

# Urinary eosinophil protein X in children with asthma: Influence of atopy and airway infections

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It has been suggested that urinary eosinophil protein X (U-EPX) can be used to monitor bronchial inflammation in childhood asthma. However, the influence of atopy and airway infections is not well elucidated. To determine the clinical value of measuring U-EPX in children with asthma and to evaluate the influence of atopy and airway infections, U-EPX was measured in 170 children with asthma (mean age 69 months, range 12–179 months), in 79 children with lower or upper respiratory tract infections (mean age 41 months, range 1–165 months), and in 64 controls. U-EPX was elevated in children with acute asthma (median 132 µg/mmol of creatinine, quartiles 77–195 µg/mmol of creatinine,  $n=51$ ,  $p<0.001$ ) and chronic asthma (median 93 µg/mmol of creatinine; quartiles 46–149 µg/mmol of creatinine,  $n=119$ ,  $p<0.01$ ) compared with controls (median 54 µg/mmol of creatinine; quartiles 40–89 µg/mmol of creatinine,  $n=39$ ). Atopic children had higher levels of U-EPX than non-atopics with acute asthma (median 155 µg/mmol of creatinine, quartiles 113–253 µg/mmol of creatinine,  $n=27$ , vs. median 102 µg/mmol of creatinine, quartiles 56–168 µg/mmol of creatinine,  $n=24$ ,  $p<0.05$ ), as well as with chronic asthma (median 110 µg/mmol of creatinine, quartiles 65–162 µg/mmol of creatinine,  $n=63$ , vs. median 60 µg/mmol of creatinine, quartiles 39–123 µg/mmol of creatinine,  $n=56$ ,  $p<0.01$ ). In chronic asthma, children without atopy had levels of U-EPX similar to values of controls; levels were similar in symptomatic and asymptomatic patients, and not influenced by treatment with inhaled corticosteroids. Moreover, U-EPX levels were higher in children with pneumonia (median 207 µg/mmol of creatinine, quartiles 111–280 µg/mmol of creatinine,  $n=35$ ,  $p<0.001$ ), laryngitis (median 109 µg/mmol of creatinine, quartiles 65–161 µg/mmol of creatinine,  $n=24$ ,  $p<0.01$ ), and rhinitis (median 172 µg/mmol of creatinine, quartiles 123–254 µg/mmol of creatinine,  $n=19$ ,  $p<0.001$ ) than in controls (median 62 µg/mmol of creatinine, quartiles 41–93 µg/mmol of creatinine,  $n=64$ ). There was significant overlap among all groups of children with disease, as well as between children with disease and controls. Hence, U-EPX may reflect differences in eosinophil involvement and activation between children with atopic and non-atopic asthma, but the individual spread within groups and the influence of airway infections limits the clinical value of U-EPX in childhood asthma.

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Asthma often starts in early childhood (1), but the diagnosis may be difficult in this age-group, partly because pulmonary function testing is not readily available. In addition, decisions on treatment are often based on clinical assessment only, which may be insufficient to reflect the inflammatory process

of asthma (2). Hence, there is a need for better diagnostic tools, as well as markers for monitoring disease activity, especially in early childhood (3–5).

During recent years, the importance of eosinophil inflammation in the pathogenesis of asthma has been clearly established (2,6). Activated

eosinophils release granule proteins, e.g. eosinophil cationic protein (ECP) and eosinophil protein X (EPX) (also known as eosinophil-derived neurotoxin), which are cytotoxic to the airway epithelium (6). Serum ECP and EPX have been measured to monitor disease activity in bronchopulmonary obstruction (7–11), and recently urinary EPX (U-EPX) has been suggested as a marker of inflammation in children with asthma (5,12–17). EPX is the only basic eosinophil granule protein that can be accurately measured in the urine (18), and U-EPX has been shown to correlate both with blood and bronchoalveolar eosinophil cell counts (19). Urine sampling is simple, non-invasive, and painless, and measurements of U-EPX may therefore be of particular value for supporting the diagnosis and monitoring disease activity in early childhood.

