

Serum eosinophil cationic protein and interleukin-5 in children with bronchial asthma and acute bronchiolitis

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The aim of our study was to evaluate the clinical applicability of serum eosinophil cationic protein (ECP), interleukin-5 (IL-5) and total eosinophil counts in childhood asthma and bronchiolitis. These parameters were measured in 44 children aged 12–84 months with moderate and mild asthma during symptomatic and asymptomatic phases of disease. Fifteen of the patients were included at the time of admission to hospital due to an acute asthmatic attack, and ten of these were also examined one month after discharge. None of the patients were treated with glucocorticoids or cromoglycate at any time during the study. Serum ECP was significantly increased in the children with acute asthma compared to children with stable moderate asthma, stable mild asthma, as well as to controls. There was no difference between the groups with stable asthma or between stable asthma and controls, and there was large overlap between all groups of asthmatics and controls. Detectable levels of circulating IL-5 were demonstrated in eight of 15 children with acute asthma, with significantly higher levels in atopic children, whereas all samples from children with stable asthma and controls were negative. The results suggest that even though serum ECP and IL-5 increases during acute asthmatic attacks, these parameters cannot alone be used to discriminate between different groups of young children with stable asthma, nor between asthmatics and healthy controls. In addition, the same parameters of eosinophil inflammation were examined in serum samples from 25 children aged 1–17 months undergoing their first episode of acute bronchiolitis. Children with acute respiratory syncytial virus (RSV) bronchiolitis had significantly higher levels of serum ECP than those with RSV negative disease, whereas the total eosinophil counts were significantly decreased in all patients with acute bronchiolitis. Serum IL-5 was only detected in two children with acute bronchiolitis. The results suggest that the inflammation in RSV bronchiolitis differs from that induced by other viruses.

Infiltration and activation of eosinophils in the bronchial mucosa are believed to be a key event in the pathogenesis of asthma (1). Interleukin-5 (IL-5), mainly produced by activated T-cells, promotes differentiation and survival of eosinophils (2, 3), and is considered to be the main mediator of eosinophil tissue infiltration and eosinophilia in asthma (2). In addition, IL-5 activates eosinophils (3), inducing the release of toxic granular proteins and oxygen radicals, as well as lipid mediators and cytokines, all of which contribute to the inflammatory process of asthma (4). Increased levels of eosinophil cationic protein (ECP) have consistently been demonstrated in the airways (5) and in serum (6–12) of children

and adults with asthma. Furthermore, a few studies have demonstrated increased levels of circulating IL-5 in a subgroup of adult asthmatics (9).

Small children with obstructive pulmonary disease (both asthma and bronchiolitis) represent a particular problem for the clinician, as measurements of airway flow and bronchial responsiveness to histamine or metacholine are usually only possible in children older than 5 years. There is a need for objective tools to document inflammation, as well as to evaluate the severity of disease, especially when the symptoms are moderate. Recently, serum ECP has been proposed to be a useful parameter of inflammation and eosinophil activation in childhood

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obstructive pulmonary disease (8, 11, 12). However, so far there are only few studies on serum ECP in young asthmatic children (11), and the role of serum ECP in bronchiolitis is still unclear (13–15). Moreover, serum IL-5 levels have not been determined in these children, although it has been suggested that this parameter may become a tool in the monitoring of allergic inflammation (12).

The aim of the present investigation was to evaluate the clinical applicability of selected parameters of eosinophilic inflammation in different groups of asthmatic children, as well as in bronchiolitis. We determined serum ECP, IL-5 and total eosinophil counts in 44 children with mild or moderate asthma, and in 25 children undergoing their first episode of acute bronchiolitis. Fifteen of the patients with asthma were studied on admission to hospital due to an acute asthmatic attack and again one month later, whereas the other 29 were studied in a stable asymptomatic phase. To reduce the possible influence of therapy, only patients who did not receive prophylactic treatment (glucocorticoids or cromoglycate) were included in the study.

