Clinical value of monitoring eosinophil activity in asthma

D Y Koller, Y Herouy, M Götz, E Hagel, R Urbanek, I Eichler

Abstract
To evaluate the use of eosinophil cationic protein (ECP) in monitoring disease activity in childhood asthma, serum ECP in 175 asthmatic children was assessed. Forty-five patients with cystic fibrosis, 23 with lower respiratory tract infections (LRTI), and 87 healthy children were used as controls.

Serum ECP concentrations (34.3 μg/l v 9.8 μg/l) were significantly higher in children with bronchial asthma than in healthy control subjects. In symptomatic patients with asthma serum ECP concentrations were increased compared with those from asymptomatic patients (40.2 μg/l v 14.4 μg/l), irrespective of treatment modalities (that is, steroids, β2 agonists, or sodium cromoglycate). Moreover, atopy and infection appeared to be factors enhancing eosinophil activity in bronchial asthma as measured by serum ECP (58.4 μg/l v 36.8 μg/l and 68.8 μg/l v 42.2 μg/l, respectively). In a longitudinal trial, anti-asthmatic treatment modalities (that is, steroids) reduced serum ECP within four weeks (42.2 μg/l v 19.0 μg/l).

In conclusion, the data indicate that (1) eosinophils also play a central part in childhood asthma; (2) serum concentrations of ECP in children with bronchial asthma are related to the disease severity and may thus be used for monitoring inflammation in childhood asthma; (3) eosinophil activity appears to be enhanced by atopy and infection; and (4) longitudinal measurements of serum ECP concentrations may be useful for optimising anti-inflammatory treatment in children with bronchial asthma.

Keywords: asthma, eosinophils, eosinophil cationic protein, infection.

Asthma is a chronic inflammatory disease, which is characterised by symptoms of airway obstruction such as cough, wheezing, and breathlessness. Bronchial inflammation in asthma can be assessed directly by pathohistological observations and indirectly by examining cells and mediators present in peripheral blood. In adults the implication of eosinophils in bronchial asthma is well accepted. A correlation between activated eosinophils and pulmonary function or non-specific bronchial hyperreactivity was observed. Moreover, activated eosinophils release granule derived proteins, the most accepted of which is eosinophil cationic protein (ECP) for estimation of eosinophil activation.

In vitro studies have shown the deleterious effect to bronchial mucosa of eosinophil derived cytotoxic proteins and assessment of serum ECP has been suggested for monitoring disease activity and bronchial inflammation in adult bronchial asthma. In childhood asthma, however, problems in diagnosing or monitoring disease activity may occur because of non-specific symptoms as well as the inability to obtain reliable pulmonary function and provocation tests in children less than 5 years of age. Thus, monitoring by the assessment of mediators, reflecting pulmonary inflammation, could support an optimal management of childhood asthma.

To evaluate the clinical value of activated eosinophils in children with bronchial asthma, serum ECP and blood eosinophil counts were determined. Sensitisation to inhaled allergens as well as infections of both the upper and the lower respiratory tract are known to cause exacerbations in asthmatic children. To differentiate between various factors assumed to influence eosinophil activities, the patients therefore were subdivided into groups due to treatment modalities, to atopy and infection. In addition, healthy non-atopic children and patients with cystic fibrosis and lower respiratory tract infections (LRTI) were used as controls.

Subjects and methods

PATIENTS AND CONTROLS
A total of 175 children (78 girls and 97 boys) with bronchial asthma (diagnosis was based on episodic cough, wheeze, and breathlessness that was responsive to β2 agonists, and after other conditions have been excluded) were enrolled for this study (mean (SD) age 9.8 (3.8) years; 52 of the patients were less than 6 years of age). At the time of examination the children were divided into different treatment groups: (1) 44 children on inhaled β2 agonists (33 with salbutamol, 11 with fenoterol); (2) 37 on inhaled steroids (29 with budesonide, eight with beclometasone); (3) 47 on inhaled steroids and β2 agonists; (4) 27 on inhaled sodium cromoglycate; and (5) 20 children with bronchial asthma who were newly diagnosed and thus without antiasthmatic medication.

