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.
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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. Atopy, atopic disease, and atopic dermatitis were common in the study group. Atopy 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 on 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 atopy 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 beclomethasone); (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 \( \beta_2 \) agonist (salbutamol 300 \( \mu \)g daily) for 10 days and thereafter with inhaled budesonide (600 \( \mu \)g daily).

The controls comprised: (1) 23 non-asthmatic 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 \textit{Pseudomonas aeruginosa} and/or \textit{Staphylococcus aureus} as proved by sputum cultures. Clinical condition was assessed by the method of Shwachman–Kulczycki\textsuperscript{10} 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.

\textbf{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 (\textit{Dermatophagoides pteronyssinus} and \textit{D farinae}, cat, dog, alternaria, cladosporum, aspergillus, birch, alder, hazel, and grass pollens).

\textbf{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.

\textbf{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.

\textbf{Results}

\textbf{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 to 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: 235 kU/l).

\begin{table}
\centering
\begin{tabular}{|l|l|l|}
\hline
 & Eosinophil (cells/\( \mu \)l) & ECP (\( \mu \)g/l) \\
\hline
Bronchial asthma & 443 (290–601) & 34·3 (22·5–55·2) \\
Cystic fibrosis & 235 (105–350) & 65·7 (39·6–82·5) \\
LRTI & 205 (125–230) & 12·3 (8·7–16·3) \\
Controls & 209 (118–328) & 9·8 (7·3–13·5) \\
\hline
\end{tabular}
\caption{Eosinophil counts as well as serum ECP concentrations in children with bronchial asthma, cystic fibrosis, and LRTI compared with healthy non-atopic subjects.}
\end{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>Ununtreated</td>
<td>43.4 (36.4-68.8)</td>
<td>22.9 (21.1-59.0)</td>
<td>p&lt;0.005</td>
<td>378 (278-545)</td>
<td>404 (254-509)</td>
<td>p=0.100</td>
<td>398 (110-400)</td>
</tr>
<tr>
<td>β2 Agonists</td>
<td>39.0 (22.1-59.0)</td>
<td>13.0 (11.2-31.0)</td>
<td>p&lt;0.005</td>
<td>404 (254-509)</td>
<td>347 (210-580)</td>
<td>p&lt;0.005</td>
<td>311 (188-411)</td>
</tr>
<tr>
<td>Steroids</td>
<td>48.5 (20.5-70.9)</td>
<td>14.3 (10.0-23.5)</td>
<td>p&lt;0.005</td>
<td>347 (210-580)</td>
<td>335 (289-481)</td>
<td>p&lt;0.001</td>
<td>360 (320-450)</td>
</tr>
<tr>
<td>Steroids and β2 agonists</td>
<td>33.0 (17.0-54.3)</td>
<td>15.0 (12.0-24.4)</td>
<td>p&lt;0.001</td>
<td>335 (289-481)</td>
<td>387 (254-467)</td>
<td>p&lt;0.001</td>
<td>365 (298-485)</td>
</tr>
<tr>
<td>Sodium cromoglycate</td>
<td>41.4 (24.6-76.4)</td>
<td>14.7 (9.8-23.9)</td>
<td>p&lt;0.001</td>
<td>387 (254-467)</td>
<td>369 (298-485)</td>
<td>p&lt;0.001</td>
<td>365 (298-485)</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 (47.9-98.9%), 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 (79.8-124.5%), 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

Eosinophil 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=1-310; p<0.001) and the Shwachman-Kulczycki score (r=1-222; 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>Atopic</th>
<th>p Value</th>
<th>Eosinophil/µl</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-atopic</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bronchial asthma</td>
<td>36.8 (15.7-48.5)</td>
<td>58.4 (42.1-80.7)</td>
<td>p&lt;0.05</td>
</tr>
<tr>
<td>Untreated</td>
<td>12.7 (9.1-21.9)</td>
<td>32.4 (13.9-43.0)</td>
<td>p&lt;0.01</td>
</tr>
<tr>
<td>β2 Agonists</td>
<td>20.0 (8.0-56.0)</td>
<td>24.5 (12.0-51.6)</td>
<td>NS</td>
</tr>
<tr>
<td>Steroids</td>
<td>19.5 (10.0-39.0)</td>
<td>19.0 (10.0-36.0)</td>
<td>NS</td>
</tr>
<tr>
<td>Steroids and β2 agonists</td>
<td>18.8 (9.8-29.4)</td>
<td>29.9 (10.8-44.7)</td>
<td>p&lt;0.05</td>
</tr>
<tr>
<td>Sodium cromoglycate</td>
<td>61.3 (9.8-71.4)</td>
<td>55.0 (13.2-65.8)</td>
<td>NS</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.
Table 4 Infection: influence on serum ECP in children with bronchial asthma (subdivided into different treatment groups), cystic fibrosis, and with LRTI

<table>
<thead>
<tr>
<th>ECP (µg/l)</th>
<th>Infection</th>
<th>No infection</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bronchial asthma</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Untreated</td>
<td>[50.0–85.5]</td>
<td>[36.3–48.5]</td>
<td>p&lt;0.05</td>
</tr>
<tr>
<td>p2 agonists</td>
<td>[32.5–58.3]</td>
<td>[12.1–37.7]</td>
<td>p&lt;0.05</td>
</tr>
<tr>
<td>Steroids</td>
<td>[58.3–76.6]</td>
<td>[15.4–26.7]</td>
<td>p&lt;0.05</td>
</tr>
<tr>
<td>Cystic fibrosis</td>
<td>[39.0–114.0]</td>
<td>[17.0–32.0]</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>Sodium cromoglycate</td>
<td>[38.2–76.4]</td>
<td>[18.9–29.8]</td>
<td>p&lt;0.005</td>
</tr>
<tr>
<td>LRTI</td>
<td>[32.5–58.3]</td>
<td>[9.8–65.8]</td>
<td>NS</td>
</tr>
</tbody>
</table>

Results are expressed as median (quartile 1–quartile 3); p values indicate significant differences between infected and non-infected patients. NS=not significant.

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.

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).

Figure 1 The change in serum concentrations of ECP and FEV1 in 14 newly diagnosed asthmatic children. Examinations were performed before starting treatment, after two weeks of treatment with budesonide and salbutamol and after the following two weeks of treatment with budesonide alone. Median serum ECP concentration before treatment: 42.2 µg/l; after 2 weeks: 23.9 µg/l; after 4 weeks: 19.0 µg/l. Median FEV1% predicted before treatment: 82.9%; after 2 weeks: 94.1%; after 4 weeks: 97.4%. No significant changes of eosinophil counts were observed.

Figure 2 The change in serum concentrations of ECP and FEV1 in 14 newly diagnosed asthmatic children treated with budesonide alone. Median serum ECP concentration before treatment: 42.2 µg/l; after 2 weeks: 23.9 µg/l; after 4 weeks: 19.0 µg/l. Median FEV1% predicted before treatment: 82.9%; after 2 weeks: 94.1%; after 4 weeks: 97.4%. No significant changes of eosinophil counts were observed.
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**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 infection. 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 μg/l; after 10 days: 38.2 μg/l (p<0.03 compared to previously); after 28 days: 13.5 μg/l (p<0.03 compared to values on day 0). Median FEV₁% predicted before treatment: 73.9% after treatment: 85.4% (p<0.05 compared with values on day 0); after 28 days: 96.0% (p<0.03 compared with 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; infection 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, it is enhanced eosinophil activation – 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?

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