

High levels of urinary eosinophil protein X in young asthmatic children predict persistent atopic asthma

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Levels of urinary eosinophil protein X (U-EPX) and eosinophil counts were measured in 32 children (12–36 months of age) who were hospitalized for acute asthma, and the U-EPX levels were measured in 20 healthy children of the same age. The ability of these parameters to predict persistent asthma (at least one wheezing episode during the last 6 months) and atopic asthma (a positive skin-prick test [SPT]), was evaluated at a follow-up 2 years later. On admission, levels of U-EPX were higher in children with asthma (median: 120 µg/mmol of creatinine; quartiles: 67–123 µg/mmol of creatinine) than in controls (60 µg/mmol of creatinine, 38–74 µg/mmol of creatinine; $p < 0.001$). The U-EPX level was higher in those with persistent atopic asthma at follow-up (173 µg/mmol of creatinine, 123–196 µg/mmol of creatinine, $n = 16$), than in those with persistent non-atopic asthma (73 µg/mmol creatinine, 46–105 µg/mmol of creatinine, $n = 8$; $p < 0.05$), and higher than in those with transient asthma (no symptoms at follow-up) (106 µg/mmol creatinine; 42–167 µg/mmol of creatinine, $n = 8$; $p < 0.05$). By multiple logistic regression analysis, U-EPX was the only parameter able to predict persistent atopic asthma; eosinophil counts, parental atopy, age or gender could not. Parental atopy was the only parameter predictive for persistent asthma, regardless of atopic status. In conclusion, levels of U-EPX, but not eosinophil counts, measured in young children hospitalized with acute asthma can predict the persistence of atopic asthma 2 years later.

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During recent years it has become clear that children with symptoms of asthma or wheezing during early childhood represent different subgroups with regard to pathophysiology and prognosis (1,2). Whereas some children with transient wheezing may have non-atopic conditions related to lower levels of airway function (2,3), children with persistent symptoms may show immune responses characteristic of atopic asthma, even at the time of the first wheezing episode (1,4). Consequently, it has been suggested that the early recognition of wheezing children with a high risk of subsequent atopic asthma may be important (1,5).

Activation of eosinophils is one of the main features of asthma (6), and levels of eosinophil

granule proteins such as eosinophil cationic protein (ECP) and eosinophil protein X (EPX) in different body fluids have been suggested as markers of eosinophil activation in asthma (7). Furthermore, a high level of ECP in serum has been suggested as a predictor of asthma in different age-groups of wheezing children (8,9), but the results are not consistent (10). In asthmatic children, levels of urinary EPX (U-EPX) are higher in atopic than in non-atopic individuals (11), and in children with wheezing episodes during the first year of life, a high level of U-EPX may predict subsequent allergic sensitization (12). However, the value of U-EPX in the prediction of persistent symptoms in young asthmatic children is not known.

Atopy (in particular atopic dermatitis) has been suggested to be the single most important factor for predicting the persistence of disease in young children with wheezing or asthma (1,5,13), and the persistence of symptoms may therefore be difficult to predict in children without clinical signs of atopy in early life. The aim of this study was to evaluate the value of U-EPX and total eosinophil counts measured in children at 1–3 years of age and without atopic dermatitis or any other allergic disease, in predicting persisting asthma and atopic asthma (as defined by a positive skin-prick test [SPT]) 2 years after hospitalization for acute asthma.

Materials and methods

Study subjects

Thirty-two children (15 boys and 17 girls, mean age 22 months, range 12–36 months) were recruited during the winter of 1997–98 as they were admitted to the Paediatric Department, Rogaland Central Hospital, because of an acute episode of wheezing. All children had experienced at least three episodes of wheezing with response to β_2 -agonists, to fulfill international criteria for having asthma (14). None of the children had received inhaled corticosteroids (ICS) or any other anti-inflammatory drug prior to hospitalization. Children with pre-existing lung disease (e.g. bronchopulmonary dysplasia), signs of bacterial infection, or children with atopic dermatitis at the time of inclusion, were not recruited to the study. A standardized questionnaire was used to interview all parents, including the history of wheezing of the child and a parental history of atopic disease (atopic dermatitis, rhinoconjunctivitis or asthma).

