Relationship between the inflammatory infiltrate in bronchial biopsy specimens and clinical severity of asthma in patients treated with inhaled steroids

Jacob K Sont, J Han J M van Krieken, Christine E Evertse, Ria Hooijer, Luuk N A Willems, Peter J Sterk

Abstract

Background – Current guidelines on the management of asthma advocate the use of anti-inflammatory treatment in all but mild disease. They define disease control in terms of clinical criteria such as lung function and symptoms. However, the relationship between the clinical control of the disease and inflammation of the airways is not clear. A cross sectional study was therefore undertaken to investigate the relationship between airways inflammation and measures of clinical control and bronchial hyperresponsiveness in asthmatic patients treated with inhaled steroids.

Methods – Twenty six atopic adults (19-45 years) with mild to moderate asthma (baseline forced expiratory volume in one second (FEV₁) ≥50% predicted, concentration of histamine causing a 20% fall in FEV₁ (PC₂₀) 0-02-7-6 mg/ml) on regular treatment with inhaled steroids entered the study. Diary card recordings during the two weeks before a methacholine challenge test and bronchoscopic examination were used to determine peak flow variability, symptom scores, and use of β₂ agonists. Biopsy specimens were taken by fibreoptic bronchoscopy from the carina of the right lower and middle lobes, and from the main carina. Immunohistochemical staining was performed on cryostat sections with monoclonal antibodies against: eosinophil cationic protein (EG₁, EG₂), mast cell tryptase (AA1), CD45, CD22, CD3, CD4, CD8, CD25, and CD45RO. The number of positively stained cells in the lamina propria was counted twice by using an interactive display system.

Results – There were no differences in cell numbers between the three sites from which biopsy specimens were taken. The PC₂₀ for methacholine was inversely related to the average number of total leukocytes, EG₁+, and EG₂+ cells, mast cells, CD8+, and CD45RO+ cells in the lamina propria. These relationships were similar for each of the biopsy sites. Symptom scores, β₂ agonist usage, FEV₁, and peak flow variability were not related to any of the cell counts.

Conclusions – Infiltration of inflammatory cells in the lamina propria of the airways seems to persist in asthmatic outpatients despite regular treatment with inhaled steroids. The number of infiltrating leukocytes such as mast cells, (activated) eosinophils, CD8+, and CD45RO+ cells in bronchial biopsy specimens from these patients appears to be reflected by airway hyperresponsiveness to methacholine, but not by symptoms or lung function. These findings may have implications for the adjustment of anti-inflammatory treatment of patients with asthma.

Keywords: atopic asthma, bronchial hyperresponsiveness, inflammatory infiltrate.

Asthma is a chronic inflammatory disorder of the airways with a characteristic infiltrate of mast cells, lymphocytes, and eosinophils in the bronchial epithelium and lamina propria. Asthma is a chronic inflammatory disorder of the airways with a characteristic infiltrate of mast cells, lymphocytes, and eosinophils in the bronchial epithelium and lamina propria. The symptoms of wheezing, chest tightness, difficult breathing, and coughing develop after exposure to bronchoconstrictor stimuli and are associated with variable airways obstruction which can be provoked in the laboratory, thereby demonstrating airway hyperresponsiveness. The disease state can therefore be assessed at different levels: the severity of symptoms, the level of obstruction and degree of airway hyperresponsiveness, and the extent of airway disease.

To date there is no “gold standard” parameter that ultimately reflects asthma severity towards which therapeutic interventions should be aimed. The present international consensus on the diagnosis and treatment of asthma is based on the working hypothesis that it is desirable to reverse or prevent airways inflammation. However, at present the treatment level is solely guided by clinical indices such as symptoms and lung function and the current therapeutic approach is based on the assumption that there is a relationship between the clinical indices of asthma severity and airways inflammation.

