Pathological features and inhaled corticosteroid response of eosinophilic and non-eosinophilic asthma

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Abstract

Introduction
Non-eosinophilic asthma is a potentially important clinicopathological phenotype since there is evidence that it responds poorly to inhaled corticosteroid therapy. However, little is known about the underlying airway immunopathology and there are no data from placebo-controlled studies examining the effect of inhaled corticosteroids.

Methods
We have investigated airway immunopathology using induced sputum, bronchial biopsies, bronchial wash and bronchioalveolar lavage in 12 patients with symptomatic eosinophilic and 11 patients with non-eosinophilic asthma and 10 healthy controls. The patients with non-eosinophilic asthma and 6 different patients with eosinophilic asthma entered a randomised, double blinded, placebo controlled cross over study in which we investigated the effects of inhaled mometasone 400µg once daily for 8 weeks on airway responsiveness and asthma quality of life.

Results
Patients with non-eosinophilic asthma had absence of eosinophils in the mucosa (median 4.4 cells/mm² vs. 23 cells/mm² in eosinophilic asthma and vs. 0 cells/mm² in normal controls; p=0.03) and normal subepithelial layer thickness (5.8µm vs. 10.3µm in eosinophilic asthma and vs. 5.1µm in controls, p=0.002). Non-eosinophilic and eosinophilic asthma groups had increased mast cell numbers in the airway smooth muscle compared to normal controls (9 vs. 8 vs. 0 cells/mm², p=0.016). Compared to placebo 8 weeks treatment with inhaled mometasone led to less improvement in methacholine PC_{20} (0.5 vs. 5.5 doubling concentrations, 95% C.I. of difference 1.1, 9.1; p=0.018) and asthma quality of life (0.2 vs. 1.0 points, 95% C.I. of the difference 0.27, 1.43; p=0.008).

Conclusions
Non-eosinophilic asthma represents a pathologically distinct disease phenotype, which is characterised by absence of airway eosinophilia, normal subepithelial layer thickness and a poor short-term response to treatment with inhaled corticosteroids.
**Introduction**

Clinicians have long regarded asthma as a heterogeneous disease \(^1,^2\) although detailed clinicopathological studies have tended to emphasise the similarities in the underlying airway pathology and disordered function between patients \(^3,^4\). The development of safe, non invasive induced sputum techniques has provided the opportunity to study airway inflammation in a diverse range of patients. Using this technique we and a number of other groups have identified a subset of adults who have clear physiological evidence of asthma but no induced sputum evidence of eosinophilic airway inflammation \(^5-^7\). This asthma phenotype is potentially clinically important since several uncontrolled studies have suggested that it is associated with a poor short term and longer term response to inhaled corticosteroid \(^5,^8,^9\).

Non-eosinophilic asthma is present in 53\% of patients presenting to an adult respiratory clinic with symptomatic asthma \(^9\). Other investigators have reported the absence of a sputum eosinophilia in up to 50\% of patients with refractory asthma \(^7\), patients studied during an asthma exacerbation \(^10\) and patients taking high doses of inhaled corticosteroids \(^6\). In a recent longitudinal study of patients with severe asthma the absence of sputum eosinophils has been reported to be a stable feature in a number of patients observed over 12 months \(^11\); another study showed that it was present in untreated symptomatic patients as well as those receiving inhaled corticosteroid therapy \(^9\). These observations suggest that, in some patients at least, non-eosinophilic asthma is a stable phenotype that is not solely explained by the effects of corticosteroid therapy.

Several studies have noted that a airway neutrophilia is often present in patients with non-eosinophilic asthma and Wenzel et al \(^7\) have reported a predominantly neutrophilic airway inflammatory response with absence of eosinophils and normal basement membrane thickness in a subgroup of patients with refractory asthma who underwent bronchial biopsy. These findings support the concept that non-eosinophilic is a pathologically distinct entity, although the extent to which these findings reflect the effects of treatment remains unclear.