In 14 of these patients treatment was initiated with budesonide (600 μg daily) and salbutamol (300 μg daily) for two weeks, and thereafter with budesonide alone. Every two weeks follow up examinations, including pulmonary function and blood sampling, were...
performed for a period of four weeks. The remaining six patients were first treated with oral antibiotics – due to LRTI – and an inhaled β₂ agonist (salbutamol 300 µg daily) for 10 days and thereafter with inhaled budesonide (600 µg daily).

The controls comprised: (1) 23 non-atopic children with LRTI (14 girls and nine boys; age 8-6 (2-5) years) before initiating antibiotic treatment. (2) Forty five patients with cystic fibrosis (22 girls and 23 boys; age 10-8 (6-5) years); 20 of these patients were colonised with Pseudomonas aeruginosa and/or Staphylococcus aureus as proved by sputum cultures. Clinical condition was assessed by the method of Shwachman-Kulczycki without reviewing the x ray resulting in a score between 0-75. The median score in our patients investigated was 55 (range 24-73). Acute pulmonary exacerbation in cystic fibrosis was defined as marked increase of C reactive protein (median concentration 129 mg/l), by weight loss, anorexia, increased cough, increased sputum production, fever with and without new lung infiltrates, deterioration of oxygen saturation and lung function. None of these patients received systemic or inhaled steroids within the month of blood drawing. (3) Eighty seven healthy children (51 girls and 36 boys; age 10-2 (4-5) years) with no history of atopy or asthma and normal total serum IgE concentrations, who were free of infection for at least two weeks before the drawing of blood were recruited as normal references.

Parental agreement to blood sampling was obtained in all cases and blood was obtained at routine sampling for clinical evaluation.

DEFINITION OF ATOPIC STATUS
Patients were defined as being atopic by having increased total serum IgE antibody concentrations and by the presence of specific IgE antibodies against at least two or more inhalant allergens tested (Dermatophagoides pteronyssinus and D farinae, cat, dog, alternaria, cladosporium, aspergillus, birch, alder, hazel, and grass pollens).

DEFINITION OF INFECTION IN CHILDREN WITH BRONCHIAL ASTHMA
Infection of the upper or lower respiratory tract was diagnosed clinically, by leucocytosis, radiographic evidence of pulmonary infiltrates and – if possible – by sputum cultures and evidence of viral antigens in nasal fluids.

CLINICAL EVALUATION AND PULMONARY FUNCTION
Physical examination and a history of wheeze, cough (nocturnal), and dyspnoea within the last two weeks before blood sampling allowed to divide the patients into symptomatic and asymptomatic children with bronchial asthma.

Pulmonary function tests were performed – as compliance of the patients and/or parents allowed – in 52 children with bronchial asthma and 38 patients with cystic fibrosis as follows.

Forced vital capacity (FVC), forced expiratory volume in the first second (FEV₁) and maximum expiratory flow at 50% (MEF50) and at 25% (MEF25) of vital capacity were recorded in the form of a maximum flow volume curve (Masterlab, Jaeger, Germany) according to the American Thoracic Society standard. The best of three efforts was used for calculations. Results were presented as percent predicted based on accepted reference standards.

BLOOD SAMPLES
Blood eosinophil counts were determined by automated counting (Sysmex NE-5500; Müller GesmbH, Austria) with a coefficient of variation about 7-0%. Serum was used for the determination of ECP. Assessments were done by using a sensitive and specific double antibody radioimmunoassay (Pharmacia Diagnostics, Sweden) in which ECP in the samples competes with a fixed amount of 125I-labelled ECP for the binding sites of specific antibodies. The interassay coefficient of variation for the assay was less than 10%.

