use of high dose ICS in patients with stable asthma can lead to a significant reduction in the daily dose of ICS without compromising asthma control (258).

1.11 - Studies assessing the feasibility of exhaled nitric oxide monitoring for asthma in UK primary care:

In a study by Gruffydd-Jones *et al.* (2007) (267), the feasibility of using FeNO as an asthma monitoring tool in primary care was assessed.

In this study, 22 adults and 15 children completed the study which involved FeNO measurements being performed by the practice nurse at two-week intervals over a period of 12 weeks. The primary aim of the study was to investigate the feasibility of measuring exhaled nitric oxide in children (over six years of age) and adults, during the standard asthma review appointment in primary care. The secondary outcome was to measure the variability of FeNO readings over time and to collect comparative data on the relationship between FeNO and measures of asthma control such as symptoms, exacerbation, medication use and asthma-related health status (267).

The study assessed the stability of FeNO measurements in individual patients, and the relationship changes between changes in FeNO readings and changes in other primary care parameters of asthma control. Previous hospital-based studies had reported weak cross-sectional and longitudinal correlations between FeNO readings and other parameters of asthma control such as lung function, symptomatic control, and asthma-related health status. In the study performed by Gryffydd-Jones *et al.* (2007) (267), correlations of a similar magnitude were observed and generally failed to reach statistical significance, probably due to the small population size. The study found weak and

inconclusive relationships between FeNO readings and both physiological and patient-centred outcome measures after cross-sectional analysis, there were weak but non-significant relationships between FeNO and lung function parameters after longitudinal analysis. There were however, statistically significant relationships between rising FeNO and worsening lung function (267). The study concluded that FeNO monitoring in primary care is feasible with most patients age six years and over being able to perform the test successfully. The primary care practitioners found the test acceptable and easy to use, but with no statistical significance between FeNO and lung function the team concluded that further studies are needed to investigate the clinical effectiveness and cost-effectiveness of such technology in the primary care diagnosis and management of asthma (267).

Hewitt *et al.* (2008) (268) performed a study to investigate whether FeNO in primary care can help GPs in the diagnosis of patients who present with non-specific respiratory symptoms. The team hypothesised that FeNO, coupled with spirometry would improve diagnostic and therapeutic decision making as well as enhancing clinician confidence when assessing patients presenting with non-specific respiratory symptoms (268).

55 patients aged from 12 to 80 years presenting with non-specific respiratory symptoms were recruited from 14 GP practices into the study. The patients were required to have a history of cough, wheeze or shortness-of-breath for at least six weeks and with no previous

respiratory diagnosis or if their previous diagnosis was deemed as uncertain. The results of the study confirmed that FeNO measurements obtained in primary care offered helpful diagnostic information. The primary outcome was to demonstrate that FeNO coupled with spirometry improves diagnostic accuracy in primary care patients. When asked, GPs deemed that FeNO improved diagnostic accuracy in 94% of cases (268). The results showed that spirometry was normal in the majority of cases (88%) and was only considered helpful in aiding the diagnosis of non-specific respiratory symptoms in 53% of cases. This is, in contrast to FeNO which clinicians deemed to be relevant in 94% of cases of uncertain diagnosis. The reason for this is likely to be because FeNO measurements are a surrogate marker for eosinophilic airway inflammation (69, 269, 270), which in turn indicates the likelihood of steroid-responsiveness (271, 272). Hence, in patients with non-specific respiratory symptoms and high FeNO levels, a positive response to inhaled corticosteroids may be anticipated (206), thus FeNO levels may be useful in guiding treatment decisions and management.

Deciding whether or not to prescribe a trial of ICS is often decided empirically, and this aspect of management was simplified in the study using FeNO. Follow-up evaluation confirmed that 14 out of the 17 patients with a high FeNO level in this study demonstrated a satisfactory clinical response when treated with inhaled corticosteroids.

The study also showed that both low and high FeNO levels proved to be meaningful in the interpretation of non-specific respiratory symptoms (271, 273). This is not the case for changes in lung function, where only low values are clinically instructive. The study also showed that access to FeNO measurements improved diagnostic confidence, the follow-up diagnosis at three-month review was different in 10 out of 51 cases (20%). In these cases the FeNO level was either intermediate (20-35ppb) or low (<20ppb) at the point of initial diagnosis. Neither FeNO measurements nor spirometry could distinguish between GORD, non-specific cough, post-viral respiratory symptoms and anxiety hyperventilation (268).

The study was not designed to confirm or complement previous studies which investigated the overall utility of FeNO measurements (224, 225, 274). Rather it was used to assess the usefulness of adding FeNO into a busy general practice. As with the Gruffyd-Jones study, 2007 (267), this study confirmed that FeNO can be successfully implemented in general practice but there is further research required to confirm the utility of FeNO for diagnostic support in primary care. The feeling is that spirometry is limited in the information it can provide (275) and whilst the use of FeNO has not been conclusively determined, the test can provide complementary data which is useful in the assessment and management of respiratory patients in primary care.

Hewitt *et al.* (2009) (231) then performed a second study to investigate the use of exhaled nitric oxide specifically in the management of asthma in primary care (231). The primary aim of the study was firstly to assess whether an open-ended FeNO based protocol could be applied in the primary care setting, and the subsequent impact this would have on asthma outcomes. The secondary outcome was to evaluate the practical issues associated with using and interpreting FeNO levels in nurse-led primary care asthma clinics. 78 patients with asthma completed the study having attended five visits at three-monthly intervals where FeNO, symptom scores and spirometry were recorded at each visit. The inhaled corticosteroid treatment was then adjusted in response to FeNO levels (231). The treatment algorithm devised for this study based on previous research which indicates that FeNO values over 47ppb are associated with steroid responsiveness (274) or that the potential for relapse in asthma control when inhaled corticosteroid therapy is reduced or withdrawn (210) and that the upper limit of “normal” for patients with stable asthma is 33ppb (152). Thus 45ppb and 30ppb were chosen as cut-offs for the study.

The results of the study showed that based on the treatment algorithm presented for FeNO cut-off values resulted in an overall increase in the number of patients with well-controlled asthma (from 40% to 70% approximately). The improved asthma control also coincided with a progressive reduction in the dose of inhaled corticosteroid medication, notably in patients already being prescribed inhaled corticosteroid treatment (a 44.6% reduction). The results recorded were similar to

those recorded in a randomised controlled trial by Smith *et al.* (2005) (274). The study team concluded that although achieving asthma control in 70% of patients is less than ideal, these results are consistent with the GOAL study in which 60-70% of patients achieved well-controlled asthma involving progressive step-up in inhaled corticosteroid dose (27). Therefore, the FeNO algorithm presented in this study appear to achieve clinical benefits of the same magnitude as other clinical processes. The research concluded that using the FeNO algorithm to support decisions surrounding asthma management in primary care decreased the proportion of patients with poorly controlled asthma as well as a progressive reduction in inhaled corticosteroid dose requirements. The outcomes obtained in patients with persistently high FeNO levels suggest that fixed cut-points from FeNO are less likely to be helpful than individually determined optimum changes (231).

In conclusion, although the weight of evidence in the literature does not support the routine use of FeNO to optimise asthma diagnosis and management, measuring FeNO levels enables factors other than airway inflammation to be identified in patients with ongoing symptoms and this can now be done cheaply and easily in UK primary care (231).

Chapter 2 - Hypothesis and aims:

Previous research has demonstrated that exhaled nitric oxide is a simple clinical biomarker which would be feasible for use in UK primary care (267). It has been shown to correlate well with measures of sputum eosinophilia in the moderate to severe asthma cohort (269), and levels change rapidly in response to steroid medication (196, 245, 276). It has also been used to guide treatment regimes in an attempt to reduce exacerbation rates (172, 210, 277).

However, there have been no exacerbation studies to date which have investigated whether exhaled nitric oxide can be used as a clinical marker to distinguish between well-controlled patients with asthma who can and cannot successfully step-down their inhaled corticosteroid medication.

Overall hypothesis:

Exhaled nitric oxide can be used as a non-invasive biomarker of airway inflammation to predict which patients with mild to moderate asthma can successfully step-down their inhaled corticosteroid medication in UK primary care.

The data presented in this thesis aimed to answer five questions:

1. Can exhaled nitric oxide (either at baseline or change within seven days) be used to predict successful step-down of inhaled corticosteroid medication in patients with mild to moderate asthma in UK primary care?

2. Do asthma symptoms (as measured by the Juniper Asthma Control Questionnaire) reflect airway inflammation in patients with mild to moderate asthma?
3. Does exhaled nitric oxide reflect airway inflammation in patients with mild to moderate asthma?
4. Can exhaled nitric oxide be used in primary care to predict the likelihood of “true” asthma in patients with a suspected diagnosis of mild to moderate asthma?
5. Can exhaled nitric oxide can distinguish between eosinophilic and non-eosinophilic sputum in a mixed cohort of patients with asthma.

Chapter 3 - Methods:

All procedures were carried out according to ATS/ERS/BTS guidelines and followed standard operating procedures outlined within the guidelines and the manufacturer's instructions.

Specific safety measures were in place throughout all the tests in accordance with guidelines, University of Nottingham, Nottingham University Hospitals NHS Trust and Leicester University Hospitals NHS Trust health and safety procedures.

3.1 - Allergen sensitisation:

Atopy was assessed by skin prick tests to *Dermatophagoides pteronyssinus* (house dust mite), cat and dog fur, tree and grass pollen and *Aspergillus fumigatus* with normal saline and histamine controls (Alk-Abello™, Berkshire, UK). A positive response to an allergen on the skin prick tests was recorded in the presence of a weal >3mm and more than the negative (saline) control.

Participants were requested not to take any antihistamine medications for a minimum of 48 hours prior to the test.

3.2 - Spirometry:

Spirometry was performed with a Vitalograph™ spirometer (Vitalograph™, Buckinghamshire, UK). The spirometer was calibrated daily by a qualified lung function technician. Bronchodilator reversibility was assessed 15 minutes after administration of 400 micrograms of salbutamol inhaled via a Volumatic® spacer. FEV₁ was recorded as the

best of three successive readings within 100ml or 5%. Percentage predicted values were calculated using the ECCS guidelines.

The same spirometer was used throughout the study.

3.3 - Airway hyperresponsiveness:

Using the standard Juniper tidal breathing method, the concentration of methacholine causing a 20% fall in FEV₁ was recorded as the PC₂₀ FEV₁ (278). In brief, following the measurement of the baseline FEV₁ subjects inhaled normal saline followed by doubling concentrations of methacholine from 0.03mg/ml to 16mg/ml via a Wright's® nebuliser (Roxon, Canada) (flow 0.13ml/min driven by dry compressed air). The subject was instructed to breath normally (tidal breathing) through the two-way valve nebuliser for two minutes with a nose clip. The FEV₁ was then measured 30, 90 and 180 seconds after the two minute nebulisation period. If the FEV₁ fell less than 20% from baseline the procedure was repeated with the next highest concentration. If the FEV₁ fell more than 20% from baseline (or the highest concentration 16ml/mg had been given), no further methacholine was given. Methacholine PC₂₀ FEV₁ concentration was calculated by linear interpolation of the log dose response curve.

At the end of the bronchial challenge test 400 micrograms of salbutamol was administered via a Volumatic® spacer to the subject.

The same Wright's® nebuliser was used throughout the study.

3.3.1 - Calibration for methacholine challenge:

The output of the Wright's[®] nebuliser was assessed at baseline by a qualified lung function technician using the following protocol: 3ml of saline was placed into the nebuliser at room temperature. The solution was then weighed and then nebulised at a flow rate of 7l/min for two minutes. This process was repeated three times for a range of flow rates and the average output at each flow rate was calculated. The necessary flow rate was determined to give an output of 0.13ml/min. The calibration process was repeated at one monthly intervals by the same individuals.

The calibration for the study varied between 4 and 6 L/min.

3.3.2 - Safety procedures during methacholine challenge:

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

3.4 - Sputum induction:

The following protocol was used to obtain sputum from patients:

1. Measure baseline FEV₁ on three occasions.
2. Give 200 micrograms of salbutamol by MDI (Metered Dose Inhaler) and Volumatic[®] spacer.

3. After 20 minutes, measure post bronchodilator FEV₁ three times.
Use the best bronchodilator FEV₁ value to calculate any subsequent fall in FEV₁ during the procedure.

NB: Do not proceed if the FEV₁ after inhalation of the short - acting β_2 agonist is less than one litre.

4. Fill the nebuliser cup with 5ml of 3% pyrogen-free hypertonic saline.
5. Hold the nebuliser upright and do not adjust from the default maximum output setting. Ask the patient to breathe tidally, whilst taking a slightly deeper breath every minute. Do not use a nose clip. Discontinue the procedure if significant symptoms occur or if the patient experiences undue discomfort. Use of the Borg breathlessness scale allows patients to quantify any symptoms they may be experiencing. A discard vessel should always be available for the patient to spit out any excessive saliva generated during the induction.
6. After five minutes, ask the patient to rinse their mouth and throat with water and blow their nose in order to reduce squamous cell contamination and post-nasal drip.
7. Ask the patient to cough any sputum into a plastic sputum pot using a deep cough. Several attempts at coughing should be made until the sound of the cough becomes dry and unproductive.
8. Measure FEV₁ (three measurements will be made).

9. Repeat the above steps on two further occasions with 4% and 5% pyrogen-free hypertonic saline, respectively, if the FEV₁ has not fallen by more than 10% or 200ml (whichever is greater) of the best post-bronchodilator FEV₁ value. If the FEV₁ falls by more than 10% or 200ml (whichever is greater) but less than 20% of 400ml (whichever is greater), repeat the steps for the same concentration of saline. Patients should not breathe saline for more than 15 minutes in total.

If the FEV₁ falls by more than 20% or 400ml (whichever is greater) of the best post-bronchodilator value, or if significant symptoms occur, stop nebulisation and administer repeat short-acting β_2 agonist.

10. At the end of any nebulisation 200 micrograms of salbutamol was administered via a Volumatic[®] spacer. FEV₁ is reassessed to ensure return to baseline in the case of a fall.

The same sputum induction nebuliser was used throughout the study (NE-U17, Omeron Healthcare™, Milton Keynes, UK).

3.4.1 - Sputum induction instruction for patients:

Prior to commencing the induction the procedure is fully explained to the patient with the emphasis of the following:

Instruction on spitting out saliva generated during the inhalation of saline into a “discard” vessel.

Instruction about blowing their nose, rinsing their mouth and swallowing the water prior to trying to expectorate sputum.

Instruction on how to expectorate effectively. It is necessary to demonstrate the technique for coughing up sputum and moving the sputum from the back of the throat, forward to the specimen container.

A reminder not to swallow sputum as it comes up the bronchial tree.

Guidance on posture: sitting straight upright during nebulisation, and leaning forward during expectoration.

3.4.2 - Calibration for sputum induction:

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

3.4.3 - Safety procedures during sputum induction:

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

3.5 - Protocol for sputum processing:

The following protocol was used to process and count the sputum samples.

Sputum is collected in a sputum sample pot, stored on ice and processed at 4°C within two hours of expectoration.

Select sputum plugs from saliva and transfer to a Petri dish. Transfer sputum free from salivary contamination into an empty (pre-weighed) polypropylene centrifuge tube (opaque) with a screw top.

Subtract the weight of the empty centrifuge tube from the weight of the centrifuge tube plus sputum to obtain the weight of the sputum portion to be processed.

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

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

Add an equal volume of Dulbecco's phosphate buffered saline (D-PBS).

Vortex for a further 15 seconds, filter the sputum suspension through a 48mm nylon gauze pre-wet flat with D-PBS, shake off excess and centrifuge at 200rpm (790g) for ten minutes.

Aliquot all of the supernatant in 0.5ml portions into 2ml microtubes, leaving behind a covering of fluid and the undisturbed pellet. There should be sufficient supernatant for 2-4 microtubes of supernatant.

Resuspend the cell pellet in 0.5ml to 1.0ml of D-PBS (depending on the size of the cell pellet) and mix gently with a wide bore plastic pipette.

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

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

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

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

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

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

Total cell count (cells/g sputum) = mean number of cells/square x 2 x 10,000 x volume of cells resuspended in (ml) / weight of selected sputum (g).

Adjust the cell suspension to $0.5-0.75 \times 10^6$ cells/ml with D-PBS.

Use 50microlitres to prepare two cytopsins and centrifuge at 450rpm (18.1g) for six minutes using a Shandon III cytocentrifuge™.

Air dry four slides for at least 15 minutes at room temperature, then fix with methanol for ten minutes.

Perform a 400 cell count (non-squamous cells) differentiating between eosinophils, neutrophils, macrophages, epithelial cells and lymphocytes.

The cell counts were all performed by trained laboratory scientists and a number of the slides were sent for quality control assessment to determine they were accurate and consistent.

3.5.1 - Cell counting: Romanowski stain preparation:

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

Dilute Romanowski stain:

62ml 10mM HEPES buffer pH 7.2

3.5ml DMSO

4.6ml Romanowski stain

3.5.2 - Cell counting: Differential cell counts:

A sputum differential cell count was obtained by counting >400 non-squamous cells on a Romanowski stained cytospin. Cell counts were performed by trained laboratory scientists and a number of the slides were sent for quality control assessment to determine they were accurate and consistent.

3.6 - Exhaled nitric oxide:

Exhaled nitric oxide concentration was measured using an online chemiluminescence analyser (NIOX FlexFlow®; Aerocrine™, Tolna, Sweden). Air was inhaled via a scrubber to ensure nitric oxide free air,

up to tidal volume and then exhaled at constant pressure ($>10\text{cmH}_2\text{O}$) to aid closure of the velopharynx. The rate of exhalation was set by a standard valve at five different flow rates (10, 30, 50, 100, 200 ml/sec) and controlled by means of visual feedback. The exhaled nitric oxide concentration was recorded as the mean of three recordings of the plateau phase. The analyser was calibrated twice weekly against a standard gas containing 200 ppb of nitric oxide, according to the manufacturer's instructions.

The same machine was used throughout the study.

Participants were blinded to their FeNO values.

Exhaled nitric oxide was the first clinical test to be performed at every visit.

3.7 - Juniper asthma control questionnaire:

The Juniper Asthma Control Questionnaire (ACQ) was used to assess asthma control. This is a validated questionnaire which was designed in consultations with 100 international experts. Each symptom was scored for its importance in evaluating asthma control. From the 91 responses, the five highest scoring symptoms were selected for the ACQ. In addition, one question on short-acting β_2 agonist use and one on airway calibre were added. The ACQ was tested in a 9-week observational study of 50 adults with symptomatic asthma. The ACQ and other measures of asthma health status were assessed at baseline, 1, 5 and 9 weeks. In patients whose asthma was stable between clinic visits, reliability of the ACQ was high (intraclass correlation coefficient

(ICC)=0.90). The questionnaire was very responsive to change in asthma control ($p<0.0001$). Cross-sectional and longitudinal validity were supported by correlations between the ACQ and other measures of asthma health status (84). The questionnaire is self-completed by the patient with the exception of question 7 which is completed by the researcher.

See appendix section 3 for copy of the ACQ.

3.8 - Blood tests:

Venepuncture was performed to obtain venous blood samples using standard NHS protocol.

5ml of blood was collected in a serum-separating tube (SST II Gold-top Vacutainer®) to determine serum IgE level using the Phadia-100 ImmunoCap™ Automated Clinical Chemistry System.

4ml of blood was collected in an EDTA tube (Lavender-top Vacutainer®) to determine full blood count and white cell differential cell counts using the Sysmex XE-2100™ Automated Haematology System.

The blood samples were all processed by the NHS pathology laboratories at Nottingham City Hospital and Leicester Glenfield Hospital.

Chapter 4 - Using exhaled nitric oxide to step-down inhaled corticosteroid therapy in patients with mild to moderate asthma in UK primary care.

Introduction:

Inhaled corticosteroids (ICS) are one of the mainstays of treatment in asthma and act to reduce airway inflammation. Although generally regarded as safe, inhaled corticosteroids are associated with side effects including adrenal suppression, bone loss, skin thinning, increased cataract formation, metabolic changes and decreased linear growth in children (279, 280).

Although ICS target airway inflammation in asthma, guidelines recommend increasing or decreasing ICS dose based on the assessment of symptoms (281, 282). Once asthma symptoms and exacerbations are controlled, ICS dose reduction is recommended and safe (283), but is often not implemented leaving some patients over-treated (282). There are few studies investigating the most appropriate way to reduce ICS dose. A study in adults receiving at least 900 micrograms per day of ICS found that for patients who are stable it is reasonable to halve the dose of ICS every 3 months (258) and this study forms the basis for the recommendation in the current British Thoracic Society (BTS) and Scottish Intercollegiate Group Network (SIGN) guideline (282).

Given the poor relationship between symptoms (17), or spirometry (284), and airway inflammation in asthma, other strategies using the measurement of airway inflammation to increase or decrease steroid treatment in asthma have been assessed. The differential induced sputum eosinophil count has been successfully used to guide steroid therapy and reduce exacerbations (39, 54) in patients with moderate to severe asthma, however induced sputum is time consuming, requires expertise and is less suited for near patient testing in primary care.