The aim of this study was to compare the immunopathology of eosinophilic and non-eosinophilic asthma with normal controls in patients with symptomatic asthma who were not treated with inhaled corticosteroids. We also set out to compare the response to 8 weeks treatment with the inhaled corticosteroid mometasone in a prospective randomised, double blind, placebo controlled cross over trial in the patients with non-eosinophilic asthma and in a subgroup with eosinophilic asthma.

**Methods**

**Subjects**

Subjects were recruited from Glenfield Hospital clinics and by local paper advertisement. All subjects with asthma had symptoms of episodic cough, wheeze or breathlessness such that their Juniper asthma control score was greater than 1.57 points, a level which is consistent with the Global Initiative for Asthma (GINA) recommendations for an increase in treatment. Subjects had at least one of the following objective measures of airway hyperresponsiveness and/or variable airflow obstruction: methacholine provocative concentration causing a 20\% fall in FEV\(_1\) (PC\(_{20}\)) <8mgml\(^1\), increase in FEV\(_1\) of 15\% or greater following inhalation of
200µg of salbutamol and/or peak flow amplitude as percent of mean over 14 days of >20%. Patients with eosinophilic asthma were recruited in two separate groups, one for the bronchoscopy study and one for the placebo-controlled study (figure 1). Normal control subjects had no respiratory symptoms, normal spirometry values and a methacholine PC_{20} >16mg/ml.

Non-eosinophilic was diagnosed in patients with asthma who had a sputum eosinophil count below our normal range (1.9%, 9) on at least two occasions separated by one month while still symptomatic with a Juniper asthma control score >1.57 points and while not receiving inhaled or oral corticosteroids. All patients with non-eosinophilic asthma had no evidence of bronchiectasis on high-resolution computerised tomography scan. We excluded patients who had symptoms due to rhinitis and gastro-oesophageal reflux disease. Our criteria for identifying these symptoms has been previously described 12.

None of the patients had ever smoked, had a respiratory tract infection within six weeks of recruitment or received inhaled or oral corticosteroids for 3 months before entering the study. The Leicestershire and Rutland ethics committee approved this study; all patients provided informed written consent.

BAL T-Cells from some of the subjects in this study were used in a separate study 13.
Measurements
Single flow NO was recorded at 50mlsec\(^{-1}\) as previously described 14. Spirometry was measured using a rolling seal spirometer (Vitalograph, UK). The methacholine PC\(_{20}\) was measured using the tidal breathing method with a maximum inhaled concentration of 16mg/ml, as previously described 15. Methacholine PC\(_{20}\) was calculated by linear interpolation of the change in FEV\(_1\)/concentration of methacholine, on a log dose response curve, as the inhaled concentration of methacholine causing a 20% reduction in FEV\(_1\). Change in methacholine PC\(_{20}\) was expressed in doubling concentrations. Symptom visual analogue score was measured using three 100mm scales representing cough, wheeze and breathlessness 16. Asthma quality of life was measured using the Juniper asthma quality of life score 17. Sputum induction was performed and samples processed as previously described 11.

Sputum IL-8, Cyseinyl-leukotriene and Histamine were measured using standard ELISA kits (BD Pharmagen, Immunotech and Cayman chemicals respectively) and eosinophilic cationic protein (ECP) was measured using a fluorescence immunoassay (UniCAP test, Pharmacia, Uppsala, Sweden). These assays have been previously validated for use in sputum supernatants 18. The sensitivity levels of the assay were 2, 13\(\times\)10\(^{-3}\), 0.8\(\times\)10\(^{-3}\), 50\(\times\)10\(^{-3}\) ng/ml for ECP, cysteinyl leukotrienes, IL-8 and histamine. The intra-assay coefficient of variability was 5-10% and the interassay coefficient of variability 3-15% across a range of concentrations of mediators measured.