When using the scoring system of Aas to assess symptoms over a one year period, it has been shown that the severity of the symptoms is related to the number and activity of eosinophils in the airways epithelium and in the bronchoalveolar lavage (BAL) fluid of patients.
with atopic asthma. The forced expiratory volume in one second (FEV\textsubscript{1}%), expressed as a percentage of the predicted value,\textsuperscript{6-10} and airway hyperresponsiveness to inhaled methacholine\textsuperscript{11-12} are positively related to the number and activity of eosinophils and activated CD4 + T cells in BAL fluid from subjects with atopic asthma. Furthermore, the provocative concentration of methacholine causing a 20% fall in FEV\textsubscript{1} (PC\textsubscript{20}) has also been shown to be related to the number of activated eosinophils in bronchial biopsy specimens.\textsuperscript{7,13,14} Hence, there is evidence that some of the clinical indices of asthma are associated with airways inflammation, at least in patients not on treatment with anti-inflammatory medications.

In order to adjust treatment in patients using anti-inflammatory drugs, the international guidelines rely on the following clinical characteristics: symptom severity, \( \beta_2 \) agonist usage, peak flow variability, and spirometric parameters.\textsuperscript{15} To date it has not been systematically investigated which of these indices is most closely related to the level of airways inflammation in asthmatics using inhaled corticosteroids. In addition, it is still unclear whether measures of airways responsiveness can provide complementary information to symptoms and lung function on the disease state of the airways in these patients.

In the present study we have investigated whether clinical asthma severity, expressed according to present guidelines, is related to inflammatory activity in the airways of patients already using regular inhaled steroids. If so, such a relationship justifies the usage of clinical indices to adjust anti-inflammatory therapy. To that end we have assessed the relationship of symptom scores, \( \beta_2 \) agonist usage, peak flow variability, FEV\textsubscript{1}, and airways responsiveness to methacholine with mast cell number and number and activity of eosinophils and (sub) populations of lymphocytes in bronchial biopsy specimens from patients with atopic asthma who were on regular treatment with inhaled corticosteroids.

**Methods**

**Subjects**

Twenty-six non-smoking atopic adults (17 men, six ex-smokers) of mean age 29 years (range 19–45) with a wide range in asthma severity were asked to participate in the study (table 1) at the outpatient clinic of the University Hospital Leiden. All patients had a history of episodic chest tightness and wheezing in the previous year and were on regular treatment with inhaled corticosteroids for a mean (SD) of 21 (26) months. Atopy was indicated by a positive skin prick test (>3 mm weal) to one or more common airborne allergen extracts (Soluprick, ALK, Denmark). Pre-bronchodilator FEV\textsubscript{1} was more than 50% of predicted\textsuperscript{16} and >1.5 l, whilst post-bronchodilator FEV\textsubscript{1} was within the normal range (>80% predicted). The PC\textsubscript{20} for histamine at the start of the study was 0.02–7.3 mg/ml (geometric mean 0.42 mg/ml). Symptoms were additionally controlled by on demand usage of short acting \( \beta_2 \) agonists that were withheld for at least eight hours before each challenge test. All subjects gave their informed consent and the study was approved by the local ethical committee.

**STUDY DESIGN**

The study was of a cross sectional design. At entry to the study the subjects were equipped with diary cards and a mini-Wright peak flow meter and were asked to record symptoms, peak flow rates (PEF), and bronchodilator usage on the diary cards during the two weeks before the methacholine challenge test. Fibre-optic bronchoscopy was carried out 3–6 days later.

**DIARY CARDS**

The symptom score parameter was calculated from the diary card scores for night time asthma, morning tightness of the chest, daytime asthma, and daytime cough.\textsuperscript{17} Each item could range from 0 to 4 on each day. The highest score for each day among these four items was then added up over a period of 14 days.