It has been discussed whether increased levels of eosinophil-derived proteins in serum reflect atopy rather than airway inflammation (9,10), but the influence of atopy as well as airway infection on U-EPX is not known (12). The aim of this investigation was to determine the value of measurements of U-EPX in children with asthma, and to evaluate the independent effect of atopy or airway infection. To study the influence of infection, EPX was measured in urine samples from children with bacterial pneumonia and viral airway infections. A group of children without atopic disease served as controls.

## Materials and methods

### Study design

In this cross-sectional study, children with acute asthma or airway infections were consecutively included as they were hospitalized for these conditions. Children with chronic asthma were included when they were examined at the out-patient clinic as part of their routine examination. Controls were recruited at the out-patient clinic among children attending for non-inflammatory conditions, or from a baby clinic. The parents of all children answered a questionnaire about atopy in the family, and a history (including infections in the previous 2 weeks) was taken.

### Study subjects

**Children with asthma.** A total of 170 children (Table 1)  $\geq 1$  year of age were diagnosed as having mild or moderate asthma, based on episodes of cough and wheeze (with response to  $\beta_2$ -agonists) persisting or recurring for at least 6 months, without a history of another diagnosis (20). By clinical assessment the children were given a symptom score from 0 to 6 (Table 2)

(21). Atopic status was defined as having at least one positive skin-prick test for common allergens (including egg, milk, pea, hazelnut, peanut, cod, shrimp, *Cladosporium herbareum*, *Dermatophagoides pteronyssinus*, cat, dog, grass pollen, and tree pollen; Soluprick, ALK, Hørsholm, Denmark). Histamine was used as a positive control and a 0.9% saline solution as a negative control. A wheal of at least 3 mm larger in diameter than the negative control was judged as a positive result.

A group of 51 children were hospitalized for acute asthma with a symptom score of 3–6. Thirteen of these children were treated with inhaled corticosteroids (ICS) before admission. Twenty-seven of the children were atopic and 24 were non-atopic. The atopic children were sensitized to aeroallergens alone ( $n=15$ ), to food allergens alone ( $n=3$ ), or to both aeroallergens and food allergens ( $n=9$ ).

Children with chronic asthma ( $n=119$ ) were examined at the out-patient clinic and were classified as having moderate asthma ( $n=90$ ) or mild asthma ( $n=29$ ), according to accepted international criteria (20). Seventy-nine of the children with chronic asthma (66%) were using ICS as anti-inflammatory treatment. Children with moderate asthma without anti-inflammatory treatment started ICS at the time of examination, according to international guidelines (20). All patients used short-acting  $\beta_2$ -agonists on demand when symptomatic. Forty-two of the children with chronic asthma were symptomatic with a symptom score of 1–4, whereas 78 children were asymptomatic. The asymptomatic children had been without symptoms of asthma or infections during the previous 2 weeks. Sixty-three of the children in this group were atopic and 56 were non-atopic. The atopic children were sensitized to aeroallergens alone ( $n=49$ ), to food allergens alone ( $n=4$ ), or to both aeroallergens and food allergens ( $n=10$ ).

Sixty children aged 6 years or older (mean age 9 years, range 6–14 years), with chronic asthma, performed pulmonary function testing at the time of the out-patient visit. Thirty of the children had a forced expiratory volume in 1 s ( $FEV_1$ )  $<85\%$  of the predicted value and 30 children had a  $FEV_1 \geq 85\%$  of the predicted value. None of these children had taken  $\beta_2$ -agonists on the day of the examination.

None of the children with asthma suffered from atopic dermatitis.

**Children with infections.** Thirty-five children were classified as having bacterial pneumonia (Table 1); this classification was based on typical symptoms (fever, tachypnoea, coughing), infil-