Bronchoscopy was performed according to British Thoracic Society guidelines. 20ml of warmed sterile saline solution was instilled into the bronchus intermedius, aspirated and analysed as the wash sample. Three sequential samples of 60ml of warmed sterile saline solution was then instilled into the middle lobe bronchus and then aspirated; the pooled aspirate from these samples was analysed as the BAL. Biopsies were taken from the middle and right lower lobe carinae. The procedures for processing BAL and bronchial biopsies are given in the online supplement.

The BAL and bronchial wash aspirates were filtered through 48\(\mu\)m gauze and diluted to a cell concentration of 0.5\(\times\)10\(^6\) cells per/ml for cytospins and 5\(\times\)10\(^6\) cells per/ml for flow cytometry. Cytospins were made with 75\(\mu\)l of aspirate and stained with Romanowski stain prior to counting by a person blind to the subjects’ details. Cell counts were given as a percentage of at least 400 inflammatory cells counted. Flow cytometry was used to measure CD4, CD8, Interleukin-4 and interferon gamma on CD3+ve lymphocytes from peripheral blood and BAL cells using commercially available antibodies as previously described 19. IL-4 and IFN-gamma were measured using intracellular staining following 4 hours stimulation with PMA and calcium ionophore as previously described.
Biopsy processing
Biopsies were taken from the middle and right lower lobe carinae and were fixed by placing immediately into ice-cold acetone containing the protease inhibitor PMSF (2mM) and maintained at –20°C for 24 hours. Following this, the fixative was replaced with first water free acetone and then methylbenzoate for 15 minutes at room temperature. Biopsies were then placed in 5% methyl benzoate in glycol methacrylate (GMA solution A, Polysciences, Northampton, UK) for 3 periods of 2 hours. Embedding was then performed by placing individual biopsies into Taab tubes and filling with a mixture of 10 mls GMA solution A, 250mcl GMA solution B (Polysciences) and 45mg benzoyl peroxide (Polysciences), replacing the lid to expel any air. Specimens were left to polymerise overnight at 4°C and then stored with silica gel at –20°C until sectioning. 2µm sections were cut and immunostaining performed for major basic protein, neutrophil elastase and tryptase followed by counter staining with haemotoxylin as previously described 20. Biopsies were counted by an individual blinded to the patients’ clinical status and were recorded as the number of cells/mm² positive for major basic protein, neutrophil elastase and tryptase in the submucosa, the number of tryptase positive cells/mm² in the airway smooth muscle and the subepithelial layer thickness. Subepithelial layer thickness was recorded as the mean of 50 measurements taken over a distance of at least 1mm as previously described 21.

Protocol
Patients were identified as eosinophilic or non-eosinophilic on sputum criteria as part of a standard clinical assessment prior to recruitment. All patients with non-eosinophilic asthma had a CT scan as part of their clinical evaluation to exclude sub-clinical bronchiectasis. Following recruitment patients attended for a screening visit at which exhaled nitric oxide concentration was measured prior to skin prick testing, spirometry, methacholine challenge testing and sputum induction. Patients with non-eosinophilic asthma attended for a second screening visit at last 4 weeks after the initial visit, at which symptom scores and sputum differential cell count was repeated. Only the patients with persistent non-eosinophilic asthma proceeded to bronchoscopy and placebo controlled study. All assessments were performed at the same time of day, at least six hours after the last dose of short acting β2-agonist. Because of the possibility that sputum induction could alter airway immunopathology bronchoscopy was performed at least 10 days following the screening visit.

At least four weeks after bronchoscopy patients with non-eosinophilic asthma were entered into a randomised, double blind, placebo controlled crossover study of inhaled mometasone; a separate group of patients with eosinophilic asthma entered this study as a comparison group (figure 1). Flow independent exhaled nitric oxide parameters, spirometry, methacholine PC20, induced sputum, symptom visual analogue scores and asthma quality of life were measured at baseline and after 8 weeks of each treatment, 24 hours after the last dose of study medication and more than 6 hours after the last dose of inhaled short acting β2-agonist. Placebo or mometasone 400µg were inhaled once daily via matched twisthaler® devices. Treatment phases were randomly allocated; they lasted for 8 weeks and were separated by a four-week wash out phase. The order of treatment was determined using a randomisation sequence prepared from a random number generator. The
study drugs and randomisation codes were stored in the hospital pharmacy and could not be accessed by the investigators.