Bronchodilator usage was recorded as the total number of puffs of salbutamol or ipratropium bromide during 14 days registered on the diary cards and was expressed per week.\textsuperscript{19} PEF values were obtained from twice daily measurements, on waking and before bedtime, and from additional measurements when a bronchodilator had been used. Diurnal variability in PEF was expressed as the mean value of 14 days calculated from the highest minus the lowest daily value, divided by the highest.\textsuperscript{31,15}

**INHALATION CHALLENGE TEST**

Methacholine inhalation challenge tests were performed according to a standardised tidal breathing method.\textsuperscript{14} The level of baseline lung function was determined as the mean of three reproducible values (within 5%) of the FEV\textsubscript{1}, and was expressed as a percentage of the predicted value (FEV\textsubscript{1}(%pred)). Doubling concentrations of methacholine in normal saline (0.03–256 mg/ml; Janssen Pharmaceutica, Beerse, Belgium) were aerosolised at room temperature by a DeVilbiss 646 nebuliser (output 130 mg/min; DeVilbiss Co, Somerset, Pennsylvania, USA) operated by oxygen and connected to the central chamber of an inspiratory and expiratory valve box with an expiratory aerosol filter (Pall Ultipor BB50T). The aerosols were inhaled by tidal breathing for two minutes at five minute intervals through the mouth with the nose clipped. The response was measured as FEV\textsubscript{1}, recorded by a dry rolling seal spirometer (Morgan Spiroflow, Rainham, UK) 90 seconds after each dose. Bronchodilators were administered after completion of the test.

The response of FEV\textsubscript{1}, was expressed as the percentage fall from mean baseline value and was plotted against the log concentration of methacholine. The dose-response curves were characterised by their position, which was expressed as PC\textsubscript{20}.\textsuperscript{18}
Table 1 Patient characteristics, clinical indices of asthma severity and airways hyperresponsiveness to methacholine

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age (years)</th>
<th>Sex</th>
<th>Inhaled steroids</th>
<th>Mean symptom score</th>
<th>β₂ agonist usage (puff/week)</th>
<th>FEV₁ (l)</th>
<th>PEF variability (%)</th>
<th>PC₂₀* (mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>21</td>
<td>M</td>
<td>BDP 400</td>
<td>2</td>
<td>9</td>
<td>51</td>
<td>18-0</td>
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</tr>
<tr>
<td>2</td>
<td>23</td>
<td>M</td>
<td>Bud 1600</td>
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<td>17</td>
<td>64</td>
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<td>F</td>
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<td>15-7</td>
<td>0.27</td>
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<td>40</td>
<td>M</td>
<td>Bud 400</td>
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<td>21</td>
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<td>12-8</td>
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<td>21-5</td>
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<td>M</td>
<td>Bud 800</td>
<td>2</td>
<td>14</td>
<td>81</td>
<td>26-6</td>
<td>0.68</td>
</tr>
<tr>
<td>7</td>
<td>40</td>
<td>M</td>
<td>Bud 1600</td>
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<td>82</td>
<td>13-3</td>
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<td>8</td>
<td>20</td>
<td>M</td>
<td>Bud 400</td>
<td>1</td>
<td>2</td>
<td>82</td>
<td>5-7</td>
<td>0.11</td>
</tr>
<tr>
<td>9</td>
<td>21</td>
<td>M</td>
<td>BDP 800</td>
<td>1</td>
<td>0</td>
<td>86</td>
<td>6-3</td>
<td>0.47</td>
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<tr>
<td>10</td>
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<td>M</td>
<td>BDP 2000</td>
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<td>12</td>
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<td>M</td>
<td>Bud 1600</td>
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<td>M</td>
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<td>96</td>
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<td>Bud 400</td>
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<td>3</td>
<td>96</td>
<td>9-7</td>
<td>0.38</td>
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<td>F</td>
<td>BDP 800</td>
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<td>0-22</td>
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<td>1-63</td>
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<td>F</td>
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<td>BDP 1200</td>
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<td>F</td>
<td>Bud 800</td>
<td>4</td>
<td>6</td>
<td>110</td>
<td>5-1</td>
<td>7-61</td>
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<td>26</td>
<td>F</td>
<td>Bud 800</td>
<td>6</td>
<td>1</td>
<td>111</td>
<td>9-1</td>
<td>0-27</td>
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<td>M</td>
<td>BDP 800</td>
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<td>2</td>
<td>113</td>
<td>8-8</td>
<td>3-16</td>
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<td>26</td>
<td>26</td>
<td>M</td>
<td>BDP 800</td>
<td>4</td>
<td>12</td>
<td>120</td>
<td>8-4</td>
<td>0-08</td>
</tr>
<tr>
<td>Mean (SD)</td>
<td>29 (7-4)</td>
<td></td>
<td>21 (26)</td>
<td>3-2 (2-3)</td>
<td>8-0 (11-5)</td>
<td>90-2 (26-1)</td>
<td>10-3 (6-8)</td>
<td>0-73 (2-1)</td>
</tr>
</tbody>
</table>