Table 1. Children included in the study

| Patient group       | No. of patients | Gender (male/female) | Age (months) (mean; range) | CRP (mg/l) (mean; range) |
|---------------------|-----------------|----------------------|----------------------------|--------------------------|
| Acute asthma        | 51              | 30/21                | 44; 12–112                 | 15; 5–49                 |
| Atopic              | 27              | 16/11                | 61; 13–112*                | 10; 5–26                 |
| Non-atopic          | 24              | 13/11                | 26; 12–97                  | 20; 5–49                 |
| Chronic asthma      | 119             | 83/36                | 80; 17–179                 | ND                       |
| Atopic              | 63              | 45/18                | 89; 20–179*                | ND                       |
| Non-atopic          | 56              | 38/18                | 69; 17–172                 | ND                       |
| Pneumonia           | 35              | 17/18                | 53; 1–165                  | 214; 100–464             |
| Laryngitis          | 25              | 20/5                 | 30; 5–129                  | 13; 5–33                 |
| Rhinitis            | 19              | 10/9                 | 32; 2–146                  | ND                       |
| All controls        | 64              | 32/32                | 45; 2–152                  | ND                       |
| Controls for asthma | 39              | 15/24                | 68; 13–152                 | ND                       |

\*p < 0.001 compared with non-atopics.

CRP, C-reactive protein; ND, not determined.

trate on an X-ray of the chest, and a C-reactive protein (CRP) concentration of >100 mg/l. Twenty-five children were considered to have acute viral laryngitis; this diagnosis was based on typical symptoms (fever, barking cough and inspiratory stridor) and a low level of CRP (Table 1). Nineteen children had rhinitis with acute secretion from the nose and no symptoms of the lower airways. None of the children with infections had clinical signs of bronchopulmonary obstruction or a history of atopic disease.

**Controls.** A total of 64 children without a history of wheezing or any other recurrent or chronic airway or inflammatory disease, and without a history of infection during the previous 2 weeks, served as controls (Table 1). These children had neither parental history of atopy or asthma nor a history of atopic dermatitis, food allergy or allergic rhinoconjunctivitis. As all children with asthma were  $\geq 12$  months of age, only children above this age (n=39) were included as controls for children with asthma (Table 1).

## Ethics

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

## Laboratory analyses

Urine from hospitalized children with acute asthma and infections was collected within 4 h of admission and kept in a refrigerator until aliquoting and freezing ( $-20^{\circ}\text{C}$ ) within 10 h of collection. Urine from controls and out-patients with chronic asthma was collected at home in the morning and frozen within 6 h.

U-EPX was analyzed by using a specific radioimmunoassay (Pharmacia, Uppsala, Sweden). The detection limit of the assay was  $<3 \mu\text{g/l}$  and the coefficient of variation was  $<5\%$  within assays and  $<10\%$  between assays. Urine creatinine levels were measured by using a Vitros 250 system (Ortho Clinical Diagnostics, Rochester, NY, USA). All measurements were performed in duplicate. U-EPX levels are presented as  $\mu\text{g/mmol}$  of creatinine.

In hospitalized patients, blood was drawn immediately after admission, and total eosinophil counts, total leucocyte counts (Technicon H\*2/H\*3, Bayer Diagnostics, Tarrytown, NY, USA or Coulter STKS, Coulter Corp., Miami, FL, USA), and CRP concentration (Immunoturbidimetric Technic, Roche, Basel, Switzerland) were determined.

Pulmonary function tests were performed by using spirometry (Vitalograph Compact, Vitalograph Ltd, Buckingham, UK).  $FEV_1$  was recorded as a maximum flow–volume curve, and the results presented as percentage of predicted. Predicted

Table 2. Symptom score by clinical assessment in children with asthma

|                                                                                                                         |
|-------------------------------------------------------------------------------------------------------------------------|
| 0. Normal                                                                                                               |
| 1. No dyspnoea. Slightly faint respiratory sounds.                                                                      |
| 2. No dyspnoea. Fine crackles or wheezing on auscultation.                                                              |
| 3. No dyspnoea at rest. Considerable wheezing on auscultation. Slight use of auxiliary respiratory muscles.             |
| 4. Considerable wheezing audible with or without stethoscope. Moderate use of auxiliary muscles. Chest wall recessions. |
| 5. Considerable wheezing. Considerable use of auxiliary muscles. Possible slight cyanosis.                              |
| 6. Poor respiratory effort. Quiet respiratory sounds. Cyanosis usual.                                                   |

Modified from reference (21).