Analysis
Data was tested for normality of distribution using the Kolmogorov-Smirnov test. Data which where log normally distributed was log transformed prior to analysis. Between group comparisons of three groups were made using one way ANOVA with Tukey’s post hoc test for individual group comparison when data was normally distributed or Kruskal-Wallis test when it was not. Between two groups comparisons were made using either independent t-tests or Mann-Whitney U-test according to distribution. Descriptive statistics are given as mean (standard error) for normally distributed data, geometric mean (log standard error) for log normally distributed data and median (interquartile range) for data that was not normally distributed. The bronchial biopsy study was a descriptive, hypothesis generating study, therefore no power calculations were performed and no adjustment was made for multiple comparisons.

Primary outcome measures for the mometasone trial were difference in doubling concentration change in methacholine PC$_{20}$ between placebo and mometasone at 8 weeks and difference in change in asthma quality of life score between placebo and mometasone at 8 weeks. Differences in primary outcomes were compared within groups using paired t-tests and between groups by unpaired t-tests; the period and order effect were analysed by analysis of covariance (ANCOVA). Secondary outcome measures were net change in post-bronchodilator FEV$_1$, symptom visual analogue score, exhaled nitric oxide concentration, sputum eosinophil count and sputum neutrophil count. This study was analysed as a mechanistic study and only data from patients who completed both treatment phases was analysed. We chose methacholine PC$_{20}$ as a co-primary outcome as it was the most responsive outcome measure in an earlier study. AQLQ was chosen as a second primary outcome as it was felt to be a more patient relevant outcome than PC$_{20}$ and potentially allowed us to evaluate a different component of the corticosteroid response. The study had >80% power to detect a 2 doubling concentration difference in net change in methacholine PC$_{20}$ between the two treatment groups.

Results
Subject characteristics are given in table 1. Patients with asthma had significantly lower FEV$_1$ as percent of predicted, FEV$_1$/FVC and methacholine PC$_{20}$ and significantly higher β2-reversibility compared to normal controls. There was no difference between eosinophilic and non-eosinophilic asthma. No patients classified as non-eosinophilic asthma at the first visit developed a sputum eosinophilia (>1.9%) at the second visit or at any other point during the investigation.

Immunopathology study
Patients with non-eosinophilic asthma had lower sputum eosinophil counts, bronchial wash eosinophil counts and BAL eosinophil counts than patients with eosinophilic asthma (table 2). There was no significant difference between sputum, bronchial wash and BAL neutrophil counts, although a trend towards higher sputum neutrophil counts in non-eosinophilic asthma was noted (table 2). There was no difference in the percentage of BAL lymphocytes positive for IL-4 or IFN-γ or in the blood or BAL lymphocyte CD4/CD8 ratio between the groups.
Following embedding, cutting and staining, adequate biopsies suitable for counting were obtained from the following number of normal controls, patients with eosinophilic asthma and patients with non-eosinophilic asthma; submucosa (9, 7, 10), airway smooth muscle (5, 7, 6) and subepithelial layer (7, 11, 7). There were no significant differences in clinical characteristics from patients from who we obtained adequate biopsies and those we did not. Patients with eosinophilic asthma had a median (IQR) 23 (29) bronchial submucosal cells positive for major basic protein per mm² which was higher than both normal controls who had 0 cells/mm² (9.4) and patients with non-eosinophilic asthma who had 4.4 cells/mm² (7.9) (p=0.03, figure 2a). There was no significant difference between the groups in the number of submucosal cells positive for tryptase (13 cells/mm² (5.7) vs. 11 cells/mm² (15) vs. 22 cells/mm² (33); p=0.52); however, the number of tryptase positive cells in the airway smooth muscle was elevated in eosinophilic asthma 8 cells/mm² (12) and non-eosinophilic asthma 9 cells/mm² (56) compared to normal controls (0 cells/mm² (1.8); p=0.016, figure 2b). There were no significant differences in the number of submucosal cells positive for neutrophil elastase. The subepithelial layer thickness was 10.3 µm (3.1) in patients with eosinophilic asthma compared to 5.8 µm (3.0) in non-eosinophilic asthma and 5.1 µm (2.1) in normal controls (p=0.002, figure 2c). Sputum supernatant interleukin-8 concentration was significantly lower in patients with non-eosinophilic asthma compared to normal controls and patients with eosinophilic asthma, table 2.