Patients and methods

Study subjects

Patients with asthma: Patients aged 12 to 84 months were diagnosed as having mild or moderate asthma based on episodic cough and wheeze with response to β_2 -agonists persisting or recurring for at least 6 months without a history of another diagnosis (16). In total, 44 patients (28 males and 16 females) were studied and divided into 3 separate groups. The first group consisted of 15 children hospitalized for acute asthma (mean age 46 months; range 12–83 months). Ten of these patients were examined one month later. At this time point four patients still had symptoms indicating mild bronchial obstruction. Five children refused to have blood samples taken at follow-up. The other patients were classified to have stable moderate asthma ($n = 17$; mean age 57 months; range 22–82 months) or stable mild asthma ($n = 12$; mean age 51 months; range 14–83 months) according to accepted international criteria (16). Children with mild asthma had less than six episodes of asthma per year, symptoms lasting less than one week and symptomfree intervals without medication for more than a month. Children with moderate asthma had more than six episodes of asthma per year or duration of symptoms for more than a week but less than a month, and symptomfree periods less than a month. No patients had severe attacks or chronic symptoms affecting growth and development. All patients used bronchodilators on demand in periods with symptoms. No patient with asthma

had been using prophylactic therapy (i.e. inhaled glucocorticoids or cromoglycate) before the study. In accordance with international recommendations (16) most of the patients with moderate asthma started prophylactic treatment after the blood sample was drawn. In addition, some patients with acute asthma started prophylactic therapy at follow-up.

None of the patients with stable asthma had had clinical symptoms of bronchopulmonary obstruction the last week before inclusion, and none had clinical signs of infection. All patients with stable asthma had C-reactive protein and total leukocyte counts in the normal range. The patients with acute asthma had no signs of bacterial infection, as evaluated by clinical examination, serum C-reactive protein, total leukocyte counts and chest X-rays.

Twenty-one of the children with asthma were atopic and 23 were non-atopic. Atopic status was defined as having at least one positive skin prick test to common allergens (egg, milk, pea, hazelnut, peanut, cod, shrimp, *Cladosporium herbarum*, *Dermatophagoides pteronyssinus*, cat, dog, grass pollen and tree pollen; Soluprick, ALK, Hørsholm, Denmark). A skin prick test was considered positive when equivalent to histamine 1 mg/ml. The median total IgE for the atopic children with asthma was 119 kU/l and for the non-atopic 14 kU/l ($p < 0.001$).

Patients with bronchiolitis: This group consisted of 25 children aged 1–17 months (mean 6.8 months) needing hospitalization for their first episode of bronchiolitis (classified on the basis of previously described criteria (14)). Nasopharyngeal mucus was examined for respiratory syncytial virus (RSV) by direct immunofluorescence in all patients (bioMérieux, Marcy-l'Étoile, France). Twelve of the patients were RSV positive and 13 RSV negative. Seven of the patients with bronchiolitis had serum concentrations of total IgE above the normal age range, and one of these had atopic dermatitis. Chest X-rays showed minor perihilar changes in seven patients, but they had no signs of bacterial infection, as evaluated by clinical examination, total leukocyte counts and normal serum C-reactive protein. Children with pre-existing lung disease (e.g. bronchopulmonary dysplasia) or signs of bacterial infection were excluded.

Controls: Ten non-atopic children without asthma or infection served as controls (mean age 62 months; range 13–86 months).

Ethics: The study was approved by the Regional Ethical Committee, and signed statements of informed consent were obtained from all parents.

Laboratory analysis

Venous blood was drawn at an outpatient visit or immediately after admission for acute asthma or bronchiolitis. Serum was prepared according to the recommendation on sample processing for ECP measurements given by Pharmacia (Uppsala, Sweden). Aliquots were stored at -70°C until analysis.

Serum ECP was measured by ECP radioimmunosorbent assay manufactured by Kabi Pharmacia Diagnostics AB, (Uppsala, Sweden). The detection limit given by the manufacturer was 2 $\mu\text{g/l}$. The highest intra-assay coefficient of variation was approximately 11%. The reference range for ECP was 2.3–16.0 $\mu\text{g/l}$.