Controls

Twenty children (mean age 24 months, range 10–51 months) referred to our out-patient clinic and who had no history of wheezing or any other recurrent or chronic airway or inflammatory disease, and no history of infection during the previous 2 weeks, served as controls. These children had neither a parental history of atopy or asthma nor a history of atopic dermatitis, food allergy or allergic rhinoconjunctivitis.

Laboratory analyses

Urine from the children with asthma was collected within 4 h of admission and stored

in a refrigerator until aliquoting and freezing (-20°C) within 10 h of sampling; controls had a urine sample taken in the morning, which was frozen within 8 h of sampling (15). U-EPX was analyzed by a specific radioimmunoassay (Pharmacia, Uppsala, Sweden). The detection limit of the assay was $<3\text{ }\mu\text{g/l}$, the within-assay coefficient of variation was $<5\%$, and the between-assay coefficient of variation was $<10\%$. Urine creatinine levels were measured by using a Vitros 250 system (Ortho Clinical Diagnostics, Rochester, NY, USA). All measurements were carried out in duplicate. U-EPX levels are presented as $\mu\text{g/mmol}$ of creatinine.

In children with asthma, blood was drawn immediately after admission and eosinophil counts were determined (Technicon H*2/H*3; Bayer Diagnostics, Tarrytown, NY, USA, or Coulter STKS; Coulter Corp., Miami, FL, USA).

Follow-up

During February and March 2000, on average 24 months (standard deviation [SD] 1.5 months) after hospitalization, the children (mean age 46 months, range 35–59 months) with asthma were re-examined and a medical history taken. To identify subsequent episodes of wheezing following hospitalization, the parents were asked if the child had demonstrated symptoms similar to those experienced when hospitalized. Wheezing episodes either recorded by a physician or reported by parents were evaluated. One or more consecutive days of wheezing, followed and preceded by a healthy period of 1 week, were counted as one wheezing episode. Furthermore, the previous or present use of ICS were recorded. Children were considered to have persistent asthma if they had experienced at least one wheezing episode during the previous 6 months (autumn and winter).

A standardized SPT 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) was performed in all children. Histamine (10 mg/ml) was used as a positive control and a 0.9% saline solution as a negative control. A wheal at least 3 mm larger in diameter than the negative control was judged as a positive result. Children were considered to have atopic asthma if they had persistent asthma and a positive SPT.

Ethics

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

Statistical methods

Data were analyzed using the SPSS 9.0 statistical package (SPSS Inc, Chicago, IL, USA). Comparisons between groups were made using non-parametric tests. The Kruskal–Wallis analysis of variance was first applied to the groups. When this was significant, each pairing was analyzed by the Mann–Whitney *U*-test. Relationships between variables were evaluated by the Spearman rank correlation coefficient.

Logistic regression models were used to calculate the odds ratios (OR) with 95% confidence intervals (CI) for estimating the associations between potential risk factors and different outcomes. Each variable was first entered separately into univariate models. Predictive accuracy, *p*-values and OR with 95% CI from the inclusion of each variable were calculated. Variables with a significance level of $p < 0.1$ were further analyzed in multiple regression analysis models. Probabilities of less than 5% were considered significant.

Results

On admission, levels of U-EPX were higher in children with asthma (median: 120 $\mu\text{g}/\text{mmol}$ of creatinine; quartiles: 67–123 $\mu\text{g}/\text{mmol}$ of creatinine) than in controls (60 $\mu\text{g}/\text{mmol}$ of creatinine; 38–74 $\mu\text{g}/\text{mmol}$ of creatinine; $p < 0.001$). In children with asthma there was no correlation between levels of U-EPX and eosinophil counts ($r = 0.09$, $p = 0.6$).

Follow-up

At follow-up, 24 children were classified as having persistent asthma (75%). Of these, 17 were receiving ICS, six had been treated with ICS in the past, and one had received no ICS treatment, past or present. Sixteen of the children with persistent asthma had a positive SPT for at least one allergen (atopic asthma). Eight of these children were positive to food allergens and 14 to inhalant allergens. Eight children had persistent asthma and a negative SPT (non-atopic asthma). Eight children had been without symptoms of wheezing and had not received ICS during the past 6 months

(transient asthma), and all these children had a negative SPT at follow-up.