Placebo controlled study
One patient with non-eosinophilic asthma withdrew from the study after bronchoscopy due to work commitments. No patients withdrew from this part of the study after enrolment. Treatment period or order did not influence values before treatment or the change in primary outcome measures. Compared to placebo 8 weeks treatment with inhaled mometasone led to a net 5.5 (95% C.I. 2.0, 9.0; p=0.01) doubling concentration improvement in methacholine PC₂₀ in patients with eosinophilic asthma and a 0.5 (95% C.I. -2.4, 3.3; p=0.72) doubling concentration improvement in the non-eosinophilic asthma group (mean difference 5.1 doubling doses, 95% C.I. 1.1, 9.1; p=0.018). There was a net 1.0 (95% C.I. 0.5, 1.5; p=0.004) point improvement in Juniper asthma quality of life following treatment with inhaled mometasone compared to placebo in the eosinophilic asthma group and a 0.2 (95% C.I. -0.3, 0.6; p=0.43) improvement in the non eosinophilic asthma group (mean difference 0.9, 95% C.I. 0.27, 1.43; p=0.008, figure 3).

Secondary outcome measures are given in table 3.

Discussion
We describe 12 patients with symptomatic asthma who had distinct sputum and bronchial biopsy pattern, characterised by the absence of eosinophilic airway inflammation and normal subepithelial layer thickness. In common with previous reports, patients tended to be non-atopic middle-aged females; some had sputum evidence of neutrophilic airway inflammation, although increased neutrophil numbers were not evident in bronchoscopy samples. Importantly airway responsiveness and asthma quality of life did not change significantly with 8 weeks treatment with inhaled mometasone when compared to placebo. This was in contrast to findings in patients with asthma and eosinophilic airway inflammation,
where inhaled steroids were associated with a marked improvement in these measures.

Our patients were sufficiently symptomatic to warrant an increase in treatment based on their Juniper asthma control score and all had clear objective evidence of asthma, so the absence of eosinophilic airway inflammation seen in our patients does not reflect remission of underlying disease. Indeed they had more symptoms than the patients with eosinophilic asthma who participated in the double blind placebo controlled study. The absence of sputum eosinophils was evident before entry into the study and was a consistent feature during the study. No patients with non-eosinophilic asthma had evidence of eosinophilic airway inflammation on bronchoscopy or in the six sputum tests done throughout the study, five of which were done off inhaled corticosteroids. These observations support the hypothesis that non-eosinophilic asthma represents a stable clinical phenotype which is not solely explained by the effects of treatment. Our finding of a normal subepithelial layer thickness in our patients with non eosinophilic asthma is consistent with the finding by Wenzel and colleagues in severe asthma. The fact that subepithelial layer thickening is found in patients with rhinitis and in patients with eosinophilic bronchitis without asthma and not in these with non eosinophilic asthma suggests that this finding may be related to eosinophilic airway inflammation rather than asthma per se. Longitudinal studies have suggested that increased subepithelial layer thickness is a longer term marker of eosinophilic airway inflammation than cell counts so the observation that subepithelial layer thickness is normal in non eosinophilic asthma increases our confidence that the absence of eosinophilic airway inflammation is a stable feature.

We acknowledge that our biopsy study observations were made on a small number of patients and that we did not adjust for multiple comparisons. Thus our findings should be regarded as hypothesis generating rather than definitive. However, biopsies were examined blind to patient status and the magnitude and consistency of the effect makes a chance finding unlikely.