Total serum IgE and specific IgE antibodies were measured by using enzyme immunoassays (Kallestad Diagnostics, USA).

STATISTICAL ANALYSIS
Results are expressed as medians (quartile 1–quartile 3) unless mentioned otherwise. Correlation coefficients were obtained using the Kendall Tau B test. Non-parametric statistical tests were used in comparative analysis. The Kruskal-Wallis analysis was applied first to the data in all groups. When this was significant, each pair was examined by means of the Mann-Whitney U test. Changes related to treatment were tested for significance using Wilcoxon signed rank test for paired samples. Probabilities of less than 5% were taken as statistically significant.

Results
CHARACTERISTICS OF PATIENTS
At the time of examination, 90 asthmatic children with bronchial asthma were asymptomatic whereas 85 patients had symptoms of airway obstruction within the last two weeks before examination; 105 children presented with specific sensitisation against two or more of tested allergens. Total serum IgE concentrations were significantly increased in the atopic group (median: 457 kU/l) compared with the non-atopic asthmatics (median:

<table>
<thead>
<tr>
<th>Table 1 Eosinophil counts as well as serum ECP concentrations in children with bronchial asthma, cystic fibrosis, and LRTI compared with healthy non-atopic subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eosinophils (cell/µl)</td>
</tr>
<tr>
<td>---------------------</td>
</tr>
<tr>
<td>Bronchial asthma</td>
</tr>
<tr>
<td>Cystic fibrosis</td>
</tr>
<tr>
<td>LRTI</td>
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<tr>
<td>Controls</td>
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</tbody>
</table>

Results are presented as median (quartile 1–quartile 3); p values in text (see results section).
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Table 2 Serum ECP and eosinophil counts in symptomatic and asymptomatic children with bronchial asthma subdivided into treatment groups

<table>
<thead>
<tr>
<th>ECP (µg/l)</th>
<th>Symptomatic</th>
<th>Asymptomatic</th>
<th>p Value</th>
<th>Eosinophil/μl</th>
<th>Symptomatic</th>
<th>Asymptomatic</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated</td>
<td>43.4 (36-4-68.8) [n=20]</td>
<td></td>
<td></td>
<td>388 (278-545)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>β2 Agonists</td>
<td>39.9 (22.1-59.5) [n=18]</td>
<td>13.4 (11-2-31.8) [n=26]</td>
<td>p&lt;0.005</td>
<td>404 (254-509)</td>
<td>398 (110-400)</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Steroids</td>
<td>48.5 (20.5-70.9) [n=17]</td>
<td>14.3 (10-0-23.5) [n=20]</td>
<td>p&lt;0.001</td>
<td>347 (210-580)</td>
<td>311 (188-411)</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Steroids and β2 agonists</td>
<td>30.0 (17.0-54.3) [n=20]</td>
<td>15.0 (12.0-24.4) [n=27]</td>
<td>p&lt;0.001</td>
<td>335 (289-481)</td>
<td>360 (320-450)</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Sodium cromoglycate</td>
<td>41.4 (24.6-76.4) [n=10]</td>
<td>14.7 (9-8-23.9) [n=17]</td>
<td>p&lt;0.001</td>
<td>387 (254-467)</td>
<td>365 (298-485)</td>
<td>NS</td>
<td></td>
</tr>
</tbody>
</table>

Results are presented as median (quartile 1–quartile 3); p values indicate significant differences between symptomatic and asymptomatic children with bronchial asthma. NS = not significant.

17 kU/l; p<0.0001). Infection of the lower or upper respiratory tract was present in 77 children with bronchial asthma at the time of examination.

Assessment of pulmonary function in 52 of the asthmatics showed for symptomatic children (n=31) a median (range) FEV1 value of 78.4% predicted (47.9-89-0%), MEF50 value of 62.9% predicted (25.7-75-0%), and MEF25 value of 58.1% predicted (20.6-72.9%). Asymptomatic asthmatics (n=21) demonstrated a median (range) FEV1 value of 95.6% predicted (88.6-120%), MEF50 value of 90.1% predicted (79.8-124%), and MEF25 value of 85.2% predicted (73.9-112.4%).