FeNO (the fraction of nitric oxide in the exhaled breath) is attractive as a non-invasive marker of airway inflammation in asthma. The test is relatively inexpensive, provides a rapid result, can be used for near patient testing and correlates with the degree of eosinophilic airway inflammation (69, 269). Although a study has recently been published demonstrating that FeNO measurements can be used to up and down titrate ICS dose in pregnant women with asthma (285), and the ATS (American Thoracic Society) guidelines currently recommend the use of FeNO as a non-invasive biomarker in patients with asthma to provide additional clinical information (252), its use is controversial. Other studies utilising FeNO measurements to guide treatment decisions, (with differing designs and outcome measures) have been less successful in guiding ICS dose (230, 236), reducing exacerbations (172) or improving asthma control (3, 229) when compared to routine management. These latter studies have been criticised for not using

individualised cut-points for FeNO levels, and not assessing change from baseline FeNO levels (243).

Given the evidence of over-treatment from treatment prescription surveys (286) and clinical trials (287), and the possible reluctance of medical staff to reduce treatment in well-controlled patients (231), we set out to explore whether FeNO measurements could be used practically to step-down ICS dose whilst addressing some of the criticism levelled at previous studies of FeNO in asthma management.

We performed a single blind cohort study in well-controlled patients with asthma recruited from primary care. It was designed to identify whether a baseline level or a change in FeNO levels following inhaled corticosteroid dose reduction could be used to step-down treatment, and the sample size needed for a definitive randomised placebo-controlled study.

Methods:

Settings and Patients:

The study was conducted across two sites: Nottingham Respiratory Research Unit (Nottingham City Hospital, UK) and Leicester Respiratory Biomedical Research Unit (Leicester Glenfield Hospital, UK).

Patients were identified with the help of the Primary Care Research Network (PCRN) from registers held in GP surgeries in Nottingham and

Leicester, UK. All patients were aged between 18 and 75 at study enrolment and had a diagnosis of asthma recorded in their GP medical notes. Patients were eligible if they had received at least one prescription for any inhaled corticosteroid medication in the previous 12 months.

The study was restricted to non-smokers with a past smoking history of less than 10 pack years. Patients were also excluded if they were considered by their GP to be poorly compliant, had experienced an asthma exacerbation requiring oral steroids in the previous 12 weeks, or if their Asthma Juniper Control Questionnaire (ACQ) (84) score at visit 1 was greater than 1.5 (see below for further details).

All suitable patients on the register who responded to an invitation from their GP to were invited to participate in the study. Ethics approval for the study was given by Leicester, Rutland and Northampton REC 2 and Nottingham City, Nottinghamshire County, Leicester City and Leicestershire County Primary Care Trusts. All patients gave written informed consent prior to starting the study. The study was funded by a grant from the National Institute for Health Research (RfPB).

Clinical tests:

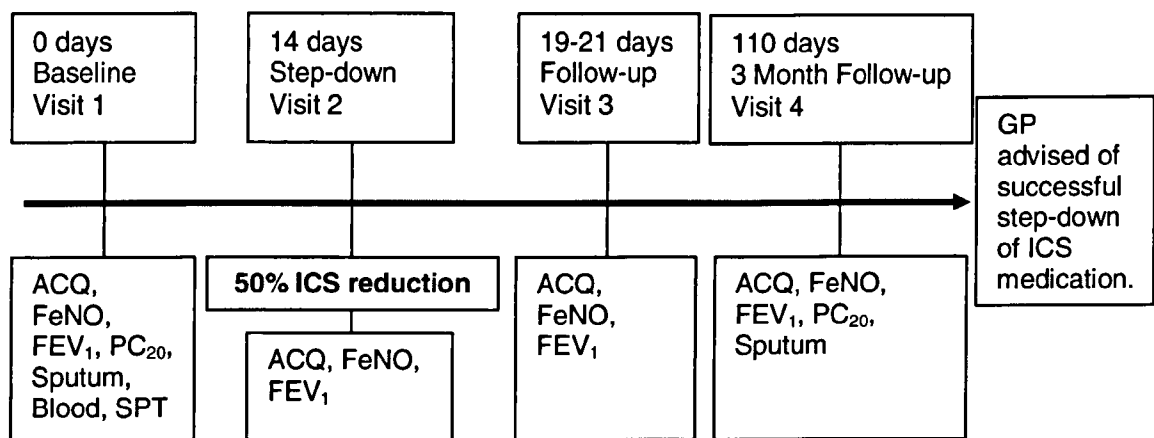
Patients were seen at visit 1 (day zero) and then at visit 2 (day 14), visit 3 (day 21) and for a final visit 4 (at three months). Each of the visit appointments occurred at the same time of day and consisted of ACQ, FeNO and Spirometry. ICS reduction occurred after the tests at visit 2

had been performed. At visits 1 and 4 sputum induction and methacholine challenge were also performed.

Tests were performed in the order listed in the flow diagram below (figure 1). Symptoms were assessed using the Juniper Asthma Control Questionnaire (ACQ) (score of <1.5 required to enter the study). Airway inflammation was measured using exhaled nitric oxide levels (FeNO) measured at flows of 10, 30, 50, 100, 200ml/sec, differential cell counts were performed on induced sputum, spirometry, airway hyperresponsiveness, skin prick tests, blood tests to determine blood differential eosinophil count ($\times 10^9/L$) and blood IgE (kIU/L) were performed. Detailed methodology for each test can be found in chapter 3. See Figure 4.1 for further details of the study regime.

Figure 4.1:

Study design



Study design. FeNO = fraction of exhaled nitric oxide at five flow rates; ACQ = Juniper Asthma Control Questionnaire; PC₂₀ = methacholine challenge; Sputum = differential sputum eosinophil count; Blood = Full blood count, IgE, eosinophils; SPT = skin prick tests

Inhaled corticosteroid step-down:

Patients were assessed at visit 2; if their ACQ score remained <1.5 and had not increased by more than 0.5, their ICS dose was reduced by 50%. Inhaler types were kept the same, by prescribing a smaller dose or halving the number of puffs. In the majority of instances we halved the dose of the inhaler prescribed and kept the number of puffs the same. However, where this was not possible (e.g. Seretide[®] 125) we halved the daily routine (number of puffs) and kept the inhaler dose the same (e.g. 2bd to 1bd). Participants who were taking less than 200 micrograms bdp equivalent daily were asked to stop their inhaled corticosteroids entirely. This was only applicable to participants who were taking inhaled corticosteroid inhalers as monotherapy.

In the case of participants who were taking combination inhalers (e.g. Seretide[®], Symbicort[®] and Fostair[®]), the inhalers were prescribed as two separate component inhalers (Seretide[®] = Flixotide[®] and Serevent[®], Symbicort[®] = Pulmicort[®] and Oxis[®], Fostair[®] = Clenil Modulite[®] and Atimos Modulite[®]).

The dose of long-acting β_2 agonist (LABA) was kept the same in all participants who were taking them. All participants were told to use their short-acting β_2 agonist (SABA) whenever they required it.

A 50% reduction in ICS dose was chosen to reflect the BTS/SIGN and GINA guidelines, both of which recommend a 50% step-down in patients with asthma after three months of good symptom control (288).

Patients were asked to take the half dose of ICS for seven days and then return for visit 3. When patients returned for visit 3 their ACQ score was re-measured. If the score had not increased by >0.5 or above 1.5 and they had not experienced an exacerbation (see definition below) they were entered into the final phase of the study, which saw patients remain on their half dose inhaled corticosteroid for a period of three months until visit 4.

Safety measures, loss-of-control and exacerbation:

Patients were given a detailed safety card explaining what to do in the event of worsening of their symptoms. All patients who contacted the emergency team were assessed by a physician within 24 hours and their ACQ score was recorded. A physician (blinded to the study data) made a decision on how to treat each based on the BTS guidelines. Every patient who contacted the emergency team with a worsening of their symptoms had their ICS dose increased back to its original level. A loss of control was defined as an increase in symptoms with an increase in ACQ score of greater than 0.5. An exacerbation was defined as an episode of increasing asthma symptoms requiring a course of antibiotics or oral steroids (17, 172).

Analysis:

Power calculation:

Based on findings from the FACET (17) and from a previous study looking at the role of FeNO in asthma we estimated that we needed 154

patients to give 80% power to show that a low FeNO, or persistently low FeNO could successfully predict stable control and lack of asthma exacerbation following a reduction in inhaled corticosteroid dose. Our power calculation was based on the assumption that within the patients with a persistently low FeNO (less than 25ppb at 50ml/sec flow rate), 10% would have a loss of control or exacerbation, compared to 30% in the high FeNO group. To counter an expected 10% drop out rate we aimed to recruit 200 patients into the study.

Statistical analyses:

Predictors of loss of control and exacerbation were sought using multiple logistic regression. Correlations were measured using Pearson correlation coefficient or Spearman rank correlation. FeNO and PC₂₀ were log transformed to normality and analysed as geometric means with 95% confidence intervals. Pack years, ICS dose, blood IgE, blood eosinophils, ACQ questionnaire, differential sputum eosinophil and sputum neutrophil count were all presented as median and IQR non-normal data. All analyses were performed using STATA 11.

In order to assess whether FeNO measurements could predict which patients would remain stable following a 50% reduction in their ICS dose from those who would deteriorate (either suffer a loss of control or exacerbation) we analysed the data in two ways. Firstly we assessed whether a low baseline FeNO could predict successful dose reduction; then we evaluated whether change in FeNO could predict a successful dose reduction. We assessed whether a change in measurements of

FeNO between visit 2 (step-down visit) and visit 3 (7 days post step-down) could distinguish between those patients who could or could not tolerate a 50% ICS dose reduction as defined by a loss of control or exacerbation.

Next we evaluated whether any clinical measures could predict stability. We assessed whether other baseline clinical measurements of spirometry, methacholine PC₂₀, blood IgE, blood eosinophil count, ACQ score, differential sputum eosinophil or sputum neutrophil count could distinguish between those patients who could or could not tolerate a 50% ICS dose reduction at three months.

We assessed whether a change in spirometry between visit 2 (ICS dose reduction) and visit 3 (7 days post ICS reduction) could distinguish between those patients who would or would not tolerate a 50% ICS dose reduction at three months.

Lastly we carried out a reanalysis in a pre-specified sub group examining the stable, loss of control and exacerbation groups separately.

A p-value of ≤ 0.05 was regarded as statistical significance.

Results:

A total of 5068 patients with a primary care diagnosis of asthma were contacted in writing by their GP to ask if they were interested in participating in the study. Of these, 3266 declined screening or failed to respond. 350 patients were recruited into the study for a screening baseline visit (visit 1), and 200 were entered into the study across the

two sites between September 2010 and December 2011. During the study 9 patients were not able to complete the study. 191 patients completed the study with a complete data set (Figure 4.2).

There were a total of 128/191 (67%) patients who completed the three month study period (post ICS reduction) with no loss of control or exacerbation. 63/191 (33%) patients suffered from either a loss of control (32 = 17%) or an exacerbation (31=16%) within the three month period following ICS dose reduction. The median and IQR baseline (visit 1) ACQ was 0.6 (0.2-1.0) for the stable group and 0.8 (0.2-1.0) for the deterioration group ($p=0.53$).

Additional graphs for results presented below can be found in appendix section 12.

Figure 4.2:

Study consort diagram

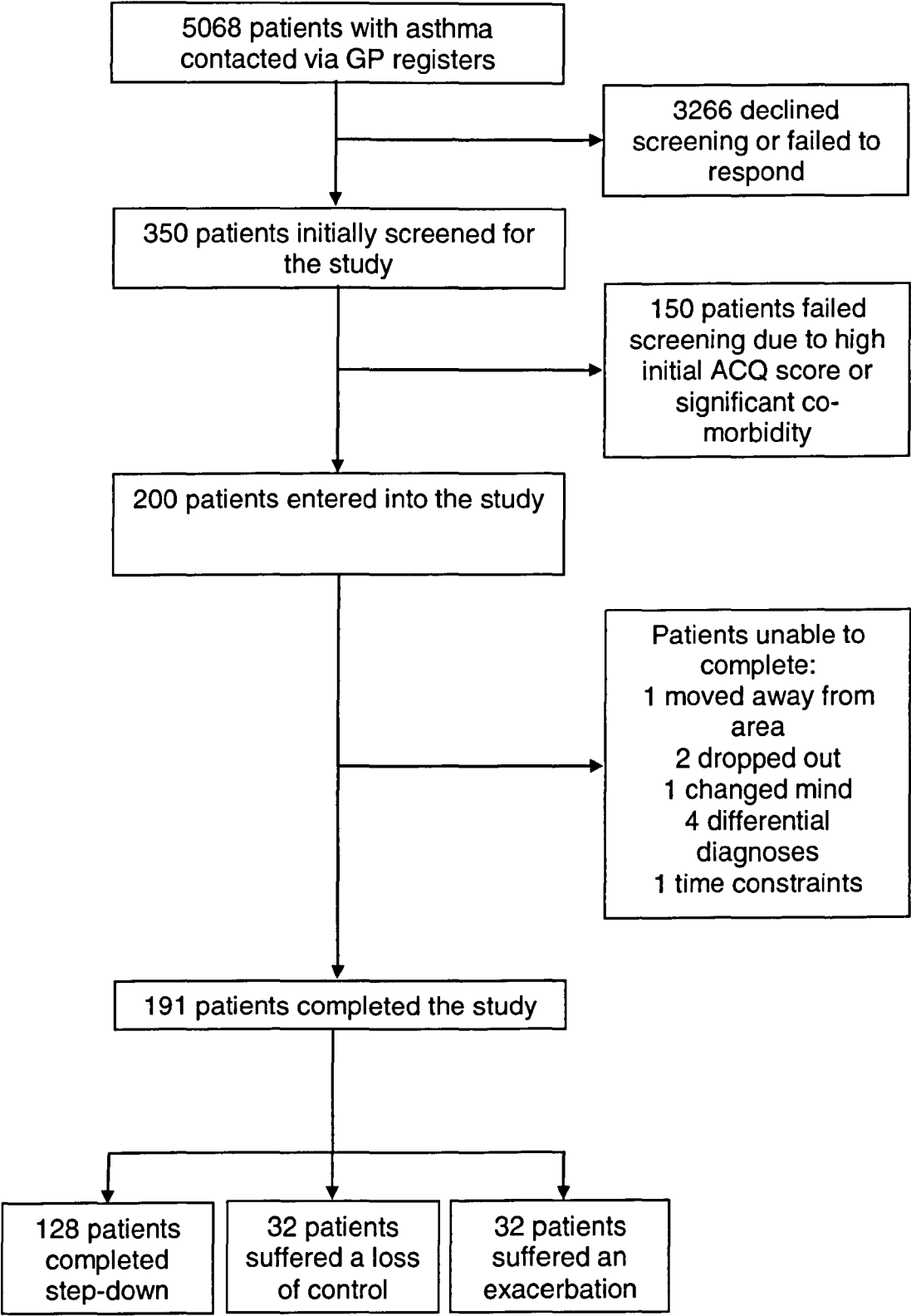


Table 4.1:

Baseline demographics presented for study population (n=191).

Data are presented as: ^{*} Mean and standard deviation, [†] Median and Interquartile range, [#] Geometric Mean and 95% confidence intervals.

VARIABLE	RESULT
Age [*]	54.15 ± 13.50
Gender	
Male	83 (43.5%)
Female	108 (56.5%)
When Diagnosed	
0-5 Years	16 (8.4%)
>5 Years	175 (91.6%)
BTS Stage	
2	57 (29.84%)
3	111 (58.12%)
4	23 (12.04%)
Pack Years [†]	0 (0-4)
Height (cm) [*]	169.24 ±9.89
Weight (kg) [*]	81.14 ± 17.31
BMI (kg/m ²) [*]	28.27 ±5.36
BDP Equivalent Daily Dose (mcg/day) [†]	400 (200-1000)
FEV ₁ (L) [*]	2.68 ± 0.85
FEV ₁ % predicted (%) [*]	89.85 ±19.15
FVC (L) [*]	3.65 ± 1.00
FVC % predicted (%) [*]	99.81 ±16.87
Airway hyperresponsiveness (PC ₂₀) [#]	8.02 (95%CI 6.31-10.17)
Blood IgE (kIU/L) [†]	91.5 (28.50 - 253.50)
Blood Eosinophils (x10 ⁹ /L) [†]	0.20 (0.12-0.32)
Sputum Eosinophils (%) [†]	0.80 (0.25-4.75)
Sputum Neutrophils (%) [†]	64.75 (42.25-84.00)
ACQ Questionnaire 1-5 [†]	0.60 (0.20-1.00)

BMI = Body Mass Index

ACQ = Juniper Asthma Control Questionnaire.

*BDP Equivalent = Beclomethasone Dipropionate Equivalent
(QVAR = 2:1BDP, Fluticasone = 2:1 BDP, Budesonide = 1:1BDP)*

Baseline FeNO:

There were no significant differences in the baseline (visit 1) FeNO between the patients who could successfully reduce their ICS dose (stable group) compared to those who suffered from a loss of control or exacerbation (deterioration group), at any of the five FeNO flows. At a flow of 50ml/sec the stable group had a mean FE_{NO} of 18.9ppb (95% CI 16.8-21.5) and the deterioration group a mean of 19.7ppb (95% CI 16.4-23.6ppb), (p=0.76).

Table 4.2 shows the baseline FeNO for these two groups of patients for the other flows studied.

Table 4.2:

The difference in baseline FeNO for successful step-down patients versus exacerbation and loss of control patients for all of the NIOX FlexFlow[®] flow rates.

<u>Exhaled Nitric Oxide Characteristic</u>	<u>Stable Group: (n=128 (67.0%))</u>	<u>Deterioration Group: (n=63 (33.0%))</u>	<u>Significance level: (T-test)</u>
FeNO 10ml/sec	59.7 (46.1-77.5)	73.7 (54.1-102.5)	p=0.34
	n=43	n=22	
FeNO 30ml/sec	26.6 (23.1-30.9)	32.1 (25.8-40.4)	p=0.13
	n=91	n=43	
FeNO 50ml/sec	18.9 (16.8-21.5)	19.7 (16.4-23.6)	p=0.76
	n=128	n=62	
FeNO 100ml/sec	11.4 (9.9-13.1)	11.5 (9.7-14.0)	p=0.95
	n=111	n=53	
FeNO 200ml/sec	6.8 (5.9-7.8)	7.5 (6.4-8.9)	p=0.31
	n=108	n=50	
	n=101	n=47	

† Data calculated as Geometric Mean with 95% confidence intervals. Presented as anti-log Geometric means and 95% confidence intervals.

Change in FeNO:

There were no significant differences in the change in FeNO (visit 2 to visit 3) between stable group and deterioration group, at any of the five FeNO exhalation rates. The mean absolute change between visit 2 and 3 was 1.58 ± 11.9 ppb for the stable group and 1.03 ± 14.88 ppb for the deterioration group ($p=0.80$). In terms of percentage change in FeNO values at 50ml/sec the changes were $17.60 \pm 69.97\%$ for the stable group and $11.01 \pm 41.05\%$, ($p=0.54$)

Table 4.3:

Change in FeNO between these two groups of patients for the other flow-rates studied.

<u>Change in FeNO between visit 2 and visit 3</u>	<u>Stable Group:</u>	<u>Deterioration Group:</u>	<u>Significance level: (T-test)</u>
<u>10ml/sec flow rate profile</u>	n=42	n=16	
Absolute Change in FeNO (ppb)	5.63 ± 58.24	5.47 ± 54.87	$p=0.99$
Percentage Change in FeNO (%)	22.31 ± 107.99	6.91 ± 51.46	$p=0.59$
Fold Change in FeNO	1.22 ± 1.08	1.07 ± 0.51	$p=0.59$
<u>30ml/sec flow rate profile</u>	n=81	n=33	
Absolute Change in FeNO (ppb)	0.06 ± 19.82	0.87 ± 25.58	$p=0.86$
Percentage Change in FeNO (%)	12.85 ± 66.88	13.39 ± 49.39	$p=0.97$
Fold Change in FeNO	1.13 ± 0.67	1.13 ± 0.49	$p=0.97$
<u>50ml/sec flow rate profile</u>	n=124	n=49	
Absolute Change in FeNO (ppb)	1.58 ± 11.9	1.03 ± 14.88	$p=0.80$
Percentage Change in FeNO (%)	17.60 ± 69.97	11.01 ± 41.05	$p=0.54$
Fold Change in FeNO	1.18 ± 0.70	1.11 ± 0.41	$p=0.54$
<u>100ml/sec flow rate profile</u>	n=103	n=42	
Absolute Change in FeNO (ppb)	0.72 ± 7.42	0.63 ± 8.98	$p=0.95$
Percentage Change in FeNO (%)	14.38 ± 76.24	11.41 ± 42.80	$p=0.81$
Fold Change in FeNO	1.14 ± 0.76	1.11 ± 0.43	$p=0.81$
<u>200ml/sec flow rate profile</u>	n=102	n=42	
Absolute Change in FeNO (ppb)	0.46 ± 4.09	0.35 ± 6.15	$p=0.90$
Percentage Change in FeNO (%)	13.31 ± 58.79	9.90 ± 52.42	$p=0.74$
Fold Change in FeNO	1.13 ± 0.59	1.10 ± 0.52	$p=0.74$

Data is presented as Mean and Standard Deviation

Baseline clinical measurements:

There were no statistically significant differences in the baseline clinical measurements between the patients who could successfully reduce their ICS dose (stable group) compared to those who suffered from a loss of control or exacerbation (deterioration group).