The cause of airway inflammation was not specifically investigated in our study. None of the patients had CT evidence of bronchiectasis, symptoms to suggest recent respiratory tract infection or a significant smoking history and atopy was unusual. The association with female gender and onset of symptoms in middle age is similar to observations made in chronic cough and it is possible that there are some similarities between these conditions. Other studies have reported increased sputum neutrophil counts and interleukin-8 concentration and have speculated that the inflammatory response is due to stimuli such as environmental endotoxin, viral infection or ozone leading to activation of the innate immune system and consequent release of proinflammatory cytokines. Our finding of a reduced IL-8 concentration in the sputum supernatants of patients with non-eosinophilic asthma was unexpected and in contrast to previously reported findings; one possible explanation is that there are different types on non-eosinophilic asthma with varying degrees of neutrophilic inflammation.

Perhaps the main point of interest in non-eosinophilic asthma is the suggestion that it represents a corticosteroid resistant form of the disease. Our study is the first double blind, randomised control trial to specifically compare the response to
inhaled corticosteroids in eosinophilic and non-eosinophilic asthma. The observations were made on a small number of patients and there was a difference in baseline severity of symptoms and airway responsiveness between and within group, which could have compromised our results. In addition the small sample size means that we had limited power to detect carry-over effects. However, there were clear differences in primary outcome measures that were above level that the study was powered to detect, suggesting that they were real differences. The significantly reduced response to inhaled mometasone in patients with non eosinophilic asthma compared to eosinophilic asthma is consistent with the findings of previous uncontrolled studies. In contrast to these findings, Godon et al. found a similar response to inhaled corticosteroids in patients who were classified as eosinophilic or non-eosinophilic on the basis of a single sputum sample. However, these observations were based on an uncontrolled study in patients with symptomatic asthma who were recruited during a period of poor asthma control and it is possible that the improvement seen in patients with non-eosinophilic asthma reflected regression to the mean. In addition, patients were younger and more likely to be atopic than the patients with non-eosinophilic asthma identified by us, so it is possible that the populations were different.

Although our study demonstrated a difference in response to inhaled corticosteroids in eosinophilic compared to non-eosinophilic asthma, we cannot conclude from our sample size that there is no significant response to inhaled mometasone in non-eosinophilic asthma. Similarly our study does not allow us to draw any conclusions about the long-term benefits of inhaled corticosteroids. However, an earlier 12 month study has suggested no increase in exacerbation frequency in non eosinophilic asthma despite substantial reductions in the dose of oral and inhaled corticosteroids. Further, appropriately powered studies, should address this important question in more detail.

Our findings suggest that eosinophilic and non-eosinophilic asthma represent distinct clinical and pathological phenotypes. They provide further evidence against a causal relationship between eosinophilic airway inflammation and variable airflow obstruction and airway hyperresponsiveness but provide support for the concept that airway hyperresponsiveness and variable airflow obstruction are causally related to the presence of tryptase positive mast cells in the airway smooth muscle. We have demonstrated that patients with non-eosinophilic asthma have a significantly reduced short-term response to inhaled corticosteroids compared to eosinophilic asthma. Longer, larger studies are required to determine whether inhaled corticosteroids can be safely withdrawn in patients with this asthma phenotype.
Acknowledgements
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Table 1
Clinical characteristics of eosinophilic asthma, non-eosinophilic asthma and normal controls

Table 2
Inflammatory characteristics of eosinophilic asthma, non-eosinophilic asthma and normal controls

Table 3
Clinical and inflammatory characteristics of patients before and after 8 weeks treatment with inhaled mometasone 400µg once daily and matched placebo
### Table 1