Serum IL-5 was measured by a 'sandwich' enzyme-linked immunosorbent assay (ELISA) using a kit from R&D Systems (Abingdon, UK). The highest intra-assay coefficient of variation was 5.7%. The lower detection limit of the ELISA used was 1 ng/l .

Serum-tryptase was analysed by a radioimmunosorbent assay produced by Kabi Pharmacia Diagnostics AB, (Uppsala, Sweden). The standard provided was calibrated against tryptase, isolated and purified from human lungs. One μg has been assigned a value of 1 U. The highest intra-assay coefficient of variation was 2.8% and the limit of detection was 0.5 U/l.

Serum total IgE was analysed using Pharmacia's CAP system. Serum-IgE levels $> 1\text{s.d.}$ above the

age related mean value was considered elevated.

Total eosinophil counts, total leukocyte counts (Technicon H*2/H*3 or Coulter STKS) and C-reactive protein (immunoturbidimetric technique; Roche, Basel, Switzerland) were determined in all patients. All patients with bronchiolitis and most with acute asthma had a chest X-ray taken after the initial treatment.

Statistical analysis

Significance of difference was determined by the Wilcoxon two sample-test, and Spearman's test was used for correlation analysis. $p < 0.05$ was considered significant. The data are given as mean, median and quartiles.

Results

Bronchial asthma

ECP: The serum concentration of ECP in children with acute asthma (23.9 $\mu\text{g/l}$; 21.1; 11.6–32.9) (mean, median, quartiles) was significantly higher than that of controls (10.9 $\mu\text{g/l}$; 11.7; 5.5–14.4, $p < 0.05$) on admission, and ten of the patients had serum ECP concentrations higher than 16.0 $\mu\text{g/l}$ (Fig. 1). One month later, the mean serum concentration of ECP was still increased (27.9 $\mu\text{g/l}$; 22.4; 9.1–54.5). Five of the patients had lower ECP levels at the follow-up than during the acute phase, whereas five patients had serum concentrations higher than

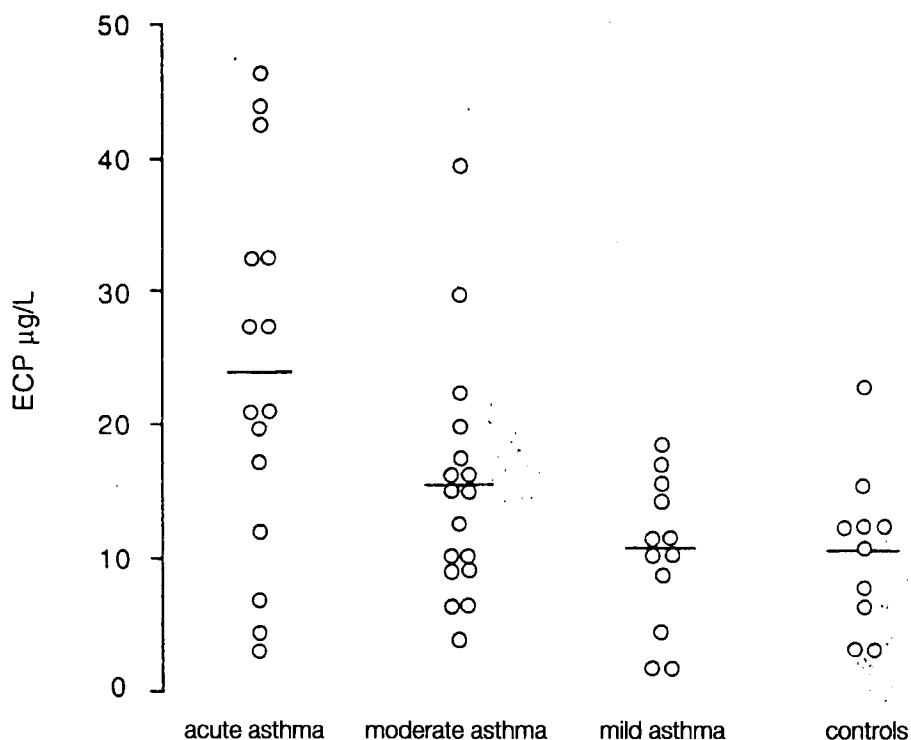


Fig. 1. Serum ECP in infants with acute, moderate and mild stable asthma and controls. The lines indicate mean values. The serum ECP levels in children with acute asthma were significantly higher than in the controls ($p < 0.05$).