Table 3. Urinary eosinophil protein X (U-EPX) and total eosinophil count in children with asthma

| Patient group     | Number of patients | U-EPX<br>( $\mu\text{g}/\text{mmol}$ of creatinine) | Eosinophil count<br>( $\times 10^9/\text{l}$ ) |
|-------------------|--------------------|-----------------------------------------------------|------------------------------------------------|
| Acute asthma      | 51                 | 132; 77–195*                                        | 0.39; 0.10–0.65                                |
| Atopic            | 27                 | 155; 113–253*†                                      | 0.42; 0.12–0.79                                |
| Non-atopic        | 24                 | 102; 56–168‡                                        | 0.29; 0.10–0.48                                |
| Chronic asthma    | 119                | 93; 46–149§                                         | ND                                             |
| Atopic            | 63                 | 110; 65–162*¶                                       | ND                                             |
| Non-atopic        | 56                 | 60; 39–123                                          | ND                                             |
| Symptomatic       | 42                 | 100; 57–158§                                        | ND                                             |
| Asymptomatic      | 77                 | 91; 41–146‡                                         | ND                                             |
| $FEV_1 < 85\%$    | 30                 | 114; 59–224* **                                     | ND                                             |
| $FEV_1 \geq 85\%$ | 30                 | 74; 46–113                                          | ND                                             |
| Controls          | 39                 | 54; 40–89                                           | ND                                             |

Results are expressed as median and quartiles.  $FEV_1$ , forced expiratory volume in 1 s; ND, not determined.

\* $p < 0.001$  compared with controls.

† $p < 0.05$  compared with non-atopic children.

‡ $p < 0.05$  compared with controls.

§ $p < 0.01$  compared with controls.

¶ $p < 0.01$  compared with non-atopic children.

\*\* $p < 0.05$  compared with children with  $FEV_1 \geq 85\%$ .

lung function values were calculated using standard reference values (22).  $FEV_1$  was inversely correlated to symptom score ( $r = -0.65$ ;  $p < 0.05$ ). Children with a  $FEV_1 < 85\%$  of predicted received an inhalation with a  $\beta_2$ -agonist. Spirometry was repeated after 15 min and the percentage improvement was recorded.

#### Statistical methods

The levels of U-EPX in different groups were not normally distributed. Comparisons between groups were made by using the non-parametric Mann–Whitney  $U$ -test, and results are presented as median and quartiles. Relationships between variables were evaluated by using the Spearman rank correlation coefficient.

Because of the skewed distribution of U-EPX, the influence of covariates in asthma was first investigated by assessment of U-EPX in atopic and non-atopic individuals, separately in acute and chronic asthma, by use of simple non-parametric

methods. The relationships between U-EPX and the determinants of interest were further estimated in a linear regression analysis after logarithmic transformation of U-EPX (normally distributed,  $p = 0.2$ ), and adjusted for potential confounders. The covariates of asthma diagnosis, atopy, and symptoms were fitted into the models.

Data were analyzed by using the SPSS 9.0 statistical package (SPSS Inc., Chicago, IL, USA). All tests were two-tailed and probabilities of less than 5% were considered significant.

## Results

### Children with asthma

Differences between groups of patients and controls are presented in Table 3. Hospitalized children with acute asthma had levels of U-EPX that were significantly higher than those of age-matched controls ( $p < 0.001$ ) (Fig. 1) or of children with chronic asthma ( $p < 0.05$ ). There was no

Table 4. Multiple regression analysis to examine the effects on urinary eosinophil protein X (U-EPX) of asthma diagnosis and atopy in acute and chronic asthma independently, and also of the effect of symptoms in chronic asthma (controls were included)

| Log U-EPX        | p-value | Beta coefficient | 95% CI     |
|------------------|---------|------------------|------------|
| Acute asthma     |         |                  |            |
| Asthma diagnosis | 0.015   | 0.18             | 0.04–0.32  |
| Atopy            | 0.004   | 0.23             | 0.08–0.39  |
| Constant         | 0.00    | 1.78             | 1.69–1.9   |
| Chronic asthma   |         |                  |            |
| Asthma diagnosis | 0.5     | 0.05             | –0.09–0.19 |
| Atopy            | 0.006   | 0.17             | 0.05–0.29  |
| Symptoms         | 0.2     | 0.08             | –0.05–0.20 |
| Constant         | 0.00    | 1.78             | 1.67–1.9   |

Multiple linear regression table of beta coefficients and 95% confidence intervals (CI) for logarithm (log) U-EPX against asthma diagnosis with the simultaneous inclusion of atopy (yes/no) and symptoms (yes/no).