In patients with cystic fibrosis (n=38) median (range) FEV1 was 74.2% predicted (22.8-135.8%), a median MEF50 50.0% predicted (5.7-92.4%), and MEF25 30.8% predicted (3.9-91.5%). In addition, atopy was observed in 24 children with cystic fibrosis as proved by specific sensitisation against inhalant allergens and increased total serum IgE concentrations (median 480 kU/l) compared with 21 non-atopic patients with cystic fibrosis (median 26 kU/l; p<0.0001).

BRONCHIAL ASTHMA, CYSTIC FIBROSIS, AND LRTI

Eosinophils counts in peripheral blood were significantly increased in children with bronchial asthma compared with control subjects (p<0.005), to patients with cystic fibrosis (p<0.005), and to children with LRTI (p<0.005; table 1). Eosinophil numbers in asthmatic children were weakly correlated with serum concentrations of ECP (r=0.316; p<0.0001). In asthmatic children, serum ECP was raised in comparison with LRTI (p<0.001) and with healthy controls (p<0.0001), but were not different to ECP concentrations in samples obtained from patients with cystic fibrosis (table 1).

Subdivision into symptomatic and asymptomatic asthmatic children demonstrated significant differences for serum ECP concentrations (table 2) whereas eosinophil counts were not different (table 2). In addition, symptomatic patients with bronchial asthma had serum concentrations of ECP within the same pathological range, irrespective if treated with steroids, β2 agonists, or sodium cromoglycate (table 2). Serum ECP and eosinophil counts did not differ significantly between the younger (less than 6 years) and the older (more than 6 years) patients.

ECP concentrations in sera of patients with bronchial asthma were not correlated with variables of pulmonary function. In patients with cystic fibrosis, however, a relationship with pulmonary function as measured by FEV1 predicted (r=0.634; p<0.001) and the Shwachman-Kulczycki score (r=−0.678; p<0.001) was given.

Table 3 Atopy: influence on serum ECP and eosinophil counts in children with bronchial asthma on different medication and in cystic fibrosis

<table>
<thead>
<tr>
<th>ECP (µg/l)</th>
<th>Non-atopic</th>
<th>Atopic</th>
<th>p Value</th>
<th>Eosinophil (cells/µl)</th>
<th>Non-atopic</th>
<th>Atopic</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bronchial asthma</td>
<td></td>
<td></td>
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<tr>
<td>Untreated</td>
<td>36.8 (15.7-48.5) [n=9]</td>
<td>58.4 (42.1-80.7) [n=11]</td>
<td>p&lt;0.05</td>
<td>367 (250-478)</td>
<td>485 (390-660)</td>
<td>p&lt;0.05</td>
<td></td>
</tr>
<tr>
<td>β2 Agonists</td>
<td>12.7 (9.1-21.9) [n=12]</td>
<td>32.4 (19.8-43.0) [n=32]</td>
<td>p&lt;0.01</td>
<td>222 (100-310)</td>
<td>386 (230-484)</td>
<td>p&lt;0.05</td>
<td></td>
</tr>
<tr>
<td>Steroids</td>
<td>20.0 (8.6-26.0) [n=15]</td>
<td>23.5 (12.0-51.6) [n=22]</td>
<td>NS</td>
<td>320 (240-423)</td>
<td>480 (300-640)</td>
<td>p&lt;0.05</td>
<td></td>
</tr>
<tr>
<td>Steroids and β2 agonists</td>
<td>19.5 (12.0-39.0) [n=22]</td>
<td>19.0 (12.0-36.0) [n=25]</td>
<td>NS</td>
<td>343 (312-450)</td>
<td>453 (320-612)</td>
<td>p&lt;0.05</td>
<td></td>
</tr>
<tr>
<td>Sodium cromoglycate</td>
<td>18.8 (9.8-29.4) [n=12]</td>
<td>29.9 (10.8-44.7) [n=15]</td>
<td>p&lt;0.05</td>
<td>334 (298-417)</td>
<td>465 (312-608)</td>
<td>p&lt;0.05</td>
<td></td>
</tr>
<tr>
<td>Cystic fibrosis</td>
<td>61.3 (9.8-71.4) [n=26]</td>
<td>55.0 (13.2-65.8) [n=19]</td>
<td>NS</td>
<td>180 (120-220)</td>
<td>160 (90-280)</td>
<td>NS</td>
<td></td>
</tr>
</tbody>
</table>