FEV₁ = forced expiratory volume in one second; PEF = peak expiratory flow; PC₂₀ = concentration of methacholine provoking a 20% fall in FEV₁; BDP = beclometasone dipropionate; Bud = budesonide.
* Geometric mean (SD) in doubling dose.

BRONCHOSCOPIC EXAMINATION

Fibreoptic bronchoscopy was carried out by an experienced investigator (LNAW) using a standardised protocol according to recent recommendations.3 In brief, the subjects were premedicated with atropine 0.5 mg subcutaneously, codeine 20 mg orally, and salbutamol 400 μg by metered dose inhaler, and the nasal passages and the oropharyngeal space were sprayed with 10% lignocaine 30 minutes before the procedure. The lower airways were anaeesthetised by nebulising a solution containing 2% lignocaine. Fibreoptic bronchoscopy was performed using a Pentax bronchoscope (outer diameter 6 mm; Pentax Optical Co, Japan). Three bronchial biopsies were taken at (sub)segmental level from the right lower lobe, the middle lobe, and the main carina using a pair of cup forceps (Olympus FB-22C, Tokyo, Japan). Throughout the procedure 100% oxygen was delivered via a nasal cannula (1 l/min) and oxyhaemoglobin saturation was monitored in all subjects using an oximeter (N-180, Nellcor Inc, Hayward, USA) with a finger probe.

PROCESSING OF ENDOBRONCHIAL BIOPSY SPECIMENS

Biopsy samples were immediately embedded in OCT medium (Miles Inc, Diagnostics Division, Elkhart, USA), and snap frozen in isopentane cooled by iced CO₂. Samples were stored at −70°C pending further processing. Six micrometer cryostat sections were air dried for one hour and fixed in a mixture of equal parts of acetone and methanol for two minutes. Immunohistochemical staining was performed using an indirect immunoperoxidase technique with monoclonal antibodies against: eosinophils staining for eosinophil cationic protein (EG₁) or the secreted form of eosinophil cationic protein (EG₂) (both Pharmacia, Sweden), mast cell tryptase (AA1, donated by Dr R Djukanović, Southampton University General Hospital, Southampton, UK), total leucocytes (CD45), B cells (CD22), T lymphocytes (CD3), T helper cells (CD4), T suppressor cells (CD8), activated T cells (CD25) (all Becton Dickinson, Mountain View, California, USA), and T cells CD45RO (UCHL1) (DAKO, Glostrup, Denmark).

QUANTITATIVE ANALYSIS OF BIOPSY SPECIMENS

All biopsy specimens were coded and sections were examined twice at 200× magnification by one investigator (JKS) in a blind fashion by means of a video interactive display analysis system (VIDAS II, Kontron Electronik GmbH, Munich, Germany) at least one week apart. A total area of 0.123 mm² was randomly chosen and presented on a video screen. The area of lamina propria was subsequently determined by delineating the widest possible 125 μm deep zone beneath the epithelial basement membrane (at least 10 000 μm²), excluding damaged tissue and BALT. The number of positively stained cells was counted within this area and expressed as the number of cells/mm² calculated on pooled data of the two occasions.