U-EPX and total eosinophil count: Comparison between groups

Children with persistent atopic asthma at follow-up had higher levels of U-EPX on admission (173 $\mu\text{g}/\text{mmol}$ of creatinine; 123–196 $\mu\text{g}/\text{mmol}$ of creatinine) than those with persistent non-atopic asthma (73 $\mu\text{g}/\text{mmol}$ of creatinine; 46–105 $\mu\text{g}/\text{mmol}$ of creatinine; $p < 0.05$), and higher levels of U-EPX than those with transient asthma (106 $\mu\text{g}/\text{mmol}$ of creatinine; 42–167 $\mu\text{g}/\text{mmol}$ of creatinine; $p < 0.05$) and controls ($p < 0.001$) (Fig. 1). Children with persistent non-atopic asthma and transient asthma had levels of U-EPX similar to those of controls (Fig. 1).

Total eosinophil counts on admission were similar between those with persistent atopic asthma ($0.42 \times 10^9/\text{l}$; $0.1\text{--}0.59 \times 10^9/\text{l}$) and those with persistent non-atopic asthma ($0.26 \times 10^9/\text{l}$; $0.1\text{--}0.61 \times 10^9/\text{l}$; $p = 0.7$) and transient asthma ($0.24 \times 10^9/\text{l}$; $0.07\text{--}0.66 \times 10^9/\text{l}$; $p = 0.5$) at follow-up.

Prediction of persistent atopic asthma and persistent asthma: Logistic regression analysis

By logistic regression analysis with each parameter included separately, we found that U-EPX and parental atopy were predictive factors for having atopic asthma at follow-up, whereas eosinophil counts, age or gender were not (Table 1). The overall value for the prediction of atopic asthma was 75% for U-EPX and 69% for parental atopy. When these two parameters were combined in a multiple logistic regression model, only U-EPX ($p = 0.03$) was a significant predictive factor for persistent atopic asthma. Including age and gender as possible confounders did not change the significance of the results.

By using univariate logistic regression analysis, parental atopy was the only factor that could predict the persistence of asthma regardless of the presence of atopy in the child (OR = 15, 95% CI = 2.2–103, $p = 0.006$), with an overall predictive value of 81%. U-EPX, eosinophil counts, age or gender were not predictive factors for persistent asthma.

Discussion

Recognition of the heterogeneity of childhood wheezing has led to the search for tools to

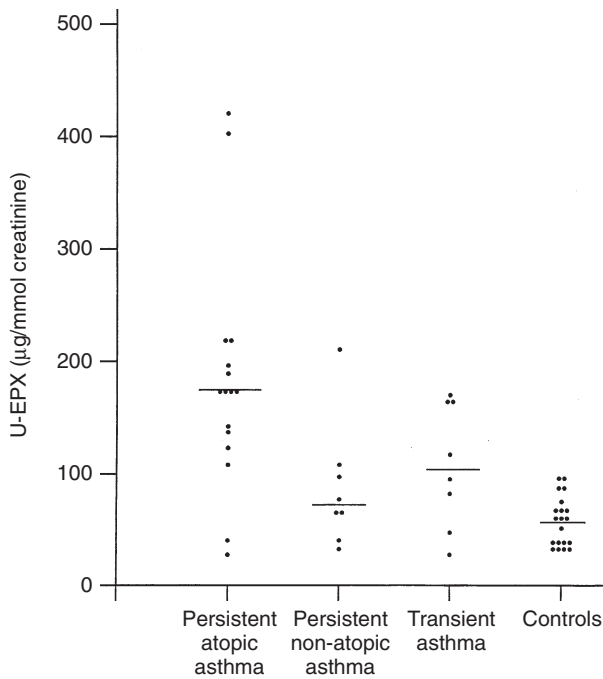


Fig. 1. Urinary EPX (U-EPX) measured in children (mean age 22 months) admitted for acute asthma, and who had persistent atopic asthma (n=16), persistent non-atopic asthma (n=8), or transient asthma (n=8), 24 months later, as well as in healthy controls (n=20). Lines indicate median value.

predict outcome and selection of subjects in whom the benefits of early intervention may outweigh the disadvantages (1,16). Eosinophil airway inflammation is one of the hallmarks of atopic asthma (6), and markers of eosinophil activation have therefore been studied as possible predictors of asthma in children with early wheezing (8,9). EPX is easily measured in the urine (17), and the level of U-EPX is thought to reflect eosinophil activation in the airways of asthmatic children (11,18,19).