<table>
<thead>
<tr>
<th></th>
<th>Normal</th>
<th>Eosinophilic asthma</th>
<th>Non-eosinophilic asthma</th>
<th>Sig. (ANOVA)</th>
<th>Sig. (Eos. vs. non eos.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number (Male)</td>
<td>10 (5)</td>
<td>12 (8)</td>
<td>11 (3)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Age (years)*</td>
<td>41 (22, 61)</td>
<td>42 (27, 69)</td>
<td>47 (19, 68)</td>
<td>0.65</td>
<td>-</td>
</tr>
<tr>
<td>FEV₁ (% predicted)</td>
<td>105 (3.9)</td>
<td>90.3 (6.2)</td>
<td>88 (4.9)</td>
<td>0.05</td>
<td>0.96</td>
</tr>
<tr>
<td>FEV₁/FVC</td>
<td>82 (1.8)</td>
<td>73.9 (2.9)</td>
<td>69 (5.6)</td>
<td>0.025</td>
<td>0.58</td>
</tr>
<tr>
<td>β₂-agonist reversibility (%)</td>
<td>1.1 (0.9)</td>
<td>9.5 (2.5)</td>
<td>12.7 (3.4)</td>
<td>0.001</td>
<td>0.67</td>
</tr>
<tr>
<td>Methacholine PC₂₀ (mg/ml) †</td>
<td>&gt;16</td>
<td>0.67 (0.2)</td>
<td>0.83 (0.2)</td>
<td>&lt;0.001</td>
<td>0.15</td>
</tr>
<tr>
<td>Atopic (%)</td>
<td>2 (20)</td>
<td>8 (66)</td>
<td>2 (18)</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Data given as mean (S.E.) except * - mean (range) and † - geometric mean (log S.E.)

FEV₁ - forced expiratory volume in 1 second, FVC - forced vital capacity, β₂-agonist reversibility – percent increase in FEV₁ following inhalation of 200μg of salbutamol, Methacholine PC₂₀ – concentration of inhaled methacholine which causes a 20% fall in FEV₁.
<table>
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<tr>
<th>Table 2</th>
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<tbody>
<tr>
<td>Normal</td>
</tr>
<tr>
<td>Sputum eosinophil count (%) †</td>
</tr>
<tr>
<td>Sputum neutrophil count (%)</td>
</tr>
<tr>
<td>Sputum IL-8 (ng/ml)*</td>
</tr>
<tr>
<td>Sputum ECP (ng/ml)*</td>
</tr>
<tr>
<td>Sputum Cys-LT (ng/ml)*</td>
</tr>
<tr>
<td>Sputum histamine (ng/ml)*</td>
</tr>
<tr>
<td>Bronchial wash eosinophil count (%) †</td>
</tr>
<tr>
<td>Bronchial wash neutrophil count (%)</td>
</tr>
<tr>
<td>BAL eosinophil count (%) †</td>
</tr>
<tr>
<td>BAL neutrophil count (%)</td>
</tr>
<tr>
<td>BAL IL-4 (% of CD3+ve lymphocytes)</td>
</tr>
<tr>
<td>BAL IFN-γ (% of CD3+ve lymphocytes)</td>
</tr>
<tr>
<td>Submucosal MBP +ve cells (/mm²)*</td>
</tr>
<tr>
<td>Submucosal NE +ve cells (/mm²)*</td>
</tr>
<tr>
<td>Submucosal tryptase +ve cells (/mm²)*</td>
</tr>
<tr>
<td>Smooth muscle tryptase +ve cells (/mm²)*</td>
</tr>
<tr>
<td>Subepithelial layer thickness (µm)*</td>
</tr>
</tbody>
</table>

Data given as mean (S.E.) except † - geometric mean (log S.E.) and * - median (interquartile range). IL-8 – interleukin-8, ECP – eosinophilic cationic protein, Cys-Lt – cysteinyl-leukotriene, BAL – bronchial alveolar lavage, IL-4 – interleukin-4, IFN-γ – interferon gamma, MBP – major basic protein and NE – neutrophil elastase, ANOVA – analysis of variance, KW – Kruskal-Wallis.
Table 3