STATISTICAL ANALYSIS

The differences in mean cell numbers between the three sites were tested by multiple analysis of variance (MANOVA). Spearman’s rank correlation coefficients were used to assess the relationships of the following clinical indices of asthma severity as independent variables: symptom scores, β₂ agonist usage, FEV₁ %pred, peak flow variability, and the methacholine PC₂₀ with the following dependent variables: average cell number/mm² for the three sites staining for CD45, EG₁, EG₂, CD3, CD4, CD8, CD25, CD22, CD45RO, and the CD4/...
Airways inflammation and clinical asthma severity

Table 2 Mean (SD) number of stained cells/mm² in the lamina propria at the (sub)segmental and central level in 26 atopic asthmatic subjects using inhaled corticosteroids

<table>
<thead>
<tr>
<th>Cells</th>
<th>Right lobe (n=22)</th>
<th>Middle lobe (n=24)</th>
<th>Main carina (n=22)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD45+</td>
<td>1354 (705)</td>
<td>1626 (861)</td>
<td>1471 (686)</td>
</tr>
<tr>
<td>EG4+</td>
<td>110 (89)</td>
<td>105 (124)</td>
<td>93 (81)</td>
</tr>
<tr>
<td>EG3+</td>
<td>118 (123)</td>
<td>86 (105)</td>
<td>81 (116)</td>
</tr>
<tr>
<td>AAI3+</td>
<td>175 (105)</td>
<td>157 (86)</td>
<td>182 (111)</td>
</tr>
<tr>
<td>CD3+</td>
<td>1111 (713)</td>
<td>1446 (878)</td>
<td>1311 (667)</td>
</tr>
<tr>
<td>CD4+</td>
<td>558 (298)</td>
<td>800 (550)</td>
<td>651 (456)</td>
</tr>
<tr>
<td>CD8+</td>
<td>583 (399)</td>
<td>750 (590)</td>
<td>766 (425)</td>
</tr>
<tr>
<td>CD22+</td>
<td>10 (22)</td>
<td>32 (89)</td>
<td>6 (18)</td>
</tr>
<tr>
<td>CD45RO+</td>
<td>1514 (1023)</td>
<td>1792 (851)</td>
<td>1614 (778)</td>
</tr>
<tr>
<td>CD4+CD8+</td>
<td>1.09 (0.83)</td>
<td>1.33 (1.29)</td>
<td>0.94 (0.51)</td>
</tr>
</tbody>
</table>

Table 3 Relationships between cell counts/mm² in the lamina propria, averaged for the three sites, and clinical indices of asthma severity (Spearman's rank correlation coefficients)