Table 4.4:

Differences in these baseline clinical measurements between the two groups studied.

<u>Baseline Demographic Characteristic</u>	<u>Stable Group: (n=128 (67.0%))</u>	<u>Deterioration Group: (n=63 (33.0%))</u>	<u>Significance level: (T-test)† (Kruskal-Wallis test)#</u>
FEV ₁ (L)	2.71 ± 0.89	2.61 ± 0.79	p=0.49†
FEV ₁ percent predicted (%)	91.0 ± 19.3	87.5 ± 18.7	p=0.23†
FVC (L)	3.67 ± 1.03	3.62 ± 0.95	p=0.74†
FVC percent predicted (%)	100.2 ± 17.3	98.9 ± 16.0	p=0.62†
FEV ₁ /FVC Ratio (%)	0.72 ± 0.09	0.72 ± 0.10	p=0.21†
AHR (PC ₂₀) doubling doses	3.14 ± 2.21	2.74 ± 2.49	p=0.28†
Blood IgE *	93.5 (26.0-222.5)	72.9 (30.8-269.0)	p=0.66#
Blood Eosinophils *	0.20 (0.10-0.32)	0.2 (0.14-0.39)	p=0.64#
JAC Questionnaire 1-5 Score*	0.60 (0.20-1.00)	0.80 (0.20-1.00)	p=0.53#
Sputum Eosinophils (%) *	0.78 (0.25-4.63)	0.50 (0.00-4.25)	p=0.62#
Sputum Neutrophils (%) *	65.88 (41.38-84.38)	58.00 (46.00-83.00)	p=0.51#

*Differences in baseline clinical measurements between the two groups studied. Data is presented as mean and standard deviation except where variable is marked * here the data is presented as median and interquartile range.*

Change in spirometry:

There was no significant difference in spirometry between visit 2 and visit 3 between the stable group and deterioration group.

Sub group analysis:

We split the deterioration group into patients with loss of control (n=32) or patients with an exacerbation (n=31). There was no significant difference in any of the measurements, at any of the flows for FeNO either at baseline or between visit 2 and visit 3.

Table 4.5:

Difference in baseline FeNO for successful step-down patients versus exacerbation patients versus loss of control patients for all of the NIOX® FlexFlow flow rates.

<u>Exhaled Nitric Oxide Characteristic</u>	<u>Stable Group: (n=128 (67.0%))</u>	<u>Exacerbation Group: (n=31 (16.0%))</u>	<u>Loss of control group: (n=32 (17.0%))</u>	<u>Sig level: ANOVA (F-test)</u>
FeNO 10ml/sec †	59.7 (46.1-77.5)	73.7 (45.2-126.5)	73.7 (46.5-122.7)	p=0.64
	n=43	n=10	n=12	
FeNO 30ml/sec †	26.6 (23.1-30.9)	32.5 (22.0-49.9)	31.8 (24.3-42.5)	p=0.31
	n=91	n=19	n=24	
FeNO 50ml/sec †	18.9 (16.8-21.5)	19.9 (15.6-26.3)	19.0 (15.0-25.0)	p=0.92
	n=128	n=31	n=31	
FeNO 100ml/sec †	11.4 (9.9-13.1)	12.3 (9.4-16.9)	10.8 (8.6-14.2)	p=0.74
	n=111	n=25	n=28	
FeNO 200ml/sec †	6.8 (5.9-7.8)	8.0 (6.2-10.7)	7.1 (5.8-8.9)	p=0.45
	n=108	n=24	n=26	

† Data calculated as Geometric Mean with 95% confidence intervals. Presented as anti-log Geometric means and 95% confidence intervals.

Conclusion:

We set out to evaluate the use of FE_{NO} to guide asthma management in primary care, a setting where the technique likely to be particularly applicable and where the majority of patients with asthma are managed. As there is evidence that clinicians are reluctant to sanction treatment reductions (287) we set out to establish whether FE_{NO} measurements, or other clinical indices, could inform medical professionals and patients about timing of ICS dose reduction. We also set out to address criticisms of previous studies by attempting to define individualised cut points for FE_{NO} measurements and by assessing change in baseline values.

We found that neither baseline FE_{NO} measurements, nor change at 7 days following ICS dose reduction, could delineate between stability or deterioration in asthma control at 3 months. Our study does confirm previous observations (258) that ICS dose reduction is safe in the majority of patients with mild to moderate asthma when well controlled. Recruitment for our study was not difficult suggesting that dose reduction does not occur despite the recommendations in the asthma guidelines and that patients think this is an important topic (see appendix section 2 for patient feedback).

In order for our study to have practical benefits for the future management of asthma we used a dose reduction period of 7 days between the two FE_{NO} measurements. Dose-dependent onset and

cessation of action of ICS on FE_{NO} levels in patients with mild asthma has been demonstrated, with levels rising after one day of treatment reduction (289). Moreover if reassessment was left for a longer time period it would defeat the object of using FE_{NO}, or any other clinical measure, to predict dose reduction. Compliance may have improved between visit 1 and 2, however there was no significant decrease in FE_{NO}, or improvement in other clinical measurements, suggesting compliance was not an issue.

We also considered the percentage ICS dose reduction needed to lead to an increase in FE_{NO} levels. The relationship between sputum eosinophilia and FE_{NO} levels is strongest between 100-800mcg of ICS (BDP) equivalent (245) and most of our patients fell within this range. Moreover previous studies have shown that only a small reduction in ICS dose is required to observe an increase in FE_{NO} levels (243) with one study demonstrating a significant increase with a dose reduction of 200mcg BDP equivalent (290), less than the mean reduction in our study. A 50% reduction of ICS dose was also the level at which the ethics committee were happiest.

Our study is the first to investigate whether FE_{NO} measurements can be used practically to guide ICS dose reduction in an unselected population of patients with asthma, and is the first to investigate different FE_{NO} flows in treatment decisions. There is uncertainty as to the use of the different flows rates of FE_{NO} measurements in asthma,

with lower flows thought to be associated with bronchial rather than alveolar inflammation (291). Our study shows there are no differences in the results of the different flow rates in predicting successful step-down. None of the baseline clinical indices measured (baseline ACQ, spirometry, blood eosinophil count, differential sputum eosinophil count, blood IgE, methacholine challenge) could delineate between future stability or deterioration in asthma control. This applied even if the patients who deteriorated were divided into patients with loss of control or exacerbation (24). This has important implications for ICS dose reduction and suggests better methods of identification of at risk patients are needed.

The study was designed as a pilot study to assess the feasibility of recruitment into a dose reduction randomised controlled trial and to evaluate FE_{NO} cut-points for this trial, consequently our study did not require a placebo controlled arm (or a non-dose reduction arm). We saw no increase in FE_{NO} following ICS withdrawal. Moreover previous evidence suggests (258) that the proportion who deteriorated following ICS reduction would not have differed from the proportion who would have deteriorated with no reduction. Taken together these findings suggest that our ICS reduction was not aggressive enough for our biomarker to become predictive and that a more aggressive step down in ICS dose is safe and needs to be studied in order to fully evaluate the added value of FE_{NO} in asthma management.

Chapter 5 - Additional analyses:

As part of the main clinical trial a selection of pre-specified additional analyses were performed to address aims 2-5 in chapter 2.

5.1:

The correlation between airway inflammation and asthma symptoms

5.2:

The correlation between eosinophilic airway inflammation and exhaled nitric oxide in asthma

5.3

The relationship between clinical diagnostic tests and exhaled nitric oxide in asthma

5.4

The relationship between elevated exhaled nitric oxide and eosinophilic inflammation in mixed granulocytic asthma

5.1 - The correlation between airway inflammation and asthma symptoms:

Hypothesis:

Asthma symptoms (as measured by the Juniper Asthma Control Questionnaire) reflect airway inflammation in patients with mild to moderate asthma.

Introduction:

Asthma diagnosis and management in UK primary care is largely based upon the assessment of symptoms and occasionally lung function (usually peak flow or spirometry). Primary care centres rarely have access to tests which measure airway inflammation without referring patients into secondary care or research. When patients suffer with deteriorating symptoms, asthma medications are typically stepped-up (2). Similarly, the BTS guidelines recommend that once a patient has experienced three months of good symptom control their medication be reduced to ensure optimum control on the lowest possible medication doses (2). However, these decisions are based upon symptoms and lung function, but there is little evidence to suggest that symptom scores accurately reflect the presence or absence of airway inflammation. Despite the fact that the guidelines define control as “absence of symptoms”, many patients will be asymptomatic but still have evidence of airway inflammation (2). If airway inflammation is left untreated it can ultimately lead to airway remodelling and permanent

damage to the airways. Conversely, there are also patients who suffer from severe symptoms but have no evidence of airway inflammation. These patients may be taking inhaled corticosteroid medication unnecessarily, exposing these people to side effects and prescription costs which may be avoided.

Methods:

The same methodology was used as is previously detailed in chapters 3 and 4.

Analysis:

STATA 11 was used to analyse the data. Non-normal data were corrected using natural logs where possible. Pearson correlation was used to assess the correlations between symptom scores and measures of lung function and airway inflammation. A p value of ≤ 0.05 was regarded as statistical significance.

Results:

The baseline variables for the participants studied were all within the normal clinical range (Table 5.1).

Table 5.1:

Baseline variables for participants studied in a correlation between airway inflammation and asthma symptoms.

Clinical Variable	Mean Baseline Score (n=191)
ACQ 1-5 Score*	0.70 (0.63 – 0.78)
ACQ 1-6 Score*	0.64 (0.58 – 0.72)
ACQ 1-7 Score*	0.72 (0.65 – 0.80)
Blood Eosinophils (mmol/L)*	0.23 (0.21 – 0.25)
Sputum Eosinophils (%)*	1.95 (1.14 – 2.71)
Exhaled Nitric Oxide (ppb)*	20.79 (18.98 – 22.89)
FEV ₁ % Predicted†	89.85 ± 19.15
*Data presented as Geometric Mean (95% confidence intervals)	
†Data presented as mean and standard deviation	

Table 5.2:

Pearson Correlation Coefficient table to show relationships between ACQ score, lung function and airway inflammation.

	ACQ1-5		ACQ 1-6		ACQ 1-7	
	R Coefficient	P Value	R Coefficient	P Value	R Coefficient	P Value
Blood Eosinophils (mmol/L)*	0.1636	0.0600	0.1444	0.0843	0.1444	0.0731
Sputum Eosinophils (%)*	0.2168	0.3028	0.0823	0.4982	0.1034	0.3707
Exhaled Nitric Oxide (ppb)*	0.0683	0.4060	0.0326	0.6858	0.1358	0.0972
FEV ₁ % Predicted	-0.0505	0.5382	-0.0730	0.3639	-0.4811	0.0000
* Data calculated as log(variables)						

There are no significant correlations between ACQ symptom scores and measures of airway inflammation.

See appendix section 10 for further detailed graphs of these correlations.

Discussion:

The study required all participants to have an ACQ score of less than 1.5 in order to be included. This is reflected in the normal mean baseline figures in Table 5.1.

Table 5.2 shows the correlations between symptom scores and measures of airway inflammation. There were no significant correlations between log10 symptom score and log10 sputum eosinophil count, log10 blood eosinophil count and log10 exhaled nitric oxide (50ml/sec). There was also no correlation between symptom score and FEV₁ percent predicted (with the exception of ACQ1-7, but this is expected as this form of the ACQ takes FEV₁ percent predicted into account).

This shows that asthma symptom scores alone do not accurately reflect the underlying inflammatory disease processes in patients with mild to moderate asthma.

This is important data as GPs make many treatment decisions in primary care based solely on the presence or absence of symptoms due to a lack of clinical assessment tools available in this setting. This can lead to patients being over-treated or mis-treated with inhaled corticosteroids when they present with symptoms (but may have no evidence of airway inflammation) thus subjecting these patients to unnecessary side effects and medication costs. Conversely, there are patients who have no symptoms but do not have evidence of airway inflammation. Leaving these patients untreated because they are asymptomatic may cause airway remodelling and long-term smooth muscle and airway damage. This analysis corroborates previous

research which highlights the disparities between symptoms and airway inflammation.

Conclusion:

Inhaled corticosteroids are used to treat airway inflammation in asthma. Guidelines recommend that patients whose symptoms are poorly controlled have their ICS medication increased and conversely patients who demonstrate three months of good symptom control should attempt to decrease their ICS dose (2). However we have shown that symptom scores do not accurately reflect the levels of underlying airway inflammation and therefore cannot be used alone to accurately guide management decisions involving anti-inflammatory medications.

5.2 - The correlation between eosinophilic airway inflammation and exhaled nitric oxide in asthma:

Hypothesis:

Exhaled nitric oxide reflects airway inflammation in mild to moderate asthma.

Introduction:

Exhaled nitric oxide is known to reflect eosinophilic airway inflammation in moderate to severe asthma (292). Less is known about the relationship between exhaled nitric oxide and other markers of airway inflammation and airway dysfunction in patients with a primary care diagnosis of mild to moderate asthma.

Decisions regarding inhaled corticosteroid treatment regimes are largely based upon the presence or absence of asthma symptoms. However in Chapter 5.1 the results showed that there is no correlation between asthma symptoms and airway inflammation in patients with mild to moderate asthma. Current tests to assess airway inflammation which are available in secondary care and research settings (such as sputum induction) are too expensive, time consuming and specialised to perform in primary care centres.

Exhaled nitric oxide is understood to be a non-invasive surrogate biomarker of airway inflammation, it also has added benefits in that it is portable, cost effective and quick. Therefore a suitable test to use in primary care. This is important as in the UK up to 80% of asthma is

managed purely in primary care and mild asthma may represent a different rather than simply “less severe” phenotype of asthma when compared to the moderate to severe spectrum of the disease. There is debate as to the role of routine exhaled nitric oxide monitoring in mild asthma.

Methods:

The same methodology was used as is previously detailed in chapters 3 and 4.

Analysis:

STATA 11 was used to analyse the data. Non-normal data were corrected using natural logs where possible. Pearson correlation was used to assess the correlations between exhaled nitric oxide and measures airway inflammation. A p value of ≤ 0.05 was regarded as statistical significance.

Results:

Table 5.3:

Pearson's Correlation Coefficient between log10 (FeNO 50ml/sec) and clinical variables of airway inflammation and airway function.

Clinical Variable	R Value	P Value
ACQ Score	-0.0040	0.9568
FEV ₁ % Predicted	-0.2278	0.0016
FVC % Predicted	-0.1018	0.1621
FEV ₁ /FVC Ratio (%)	-0.3155	<0.0001
Sputum Eosinophils (%)*	0.2283	0.0392
Blood Eosinophils (mmol/L)*	0.3543	<0.0001
Blood IgE (kU/L)*	0.2643	0.0005
PC ₂₀ (mg/ml)*	-0.1248	0.0988
<i>*Data calculated as log values</i>		

There was no significant correlation between baseline log10 (mean FeNO 50ml/sec) and ACQ, FVC percent predicted and PC₂₀. However there was a significant correlation between baseline log10 (mean FeNO 50ml/sec) and FEV₁ percent predicted, FEV₁/FVC ratio, log10 sputum eosinophils, log10 blood eosinophils and log10 blood IgE.

See appendix section 9 for further detailed graphs of these correlations.

Discussion:

Inhaled corticosteroid medications are used to treat airway inflammation in asthma. GPs currently make treatment decisions based upon the presence or absence of symptoms because they have limited access to

clinical tools which measure airway inflammation in their practices. However the research and the analysis performed in Chapter 5.1, shows there is no correlation between airway inflammation and asthma symptoms in patients with mild to moderate asthma.

This corroborates previous research which has shown similar findings. Exhaled nitric oxide is shown to correlate well with other clinical markers of airway inflammation in patients with mild to moderate asthma including sputum eosinophils (currently the “gold standard” in airway inflammation measurement).

Although the relationship is relatively weak between sputum eosinophils and FeNO ($r=0.2283$, $p=0.0392$), we are not considering the use of FeNO as a replacement for sputum induction, rather as an additional tool for clinicians to have access to which may provide helpful information when faced with uncertain diagnosis and treatment decisions.

Further research is needed to find the best use for this biomarker.

Conclusion:

Exhaled nitric oxide could be used as a non-invasive surrogate biomarker of airway inflammation in primary care to aid treatment and management decisions in patients with mild to moderate well-controlled asthma.

5.3 - The relationship between clinical diagnostic tests and exhaled nitric oxide in asthma:

Hypothesis:

Exhaled nitric oxide be used in primary care to predict the likelihood of “true” asthma in patients with a suspected diagnosis of mild to moderate asthma.

Introduction:

In primary care, accurate diagnosis of mild to moderate asthma can prove difficult due to the lack of clinical tests which are available or feasible.

In secondary care and research centres there are numerous clinical tests available which can help to confirm or exclude a diagnosis of asthma. The two tests of choice are airway hyperresponsiveness as measured by methacholine bronchial challenge test and airway inflammation as measured by sputum induction.

Patients who present with a positive sputum eosinophilia and / or a positive methacholine challenge test are more “likely” to have asthma which will respond to inhaled corticosteroid medication.

GPs cannot refer every patient they suspect may have asthma into secondary care for additional testing. Therefore it would be useful for GPs to have a simple test which they could use to help them make quantitative decisions about inhaled corticosteroid treatment regimes, and to help determine who may benefit from a referral into secondary

care for additional testing. It may also help GPs exclude asthma in patients who present with some “asthma-like” symptoms.

This analysis set out to investigate whether exhaled nitric oxide can be used to predict whether patients may have other positive clinical tests and thus that a diagnosis of “true” asthma is more likely and would be more likely to be responsive inhaled corticosteroid medication.

Methods:

The same methodology was used as is previously detailed in chapters 3 and 4.

Methacholine challenge result of less than 8mg/ml was defined as a positive test. Sputum eosinophils of greater than 3% was chosen as the cut-off for predicting likelihood of asthma and future exacerbation risk (293). An FEV₁ percent predicted of <80% was the chosen cut-off for spirometry to reflect reduced lung function.

Analysis:

The analysis set out to assess the mean baseline FeNO levels for participants who had a positive or negative test (spirometry, methacholine challenge and sputum eosinophilia). T-test was used to determine differences within the clinical test groups. The three clinical tests were then combined together and one way ANOVA in STATA 11 was used to calculate if there was a significant difference in baseline

FeNO based upon the number of normal and abnormal clinical tests. This was to determine whether FeNO reflects the “likelihood” of having other abnormal lung function tests and thus the likelihood of having asthma which will respond to inhaled corticosteroid treatment. A p value of ≤ 0.05 was regarded as statistical significance.

Results:

The mean baseline FeNO was significantly higher in participants who had a positive PC₂₀ (30.26ppb 50ml/sec) versus a negative PC₂₀ (23.68ppb 50ml/sec) (p=0.0279).

The mean baseline FeNO was significantly higher in participants who had a positive sputum eosinophilia (40.92ppb 50ml/sec) versus a negative sputum eosinophilia (23.06ppb 50ml/sec) (p<0.0001).

The mean baseline FeNO was significantly higher in participants who had an FEV₁ percent predicted <80% (35.02ppb 50ml/sec) versus FEV₁ percent predicted >80% (22.66ppb 50ml/sec) (p=0.0001). These results are shown in table 5.4 and illustrated graphically in Figure 5.1.

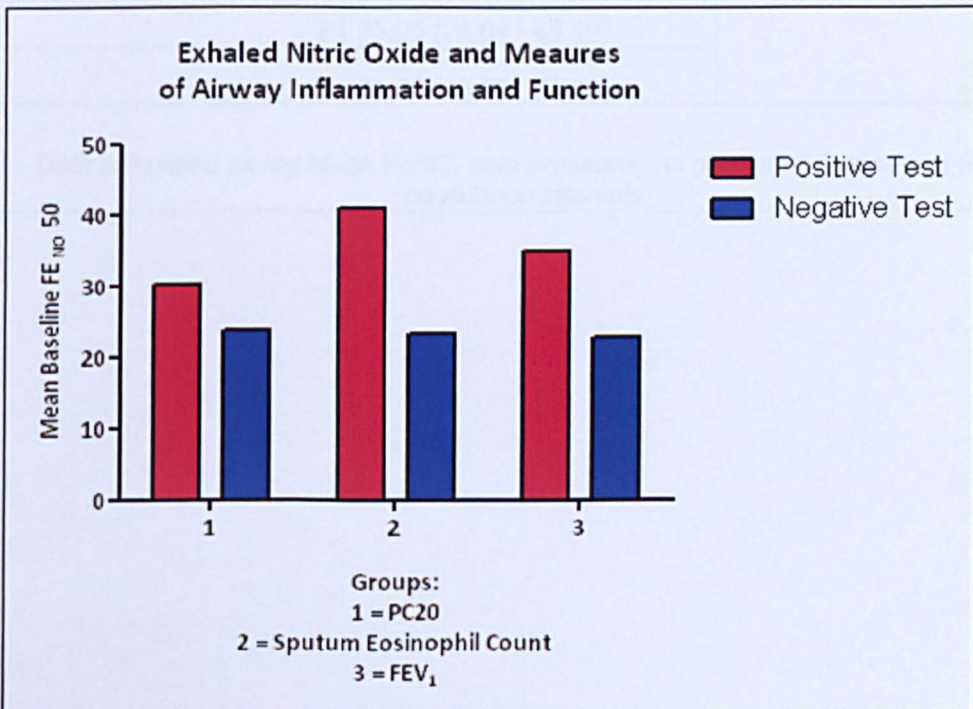
Table 5.4:

The relationship between exhaled nitric oxide and variables of airway inflammation and lung function.