Table 5. Urinary eosinophil protein X (U-EPX) in children with infections

| Patient group | No. of patients | U-EPX ( $\mu\text{g}/\text{mmol}$ of creatinine) |
|---------------|-----------------|--------------------------------------------------|
| Pneumonia     | 35              | 207; 111–280*†                                   |
| Laryngitis    | 25              | 109; 65–161‡                                     |
| Rhinitis      | 19              | 172; 123–254*                                    |
| Controls      | 64              | 62; 41–93                                        |

Results are expressed as median and quartiles.

\* $p < 0.001$  compared with controls.

† $p < 0.01$  compared with laryngitis.

‡ $p < 0.01$  compared with controls.

correlation between U-EPX and total eosinophil count ( $r = -0.22$ ) or between U-EPX and symptom score (not shown) for children with acute asthma.

Out-patients with chronic asthma had higher levels of U-EPX compared with controls ( $p < 0.01$ ), regardless of symptoms or treatment. Children with chronic asthma and symptoms had levels of U-EPX that were similar to those who were asymptomatic, and there was no correlation between symptom score and U-EPX ( $r = 0.11$ ,  $p = 0.25$ ). The asymptomatic children treated with ICS had levels of U-EPX ( $n = 57$ ; median  $97 \mu\text{g}/\text{mmol}$  of creatinine, quartiles  $42$ – $160 \mu\text{g}/\text{mmol}$  of creatinine) that were higher than controls ( $p < 0.05$ ). There was no correlation between the dose of ICS used and level of U-EPX (results not shown).

Atopic children had higher levels of U-EPX than those who were non-atopic with acute asthma ( $p < 0.05$ ), as well as those with chronic asthma ( $p < 0.01$ ) (Fig. 1). This was true even when children with chronic mild asthma and atopy ( $n = 12$ ; median  $108 \mu\text{g}/\text{mmol}$  of creatinine, quartiles  $59$ – $142 \mu\text{g}/\text{mmol}$  of creatinine) were compared to those with mild asthma without

atopy ( $n = 17$ ; median  $55 \mu\text{g}/\text{mmol}$  of creatinine, quartiles  $33$ – $88 \mu\text{g}/\text{mmol}$  of creatinine;  $p < 0.05$ ). Children with both mild and moderate chronic asthma without atopy had levels of U-EPX that were similar to those of controls (Fig. 1).

In chronic asthma, children with a  $FEV_1$  that was  $< 85\%$  of predicted had higher levels of U-EPX than children with a  $FEV_1 \geq 85\%$  ( $p < 0.05$ ), but there was no correlation between the  $FEV_1$  and U-EPX ( $n = 60$ ;  $r = -0.13$ ,  $p = 0.3$ ). In the children with a  $FEV_1 < 85\%$  of predicted, there was no correlation between U-EPX and percentage improvement of  $FEV_1$  after inhalation of  $\beta_2$ -agonist ( $n = 30$ ;  $r = 0.16$ ,  $p = 0.4$ ).

The results of multiple linear regression models with the logarithm for U-EPX as an independent variable are presented in Table 4. It was found that for children with acute asthma, the diagnosis of asthma and atopy both corresponded significantly to increased U-EPX. For children with chronic asthma, having atopy corresponded significantly to increased U-EPX, whereas the diagnosis of asthma or symptoms did not. Adding age or gender as covariates did not change the information to the regression models.