<table>
<thead>
<tr>
<th>Cells</th>
<th>Mean symptom score</th>
<th>β2 agonist usage</th>
<th>FEV1 (SD)</th>
<th>PEF</th>
<th>PC20 (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD45+</td>
<td>0.15</td>
<td>-0.14</td>
<td>-0.10</td>
<td>0.29</td>
<td>-0.43**</td>
</tr>
<tr>
<td>EG4+</td>
<td>0.17</td>
<td>0.03</td>
<td>-0.21</td>
<td>0.06</td>
<td>-0.56***</td>
</tr>
<tr>
<td>EG3+</td>
<td>0.14</td>
<td>0.01</td>
<td>-0.23</td>
<td>0.09</td>
<td>-0.50**</td>
</tr>
<tr>
<td>AAI3+</td>
<td>0.15</td>
<td>0.12</td>
<td>0.09</td>
<td>0.38*</td>
<td>-0.41***</td>
</tr>
<tr>
<td>CD3+</td>
<td>-0.03</td>
<td>-0.30</td>
<td>0.01</td>
<td>0.04</td>
<td>-0.33**</td>
</tr>
<tr>
<td>CD4+</td>
<td>0.13</td>
<td>0.06</td>
<td>-0.01</td>
<td>0.15</td>
<td>-0.28</td>
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<tr>
<td>CD8+</td>
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<td>0.03</td>
<td>-0.34*</td>
<td>0.25</td>
<td>-0.47**</td>
</tr>
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<td>-0.01</td>
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<td>-0.28</td>
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<td>CD45RO+</td>
<td>0.14</td>
<td>-0.26</td>
<td>0.18</td>
<td>0.11</td>
<td>-0.43***</td>
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<tr>
<td>CD4+CD8+</td>
<td>0.28</td>
<td>0.12</td>
<td>0.13</td>
<td>0.02</td>
<td>0.03</td>
</tr>
</tbody>
</table>

FEV1 = forced expiratory volume in one second; PEF = peak expiratory flow; PC20 = ln(PC20) to methacholine
*p<0.01; **p<0.05; ***p<0.005.

CD8 ratio. MANOVA was used on the rank numbers to assess whether a significant relationship between the average number of a particular cell and clinical index was different among the three biopsy sites. The interaction term of the biopsy site and the clinical index was tested against the between subjects error term.21 Results were considered significant when the chance of a type I error was <5% (p<0.05). All analyses were performed using the SPSS-PC+.

Results

Results of the diary card recordings and lung function measurements are presented in table 1. The average symptom score was 3.2, ranging from 0 to 9 (16 = maximum possible) and the average β2 agonist usage was 8.0 puffs/week (range 0–5). The mean FEV1 before the methacholine challenge was 90% of predicted (range 51–120% predicted). The mean FEV1 variability was 10.3% (range 2.1–27.1) and the geometric mean methacholine PC20 was 0.73 mg/ml (range 0.02–18.2 mg/ml).

The mean (SD) numbers of (sub)populations of leucocytes per area (cells/mm²) in the lamina propria of biopsy specimens at the subsegmental level of the right lower lobe, the segmental level of the middle lobe, and the central level of the main carina are shown in table 2. No significant differences in cell numbers were seen between the three sites. CD25+ cells were only found in one subject and were therefore excluded from statistical analysis. The relationships between the clinical indices of asthma severity and the average cell counts at the three sites are shown in table 3. The methacholine PC20 was inversely related to the mean number of total leucocytes (rS = -0.43, p=0.05), EG4+(rS = -0.56, p<0.005) and EG3+ cells (rS = -0.58, p<0.005), mast cells (rS = -0.41, p=0.05), CD8+(rS = -0.47, p<0.05), and CD45RO+ cells (rS = -0.43, p<0.05) (figs 1 and 2). There was a trend towards a negative relationship between the methacholine PC20 and CD3+ cells (rS = -0.33, fig 2). There were no significant differ-

Figure 1 Relationship between airways responsiveness to methacholine and the number of (A) eosinophils (EG4+), (B) activated eosinophils (EG3+), (C) mast cells (AAI3+), and (D) total leucocytes in the bronchial lamina propria averaged for three sites. Spearman's rank correlation coefficients are indicated.
ences in the relationships between methacholine PC_{20} and the cell counts among the three biopsy sites.

Symptom scores, β_{2} agonist usage, FEV_{1}, and peak flow variability were not significantly related to any of the cells (table 3). However, there was a trend towards a negative relationship between FEV_{1} %pred and CD8+ cells ($r = -0.34$) and between peak flow variability and the number of mast cells ($r = 0.38$).