Mean Baseline Exhaled Nitric Oxide (FeNO) and Baseline Variable of Airway Inflammation and Function			
Clinical Variable	Mean FeNO (ppb) for a positive clinical test	Mean FeNO (ppb) for a negative clinical test	P Value (T-Test)
Airway Hyperresponsiveness: (PC ₂₀ - mg/ml) (+ test <8mg/ml, - test >8mg/ml)	30.26 (24.77 – 35.76)	23.68 (20.55 – 26.82)	0.0279
Sputum Eosinophils: (%) (+ test >3%, - test <3%)	40.92 (30.62 – 51.21)	23.06 (19.76 – 25.69)	<0.0001
FEV ₁ Percent Predicted: (%) (+ test <80%, - test >80%)	35.02 (28.40 – 41.64)	22.66 (19.97 – 25.35)	0.0001
<i>Data were calculated using Log mean values, the values presented in the table above are geometric means and 95% confidence intervals</i>			

Figure 5.1:

Bar chart to illustrate the difference in Mean Baseline FeNO values associated with clinical tests measuring airway inflammation and lung function.



There was a significant difference in mean baseline FeNO associated with no positive variables (18.75ppb 50ml/sec), 1 positive variable (28.44ppb 50ml/sec), 2 positive variables (35.05ppb 50ml/sec) and 3 positive variables (51.65ppb 50ml/sec). ANOVA (p=0.0004). These results are presented in table 5.5 and illustrated graphically in Figure 5.2.

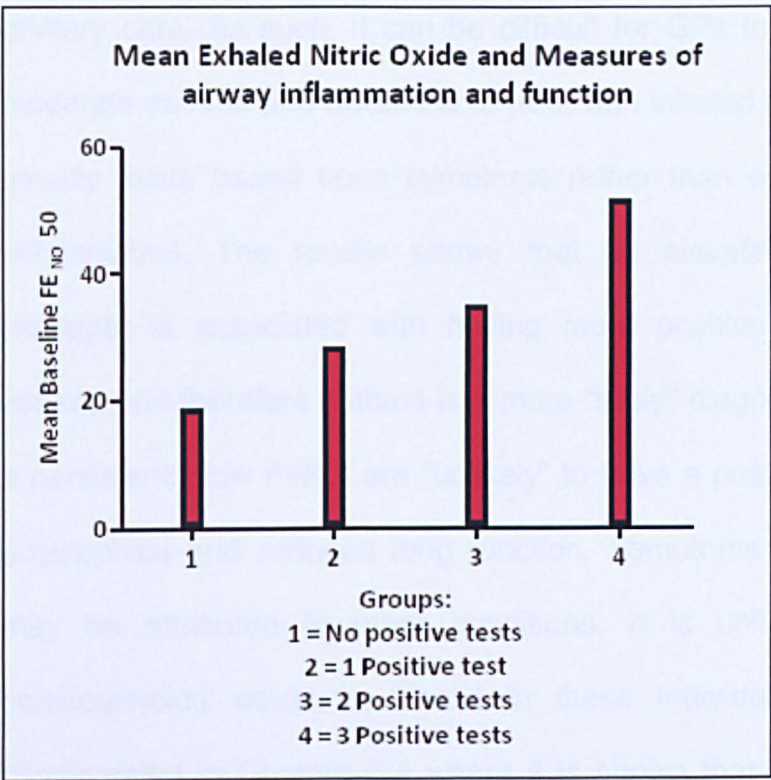
Table 5.5:

Mean Baseline FeNO associated with number of positive clinical variables.

<u>Baseline Mean FeNO according to number of positive clinical variables</u>		
Number of Positive Clinical Variables	Mean Baseline FeNO (ppb)	P Value (ANOVA)
0	18.75 (14.39 - 23.11)	0.0004
1	28.44 (22.41 - 34.46)	
2	35.05 (24.64 - 45.49)	
3	51.65 (18.96 - 84.34)	
<i>Data calculated as log Mean FeNO, data presented as geometric means and 95% confidence intervals</i>		

Figure 5.2:

Bar chart to illustrate the difference in Mean Baseline FeNO values associated with the number of positive clinical tests.



See appendix section 11 for further detailed graphs of these correlations.

Discussion:

GPs do not have access to detailed clinical lung function tests which measure airway hyperresponsiveness and airway inflammation in primary care. As such, it can be difficult for GPs to diagnose mild to moderate asthma and decisions to treat with inhaled corticosteroids are usually made based upon symptoms rather than evidence of airway inflammation. The results shows that an elevated exhaled FeNO (>25ppb) is associated with having more positive clinical tests for asthma and therefore asthma is a more “likely” diagnosis. Patients with a persistently low FeNO are “unlikely” to have a positive PC₂₀, sputum eosinophilia and reduced lung function. Symptoms in these patients may be attributed to other conditions, it is unlikely that inhaled corticosteroids would be useful in these individuals. This is also corroborated in Chapter 5.4 where it is shown that a high FeNO can distinguish between eosinophilic and neutrophilic airway inflammation and the likelihood of a response to steroids. Further investigations may be required in order to accurately determine the cause of the symptoms. Conversely a patient who presents with a persistently high FeNO and symptoms of asthma is more “likely” to have a positive PC₂₀, sputum eosinophilia and reduced lung function. In these instances inhaled corticosteroid medications are likely to be useful in reducing airway inflammation. Patients who present to their GP with symptoms of asthma and an elevated FeNO are more “likely” to have additional evidence of asthma (should these tests be performed). GPs could initiate an inhaled corticosteroid trial in these patients before referring

into secondary care. The patients who present with an intermediate level FeNO may prove more problematic. Despite the mean baseline FeNO for one positive clinical test (28.44ppb) and two positive clinical tests (35.15ppb) being elevated the confidence intervals for the two groups range from 22.41-45.49ppb. One positive clinical test may not be enough to accurately diagnose asthma. For example a reduced FEV₁ percent predicted alone may not be a reliable indicator of asthma. Using FeNO in primary care may help GPs to determine whether to prescribe an inhaled corticosteroid and whether to refer into secondary care.

Conclusion:

Exhaled nitric oxide is a non-invasive biomarker of airway inflammation. It is a quick and simple test which is feasible in primary care. When patients present to their GP with symptoms of asthma, using FeNO could provide important additional clinical information in primary care centres. FeNO is likely to be most useful in patients where it is persistently high or persistently low to help GPs determine whether a patient is “likely” to have other positive clinical tests (in the case of a high FeNO), or “unlikely” to have other evidence of asthma (in the case of a persistently low FeNO). This could help prevent unnecessary inhaled corticosteroid treatment and help guide referrals into secondary care more accurately. Thus saving NHS prescription and referral costs and preventing unnecessary exposure to inhaled corticosteroids and their side effects.

5.4 - The relationship between elevated exhaled nitric oxide and eosinophilic inflammation in mixed granulocytic asthma:

Hypothesis:

Exhaled nitric oxide can distinguish between eosinophilic and non-eosinophilic sputum in a mixed cohort of patients with asthma.

Introduction:

This section refers to a separate analysis and study population. We were interested in determining whether FeNO measurements could distinguish between eosinophilic and non-eosinophilic sputum in a large mixed cohort of patients with asthma. In order to do this analysis we combined three separate study populations together into one data set. Population one was the mild to moderate study population described in chapter 4. Population 2 was taken from a study looking at asthma patients who were taking SABA medication and/or ICS medication only (no LABA). Population 3 was taken from a study looking at patients with severe asthma taking high dose ICS and/or oral steroids.

The sputum and FeNO from all the patients was combined and analysed as a separate data set (n=191).

Exhaled nitric oxide (FeNO) and induced sputum differential cell counts can be used to classify the heterogeneous airway inflammation observed in asthma. Airway eosinophilia is associated with elevated FeNO, while neutrophilic inflammation is associated with a reduction in FeNO. Because neutrophils produce superoxide which can reduce nitric

oxide, we hypothesised that FeNO would be reduced in mixed granulocytic inflammation when compared to pure eosinophilic inflammation. This may be clinically relevant as the detection of eosinophilia by FeNO alone may be impaired by the presence of airway neutrophilia.

Methods:

We performed a retrospective, cross-sectional study of 191 subjects with asthma recruited to our respiratory research database between August 2009 and October 2011. Subjects with a clinical diagnosis of asthma, aged 18-80 years, prescribed as-required bronchodilators, long-acting β_2 agonists, 0-4000 micrograms inhaled beclomethasone dipropionate equivalent or maintenance oral steroids (0-20mg Prednisolone) were selected.

Detailed methodology of the techniques used can be found in chapter 3. Current smokers were excluded. Spirometry, FeNO 50ml/sec flow, sputum induction and Asthma Control Questionnaire (ACQ) were recorded.

Analysis:

Groups were based on differential cell counts and classified as neutrophilic (neutrophils $\geq 61\%$), eosinophilic (eosinophils $\geq 3\%$), mixed granulocytic (neutrophils $\geq 61\%$ and eosinophils $\geq 3\%$) and paucigranulocytic (eosinophils $< 3\%$ and neutrophils $< 61\%$). SPSS v16.0 was used to log transform FeNO 50ml/sec for one way ANOVA

with Tukey post-hoc test and Kruskal Wallis used for non-normal data. A p-value of ≤ 0.05 was regarded as statistical significance.

Results:

One way ANOVA demonstrated there was a statistically significant difference in mean log₁₀ FeNO between the four sputum groups ($F_{[3,187]} = 14.61, p < 0.001$). Post hoc analysis revealed this was not due to differences in the log₁₀ FeNO between the eosinophilic and mixed granulocytic groups (41.11ppb versus 35.47ppb, $p = 0.84$) or differences in the log₁₀ FeNO 50 between the paucigranulocytic and neutrophilic groups (17.46ppb versus 22.10ppb, $p = 0.26$).

The significant differences in the log₁₀ FeNO occurred between eosinophilic and neutrophilic groups (41.11ppb versus 22.10ppb, $p < 0.001$) and between the eosinophilic and paucigranulocytic groups (41.11ppb versus 17.46ppb $p < 0.001$). This was replicated between the mixed and neutrophilic groups (35.47ppb versus 22.10 ppb, $p = 0.02$) and between the mixed and paucigranulocytic groups (35.47ppb versus 17.46ppb, $p < 0.001$).

Table 5.6:

Baseline demographics for study population classified by sputum phenotype

	Neutrophilic	Mixed granulocytic	Eosinophilic	Pauci-granulocytic	Between Group difference p value
Number %	69 (36%)	25 (13%)	42 (22%)	55 (29%)	-
Age yrs¹	58.61 (13.56)	55.28 (12.17)	51.59 (13.23)	55 (14.61)	0.072
Sex M/F (%)	34/35 (49.3%/50.7%)	11/14 (44%/56%)	19/23 (45.2%/54.8%)	33/22 (60%/40%)	-
BMI¹	29.12 (6.39)	28.59 (4.15)	27.71 (4.02)	29.22 (4.57)	0.700
Smoking history² (pack years)	0 (0-20)	0 (0-15)	0 (0-10)	0 (0-19)	0.069
BDP equivalent² mcg	400 (0-3000)	800 (0-2200)	800 (0-3200)	400 (0-2000)	0.020 [*]
Oral prednisolone² (mg)	0 (0-15)	0 (0-10)	0 (0-10)	0 (0-15)	0.079
FEV₁¹ (L)	2.22 (0.70)	2.15 (0.85)	2.24 (0.74)	2.47 (0.92)	0.666
FVC¹ (L)	3.2 (0.90)	3.18 (1.10)	3.45 (0.85)	3.50 (1.24)	0.944
FeNO 50 (ppb)³	22.10 (18.88-25.69)	35.47 (25.18-49.97)	41.11 (31.77-53.19)	17.46 (14.83-20.56)	<0.001 [*]
Sputum eosinophils³(%)	0.64 (0.51-0.81)	7.76 (5.58-10.79)	13.87 (9.36-20.55)	0.89 (0.64-1.25)	<0.001 [*]
Sputum neutrophils²(%)	83.75 (46.00-99.50)	72.00 (62.00-88.50)	38.05 (7.00-56.50)	47.00 (3.00-60.00)	<0.001 [*]
Blood eosinophils³(x10⁹/L)	0.21 (0.18-0.26)	0.47 (0.35-0.61)	0.41 (0.33-0.51)	0.19 (0.15-0.24)	<0.001 [*]
ACQ²	1.14 (0-4)	2.29 (0-5)	1.5 (0-5)	1.14 (0-4)	0.020 [*]

Notes: ¹Mean (Standard Deviation), ²Median (Range), ³Geometric Mean (95% Confidence Interval),

*Significant at 0.05 level

The baseline demographic data for the four groups is summarised in Table 5.6. There were no significant differences overall between

sputum groups for age ($p=0.072$), BMI ($p=0.67$), FEV₁ ($p=0.53$), FVC ($p=0.94$) or smoking history ($p=0.056$).

Discussion:

The data presented show that in a mixed, un-selected cohort of patients with asthma exhaled nitric oxide can be used to differentiate between different sputum inflammatory phenotypes.

Patients with sputum eosinophilia (eosinophilic asthma) are more likely to be steroid responsive than patients with sputum neutrophilia (neutrophilic asthma) (294).

In primary care sputum analysis is not routinely available due to time and cost constraints. However, exhaled nitric oxide is a clinical test which we know to be feasible in primary care settings.

If GPs could use exhaled nitric oxide to distinguish between eosinophilic and neutrophilic asthma in without sputum induction, they would be better placed to make informed decisions about which patients to treat with inhaled and oral steroids and which patients to refer into secondary care for additional testing.

Conclusion:

Our data suggests FeNO can differentiate eosinophilic inflammation from both neutrophilic and paucigranulocytic asthma. Significantly, FeNO can detect eosinophilic inflammation even in the presence of the coexisting neutrophilic inflammation observed in mixed granulocytic asthma.

Chapter 6 - Overall discussion and conclusions:

In order to establish whether exhaled nitric oxide can be used as a non-invasive surrogate biomarker of airway inflammation to predict which patients with mild to moderate asthma could reduce their inhaled corticosteroid medication without suffering from a loss of control or exacerbation, certain assumptions were made.

Firstly, it must be assumed that FeNO measurements reflect an important aspect of the asthma disease process acting either directly or as a surrogate marker, secondly that FeNO is modifiable and responds to changes in treatment regime, and thirdly that FeNO measurements provide clinically important information that cannot be discerned by simpler methods. The background introduction to this thesis explores the previous research which quantifies these assumptions.

The research to determine the model for using FeNO measurements as a biomarker of airway inflammation was induced sputum differential eosinophil counts (the current “gold-standard” in non-invasive quantifying of airway inflammation).

Exhaled nitric oxide has been shown to be a good predictor of airway inflammation in moderate to severe patients with asthma (295), but the research is less robust for the mild to moderate asthma cohort (296). Exhaled nitric oxide has been shown to respond to changes in inhaled corticosteroid medication dose and act as a biomarker with which to titrate medication in patients with asthma (172, 230). This is an

important feature of exhaled nitric oxide as asthma is a highly variable disease and treatment requirements are likely to change over time.

Clinical decisions are largely based around the presence or absence of asthma symptoms; however, inhaled corticosteroids are used to treat airway inflammation. The research presented in Chapter 5.1 found no correlation between symptoms and airway inflammation in the analyses performed. These results corroborate similar previous research which also concludes that there is no evidence of a correlation between airway inflammation and asthma symptoms. All patients were recruited into the clinical trial on the basis that they were mild-to-moderate, well-controlled patients with asthma (as defined by a Juniper Asthma Control Questionnaire symptom score <1.5).

However, despite the overall well-controlled, asymptomatic nature of the study population, there was great disparity in the levels of airway inflammation present. There were no significant correlations between measures of inflammation (Blood eosinophils ($r=0.1636$, $p=0.06$), Sputum eosinophils ($r=0.2168$, $p=0.3028$), exhaled nitric oxide ($r=0.0683$, $p=0.4060$) and symptoms.

Participants who suffered from a loss of control (as defined by an increase in asthma symptoms of >0.5 according to the Juniper Asthma Control Questionnaire) did not show a corresponding significant increase in levels of airway inflammation.

Patients who suffered from an exacerbation showed an increase in FeNO and sputum eosinophilia however there were only three sputum

samples and five FeNO readings at the time of exacerbation from the 31 (17%) patients who suffered from an exacerbation. The low numbers of exacerbation samples mean that accurate conclusions are difficult to draw and thus these results are difficult to interpret.

Despite the fact that symptoms have been repeatedly shown to not correlate with measures of airway inflammation, primary care centres still continue to make ICS treatment decisions based on the presence or absence of symptoms. This is likely to be due to the lack of clinical tools which are available, affordable and feasible in primary care. GPs need a test which can be quickly performed with ease and produce an instant result to quantify levels of airway inflammation in a 3-5 minute consultation. Exhaled nitric oxide is a clinical tool which fulfils these criteria and whilst it has been shown to have positive correlations with other measures of airway inflammation (sputum eosinophils) in moderate to severe patients with asthma, the results in the mild to moderate population remain debated.

An analysis assessing the relationship between measures of airway inflammation and FeNO (Chapter 5.2), determined that there were significant correlations between FeNO and sputum eosinophils ($r=0.2283$, $p=0.0392$), blood eosinophils ($r=0.3543$, $p<0.001$) and blood IgE ($r=0.2643$, $p=0.0005$).

The results show that in patients with mild to moderate asthma FeNO does reflect airway inflammation and could therefore potentially be used

as a simple measureable biomarker in primary care to add quantitative clinical data to the subjective assessment of symptoms.

An additional analysis (chapter 5.3) also assessed whether exhaled nitric oxide levels change in patients depending on the number of other tests of airway (dys)function which indicate asthma each patient may have. Thus whether FeNO alone can help GPs decide which patients may need to be referred into secondary care or research centres for additional testing.

The three tests of airway function which were used in the model were: airway hyperresponsiveness (PC₂₀: positive indicator test <8mg/ml), sputum eosinophil count (Eosinophils: positive indicator test >3%) and FEV₁ percent predicted (% predicted: positive indicator test <80%).

The analysis concluded that participants who had no positive indicator tests had a mean baseline FeNO (50ml/sec) of 18.75ppb (normal cut-off for the Aerocrine™ FlexFlow® = <25ppb = normal/not raised). Participants who had all three positive indicator tests had a mean baseline FeNO (50ml/sec) of 51.65ppb (normal cut-off for the Aerocrine™ FlexFlow® = >50ppb = high/raised).

The four groups (0,1,2,3 positive indicator tests) were significantly different by ANOVA analysis (p=0.0004).

This relationship may help GPs decide how treatment regimes should be changed/implemented in their patients with asthma. For example if a patient presents with a persistently low FeNO and “symptoms of asthma” which do not change/improve despite medication, the GP may

wish to refer this patient for further investigation. The patient may have neutrophilic asthma which is typically unresponsive to inhaled corticosteroid medication and presents with a lower FeNO value or they may have a different medical condition. In the abstract presented in Chapter 5.4 the results show that FeNO can distinguish between eosinophilic and neutrophilic airway inflammation, which further supports the use of FeNO to help guide treatment and referral decisions.

Using FeNO in primary care could provide important additional clinical information in addition to airway function (where performed) and symptom assessment.

The analyses concluded that FeNO does correlate with airway inflammation, does not correlate with symptoms and can potentially provide important information on underlying disease pathophysiology and likelihood of having other positive indicator tests of asthma. Despite this, unfortunately the results of the pilot study showed that FeNO cannot be used to predict which well-controlled patients with asthma can step-down their inhaled corticosteroid medication without suffering from a loss of control or exacerbation. Despite 67% of the study population achieving a successful 50% ICS step-down, FeNO cannot help predict who these patients from their baseline measurements.

An important part of good asthma management is ensuring that symptom control is maintained on the lowest doses of inhaled corticosteroid medication possible (2). In well-controlled patients with

asthma inhaled corticosteroid step-down is recommended after three months of good control. However, this generally does not routinely happen in primary care. This is likely to be for three reasons: firstly; GPs do not have access to any clinical measurements to quantify a step-down decision. Secondly; GPs fear they may provoke an exacerbation in the patients who step-down. Thirdly; patients can be reluctant to step-down. This means that many patients with asthma remain over-treated with inhaled corticosteroids and thus they are exposed to unnecessary side effects and prescription costs.