#### Children with infections

Differences between groups and controls are presented in Table 5. Children with bacterial pneumonia had higher levels of U-EPX than age-matched controls ( $p < 0.001$ ), but there was no correlation between U-EPX and CRP. Children with laryngitis had levels of U-EPX that were higher than in controls ( $p < 0.01$ ), but lower than in children with pneumonia ( $p < 0.01$ ). Children with rhinitis had levels of U-EPX that were higher than in controls ( $p < 0.001$ ) or in children with laryngitis ( $p < 0.05$ ).

#### Discussion

This study has demonstrated that U-EPX was elevated both in children hospitalized for acute asthma and in children with chronic asthma, compared with controls of the same age. In both

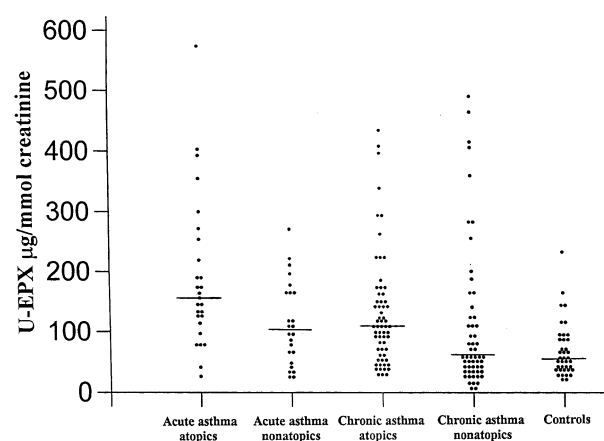


Fig. 1. Urinary eosinophil protein X (U-EPX) in children with acute asthma with atopy ( $n = 27$ ) and without atopy ( $n = 24$ ); in children with chronic asthma with atopy ( $n = 63$ ) and without atopy ( $n = 56$ ); and in controls ( $n = 39$ ). Lines indicate the median value.

acute and chronic asthma, atopic children had higher levels of U-EPX than the non-atopics, whereas in chronic asthma only atopic children had higher levels than controls. Furthermore, in chronic asthma the U-EPX concentration was not influenced by the degree of symptoms or treatment with ICS.

In asthma, eosinophils and their granule proteins contribute significantly to the airway inflammation (2,6), and several studies have demonstrated a relationship between eosinophil-derived proteins in serum and bronchial inflammation (8,16). We and others have demonstrated elevated serum concentrations of ECP in children with acute asthma (8,11,15,23), and recently two studies showed an increased U-EPX concentration during acute attacks (15,17). In the present study, the highest levels of U-EXP were observed in children with acute asthma, and in chronic asthma U-EPX was higher in children with a  $FEV_1 < 85\%$  of predicted compared to those with a  $FEV_1 \geq 85\%$ . Hence, U-EPX may, to some extent, reflect the airway inflammation in childhood asthma. There was, however, significant overlap between U-EPX in patients with acute asthma and controls, and U-EPX did not correlate well with symptom score. Furthermore, in chronic asthma moderate symptoms did not significantly influence the levels of U-EPX. The results demonstrate that in childhood asthma, U-EXP alone is not sufficient to describe inflammatory activity or symptoms.

Our study suggests that atopy is a major contributor to increased levels of U-EPX in children with acute, severe asthma as well as in chronic disease. Even though earlier reports have shown conflicting results, it has been suggested that the inflammatory mechanisms are different in atopic and non-atopic asthma (24,25). Consistently, serum-ECP and EPX have in some studies been higher in children with atopic asthma than in non-atopic disease (10,26). Two other studies have demonstrated increased U-EPX concentrations in children with chronic stable asthma (13,17), but in these studies only atopic individuals were included, and the relative contribution of atopy could not be determined.

The child's age may also play a role, and in our study the non-atopic children were younger than the atopic individuals, with a mean age of 26 months in acute asthma. Our results contrast those of Lugosi et al., who reported that U-EPX was similar in atopic and non-atopic children with asthma (12). However, the children in their study had a mean age of 10.1 years, which is significantly older than those included in the present study (Table 1). Many children <3 years

of age will outgrow their asthma, and this is particularly true for non-atopic children (1,3,27). In a study by Stevenson et al., eosinophil numbers in bronchoalveolar lavage were elevated in a group of children with atopic asthma (mean age 8 years), but not in younger children with viral-associated wheezing (25). It is possible that the levels of U-EPX in the present study reflect differences in the pathophysiological mechanisms in atopic and non-atopic asthma, and that the inflammation in young, non-atopic children with asthma is more similar to that of children with viral-associated wheezing than that of older children with atopic asthma (10,25).