**Discussion**

This study shows that infiltration of inflammatory cells in the lamina propria of the airways seems to persist despite regular treatment with inhaled steroids in asthmatic outpatients. In addition, it appears that, among the clinical indices of asthma severity, the degree of airway hyperresponsiveness to methacholine is most closely related to the number of leucocytes, (activated) eosinophils, mast cells, CD8+, and CD45RO+ lymphocytes in bronchial biopsy specimens from atopic asthmatic subjects who are receiving treatment with inhaled steroids. This inverse relationship is a consistent one and is observed at the (sub)segmental level in the right lower lobe, the middle lobe, and the main carina. It is remarkable that the most commonly used clinical indices such as symptom severity, β_{2} agonist usage, peak flow variability, and FEV_{1} are not significantly correlated with inflammatory cell counts in the lamina propria of the airways. These findings may have implications for the adjustment of anti-inflammatory treatment in asthma.

There have been a number of conflicting reports on the relationship between mast cell or eosinophil number in bronchial biopsy specimens and the clinical indices in atopic asthma. It has been reported that symptom severity over a one year period is related to the number and activity of eosinophils in the airway epithelium and in the BAL fluid in patients not on treatment with anti-inflammatory medications. However, in our group of atopic asthmatic subjects using inhaled corticosteroids we could not establish a positive relationship between symptom scores and (activated) eosinophils. Our results confirm and extend those of Djukanovic et al who could not demonstrate a correlation between symptom scores, β_{2} agonist consumption, or peak flow variability and submucosal AA1+ mast cells or EG_{2}+ eosinophils in subjects with asthma who were not treated with inhaled steroids. Our findings therefore further confirm that the currently recommended clinical indices of asthma severity do not reflect the extent of airways inflammation in this disease.

In contrast to symptoms and lung function, the degree of airway hyperresponsiveness appeared to be associated with the histopathology in our patients. Again, this has been a matter of controversy. An inverse relationship between the PC_{20} for methacholine and the number of activated eosinophils and mast cells in bronchial biopsy specimens has been reported previously in asthmatic patients without steroid treatment. However, other investigators were unable to confirm this. This failure might have been due to a small sample size, or to a relatively narrow range in airways hyperresponsiveness compared with our study. The relatively weak relationship between PC_{20} and CD45RO+ cells in our study confirms previous findings in a group of atopic asthmatic...
subjects who were not using inhaled cortico-
steroids. Thus, a relationship between air-
ways hyperresponsiveness and histopathology
may only be revealed by using a sufficiently
large group of atopic asthmatics characterised
by a wide range of airways hyperresponsiveness
as in the present study.

The absence of a relationship between clinical
indices of asthma severity and histo-
pathology of the airways does not seem to
be caused by patient selection, study design,
statistical power, or the processing and analysis
of bronchial biopsy specimens for the following
reasons. Firstly, in order to ensure that time
related variability in the measurements did not
obscure potential associations, the bron-
choscopy was carried out within six days of the
FEV1 and PC20 measurements and last diary
card registrations. Secondly, the patients in
this study were carefully selected to be non-
smokers, having atopic asthma of mild to mod-
erate severity with a wide range in FEV1 be-
tween 51% and 120% of the predicted value.
Thirdly, with the present number of subjects a
correlation coefficient of ≥0.40 or ≤−0.40
yields a p value <0.05. Therefore, it seems
unlikely that the absence of a significant cor-
relation of modest strength can be explained by
a problem with the power of the study. It
can be argued that the use of inhaled steroids by
these patients might have masked a potential
relationship between clinical indices and cell
counts.24 Due to the method of patient selection
we cannot exclude such a possibility, because
this study was designed to assess which param-
eter among the clinical indices best reflects
the degree of airways inflammation in patients

The range and mean numbers of infiltrating
inflammatory cells in the bronchial biopsy
specimens in our study were slightly higher
than those in other studies of asthmatic patients.232526 In those studies ≤1 µm sections of
specimens embedded in plastics were used,
whereas our cell numbers were similar to those
reported by Trigg et al who used 5 µm cryostat
sections.24 The present histopathological
methods are further validated by the highly
consistent findings between the three sites in
the tracheobronchial tree. We therefore believe
that our results are unlikely to be explained by
methodological errors.