Exhaled nitric oxide has been shown to be a simple measure which is feasible in primary care. Whilst FeNO has been shown to have positive uses, this study concluded that it cannot be used to predict successful step-down of inhaled corticosteroids in well-controlled patients with asthma.

Step-down was achieved in the majority of patients (67%) and the exacerbation rate was no higher than in background control groups in the literature (17, 277). This indicates that step-down in the mild to moderate asthma population is safe and worthwhile. However there are no clinical measures of airway (dys)function or inflammation which can be used to predict successful step-down. This lack of a quantitative clinical tool may mean that GPs remain reluctant to step-down ICS therapy in patients with mild to moderate well-controlled asthma. However the study results should give confidence to GPs and patients

who do with to trial ICS step-down, that it is possible even without a quantitative monitoring tool.

Many of the patients who participated in the ICS step-down study indicated that they wanted to step-down their ICS dose in a “controlled” environment as they were unsure if they were taking the correct dose of ICS while others were concerned with the side-effects and the “bad press” associated with taking steroids. Despite the fact that the study concluded that there were no tests which can predict successful step-down, the study was exceptionally well received and easy to recruit. 14% of the participants who remained well-controlled at three months attempted to decrease their ICS dose further after the study (appendix section 1). In the appendix section 2 I have included some of the feedback questionnaires from the study which clearly indicate that step-down is wanted by the patients.

6.1 - Study weaknesses:

This study was designed as a pilot study to determine the FeNO cut-off values and sample size required for a randomised placebo-controlled trial. As such the study did not require a placebo (non-step-down) arm.

The study also suffers from the same problem as other pragmatic clinical asthma studies; asthma is a heterogeneous disease and because of that the possible GP “asthma” diagnosis may not have been correct in some patients. In this study population 106/191 (55%) had a normal PC₂₀ at baseline (>8mg/ml).

It could be argued that the 7 day duration of step-down was not long enough. However, this decision was made for two reasons: Firstly, it is a practical time period to perform two FeNO measurements (without losing patients to drop-out). Secondly, it has been demonstrated that FeNO levels rise after one day of treatment reduction (289).

Another problem may be that the step-down of 50% was not big enough to see a response in the 7 day time period in patients with mild to moderate well-controlled asthma. Previous studies have shown that only a small reduction in inhaled corticosteroid dose leads to an increase in FeNO (243). Also a 50% step-down is the recommended dose reduction in the ATS/BTS/SIGN/GINA guidelines and was also the maximum reduction in dose which the regional ethics committee would approve.

The exacerbation rate in the study was low and consequently the study was completed being under-powered. This is not necessarily a weakness as this was a pilot study and the study was designed to assess the level of exacerbation to determine a sample size for a full randomised controlled trial. Many other exacerbation studies are 12 months in durations (this study was only 3 months) to allow for seasonal variation. The reason for choosing a 3 month study was for practical purposes in primary care and also to follow BTS guidelines which recommend a three month follow-up (2). A questionnaire was sent to all patients in the study after 12 months of step-down. There were only an additional four patients who suffered from a loss of control or an exacerbation in this time period. The completed results of the questionnaire can be found in appendix section1.

The fact that the exacerbation rate was so low could be due to the fact that the exacerbations which did take place may have been “chance” exacerbations. Exacerbation frequency is typically around 0.5 exacerbations / patient / year (17, 277) (in the well-controlled, mild to moderate control cohort) and the low number of exacerbations recorded in this study could have been “routine” exacerbations rather than step-down driven.

The low levels of exacerbation could also be attributed to an improvement in medication compliance due to being in a research study. Compliance is difficult to assess but measures were put in place to ensure compliance monitoring where possible. GP surgeries were

contacted to ensure all patients had been collecting their inhaled corticosteroid prescriptions at regular intervals for the previous year. Devices with counters (Seretide® and Symbicort®) were also checked to ensure compliance across the four visits. This was not possible for inhalers without counters. However, the FeNO and spirometry values did not change between visit one and visit two indicating that compliance did not change.

The final problems which need to be addressed when planning studies of this nature are the recurrent problems experienced with the Aerocrine™ NIOX FlexFlow® equipment. These problems have recently been raised by Gibson (2009) in a review article (243). The Aerocrine™ NIOX MINO® system appears to be much more robust than the Aerocrine™ NIOX FlexFlow®. Primary care centres would be most likely to use the Aerocrine™ NIOX MINO® system whereas secondary care and research centres are more likely to use the Aerocrine™ NIOX FlexFlow® system. Technical difficulties need to be addressed if more research is to be performed.

The other technical issue experienced was that well-controlled participants found it difficult to produce sputum. This should be taken into account when designing studies which require sputum results. This study obtained 105 viable sputum samples at baseline from a total of 191 patients (55%).

Despite these weaknesses, the study has significant strengths as discussed below.

6.2 - Study strengths:

The study was designed well to maximise recruitment and ease of collecting clinical data. The data collected was complete and accurate and served to produce robust statistical analysis.

The study confirmed previous observations (258) that step-down is safe in the majority of mild to moderate, well-controlled patients with asthma. The study completed with a 67% successful step-down rate. Recruitment was straight forward; GPs were supportive of the study as were the participants. Initially there was a concern that participants may be worried that stepping-down would lead to a loss of control. However, the feedback received from the participants was positive. Even the patients who could not successfully step-down were reassured to know that they were taking the correct dose of medication as many had never attempted a step-down. The participants and GPs all wanted this study and it was welcomed with ease in Nottingham and Leicester.

The study was the first collaboration study to recruit 191 patients into a study to assess the feasibility of using FeNO to predict step-down in primary care. It was also the first study to assess all five flow rates of exhaled nitric oxide but this study showed no differences in the results of different flow rates in predicting successful step-down.

The study was also designed to reinforce previous research which shows there is no relationship between airway inflammation and symptoms in asthma and investment is needed into research for tools

which GPs can use to improve clinical outcomes in primary care patients with asthma. The study confirmed the relationship between FeNO and sputum eosinophils which was previously shown in moderate to severe patients with asthma. The study also showed that FeNO is higher in patients who have multiple positive indicator clinical tests.

Finally, many participants may not have had a “true diagnosis” of asthma, but the cohort represents the primary care asthma population accurately. It also highlights the need to educate GPs to increase the rate of step-down in well-controlled patients to prevent unnecessary exposure to side effects and to save NHS costs.

6.3 - Unanswered questions:

Using exhaled nitric oxide to guide step-down of inhaled corticosteroid medication was no worse than using the clinical guidelines. In all instances there is no clinical tool or biomarker which can be used to determine who can successfully step-down their inhaled corticosteroid medication. It remains a “trial and error” process, however, the study reaffirmed, that despite the lack of backing by a clinical test, step-down is successful in the majority of well-controlled primary care patients with asthma, and therefore step-down should be attempted. It may become more routinely implemented if step-down became part of the primary care QOF scheme.

The first question is whether FeNO is an individual value in diagnosis, treatment decisions, exacerbation risk and adherence? It is possible to generate a FeNO for each patient and rather than using “whole-population” cut-off values, individual change around a personal value and variability over time may better reflect underlying changes in airway inflammation. Would it be possible to give people individualised treatment plans based upon their weekly/daily FeNO values?

Whilst the study did examine whether a change in FeNO over 7 days could predict step-down, personalised FeNO asthma management plans were not examined and they may be useful in order to track deterioration (e.g. seasonal variation).

The second question is whether using FeNO to step-up/step-down inhaled corticosteroid treatment is the best use of the tool?

FeNO may have a better place in using a low value as a negative predictive tool (as with a D-Dimer assay for VTE).

Thirdly, should we make the same assumptions across all patients with asthma? Mild asthma may actually represent a “different” disease to severe asthma rather than varying severities of the same disease. Treatment and management may need to be completely different.

Finally, a significant number of patients with the diagnostic “label” of asthma have no clinical evidence of the disease, yet these patients are receiving ICS treatment. This may suggest that earlier and more intensive assessment of airway physiology may prevent unnecessary treatment. More intensive work is required to answer these questions, but non-invasive assessment of airway inflammation serves to provide more clinical information into asthma and the diseases processes.

6.4 - Overall Conclusion:

Despite the fact that asthma affects 9% of the UK population and is a highly prevalent disease worldwide, we still know relatively little about the phenotypic variation which exists between and within patients with different severities and clinical presentations of the disease.

The majority of patients with asthma in the UK are managed solely in primary care and with the shift in medical services from secondary care and primary care and community increasing rapidly, the role of asthma diagnosis and management in primary care is set to become even more important.

Providing GPs with the clinical tools they need to manage asthma more effectively is difficult, expensive and time consuming.

This thesis has explored the possibility of using exhaled nitric oxide as a biomarker of airway inflammation to improve asthma diagnosis and management in primary care. The results of the studies in this thesis confirm the findings of previous research that asthma symptoms do not correlate with airway inflammation and that in a mild to moderate population FeNO correlates with other measures of airway inflammation. However, this research concludes that FeNO cannot be used to predict successful step-down of inhaled corticosteroid medication in patients with well-controlled mild to moderate asthma.

There is no doubt that FeNO can provide beneficial additional clinical information but the best use for this biomarker is yet to be determined. Further research is needed into the underlying cell biology of asthma

and FeNO to understand more fully the potential uses for FeNO in asthma management.

In the mean time, good relationships between primary care, secondary care and research need to be developed and fostered to support and improve shared patient care, research progression and the treatment of asthma in the community. A detailed step-down guideline needs to be implemented in primary care to reduce unnecessary side effects in patients who are over-treated and to reduce NHS and prescription costs.

Future research into asthma needs to focus on improving primary care diagnosis, management and medication review over time (step-down and step-up) and improvement of the annual review process.

The focus of future research should ultimately provide better understanding, successful diagnostic accuracy, improved patient outcomes and lower costs.

Chapter 7 - Suggestions for future work:

The data presented in this thesis and previous research studies have shown that exhaled nitric oxide correlates with airway inflammation in asthma. It has also been shown to successfully distinguish between different types of airway inflammation (neutrophilic and eosinophilic) in asthma. However, it is a poor predictor of exacerbation risk and outcome; as such the use of exhaled nitric oxide to predict future exacerbation risk is probably not the right use for this biomarker at this time.

This thesis has also presented the importance of step-down of inhaled corticosteroid medication. Despite step-down being safe in the majority of patients it does not routinely occur in primary care asthma management regimes.

From the background information and the novel research presented in this thesis ideas for that future research into exhaled nitric oxide in asthma and primary care asthma management may focus upon the following areas:

1. FeNO as an aid to diagnosis and management of asthma in steroid-naïve patients.

FeNO has been shown to change in relation to inhaled corticosteroid medication dose. However, steroids could be masking the true nature and use of FeNO. It may be more accurate to measure FeNO in steroid-naïve individuals, who are

suspected of having asthma, as an aid to diagnosis. This would prevent steroid use being a confounding factor in the measurement of FeNO values. It may also be beneficial in helping GPs to determine which patients need inhaled corticosteroids and which patients need referring into secondary care for additional diagnostic support.

2. FeNO as a tool to exclude asthma.

FeNO may be best used as a tool to exclude asthma rather than as a diagnostic tool. In a similar way to D-Dimer being used to “rule-out” VTE rather than confirm it. This would require a large population cohort study to determine the lower limit cut-off values of FeNO to accurately exclude a diagnosis of asthma.

3. FeNO as a tool to monitor long-term medication response.

FeNO has been shown to fluctuate in response to changed inhaled corticosteroid medication doses. Use of FeNO in a monitoring capacity could prove useful in patients who are non-compliant and also in patients who are over-treated. Regular monitoring may prove difficult unless a portable, low-cost “home” version of the exhaled nitric oxide analyser was introduced into the market. GPs would not have time to see all their patients with asthma in their surgery for routine FeNO monitoring.

4. The importance of “personalised” FeNO values as an alternative to whole-population cut-off values.

Further research is needed into the role and importance of personalised FeNO values. The fluctuation in an individual's baseline FeNO value may be more important in diagnosis, management and outcome than using whole population values. A certain percentage change in FeNO from baseline may be more important in assessing exacerbation risk than an initially high or low FeNO. Whilst our research did analyse whether a change in FeNO from baseline was useful in predicting exacerbation risk, the number of exacerbations was too low to detect a signal in change in FeNO. A large population study may help answer these questions.

5. The importance of FeNO variability over time.

It has become widely understood that asthma is a highly variable disease both between patients but also within patients. It is known that the disease presentation and severity can change in patients with asthma over time. This variability can range from short-term seasonal variation to long-term change in disease characteristics. Monitoring a highly variable disease only once a year and being treated continuously with the same medication may not be the best approach.

FeNO may prove to be a useful tool in personalising asthma treatment over time. For example: a patient could measure their

FeNO at home on a daily basis and then take a pre-determined dose of their inhaled corticosteroid medication based on the FeNO result. This could potentially prevent over-treatment during well-controlled periods and increase inhaled corticosteroid dose intake in response to a rise in FeNO / inflammation thus potentially preventing an exacerbation in the future.

6. The design of a model which includes FeNO, spirometry and symptoms.

It may be possible to incorporate FeNO into a multi-factorial model along with spirometry and symptoms.

The study demonstrated that participants were more likely to have a positive PC₂₀ and sputum eosinophilia (therefore more likely to have asthma) if their FEV₁ percent predicted was reduced and their FeNO was high. The Juniper Asthma Control Questionnaire incorporates symptoms, FEV₁ percent predicted and short-acting β_2 agonist use. If a model was developed which incorporated FeNO also, more clinical benefit may be ascertained in a short GP consultation.

7. To improve the understanding of underlying cell biology in asthma and FeNO production.

Another important aspect of future research is to try and fully understand the underlying cell biology of asthma, airway inflammation and FeNO production. It seems more likely that

there may be different forms / variants of the disease rather than just varying severities of the disease.

For example: Rheumatoid Arthritis (like asthma) is characterised as being an inflammatory disease. In Rheumatoid Arthritis (at the time of first presentation with joint stiffness) 60% of patients will have a normal Erythrocyte Sedimentation Rate (ESR) and C-Reactive Protein (CRP), over 60% will be seronegative for Rheumatoid Factor (RF) and 70% will have normal radiographs (297). However, these are the tests used to “diagnose” Rheumatoid Arthritis, thus negative test results cannot always reliably exclude the diagnosis. The disease is “relatively” slow onset and routine monitoring of these patients is vital in order to establish a correct diagnosis as early as possible. Other patients with Rheumatoid Arthritis present with positive tests from onset.

A similar phenomenon may be likely in asthma. There may be some presentation of symptoms but all biochemical and lung function tests are normal. Further assessment and monitoring of the symptoms should occur before excluding the diagnosis of asthma. Further research into these underlying processes is vital if asthma presentation and progression is to be fully understood.

8. To determine whether there is a genetic component to asthma and FeNO production levels.

There could be a genetic component to FeNO production which has not yet been determined and could alter treatment response

in these patients. There may be patients with asthma who are genetically “high FeNO” producers.

For example: There are two reasons why a person may have a high cholesterol, they may have a poor diet and lifestyle (smoking, alcohol consumption and lack of exercise) or they may have a genetic disorder known as Familial Hypercholesterolaemia (FH) which means they lack a receptor which removes cholesterol from the bloodstream. However, the “lifestyle” induced patients with high cholesterol have a much better response to statins (cholesterol lowering medication) than the patients with FH. This could be the same in asthma; there may be genetic and non-genetic variations of FeNO production and inflammation which respond differently to the same treatments.

9. To introduce a standardised step-down guideline into primary care.

Future research should also begin to focus on why GPs are not routinely stepping-down inhaled corticosteroid dose in their well-controlled patients with asthma. More support for GPs and patients may be needed in addition to a standardised step-down guideline (rather than just a recommendation). Step-down should ideally be introduced into primary care QOF guidelines in order to maximise compliance.

Research also needs to focus upon developing tools and methods of assessing step-down, however, the evidence

suggests that step-down is safe in the majority of well-controlled patients with asthma even without these tools. Guideline changes could have the most immediate impact in preventing unnecessary exposure to side effects and reducing NHS prescription costs. Both are valuable reasons to promote research into step-down.

10.To better educate GPs and patients about asthma through the use of digital media and mobile technology.

Developing new drugs and new assessment tests is useless if GPs and patients are not educated effectively about the basic topics such as medication compliance and inhaler technique. There have been recent ideas with regard to introducing more asthma education tools into digital media and mobile technology. Some centres already use electronic feedback (such as blood sugar levels in patients with diabetes) to monitor their patients more regularly without the need for face-to-face appointments. This not only gives the clinicians regular daily diaries of clinical information, but also encourages the patients to take more responsibility for their own condition as they know the GP has access to the data at anytime and not just at the annual review appointment. Similar systems may be feasible in asthma to encourage compliance but also to monitor medication responses, step-down and disease progression remotely.

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Appendix:

Appendix Section 1:

Results of 12 month follow-up questionnaire:

This questionnaire was posted to the 128 patients who completed the three month step-down successfully, to assess their symptoms and medication after 12 months. 121 questionnaires were returned. The results are included.

1. Since completing the study how would you describe your asthma symptoms?

Better: 25%

Worse: 4%

The same: 71%

2. Since completing the study have you needed to increase your ICS medication?

No: 84%

Yes Temporarily: 14%

Yes Permanently: 2%

3. Since completing the study has your ICS medication changed?

Yes Increase: 2%

Yes Decrease: 14%

Yes Change: 3%

No: 81%

4. Since completing the study have you needed any oral steroids?

Yes: 2%

No: 98%

5. Since completing the study have you been involved in any other research studies?

Yes: 26%

No: 74%

6. Since completing the study have you had any difficulty in obtaining your new ICS prescription from your GP?

Yes: 8%

No: 92%

Appendix Section 2:

Step-down study patient feedback:

Below are the comments which were received from the patient feedback questionnaire.

Please use this space to make any further comments you feel may help our research:

Apart from during the past week (when I have had a cold) I have been absolutely fine on lower dose of inhalers.

Please use this space to make any further comments you feel may help our research: I continued with 1 puff of both inhalers morning and evening, as during the trial (4 months period). The reduced amount of medication has helped me with digestive problems.

I have also drastically reduced the intake of wheat (bread, pasta, cakes) and dairy products (cheese and yogurts) in my daily diet, with many benefits including loss of weight (1 1/2 stone) without dieting and feeling less congested.

Please use this space to make any further comments you feel may help our research:

Because of the study my medication has been reduced from 2 puffs of Salmetrol twice daily & 2 puffs of fluticasone twice daily to 1 puff of each twice daily & I am delighted because I feel just as well as before.

Please use this space to make any further comments you feel may help our research:

Taking part in this research does not appear to have affected me. Very pleased to have helped.

Please use this space to make any further comments you feel may help our research:

DEFINITELY GLAD MY MEDICATION WAS REDUCED, AS I CAN STILL DO THE SAME AMOUNT OF PHYSICAL ACTIVITY, WITHOUT THE NEED OF VENTOLIN. ~~AND~~ I HAVE NOTICED A CHANGE IN MY ASTHMA AT ALL. THANKS TO ALL AT BIOMEDICAL RESPIRATORY MEDICINE!! 😊

P.S CONGRATULATIONS EMMA ON WINNING AN AWARD.

Please use this space to make any further comments you feel may help our research:

I was very impressed with the staff at Gtfield. They were so professional and yet so very friendly and dedicated to the research.

Please use this space to make any further comments you feel may help our research:

Very happy to be using what is necessary to keep asthma under control & not take medication because it was a guess as to what would control symptoms.

Please use this space to make any further comments you feel may help our research:

Since completing the study I have only used the Ventolin during a cold, and usually have no symptoms!

Please use this space to make any further comments you feel may help our research:

I FEEL THAT DEEP BREATHING EXERCISES HELP

Please use this space to make any further comments you feel may help our research:

ALREADY THERE WERE A LOT OF PEOPLE WHOSE RESEARCH WAS PUT INTO EACH INDIVIDUAL CASE AS TO WHAT PEOPLE'S ALLERGIES AND TRIGGERS TO THEIR ASTHMA.

Please use this space to make any further comments you feel may help our research:

I would be interested in being involved in any further controlled research that may reduce my intake of medicines further without the fear that I may be doing the 'wrong' thing and doing myself harm.

Please use this space to make any further comments you feel may help our research:

I would like to congratulate you on the success of the study and recognition you have received. I would also like to thank you as without the research study my inhaler would not have been reduced dosage wise. If there is anything else I could do, do not hesitate to contact me.

Please use this space to make any further comments you feel may help our research:

I enjoyed taking part and was pleased to reduce my inhaler dose.

Please use this space to make any further comments you feel may help our research:

I feel my asthma is under control at the moment with the medication I am taking.

Please use this space to make any further comments you feel may help our research:

I HOPE YOUR WORK & RESEARCH IS
SUCCESSFUL & REWARDING
THANKS

Please use this space to make any further comments you feel may help our research:

Thank you for letting me take part + congratulations on your achievement Emma. Good luck for the future. I am happy to take part in any future studies.