<table>
<thead>
<tr>
<th></th>
<th>Eosinophilic asthma</th>
<th>Non-eosinophilic asthma</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Placebo 0 8</td>
<td>Mometasone 0 8</td>
</tr>
<tr>
<td>Week</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Methacholine PC&lt;sub&gt;20&lt;/sub&gt; (mg/ml)</td>
<td>1.53 (0.17) 0.16 (0.32) 0.46 (0.22) 2.13 (0.27)</td>
<td>0.01 1.42 (0.18) 1.07 (0.27) 1.69 (0.22) 1.74 (0.18)</td>
</tr>
<tr>
<td>Asthma QOL score</td>
<td>5.5 (0.3) 5.5 (0.4) 5.6 (0.3) 6.6 (0.2)</td>
<td>0.004 5.0 (0.3) 5.0 (0.3) 5.1 (0.3) 5.2 (0.4)</td>
</tr>
<tr>
<td>FEV&lt;sub&gt;1&lt;/sub&gt; (%)</td>
<td>94 (7.9) 93 (8.4) 94 (7.3) 94 (7.9)</td>
<td>0.62 86 (3.8) 84 (4.1) 87 (4.7) 82 (3.2)</td>
</tr>
<tr>
<td>Symptom VAS (mm)</td>
<td>59 (27) 76 (29) 28 (7.6) 12 (7.1)</td>
<td>0.33 141 (41) 122 (39) 151 (39) 115 (50)</td>
</tr>
<tr>
<td>Exhaled NO (ppb)</td>
<td>56 (0.1) 79 (0.1) 65 (0.1) 21 (0.1)</td>
<td>0.003 13 (0.1) 15 (0.1) 13 (0.1) 11 (0.2)</td>
</tr>
<tr>
<td>Alveolar NO (ppb)</td>
<td>9.2 (1.2) 12 (2.3) 5.7 (1.2) 4.8 (1.3)</td>
<td>0.27 3.2 (0.5) 2.4 (0.6) 3.1 (0.3) 3.9 (0.9)</td>
</tr>
<tr>
<td>Sputum eosinophils (%)</td>
<td>7.1 (0.1) 9.9 (0.1) 11 (0.2) 2.3 (0.2)</td>
<td>0.001 0.6 (0.2) 0.4 (0.2) 0.4 (0.2) 0.5 (0.1)</td>
</tr>
<tr>
<td>Sputum neutrophils (%)</td>
<td>26 (5.6) 33 (7.2) 40 (8.9) 45 (11)</td>
<td>0.93 63 (7.7) 67 (7.6) 65 (5.7) 55 (6.7)</td>
</tr>
</tbody>
</table>

Data given as mean (standard error) except where marked † - geometric mean (log standard error) and * - median (range). FEV<sub>1</sub> – forced expiratory volume in one second, PC<sub>20</sub> - dose of inhaled methacholine causing a 20% fall in FEV<sub>1</sub>, QOL – quality of life, VAS – visual analogue score, NO – nitric oxide, IL-8 – interleukin-8, ECP – eosinophilic cationic protein and Cys-Lt – cysteinyl-leukotriene.
Figure 1
Study plan.

Figure 2
Inflammatory cell counts in bronchial biopsies of patients with eosinophilic asthma, non-eosinophilic asthma and normal controls

Figure 3
Primary outcome measures in study of inhaled mometasone versus placebo in eosinophilic and non-eosinophilic asthma
Figure 1. Study plan.
**Figure 2**

a) Submucosal T-cells per mm²
- Bars represent medians.
- Kruskal Wallis p=0.03.

b) Smooth muscle AIV cells per mm²
- Bars represent medians.
- Kruskal Wallis p=0.016

- Normal
- Eosinophilic asthma
- Non eosinophilic asthma

- p=0.043
- p=0.016
- p=0.2
- p=0.024
- p=0.38
- p=0.003
- p=0.002

For the smooth muscle AIV cells per mm², there is a significant difference between the groups (p=0.016). For the submucosal T-cells per mm², the difference is not significant (p=0.043).
Figure 3

Non eosinophilic asthma

Eosinophilic asthma

◇ Represents mean/geometric mean