Atopic disorders are generally associated with blood eosinophilia, and allergic stimulation in sensitive children may induce eosinophilopoiesis in the bone marrow and activation of eosinophils in the periphery (28). In a recent study, EPX in serum was significantly associated with atopy, even in non-asthmatic children (9), and it is therefore possible that mechanisms other than differences in airway inflammation could have contributed to the higher levels of U-EPX, as demonstrated in this study in the atopic compared with the non-atopic asthmatics. However, others have reported a decrease in U-EPX after treatment of asthmatic children with ICS, suggesting that the concentration of U-EPX at least partly reflects airway inflammation (13,17).

There are different methods for assessment of atopy, but there exists no clear definition (29). Skin-prick testing is considered to be a sensitive assay for antigen-specific immunoglobulin E (IgE), even in young children (27), but may not identify all atopic children in the youngest age-groups (29). However, children with atopic dermatitis were not included in this study, and the children with negative skin-prick tests had no history of any other atopic disease such as allergic rhinitis or conjunctivitis (9). As in this study the influence of atopy on U-EPX in asthmatic children seems clear, we have no reason to believe that using another definition of atopy would change the significance of the results.

U-EPX was significantly increased in non-atopic children with bacterial pneumonia and viral laryngitis or rhinitis, with the highest levels of U-EPX detected in children with pneumonia. In a recent study, the concentration of serum ECP was significantly raised in subjects with bacterial infections but not in viral infections (30). The difference in these results may reflect the proposed selective release of the eosinophil granule proteins (30). Both bacterial and viral infections induce secretion of cytokines capable of recruiting eosinophils and other proinflamma-

tory leucocytes from the circulation to the airway mucosa (31), and in the infected mucosa, activated eosinophils may degranulate, releasing granular proteins, e.g. EPX.

Symptoms in asthmatic children are often triggered by infections, especially in the younger age-groups (31), and in the present study, high levels of U-EPX in children with acute and chronic symptomatic asthma may have been influenced by respiratory infections initiating the symptoms. The present results therefore demonstrate that the possible effect of bacterial or viral airway infections has to be considered when evaluating levels of U-EPX, and that this influence may limit the clinical utility of U-EPX in childhood asthma. As eosinophil degranulation is followed by release of both EPX and ECP, our results may also suggest that the influence of airway infections has to be considered in studies assessing serum ECP in asthmatic children (30).

A circadian variation in U-EPX has recently been demonstrated, with higher levels in the morning compared to the afternoon (17). In our study, the urine samples from the controls and patients with chronic asthma were taken in the morning, and therefore the circadian variation did not influence the comparison between these groups. The samples taken from hospitalized patients were evenly distributed throughout the 24-h period of daytime/night-time, and the difference between these groups and controls could have been further increased if all samples had been collected in the morning. The wide spread of U-EPX levels within groups of hospitalized patients may have been influenced by the diurnal variation.

The ultimate goal when studying inflammatory markers in asthma is to find parameters that are useful in clinical practice and for the individual child. It has been suggested that U-EPX may be employed in the management of childhood asthma (5,12,13,17). In the present study, a significant difference in levels of U-EPX was observed between those with atopic and non-atopic asthma, which may reflect differences in eosinophil involvement and activation between these two groups (25). A possible clinical role for U-EPX may therefore be to assess the importance of eosinophil airway inflammation in the individual child with asthma. This could be of special relevance in the youngest age-groups, as asthma during the first years of life is thought to represent groups with differences in airway pathogenesis and outcome, and may have further implications for the implementation of anti-inflammatory treatment (1,3,4). However, the possible influence of infections, the significant spread of individual

U-EPX levels, as well as the overlap between patients and controls, may limit the clinical value of U-EPX in childhood asthma, and demonstrates that U-EPX alone is not sufficient to describe the airway inflammation or symptom activity in the individual child.

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