The present study demonstrated higher levels of U-EPX in children with symptomatic asthma than in controls, consistent with studies in older children (11,18,19). In a recent study, EPX in serum was significantly associated with atopy, even in non-asthmatic children (20), and high levels of U-EPX have also been demonstrated in children with atopic dermatitis (21,22). However, in the present study the children with asthma had no clinical signs of atopic dermatitis or any other allergic disease at the time of inclusion, suggesting that high levels of U-EPX reflect eosinophil activation in the airways of these children.

Moreover, this study demonstrated that U-EPX measured during an episode of acute asthma at early age, was predictive for persistent atopic asthma, but not for non-

Table 1. Predictive factors for persistent atopic asthma*

Variable	p-value	OR	95% CI
U-EPX ($\mu\text{g}/\text{mmol}$ of creatinine)	0.02	1.02	1.0–1.03
Parental atopy†	0.03	7.0	1.2–41.4
Age in months	0.6	1.0	0.9–1.10
Eosinophil counts ($\times 10^9/\text{l}$)	0.9	1.1	0.1–10.3
Male gender	0.3	2.1	0.5–8.80

*Thirty-two children were evaluated 24 months after hospitalization for acute asthma (age 12–36 months at admission), and p-values, odds ratios (OR), and 95% confidence interval (CI) for the prediction of persistent atopic asthma were determined by logistic regression. Each risk factor was analyzed separately.

†A history of atopic disease (atopic dermatitis, rhinoconjunctivitis or asthma) in one or both of the parents.

atopic asthma. Children with atopic asthma at follow-up had higher levels of U-EPX at the time of the first hospitalization than children with persistent non-atopic asthma, and higher levels of U-EPX than children with transient asthma. In young wheezing children, allergic sensitization is predictive for the persistence of symptoms (5,13), and in school-age children asthma is highly associated with allergy (23). In the present study the mean age of the children was 46 months at follow-up, and some of the children with persistent asthma and a negative SPT may have had transitory conditions not associated with atopic asthma (2,5). It is therefore possible that high levels of U-EPX measured during acute asthma in young children may predict persistent atopic asthma in later childhood. The results are consistent with a recent study demonstrating that in children with wheezing during the first year of life, high levels of U-EPX were predictive for persistent wheezing and concurrent allergic sensitization 2 years later, but not for the persistence of wheezing alone (12).

It has been suggested that differences in the pathophysiology and outcome between groups of young wheezing children may be related to differences in eosinophil involvement and activation in the airways during wheezing episodes. Studies of bronchoalveolar lavage have suggested two different forms of childhood asthma with higher levels of eosinophil and mast cell numbers in children with atopic asthma compared to children with viral-associated wheezing without atopy (24). Moreover, differential pathways in the inflammation cascade may be involved in atopic and non-atopic asthma, even at an early age (25). The ability of eosinophils to secrete granule proteins is enhanced by priming with cytokines involved in allergic inflammation (6,7). Although most wheezing episodes during early childhood are caused by viral infections and not by allergic

stimuli (4), the present study suggests that levels of U-EPX may reflect differences in eosinophil activation between atopic and non-atopic asthma, even at an early stage of disease. Consistently, we have recently demonstrated that in young children with acute or chronic asthma, those with atopy had higher levels of U-EPX compared to those with non-atopic asthma (11).

In contrast to the results of U-EPX, total eosinophil counts did not differ significantly between the groups and could not predict persistent asthma in children either with or without a positive SPT. It has been demonstrated that in children with wheezing before the age of 1 year, total eosinophil counts were predictive for persistent wheezing (4,12,26). However, children with wheezing episodes requiring hospitalization during the first year of life may represent a different population, with a higher proportion of children experiencing transient symptoms, compared to those with severe wheezing during the next few years of life (3,13,27).

The present study included only a small number of children, and larger studies with a longer follow-up are needed to confirm the results. However, the results suggest a possible role for U-EPX in the prediction of persistent atopic asthma when measured during active symptoms in young asthmatic children. Low levels of specificity and sensitivity may limit the utility of U-EPX as a single predictive parameter, but it might be possible for U-EPX to be employed in combination with other parameters to predict the outcome of asthma in early childhood (28).

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