Please use this space to make any further comments you feel may help our research:

THANK YOU FOR INVITING ME TO TAKE PART IN THE STUDY
I WAS NEVER INFORMED OF THE PROBLEMS AFFECTING MY
ASTHMA UNTIL NOW. THANK YOU.

Please use this space to make any further comments you feel may help our research:

No comments, Research seemed to be well organised.
Thank you for helping get more control of my asthma.

Please use this space to make any further comments you feel may help our research:

This trial has allowed my asthma to be controlled more effectively by changing my medication as a result. Thank you.

Please use this space to make any further comments you feel may help our research:

I found the visits very useful and they gave me a better understanding about my condition
Thank you for your help and encouragement

Please use this space to make any further comments you feel may help our research:

I went for a 3-4 mile brisk walk in the countryside during the week and my peak flow went up to 267 afterwards which is quite a bit higher than my normal reading. Exercise and fresh air do seem to help my asthma but the effects don't last long unfortunately.

Please use this space to make any further comments you feel may help our research:

I BELIEVE THE INTRODUCTION OF A DAILY ANTI HISTAMINE HELPS + MY SYMPTOMS WORSEN IF I RUN OUT OF THEM.
A GREAT SERVICE TO ME THANKYOU!

Please use this space to make any further comments you feel may help our research:

THE STUDY WAS ALSO AN EDUCATION FOR ME AND SINCE THE STUDY AND USING THE DRY POWDER INHALER MY ASTHMA HAS BEEN MORE CONTROLLED THAN EVER BEFORE.

Please use this space to make any further comments you feel may help our research:

Your advice to reduce my seretide to 125 and take a daily antihistamine has been very successful. I have very few bad days now and all can be linked to allergen reactions as far as I can tell. It was really interesting to me to find out about my allergies. Thanks you and good

Please use this space to make any further comments you feel may help our research:

I have purchased a Flutter device which helps to keep my chest clear - have found it much easier ~~than~~ than the breathing exercises.

Please use this space to make any further comments you feel may help our research:

Hi Emma still on Seretide one puff night and morning. Trial stopping altogether. but it showed I still need to take it.

Appendix Section 3:

Juniper Asthma Control Questionnaire:

Question 1:

On average, during the past week, how often were you woken by your asthma during the night?

0. Never
1. Hardly ever
2. A few times
3. Several times
4. Many times
5. A great many times
6. Unable to sleep because of asthma

Question 2:

On average, during the past week, how bad were your asthma symptoms when you woke up in the morning?

0. No symptoms
1. Very mild symptoms
2. Mild symptoms
3. Moderate symptoms
4. Quite severe symptoms
5. Severe symptoms
6. Very severe symptoms

Question 3:

In general, during the past week, how limited were you in your activities because of your asthma?

0. Not limited at all
1. Very slightly limited
2. Slightly limited
3. Moderately limited
4. Very limited
5. Extremely limited
6. Totally limited

Question 4:

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

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

Question 5:

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

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

Question 6:

On average, during the past period, how many puffs of short-acting bronchodilator (e.g. Ventolin[®]) have you used each day?

0. None
1. 1-2 puffs most days
2. 3-4 puffs most days
3. 5-8 puffs most days
4. 9-12 puffs most days
5. 13-16 puffs most days
6. More than 16 puffs most days

Question 7:

FEV₁ pre-bronchodilator (L)

FEV₁ predicted (L)

FEV₁ % predicted (%)

0. >95% predicted
1. 95-90% predicted
2. 89-80% predicted
3. 79-70% predicted
4. 69-60% predicted
5. 59-50% predicted
6. <50% predicted

Appendix Section 4:

Letter of invitation:

Dear patient,

I am writing to ask if you would be interested in participating in an Asthma trial of a new method of monitoring asthma run by a research group based at Nottingham Respiratory Biomedical Research Unit (based at Nottingham City Hospital). Please find enclosed some more information about this trial. If you decide to participate your asthma care will be taken over by the team at Nottingham City Hospital for the next 3 months, and I will be informed of your progress throughout. I must emphasise that this trial is not looking at a new treatment for asthma, but is investigating whether a new method of monitoring the disease results in an improved outcome and reduced medication doses. The team at Nottingham City Hospital / Leicester Glenfield Hospital will make every effort to fit in with you, and endeavour to make it easy for you to park. They will also meet reasonable travel expenses. If you are interested please contact them or return the pre-paid envelope with the form inside to them.

Thank you for your time,

Yours sincerely,

Practice Manager

Appendix Section 5:

Consent form:

CONSENT FORM (Final version 1: 25.01.2010)

Title of Study: Using exhaled nitric oxide to step-down inhaled corticosteroid therapy in asthma

REC ref: 10/H0402/11

Name of Researcher: Dr. Dominick Shaw (P.I) and Miss Emma Wilson
(PhD student)

Name of Participant:

Please initial box

1. I confirm that I have read and understand the information sheet version number 2 dated 07.03.2010 for the above study and have had the opportunity to ask questions. ☐
2. I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason, and without my medical care or legal rights being affected. I understand that should I withdraw then the information collected so far cannot be erased and that this information may still be used in the project analysis. ☐
3. I understand that relevant sections of my medical notes and data collected in the study may be looked at by authorised individuals from the University of Nottingham, the research group and regulatory authorities where it is relevant to my taking part in this study. I give permission for these individuals to have access to these records and to collect, store, analyse and publish information obtained from my participation in this study. I understand that my personal details will be kept confidential. ☐
4. I understand and agree that a blood sample will be taken for analysis of IgE antibodies and eosinophils and a sputum sample will be taken for cell counts. ☐
5. I agree to my GP being informed of my participation in this study. ☐
6. I agree to take part in the above study. ☐

_____ Name of Participant	_____ Signature	_____ Date
_____ Name of Person taking consent	_____ Signature	_____ Date
_____ Principle Investigator	_____ Signature	_____ Date

3 copies: 1 for participant, 1 for the project notes and 1 for the medical notes

Appendix Section 6:

Patient Information Sheet:

PATIENT INFORMATION SHEET

“Using exhaled nitric oxide to step-down inhaled corticosteroid therapy in asthma”

Research Student: Emma Wilson
Chief Investigator: Dr Dominick Shaw

Introduction

You are being invited to take part in a research study. Before you decide, it is important for you to understand why the research is being done and what it will involve. Please take the time to read the following information carefully and discuss it with others if you wish. Please feel free to ask us if there is anything that is not clear or if you require any further information. You may keep this information for future reference.

Purpose of the study

In asthma the breathing tubes become very congested and inflamed; this can lead to shortness of breath and wheeze or exacerbations. Inflammation in the lower breathing tubes can be measured by monitoring a gas present in your breath called nitric oxide. This research study proposes to evaluate whether tailoring long-term asthma therapy based on exhaled nitric oxide values is better than conventional care, in which therapy is based on symptoms and the results of lung function tests.

Why have I been chosen?

You have been chosen because you have been diagnosed with asthma and are receiving inhaler medications on prescription or because you have responded to our advertisement and expressed an interest in this research project

Do I have to take part?

It is entirely up to you whether or not you decide to take part. If you do decide to take part you will be given this information sheet to keep and be asked to sign a consent form (you will be given a copy of this as well). If you decide to take part you are still free to withdraw at any time and without giving a reason. You will also be asked to sign a consent form which allows us to store your details on our secure BRU database; this is for the purposes of inviting you back for further studies and continuing our research. This is optional and it is entirely your choice whether we store your details or not. A decision to withdraw, or a decision not to take part, will not affect the standard of care you already receive.

What will happen to me if I take part?

If after reading this information sheet and talking to a member of the research team, you would like to take part in this study, you will be seen by a respiratory researcher at Nottingham City Hospital, where you will be asked to sign a consent form and fill out a questionnaire. At this point you will undergo a screening visit; however, you may not be eligible to continue in this study depending on the outcome of this visit. You would also undergo a variety of breathing tests, which include the following:

1. **Spirometry**; which will measure the amount (volume) and/or speed (flow) of air that can be inhaled and exhaled. You will be asked to blow into a tube which is connected to a recording device and repeat this three times. Please be aware that if your spirometry results are slightly lower than normal you will not have a methacholine challenge test (see test 2).
2. **Methacholine Challenge Test**; this measures how reactive your airways are and involves inhaling increasing concentrations of a substance called methacholine, after which your spirometry breathing tests are repeated. It is a simple and safe test widely used in the assessment of asthma.
3. **Sputum Induction**; this test measures the amount of inflammation in the breathing tubes. By taking a sample of sputum we are able to measure the inflammatory cells present in that sputum. If you cannot produce sputum readily we can 'induce sputum' by asking you to inhale increasing concentrations of a salt solution called hypertonic saline. The methacholine challenge test and the sputum induction can sometimes make you feel a bit tight chested but this can be rapidly reversed with an inhaler or nebuliser
4. **Skin Prick Test**; this simple test is used to diagnose any allergies (e.g. grass pollen). A tiny scratch will be made on your forearm and the degree of redness and swelling will be measured.
5. **Exhaled Nitric Oxide Test**; this simple test measures the amount of inflammation in the breathing tubes by measuring the concentration of exhaled nitric oxide. It involves breathing into a tube connected to an analyser for a few seconds at various flow rates.
6. **Blood Test**; a standard blood sample will be taken and analysed for inflammatory cells (eosinophils) and levels of antibody (IgE). Blood samples will not be stored for further use.

After this visit you will be asked to re-attend at 14 (visit 2) and 21 (visit 3) days, at which times we will perform another exhaled nitric oxide and spirometry test and ask you to complete another questionnaire (this questionnaire will be the same as the one completed at visit one).

Finally, one further visit is planned at 3 months (visit 4) at which time we will perform further spirometry, induced sputum, questionnaires, methacholine challenge tests, and exhaled nitric oxide tests. Please be

aware these visits will be brief and at your own convenience as far as possible.

We will ask you if you would be willing to have your details added to our Respiratory database, this means that we can contact you if we have any further studies which we believe you may be interested in. You will sign a separate consent form if you agree to this, but it is completely optional.

What do I have to do?

In this study you will have your asthma steroid treatment reduced by 50% this may involve you stopping your steroid treatment if you are on initially low doses. You should carry on as normal; this study should not affect your lifestyle and you should continue to take any other medication as normal. We also ask that you attend the scheduled study visits although there is some flexibility in terms of the days and times when these occur.

What is the procedure being tested?

This research study proposes to evaluate whether tailoring long-term asthma therapy based on exhaled nitric oxide values is better than conventional care, in which therapy is based on symptoms and the results of lung function tests.

What are the possible disadvantages of taking part?

The methacholine challenge test and the sputum induction can sometimes make you feel a bit tight chested but this can be rapidly reversed with an inhaler or nebuliser.

There is the risk that you will experience an asthma exacerbation, however, 24hour help will be available from our physicians should you require any assistance. You will be provided with emergency contact details for this service. Your GP will also be aware of your involvement in the trial and you may seek advice from them also.

Will any genetic tests be carried out?

No.

What will happen to any samples I give?

A sputum sample will be analysed for the level of inflammatory cells. This gives us a good idea about what type of asthma you may be suffering from and also whether you have a chest infection. The results of the sputum induction will be recorded but the sample will not be stored or kept for future use.

A blood sample will be tested for blood inflammatory cells and IgE (antibodies). The results of the sample will be recorded but the sample will not be stored or kept for future use.

What are the possible benefits of taking part?

We cannot promise the study will help you, but it is hoped that we will be able to assess and control asthma symptoms more accurately and sensitively. This will hopefully lead to a decrease in asthma exacerbations and inappropriate medication use, as well as identifying some patients whose symptoms may not be due to asthma.

Will travel expenses be reimbursed?

Yes, taxi fares or a refund for mileage allowance will be available (maximum £10 allowance).

What will happen to the results of the research study?

We intend to publish the results in a medical respiratory journal. A summary of the results will be available to you should you wish.

Who is organising and funding the research?

The research is being organised and funded by the NIHR Nottingham Respiratory Biomedical Research Unit, which is part of Nottingham University Hospitals NHS Trust and the University of Nottingham. Please be aware that the research team involved in the study are not being paid for including you in this study.

Will my taking part in this study be kept confidential?

All information which is collected about you during the course of this study will be kept strictly confidential. Your G.P. will be informed of your participation in the study.

What if there is a problem?**Complaints:**

if you wish to complain or have any concerns about the way in which you have been treated, please get in touch with the research team (see below), who will do their best to answer any problems you might have. In addition, the normal NHS complaints procedures are also available to you (e.g. Patient Advice and Liaison Service – PALS).

Your first point of contact should be the Chief Investigator *Dr. Dominick Shaw*.

Nottingham Respiratory BRU, Clinical Sciences Building, Nottingham City Hospital, NG5 1PB (0115) 82 31709.

Harm:

In the event that something does go wrong and you are harmed during the research study there are no special compensation arrangements. If you are harmed and this is due to someone's negligence then you may have grounds for legal action for compensation against (Nottingham University, Nottingham University Hospitals NHS Trust) but you may still have to pay your legal costs. The normal National Health Service complaints mechanisms will still be available to you.

Clinical problems:

If at any time you feel unwell or that you are suffering with your asthma more severely than usual, you will be able to contact our 24hour physicians for advice. You will be provided with all emergency contact details. Your GP will also be able to help you should you have any problems.

What if I do not want to continue with the study?

Your participation is voluntary and you are free to withdraw at any time, without giving any reason, and without your legal rights being affected. If you withdraw then the information collected so far cannot be erased and that this information may still be used in the project analysis.

How will I obtain a summary of the results?

When you come for your first visit we will make a note of whether you would like a copy of the results of the study sending to you.

Who has reviewed this study?

All research in the NHS is looked at by an independent group of people, called a Research Ethics Committee, to protect your interests. This study has been reviewed and given favourable opinion by Leicestershire, Northamptonshire and Rutland Research Ethics Committee 2.

Contact for further information:

Emma Elizabeth Wilson

Tel: 0115 82 31935

E-Mail: enquiries@nrbru.org

Thank you for reading this information sheet.

Appendix Section 7:

Asthma drug costs England and Wales:

Drug Name	Brand Name	Inhaler Device Type	Dose Per Inhalation (mcg)	Number Of Inhalations	Cost
Salbutamol	Generic	MDI	100	200	£1.50
Salbutamol	IVAX®	Aerosol Inhalation	100	200	£1.97
Salbutamol	IVAX®	Autohaler	100	200	£6.02
Salbutamol	UCB Pharma®	Asmasal Clickhaler®	95	200	£5.65
Salbutamol	Orion®	Easyhaler® Salbutamol	100	200	£3.31
Salbutamol	Orion®	Easyhaler® Salbutamol	200	200	£6.63
Salbutamol	Chiesi®	Pulvinal® Salbutamol	200	100	£4.85
Salbutamol	IVAX®	Salamol Easi-Breathe®	100	200	£6.30
Salbutamol	Meda®	Salbulin Novolizer® MDI	100	200	£4.95
Salbutamol	A&H®	Ventolin® Accuhaler	200	60	£4.92
Salbutamol	A&H®	Ventolin® Evohaler	100	200	£1.50
Terbutaline	Astra Zenica®	Bricanyl Turbohaler®	500	100	£6.92
Beclometasone dipropionate	Generic	MDI	100	100	£5.36
Beclometasone dipropionate	Generic	MDI	200	100	£9.89
Beclometasone dipropionate	Generic	MDI	200	200	£14.93
Beclometasone dipropionate	Generic	MDI	400	100	£19.61
Beclometasone dipropionate	UCB Pharma®	Asmabec Clickhaler®	100	200	£9.81
Beclometasone dipropionate	UCB Pharma®	Asmabec Clickhaler®	250	100	£12.31
Beclometasone dipropionate	A&H®	Becodisks® Diskhaler	100	120	£11.30
Beclometasone dipropionate	A&H®	Becodisks® Diskhaler	200	120	£21.54
Beclometasone dipropionate	A&H®	Becodisks® Diskhaler	400	120	£42.52
Beclometasone dipropionate	Chiesi®	Clenil Modulite® MDI	50	200	£3.70

Beclometasone dipropionate	Chiesi®	Clenil Modulite® MDI	100	200	£7.42
Beclometasone dipropionate	Chiesi®	Clenil Modulite® MDI	200	200	£16.17
Beclometasone dipropionate	Chiesi®	Clenil Modulite® MDI	250	200	£16.29
Beclometasone dipropionate	Teva UK®	QVAR® Aerosol Inhalation	50	200	£7.87
Beclometasone dipropionate	Teva UK®	QVAR® Aerosol Inhalation	100	200	£17.21
Beclometasone dipropionate	Teva UK®	QVAR® Autohaler	50	200	£7.87
Beclometasone dipropionate	Teva UK®	QVAR® Autohaler	100	200	£17.21
Beclometasone dipropionate	Teva UK®	QVAR® Easi-Breathe	50	200	£7.74
Beclometasone dipropionate	Teva UK®	QVAR® Easi-Breathe	100	200	£16.95
Budesonide	Generic	MDI	100	200	£8.86
Budesonide	Generic	MDI	200	200	£17.71
Budesonide	Generic	MDI	400	100	£17.71
Budesonide	Meda®	Budelin Novolizer® MDI	200	100	£14.86
Budesonide	Astra Zenica®	Pulmicort Turbohaler®	100	200	£11.84
Budesonide	Astra Zenica®	Pulmicort Turbohaler®	200	100	£11.84
Budesonide	Astra Zenica®	Pulmicort Turbohaler®	400	50	£13.86
Ciclesonide	Takeda®	Alvesco® Aerosol Inhalation	80	120	£32.83
Ciclesonide	Takeda®	Alvesco® Aerosol Inhalation	160	60	£19.31
Ciclesonide	Takeda®	Alvesco® Aerosol Inhalation	160	120	£31.62
Fluticasone dipropionate	A&H®	Flixotide Accuhaler®	50	60	£6.38
Fluticasone dipropionate	A&H®	Flixotide Accuhaler®	100	60	£8.93
Fluticasone dipropionate	A&H®	Flixotide Accuhaler®	250	60	£21.26
Fluticasone dipropionate	A&H®	Flixotide Accuhaler®	500	60	£36.14
Fluticasone dipropionate	A&H®	Flixotide Evohaler®	50	120	£5.44
Fluticasone dipropionate	A&H®	Flixotide Evohaler®	125	120	£21.26

Fluticasone dipropionate	A&H®	Flixotide Evohaler®	250	120	£36.14
Mometasone furoate	Schering-Plough®	Asmanex Twisthaler®	200	30	£15.70
Mometasone furoate	Schering-Plough®	Asmanex Twisthaler®	200	60	£23.54
Mometasone furoate	Schering-Plough®	Asmanex Twisthaler®	400	30	£21.78
Mometasone furoate	Schering-Plough®	Asmanex Twisthaler®	400	60	£36.05
Salmeterol	Generic	MDI	25	120	£27.80
Salmeterol	A&H®	Serevent Accuhaler®	50	60	£29.26
Salmeterol	A&H®	Serevent Evohaler®	25	120	£29.26
Salmeterol	A&H®	Serevent Diskhaler®	50	60	£35.79
Formoterol fumarate	Generic	MDI	12	120	£23.75
Formoterol fumarate	Chiesi®	Atimos Modulite® Aerosol Inhalation	12	100	£30.06
Formoterol fumarate	Novartis®	Foradil® Capsule Inhaler	6	60	£23.38
Formoterol fumarate	Astra Zenica®	Oxis Turbohaler®	6	60	£24.80
Formoterol fumarate	Astra Zenica®	Oxis Turbohaler®	12	60	£24.80
Beclometasone dipropionate and Formoterol fumarate	Chiesi®	Fostair® MDI	100/6	120	£29.32
Budesonide and Formoterol fumarate	Astra Zenica®	Symbicort Turbohaler®	100/6	120	£33.00
Budesonide and Formoterol fumarate	Astra Zenica®	Symbicort Turbohaler®	200/6	120	£38.00
Budesonide and Formoterol fumarate	Astra Zenica®	Symbicort Turbohaler®	400/12	120	£38.00
Fluticasone dipropionate and Formoterol fumarate	Napp®	Flutiform® Aerosol Inhalation	50/5	120	£18.00
Fluticasone dipropionate and Formoterol fumarate	Napp®	Flutiform® Aerosol Inhalation	125/5	120	£29.26
Fluticasone dipropionate and Formoterol fumarate	Napp®	Flutiform® Aerosol Inhalation	250/10	120	£45.56
Fluticasone dipropionate and Salmeterol	A&H®	Seretide Accuhaler®	100/50	60	£31.19
Fluticasone dipropionate and Salmeterol	A&H®	Seretide Accuhaler®	250/50	60	£35.00
Fluticasone dipropionate and Salmeterol	A&H®	Seretide Accuhaler®	500/50	60	£40.92

Fluticasone dipropionate and Salmeterol	A&H®	Seretide Evohaler®	50/25	120	£18.00
Fluticasone dipropionate and Salmeterol	A&H®	Seretide Evohaler®	125/25	120	£35.00
Fluticasone dipropionate and Salmeterol	A&H®	Seretide Evohaler®	250/25	120	£59.48

Costs of inhalers for the treatment of asthma in the UK, information taken from www.bnf.org (valid until September 2013) (298).