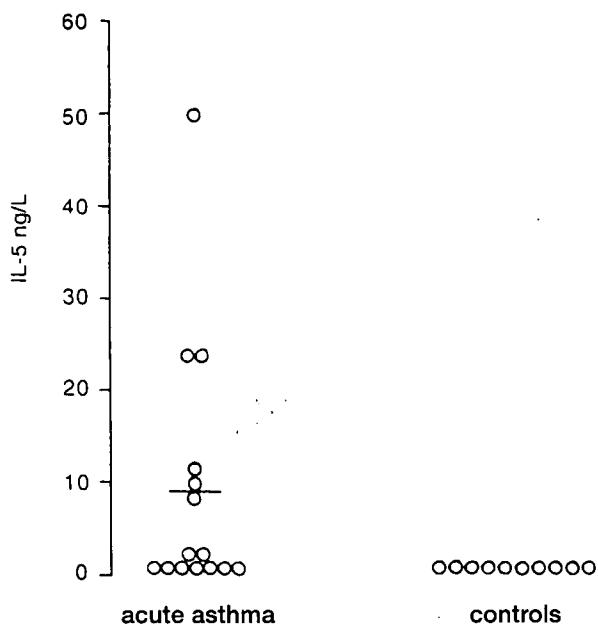


Fig. 2. Serum IL-5 in infants with acute asthma and in controls.

those at admission one month earlier. In the four patients who still had symptoms of bronchial obstruction at the follow-up, the serum ECP concentration ranged from 31 to 58 ug/l. Only one of them was atopic.

Although there was a tendency towards higher levels of serum ECP in the children with stable moderate asthma (15.5 ug/l; 14.7; 8.6–22.5), as compared to those with stable mild asthma (10.9 ug/l; 11.0; 9.8–13.2) and the controls, none of these differences reached significance. There was a large overlap of serum ECP concentrations between the groups with asthma, as well as between the asthmatics and the controls (Fig. 1). Consistently, the serum concentration of ECP was higher than 16.0 ug/l in seven children with stable moderate asthma, in three children with stable mild asthma and in two of the controls (Fig. 1).

There was a weak correlation between serum ECP concentration and total eosinophil count ($r = 0.41$; $p < 0.01$) in the asthmatic children. The serum levels of ECP were similar in atopic and non-atopic asthmatics (17.3 ug/l; 14.8; 11.1–21.8 and 16.8 ug/l; 12; 7.4–27.5), and no significant correlation was found between the serum concentrations of ECP and total IgE in them, nor did age influence the levels.

IL-5: Detectable levels of serum IL-5 were demonstrated on admission in eight of the 15 children with acute asthma (8.9 ng/l; 2.1; 0–18.6)(Fig. 2). Further,

the levels were higher in atopic children (21.1 ng/l; 12; 5.6–18.8) with acute asthma compared to non-atopics (2.8 ng/l; 0; 0–2.4, $p < 0.01$). Only three of ten non-atopics with acute asthma had measurable serum levels of IL-5. Only one of ten patients had measurable IL-5 (2.5 ng/l) at follow-up. Circulating IL-5 was not demonstrated in the children with stable moderate asthma, mild asthma or in any of the controls.

Total eosinophil count: The total eosinophil counts were similar in the children with asthma ($0.32 \times 10^9/l$; 0.28; 0.13–0.42) and the controls ($0.26 \times 10^9/l$; 0.26; 0.18–0.37), as well as between the groups with different asthma severity. The eosinophil counts were also similar in children with atopic and non-atopic stable asthma ($0.32 \times 10^9/l$; 0.32; 0.23–0.45 and $0.33 \times 10^9/l$; 0.25; 0.16–0.45). In the patients with acute asthma, the total eosinophil count tended to be higher in atopics ($0.65 \times 10^9/l$; 0.45; 0.29–0.62) than in non-atopics ($0.17 \times 10^9/l$; 0.13; 0.02–0.34), but this difference did not reach significance.