Results are presented as median (quartile 1–quartile 3); p values indicate significant differences between atopics and non-atopics. NS = not significant.
patients with bronchial asthma treated with β2 agonists and sodium cromoglycate had significantly higher concentrations of ECP than non-atopic subjects treated with these agents whereas atopic patients treated with inhaled steroids had similar concentrations of ECP to non-atopic children treated with inhaled steroids (table 3). Total eosinophil counts, however, were unaffected by inhaled steroids, thus no differences for eosinophil counts in each treatment group between atopic and non-atopic asthmatic children could be observed (table 3).

**Eosinophil Counts and Serum ECP in Infection**

Infection caused an increase of ECP in childhood asthma but not in patients with cystic fibrosis and with LRTI (table 4). Eosinophil counts, however, were not altered (median 378 cells/μl v 328 cells/μl).

**EFFECT OF INHALED Budesonide on Serum ECP**

Treatment with budesonide (600 μg/daily) for four weeks and salbutamol (300 μg/daily) for two weeks in 14 children with newly diagnosed bronchial asthma resulted in a significant decrease of serum ECP and improvement of pulmonary function (fig 1). Eosinophil counts (median eosinophil count before treatment: 388 cells/μl; after two weeks treatment with budesonide and salbutamol: 400 cells/μl; after additional two weeks with budesonide alone: 364 cells/μl) did not change significantly.

In children (n=6) with bronchial asthma presenting with LRTI oral antibiotic and inhaled β2 agonist treatment resulted in no significant change of ECP concentrations within 10 days (fig 2). Only inhalation of budesonide (600 μg/l) caused a significant reduction of serum ECP concentrations (p<0.03) in these patients (fig 2).

**Discussion**

Inflammation is being increasingly recognised as a major factor in childhood asthma. One of the predominant inflammatory cells in the asthmatic lung is the eosinophil granulocyte, which has been suggested to contribute to bronchial hyperreactivity and tissue injury. The activity of eosinophils could be determined by the measurement of eosinophil granule proteins, such as ECP. In addition, assessment of serum ECP has been assumed to reflect pulmonary inflammation in bronchial asthma.

We have found increased blood eosinophil counts and more significantly increased serum concentrations of ECP in children with bronchial asthma compared with a group of age and sex matched normal healthy control subjects. ECP concentrations were not different between children less or more than 6 years of age, as previously reported. The median serum ECP concentrations were raised, however, with many patients having normal values. This could be explained partly by the fact that inhaled steroids reduce the concentrations of ECP due to the direct inhibitory effect on eosinophil activation. However, many children on β2 agonists alone, also demonstrated low ECP despite published data, showing no in vitro effect of β2 agonists on eosinophil activation. By dividing children with bronchial asthma into symptomatic and into asymptomatic patients we were able to demonstrate that symptomatic children had higher ECP concentrations than asymptomatic asthmatics, independent of treatment modalities (that is inhaled steroids, β2 agonists, combination of both, or sodium cromoglycate).