The current prescription cost in England and Wales is £7.65 per item (298).

Appendix Section 8:

QOF Framework indicators for respiratory disease:

QOF (Quality and Outcomes Framework) indicators for respiratory disorders		
<p><i>Points are awarded for achieving the listed indicators (staged in the case of proportional indicators) which are rewarded by payments adjusted to account for the practice disease prevalence. Patients can be reported as 'exceptions' for clinical reasons (such as terminal illness or if an intervention were contra-indicated) or if a patients declines care (e.g. does not respond to three invitations to attend a review).</i></p>		
Indicator	Points	Payment stages
Asthma		
Records:		
Asthma 1. The practice can produce a register of patients with asthma, excluding patients with asthma who have been prescribed no asthma-related drugs in the previous twelve	4	
Initial Management:		
Asthma 8. The percentage of patients aged eight and over, diagnosed as having asthma from 1 April 2006 with measures of variability or reversibility.	15	40-80%
Ongoing Management:		
Asthma 3. The percentage of patients with asthma between the ages of 14 and 19 in whom there is a record of smoking status in the previous 15 months.	6	40-80%
Asthma 6. The percentage of patients with asthma who have had an asthma review in the previous 15 months.	20	40-70%
Chronic Obstructive Pulmonary Disease		
Records:		
COPD 1. The practice can produce a register of patients with COPD.	3	
Initial Management:		
COPD 12. The percentage of all patients with COPD diagnosed after 1 April 2008 in whom the diagnosis has been confirmed by post bronchodilator spirometry.	5	40-80%
COPD 10. The percentage of patients with COPD with a record of FEV1 in the previous 15 months.	7	40-70%
COPD 13. The percentage of patients with COPD who have had a review undertaken by a healthcare professional, including an assessment of breathlessness using the MRC dyspnoea score in the previous 15 months.	9	50-90%
COPD 8. The percentage of patients with COPD who have had influenza immunisation in the preceding 1 September to 31 March.	6	40-85%
Smoking Indicators		
Smoking 3. The percentage of patients with any or any combination of the following conditions: coronary heart disease, stroke or TIA, hypertension, diabetes, COPD, CKD, asthma, schizophrenia, bipolar affective disorder or other psychoses whose notes record smoking status in the previous 15 months.	30	40-90%
Smoking 4. The percentage of patients with any or any combination of the following conditions: coronary heart disease, stroke or TIA, hypertension, diabetes, COPD, CKD, asthma, schizophrenia, bipolar affective disorder or other psychoses whose notes record that smoking cessation advice or referral to a specialist service, where available, has been offered within the previous 15 months.	30	40-90%
Palliative Care Indicators		
Palliative Care 3. The practice has a complete register available of all patients in need of palliative care/support irrespective of age.	3	
Palliative Care 2. The practice has regular (at least 3 monthly) multidisciplinary case review meetings where all patients on the palliative care register are discussed.	3	

Appendix Section 9:

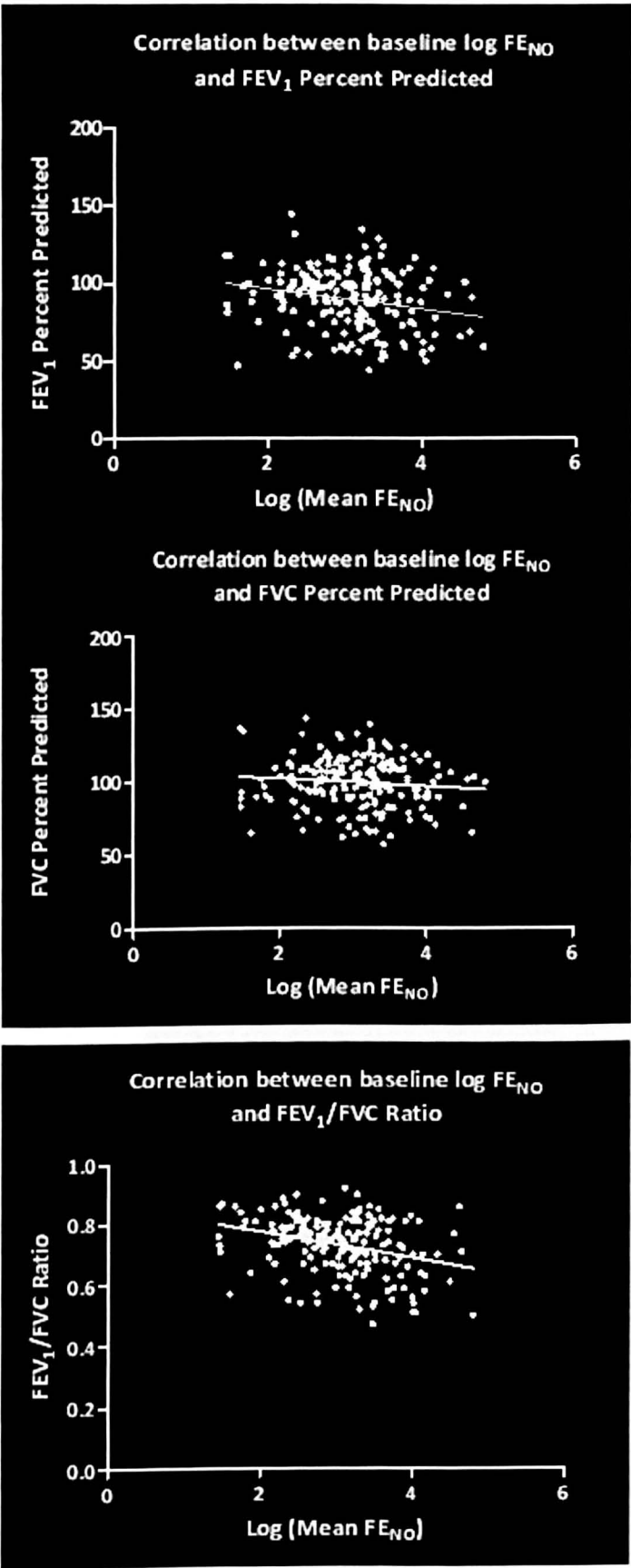


Figure 1:

Scatter graph showing the correlation between log(FeNO) and FEV₁ percent predicted.
(R= -0.2278. p=0.0016)

Figure 2:

Scatter graph showing the correlation between log(FeNO) and FVC percent predicted.
(R= -0.1018. p=0.1621)

Figure 3:

Scatter graph showing the correlation between log(FeNO) and FEV₁/FVC ratio.
(R= -0.3155. p<0.0001)

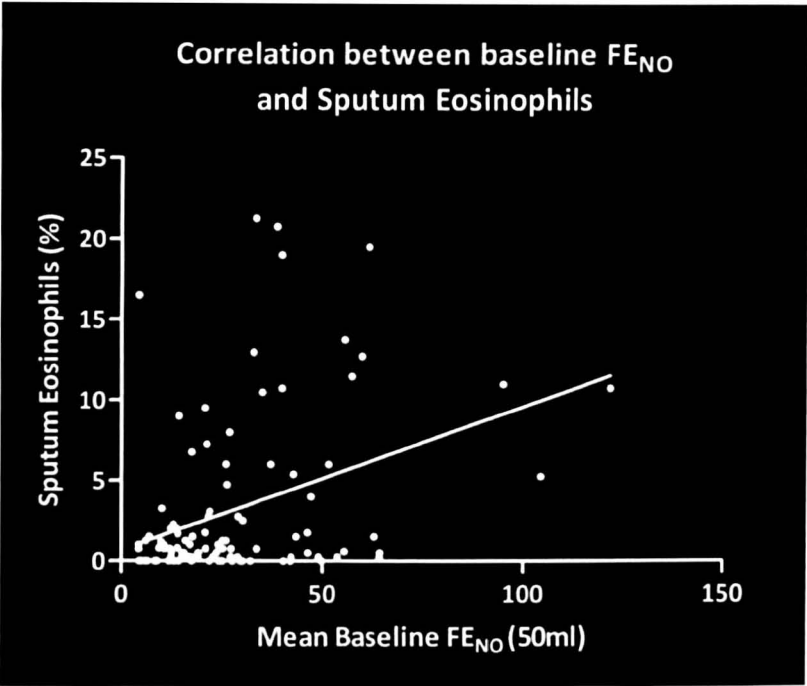


Figure 4:

Scatter graph showing the correlation between FE_{NO} and sputum eosinophils. Data have been anti-logged in order to display clinically relevant data on x/y axis.

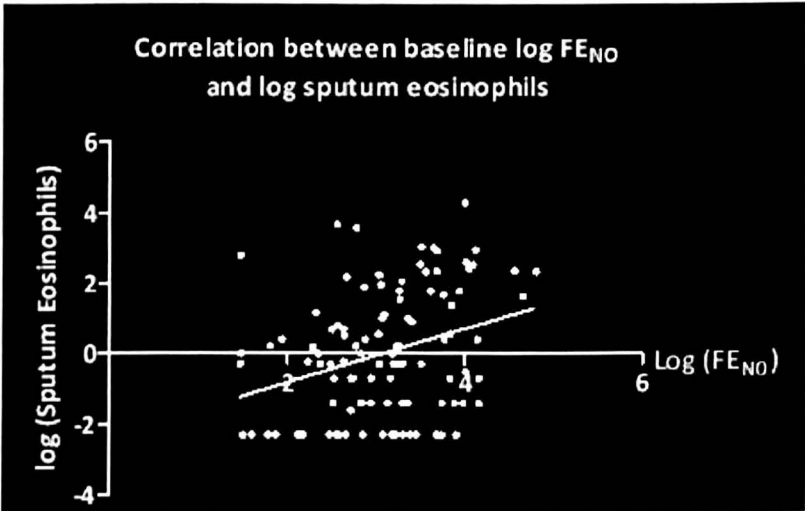


Figure 5:

Scatter graph showing the correlation between $\log(FE_{NO})$ and $\log(\text{sputum eosinophils})$.
 $R=0.2283$, $p=0.0392$.

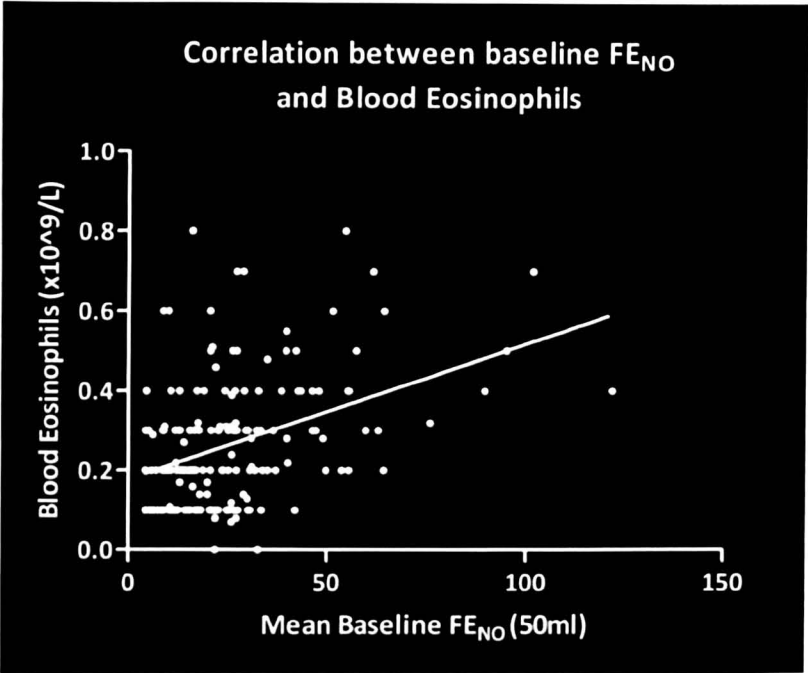


Figure 6:

Scatter graph showing the correlation between FeNO and blood eosinophils. Data have been anti-logged in order to display clinically relevant data on x/y axis.

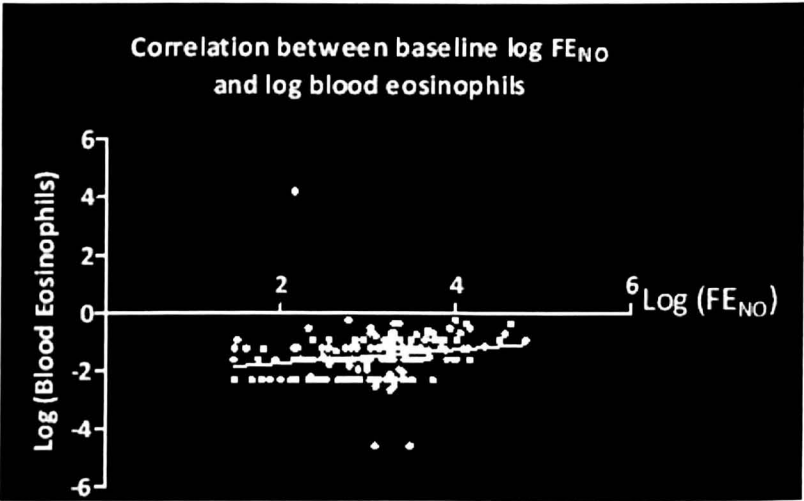


Figure 7:

Scatter graph showing the correlation between log(FeNO) and log(blood eosinophils).

$R=0.3543$, $p<0.0001$.

Appendix Section 10:

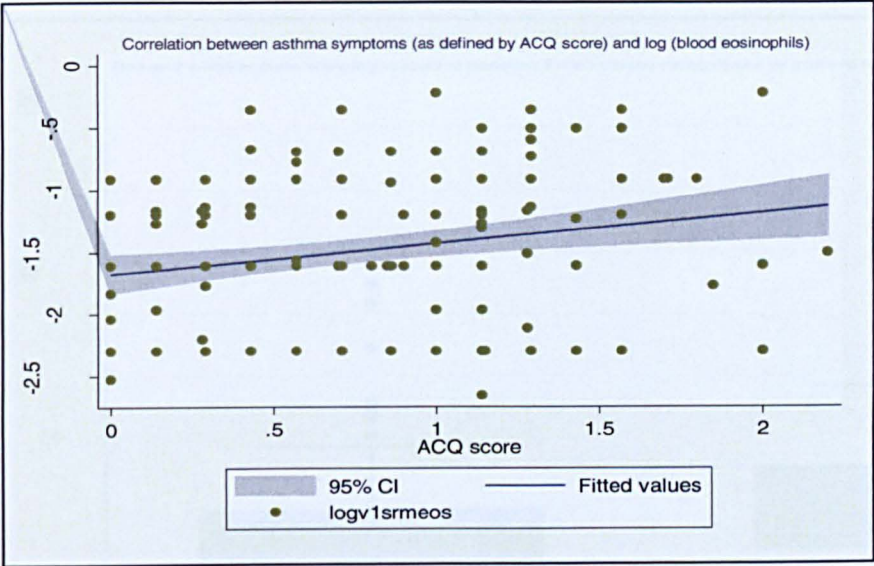


Figure 1:

Scatter graph showing the correlation between ACQ score and log(blood eosinophils).

$R=0.1444$, $p=0.0731$.

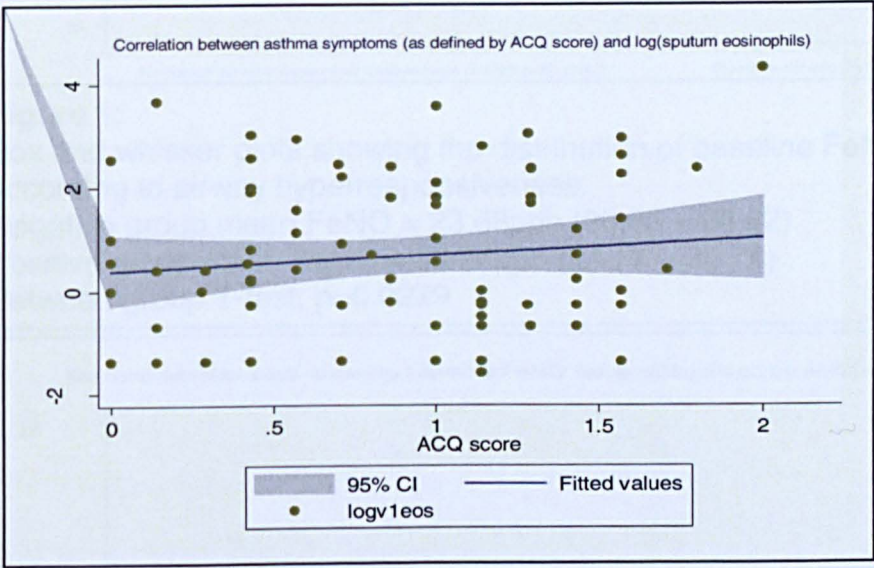


Figure 2:

Scatter graph showing the correlation between ACQ score and log(sputum eosinophils).

$R=0.1034$, $p=0.3707$.

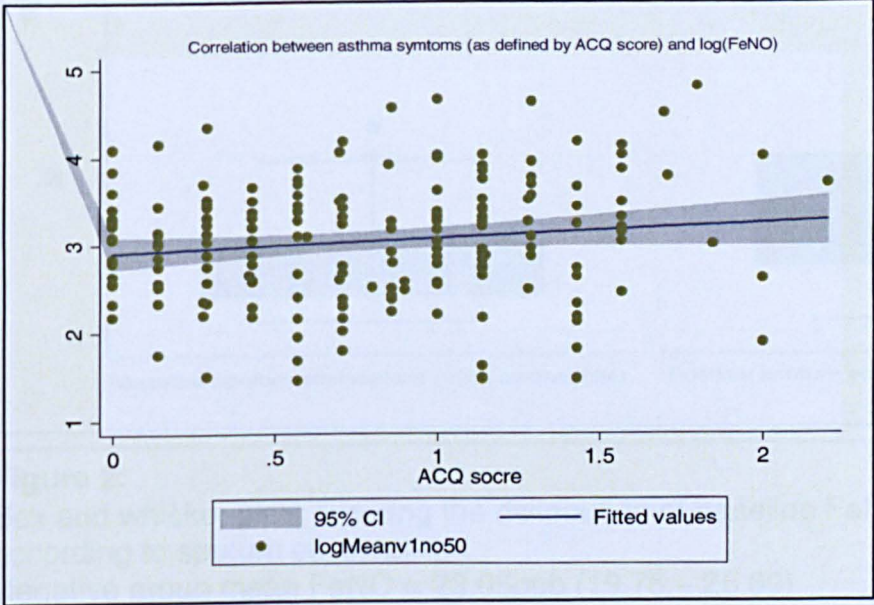


Figure 3:

Scatter graph showing the correlation between ACQ score and log(FeNO).

$R=0.1358$, $p=0.0972$.