Trypsase: Trypsase was detected in serum samples from two patients with stable mild asthma (0.2 and 0.7 U/l). All other patients and controls samples were negative for trypsin.

Acute bronchiolitis

ECP: The children with RSV bronchiolitis had higher circulating levels of ECP than those with bronchiolitis caused by other agents (14.1 ug/l; 15.2; 10.4–17.3 and 7.9 ug/l; 5.5; 3.2–10.9; $p < 0.05$). The serum concentrations of ECP were similar in bronchiolitis patients taken together and the controls (10.9 ug/l; 11.7; 5.5–14.4). Furthermore, the levels were similar in patients with high or normal IgE, as well as in girls and boys, and there was no significant change with age.

Total eosinophil count: The total eosinophil count was significantly lower in children with RSV bronchiolitis ($0.13 \times 10^9/l$; 0.07; 0.03–0.20) and in those with RSV negative disease ($0.15 \times 10^9/l$; 0.09; 0.02–0.16) as compared to the controls ($0.26 \times 10^9/l$; 0.26; 0.18–0.37) ($p < 0.05$ for both).

Other parameters: Circulating IL-5 was detected in two children with acute bronchiolitis (6.1 ug/l and 7.4 ug/l), whereas low levels of tryptase was demonstrated in serum samples from three patients (0.8, 0.8 and 1.0 U/l). Neither IL-5 nor tryptase were demonstrated in the serum of the controls. Children with RSV positive bronchiolitis needed significantly longer hospitalization (8.0 days; 7.5; 3.5–12) than those having bronchiolitis caused by other agents (3.5 days; 4; 3–4) ($p < 0.005$).

Discussion

It is now recognized that even in mild asthma there is a chronic inflammation in the airways that is responsible for the bronchial reactivity (1, 12). Consistently, anti-inflammatory medication is increasingly advocated for children with asthma (17, 18). Measurements of airway flow and reactivity are difficult to perform and tend to be unreliable in children less than about 5 years old. Hence, evaluation of disease severity and decisions on therapeutic strategy in younger children are based on history and clinical examination. Recently, measurements of tidal breathing flow volume loops have been described as a promising tool for the evaluation of small children with bronchopulmonary obstruction (19). However, such equipment is not generally available. Others have proposed that serum ECP, and possible IL-5, may be useful parameters of airway inflammation in asthma (6, 8, 10, 12). Several workers have reported that changes in serum ECP reflect changes in disease severity and therapeutic success in adults and older children (7, 10, 20). As there are few reports on serum ECP and other eosinophil-related inflammatory parameters in young children with asthma (11), we measured serum ECP and IL-5 in 44 children from 12–84 months with mild or moderate asthma in symptomatic or asymptomatic phases of their disease.

In children undergoing an acute asthmatic attack, the serum ECP was significantly higher than in patients with stable asthma or in healthy controls. However, there was significant overlap between all the groups of asthmatic children. Based on this study a value of ECP > 16 µg/l would not have a high specificity for having asthma, as two of the controls had ECP levels above the upper reference limit. In addition, the sensitivity would be low as most concentrations of ECP in stable asthma were within the presumed normal range. A higher cut-off value could give a better specificity, but the sensitivity would be very low, even for those with moderate asthma. A recent study in older children has demonstrated similar results (21).

Other authors have reported higher values of serum ECP in asthmatic children than those observed by us (8, 11). This difference could be due to the relative mild asthma in our children. Moreover, serum ECP may be increased in other allergic diseases, e.g. nasal inflammation (21) and atopic dermatitis (8, 22). Some authors even suggest that serum ECP in children is more dependent on the status of allergic sensitization than asthma itself (23). Thus, the diagnostic efficacy of serum ECP seems to be low, both in discriminating between children with stable moderate asthma and stable mild asthma, as well as between asthmatics and controls.