These results suggest that determination of serum ECP may be useful for assessing asthma severity. In addition, measurement of raised serum ECP concentrations may reflect the ongoing inflammation in the asthmatic lung and may therefore be used to predict the need for intensified anti-inflammatory treatment modalities. Similar findings were reported only recently. To emphasise this hypothesis, we were able to demonstrate the effectiveness of inhaled steroids in suppressing eosinophil activity in a four week prospective trial. Improvement of pulmonary function was strongly associated with a fall of serum ECP, in all subjects investigated, suggesting that
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With bronchial asthma, eosinophils accumulate in the airways, and their activation is thought to contribute to airway inflammation and remodeling. The measurement of eosinophil cationic protein (ECP) in bronchoalveolar lavage fluid (BALF) has been used to monitor eosinophil activity in asthma.

Figure 2 The change in serum concentrations of ECP and of FEV1 in six newly diagnosed asthmatic children with infection of the lower respiratory tract. Examinations were done before starting treatment, after 10 days of treatment by oral antibiotics and inhaled salbutamol, and after 18 days of treatment by budesonide and salbutamol. Median serum ECP concentrations before treatment: 68.8 ng/ml; after 10 days: 38.2 ng/ml (p<0.05 compared to values on day 0); after 28 days: 13.5 mg/ml (p<0.05 compared to values on day 0). Median FEV1% predicted before treatment: 73.9% after 10 days: 85.4% (p<0.05 compared to values on day 0); after 28 days: 96.0% (p<0.05 compared to values on day 0).

Longitudinal measurements of serum ECP might be used to predict the outcome of treatment. The eosinophil counts did not fall, suggesting that serum ECP is not dependent on the total eosinophil count and, in addition, ECP appears to correlate better with the severity of asthma than does the eosinophil count.

The main factors causing exacerbation of asthma in childhood are allergens and infections of either the upper or lower respiratory tract. To investigate whether atopy and infection influence the activity of eosinophil granulocytes, children with bronchial asthma were divided into different groups (atopic v non-atopic; asthma v asthma children without infection). Children with bronchial asthma (without anti-inflammatory treatment) had significantly higher ECP concentrations than non-atopic asthmatic patients. Thus, it could be speculated that atopy is a factor 'boosting' inflammatory processes in childhood asthma, consecutively increasing bronchial hyperreactivity.

Our data also clearly demonstrated that inhaled steroids appeared to suppress significantly inflammation in atopic asthma, as ECP concentrations in steroid treated atopic asthmatics were no different to those in non-atopic asthmatic children.

Infections of the respiratory tract raised serum ECP concentrations in patients with bronchial asthma. As infection caused an increase of ECP in children with asthma only, and not in patients with LRTI and cystic fibrosis, it might thus be suggested specific for bronchial asthma.

In a previous study we reported increased serum ECP concentrations in children with cystic fibrosis. In this study, our previous findings were confirmed, but—in contrast to bronchial asthma—no influence of atopy or acute infection on serum ECP concentrations could be detected. Normal eosinophil counts in patients with cystic fibrosis in the presence of increased serum ECP thus suggest different mechanisms of eosinophil activation than in patients with bronchial asthma. Another explanation might be that in cases with normal or moderately high eosinophil numbers, the reduced availability may be compensated for by the increased activity of cells, which is enough to achieve sufficient concentrations of the inducers of the inflammatory process.

In conclusion, our findings demonstrated increased serum ECP concentrations in children with bronchial asthma. By these results, it has become likely that the eosinophil plays a central part in the asthmatic inflammation in children, as it does in adults. In addition, our data suggest that the determination of serum ECP may be used for monitoring the disease activity. As both atopy and infection caused increased ECP concentrations, we hypothesise that both may act as 'boosting factors' for inflammatory processes in bronchial asthma. Finally, our results suggest that serum ECP measurements may be useful to predict the need for intensified anti-inflammatory treatment.

It may be that assessment of mediators will allow further investigation of important and until now unanswered questions in childhood asthma, namely: When is the onset of eosinophilic inflammation in the asthmatic child? And what is first: bronchial hyperreactivity or inflammation?