Appendix Section 11:

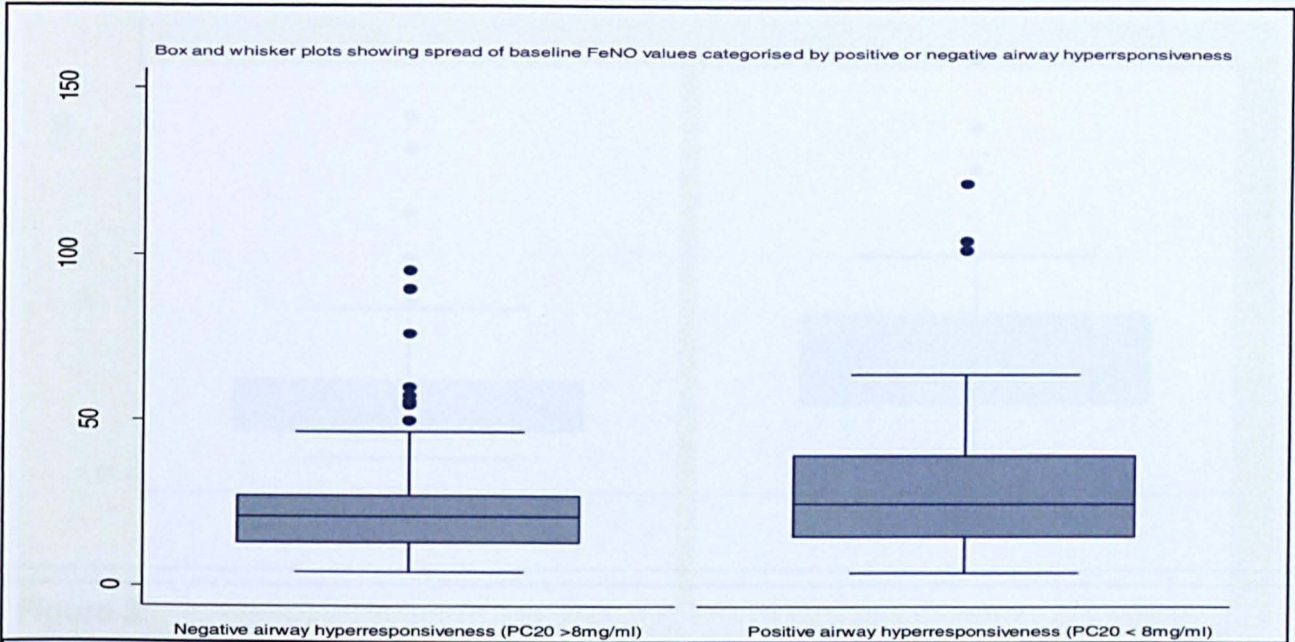


Figure 1:

Box and whisker plots showing the distribution of baseline FeNO values categorised according to airway hyperresponsiveness.
Negative group mean FeNO = 23.68ppb (20.55 – 26.82)
Positive group mean FeNO = 30.26ppb (24.77 – 35.76)
Between group T-test: p=0.0279

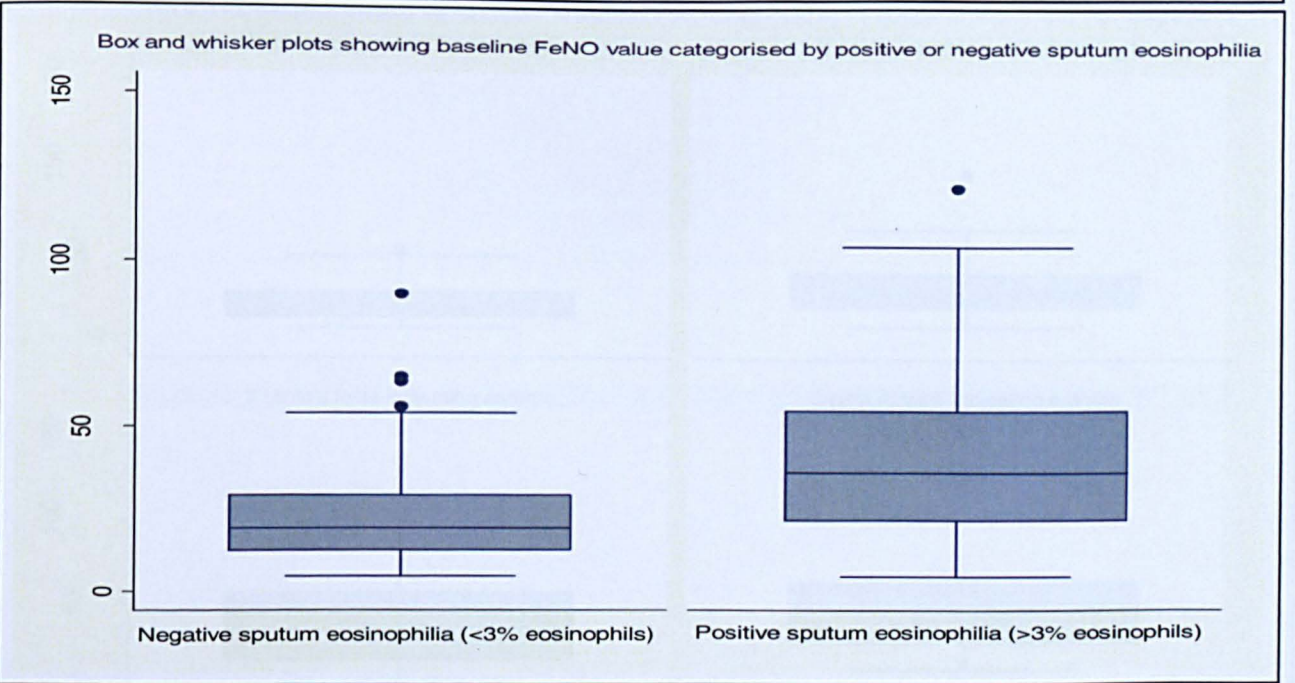


Figure 2:

Box and whisker plots showing the distribution of baseline FeNO values categorised according to sputum eosinophilia.
Negative group mean FeNO = 23.06ppb (19.76 – 25.69)
Positive group mean FeNO = 40.92ppb (30.62 – 51.21)
Between group T-test: p<0.0001

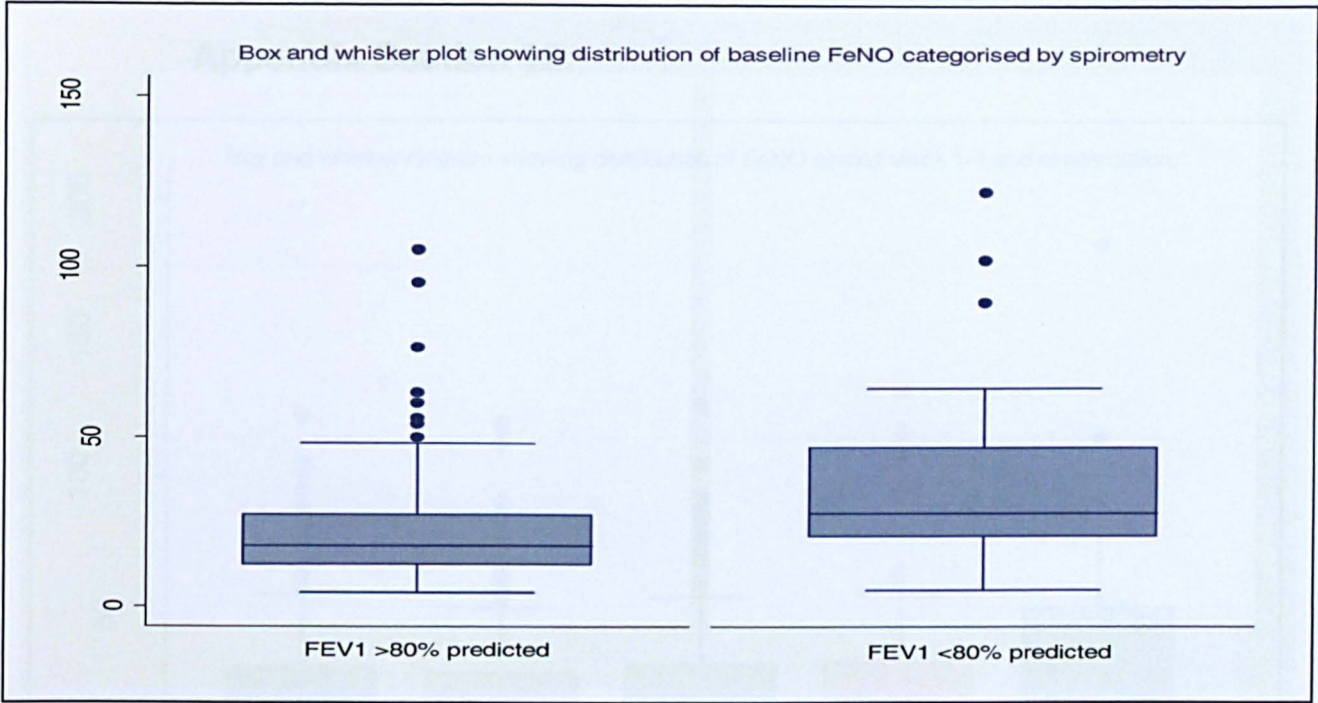


Figure 3:

Box and whisker plots showing the distribution of baseline FeNO values categorised according to spirometry.

>80% predicted group mean FeNO = 22.66 (19.66 – 25.35)

<80% predicted group mean FeNO = 35.02 (28.40 – 41.64)

Between group T-test: $p < 0.0001$

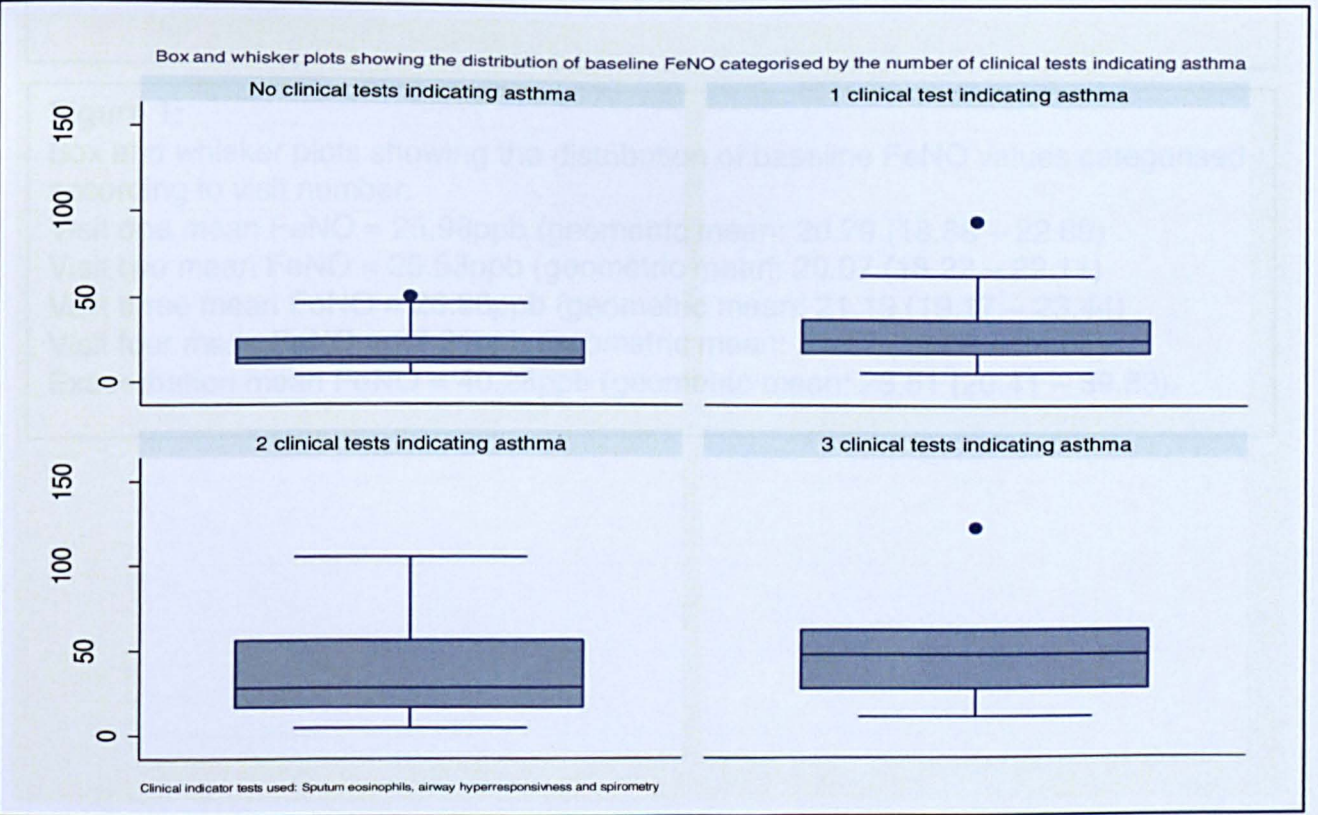


Figure 4:

Box and whisker plots showing the distribution of baseline FeNO values categorised according to number of clinical indicators of asthma.

0 tests mean FeNO = 18.75ppb (14.39 – 23.11), 1 test mean FeNO = 28.44 (22.41 – 34.46), 2 tests mean FeNO = 35.05ppb (24.64 – 45.49), 3 tests mean FeNO = 51.65ppb (18.96 – 84.34). Between groups ANOVA, $p = 0.0004$.

Appendix Section 12:

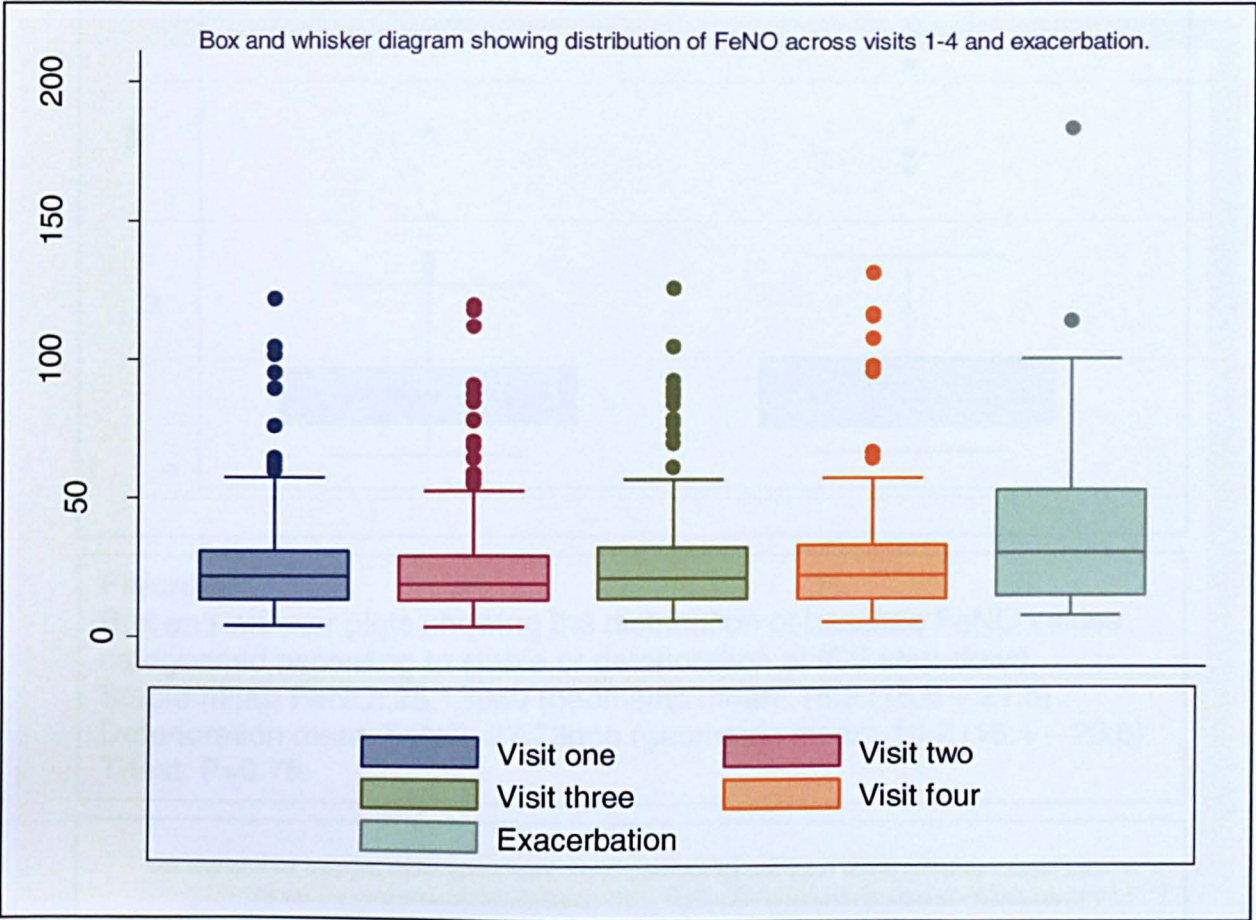


Figure 1:
Box and whisker plots showing the distribution of baseline FeNO values categorised according to visit number.
Visit one mean FeNO = 25.98ppb (geometric mean: 20.79 (18.88 – 22.89)
Visit two mean FeNO = 25.53ppb (geometric mean: 20.07 (18.22 – 22.11)
Visit three mean FeNO = 26.96ppb (geometric mean: 21.19 (19.17 – 23.44)
Visit four mean FeNO = 27.21ppb (geometric mean: 21.67 (19.53 – 24.05)
Exacerbation mean FeNO = 40.28ppb (geometric mean: 28.51 (20.41 – 39.83)

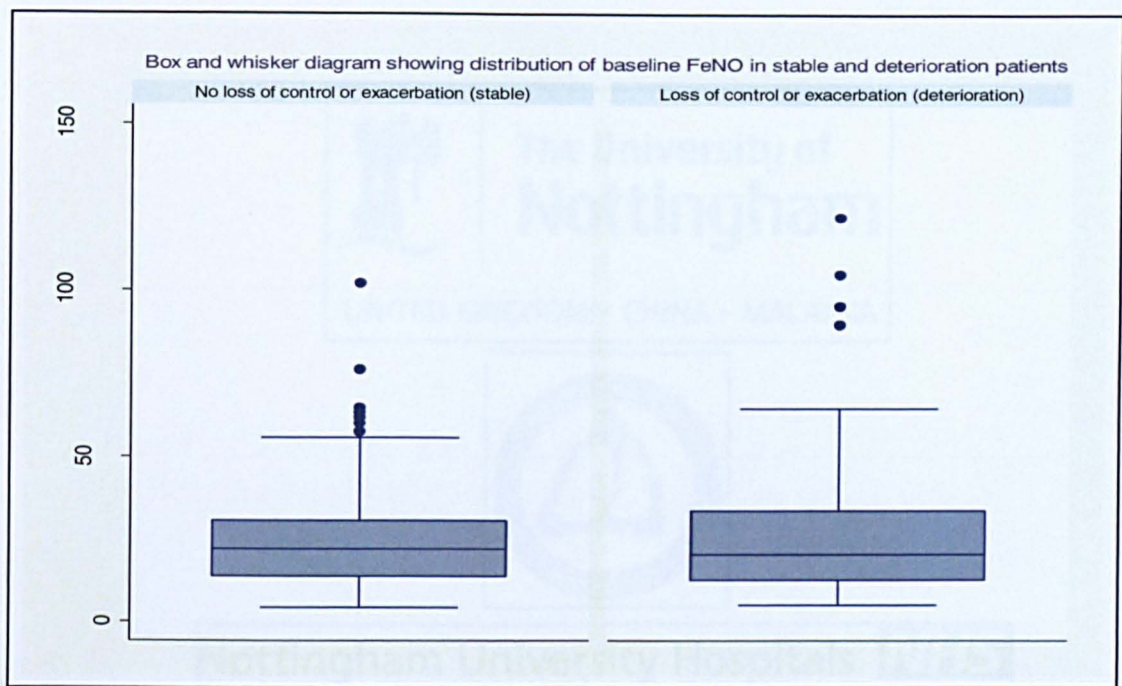


Figure 2:

Box and whisker plots showing the distribution of baseline FeNO values categorised according to stable or deterioration at ICS step-down.
 Stable mean FeNO: 25.13ppb (geometric mean: 18.9 (16.8 – 21.5))
 Deterioration mean FeNO: 27.73ppb (geometric mean: 19.7 (16.4 – 23.6))
 T-test: P=0.76

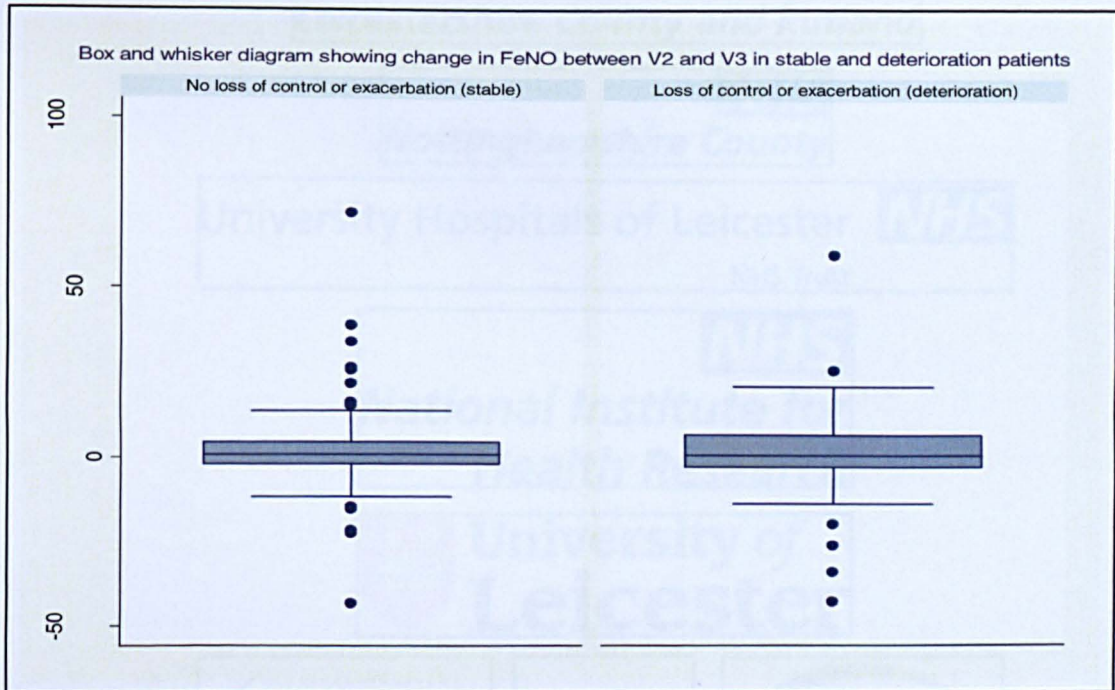


Figure 3:

Box and whisker plots showing the absolute change in FeNO values between visit two and visit 3 (post-step down) categorised according to stable or deterioration at ICS step-down.
 Stable change FeNO: 1.58 ± 11.9 ppb
 Deterioration change FeNO: 1.03 ± 14.88 ppb (T-test: P=0.80)