How can the present results be interpreted?
The association between eosinophils and mast
cells in the lamina propria and methacholine
responsiveness fits in with their role in the
development of airways hyperresponsiveness.27
This includes secretion of a variety of in-
flammatory mediators capable of inducing epi-
thelial desquamation, plasma extravasation,
and hypersecretion, thereby increasing the
thickness of the airway wall.1224 The complexity
of the pathophysiology of asthma is well il-
ustrated by our present findings. In four sub-
jects who reported symptoms we were unable
to detect an infiltrate of eosinophils in the
lamina propria. In contrast, some other subjects
had clear evidence of airway inflammation in
spite of the absence of symptoms and with lung
function parameters within the normal range.
The latter finding extends observations in acute
severe asthma indicative of a dissociation be-
tween clinical recovery and bronchial hyper-
responsiveness29 and is in keeping with a
persistent increase in airways responsiveness in
subjects who are clinically considered to be in
remission of asthma.3031 Our findings seem to
implicate that the presence of an inflammatory
infiltrate does not in itself lead to symptoms of
asthma or airways obstruction, but might point
to long term disease activity.

According to the current international re-
commendations for the treatment of asthma2432
the level of anti-inflammatory treatment is
chosen to minimise symptoms, optimise lung
function, and to prevent exacerbations. The
dosage of regular treatment should be kept to
the minimum level that fulfils these objectives.
The question is whether this approach leads to
the optimal long term outcome of asthma. A
number of studies have shown that treatment
with inhaled steroids up to four months is very
effective, particularly in reducing the number
of mast cells and eosinophils in the airway wall
which is often paralleled with improvements
in symptoms, lung function, and airways hyper-
responsiveness to methacholine.23243334 Longer
term studies on the effect of inhaled steroids
on the histopathology of the airways have not
yet been reported. However, the observation
that airways hyperresponsiveness continues to
improve even after six months of treatment,
when no further increase in FEV1 occurs,2532
suggests that it may take relatively longer to
resolve the airway histopathology.

It can be argued that maintenance of an
inflammatory state of the airways may result in
anatomical changes and functional disorders.1538
This might be reflected by abnormal
PC20 levels since two recent population based
prospective studies have shown that air-
ways hyperresponsiveness is a significant pre-
dicator of subsequent accelerated decline in
pulmonary function.39 40 We therefore postulate
that, in addition to the current guidelines, the
treatment of asthma should also be directed
towards reducing airways hyperresponsiveness.
This might benefit the long term goal of asthma
management as formulated in the international
guidelines – that is, the prevention of the
development of irreversible airways ob-
struction.153961 Clearly, such a treatment
regimen needs to be tested in long term
prospective studies.

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1 Dijanakovic R, Roche WR, Wilson JW, Beasley CRW,
Mucosal inflammation in asthma. Am Rev Respir Dis 1990;
142:434–37.
2 Corrigan CJ. Immunological aspects of asthma. Implications
3 National Heart, Lung, and Blood Institute. National In-
stitutes of Health Bethesda, Maryland. International con-
sensus report on diagnosis and treatment of asthma. Eur
4 British Thoracic Society. Guidelines on the management of
5 Cockcroft DW. Therapy for airway inflammation in asthma.
29 Whyte MKB, Choudry NB, Ind PW. Bronchial hyperresponsiveness in patients recovering from acute severe asthma. Respir Med 1993;87:29-35.
Relationship between the inflammatory infiltrate in bronchial biopsy specimens and clinical severity of asthma in patients treated with inhaled steroids.

J. K. Sont, J. Han, J. M. van Krieken, C. E. Evertse, R. Hooijer, L. N. Willems and P. J. Sterk

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