In our patients with asthma, serum ECP was similar in atopic and non-atopic children. Moreover, the

serum ECP did not correlate with total IgE. Atopy is less common in young children with asthma than in older children. Zimmermann et al. have reported higher levels of serum ECP in atopic children than in non-atopics, suggesting that atopy contributes to childhood asthma by mobilization and activation of eosinophils (11). Our data do not support this.

IL-5 plays a major role in asthmatic inflammation (2, 3), and serum IL-5 has been postulated to be a possible tool in the monitoring of allergic inflammation (12). In our asthmatic children, serum IL-5 was detectable in eight of 15 with acute asthma, whereas in all patients with stable moderate or mild asthma circulating IL-5 was below the limit of detection. Others have reported increased levels of IL-5 in serum in some adult asthmatics, particularly in those who are symptomatic (9). Thus, serum IL-5 seems to increase with exacerbations, but does not seem to be helpful in evaluating the severity of inflammation in stable moderate and mild asthma in children.

There was no significant difference in total eosinophil counts between any of the groups of asthmatics and the controls. As confirmed by other studies, total eosinophil count does not seem to be a good parameter of inflammatory activity in asthma (7, 10).

Acute bronchiolitis caused by RSV induces an inflammatory response in the airways that has been claimed to induce a lasting bronchial hyperreactivity (24). On the other hand, others have held that the clinical picture of RSV positive bronchiolitis is more a marker of pre-existing hyperreactivity of the airways (25). Increased serum concentrations of inflammatory mediators, e.g. leukotrienes (26) and histamine (27), as well as specific anti-RSV IgE (27), have been demonstrated in acute bronchiolitis, and recently increased levels of ECP in nasopharyngeal secretions have been reported (13).

In our study, most serum ECP concentrations of children undergoing their first episode of acute bronchiolitis were < 16 µg/l irrespective of the agent causing the bronchiolitis. These findings are consistent with the data of both Colocho Zelaya et al. (13) and Sigurs et al. (14), but contrasts those of Lødrup Carlsen et al. who studied children with recurrent wheeze (15). The serum ECP was significantly higher in our children with RSV positive bronchiolitis as compared to those with RSV negative disease with the same degree of bronchiolitis, whereas the total eosinophil count was significantly depressed in all children regardless of causative virus. This suggests that RSV activate the eosinophils to a higher extent than the other agents, leading to a more pronounced inflammation. This may also be supported by the finding that children with RSV positive bronchiolitis had to be hospitalized for a significantly longer time than those with RSV negative disease.

It has recently been shown in a mouse model of bronchiolitis that RSV, via its major surface glycoprotein G, activated TH2 antiviral lymphocytes with subsequent appearance of lung eosinophilia (28). In contrast, other viruses mainly stimulated TH1 cells. Thus, the promotion of immune responses along TH2 cell pathways (associated with IgE production), could be a possible explanation for how RSV infection could trigger an atopic diathesis in childhood. In a recent study, Sigurs et al. found that children who had been hospitalized for RSV bronchiolitis were more likely to have a diagnosis of asthma and evidence of immediate hypersensitivity to allergens 3 years after hospitalization compared to controls (29). Our finding of higher ECP levels in RSV positive bronchiolitis suggest that RSV promotes a more pronounced eosinophilic inflammatory response than other viruses, possible through a TH2 dependent mechanism.

In spite of severe symptoms in some of our patients with acute bronchiolitis, serum IL-5 was only detected in two patients with RSV negative bronchiolitis. Thus, the role of IL-5, and especially the role of circulating IL-5, remains to be determined in acute bronchiolitis. This was also true for tryptase which was demonstrated in only two of 44 children with asthma and three of 25 with bronchiolitis. Thus, serum tryptase does not seem to reflect mast cell degranulation as long as it is restricted to the airways (30, 31).

In conclusion, this study has shown significantly increased levels of serum ECP and IL-5 in children with acute asthma. Neither ECP nor IL-5 could be used to discriminate between children with stable moderate and stable mild asthma, suggesting that both parameters are poor indicators of disease activity in these children. Children with acute RSV bronchiolitis had significantly higher levels of serum ECP than those with RSV negative disease, suggesting that RSV induces an inflammatory process that is different from that induced by other viruses.

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