Clinical Study

Urinary Eosinophil Protein X in Children with Atopic Asthma

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The aim of this study was to investigate the relationship between urinary eosinophil protein X (uEPX) and asthma symptoms, lung function, and other markers of eosinophilic airway inflammation in asthmatic school children. Methods. A cross-sectional study was performed in 180 steroid dependent atopic children with stable moderately severe asthma, who were stable on 200 or 500 μg of fluticasone per day. uEPX was measured in a single sample of urine and was normalized for creatinine concentration (uEPX/c). Symptom scores were kept on a diary card. FEV1 and PD20 methacholine were measured. Sputum induction was performed in 49 and FE NO levels measured in 24 children.

Results. We found an inverse correlation between uEPX/c and FEV1 (r = −0.20, P = 0.01) and a borderline significant correlation between uEPX/c and PD20 methacholine (r = −0.15, P = 0.06). Symptom score, %eosinophils and ECP in induced sputum and FE NO levels did not correlate with uEPX/c.

Conclusion. uEPX/c levels did not correlate with established markers of asthma severity and eosinophilic airway inflammation in atopic asthmatic children.

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1. INTRODUCTION

Eosinophilic airway inflammation is the pathological substrate of allergic asthma both in adults and in children [1, 2]. The severity of airway inflammation correlates poorly with symptoms and lung function [3]. As asthma treatment with inhaled steroids aims at reducing inflammation, there is a need to monitor the disease with a marker of inflammation [4, 5]. Potential markers are serum eosinophilic cationic protein (ECP), induced sputum cellularity and soluble markers [6], and the concentration of nitric oxide in exhaled air (FE NO) [7, 8].

Eosinophil protein X (EPX) is one of the toxic proteins present in eosinophil granules and is released by activated eosinophils. EPX can be measured accurately in urine (uEPX) [9]. Therefore, uEPX can be regarded as a marker of eosinophil degranulation in vivo [10]. uEPX levels in allergic asthmatic children were found to be significantly higher than in healthy controls [11–14]. Treatment with inhaled steroids reduced uEPX levels [14]. We hypothesized that measuring EPX in urine could potentially prove to be useful for monitoring eosinophilic airway inflammation in children and may complement other markers of asthma control such as symptom scores and lung function.

The aim of this study was to evaluate the relationship between uEPX and current symptoms and lung function parameters, and the relation between uEPX, induced sputum eosinophilia, and FE NO. For this purpose, we analyzed cross-sectional data obtained at enrolment for a multicentre trial.

2. METHODS

2.1. Subjects

Data were obtained from steroid-dependent asthmatic children who took part in a large randomized controlled multicentre trial (CATO: Children Asthma Therapy Optimal). One hundred and eighty atopic (RAST ≥ class 1 for at least one airborne allergen) children, median age 10.3 years (range 6–16 years), with a documented clinical history of
moderately severe asthma were recruited from paediatric clinics in 8 general hospitals and 7 university hospitals in The Netherlands. All had been treated with inhaled corticosteroids (ICS) for at least 4 weeks. Data were obtained during a clinic visit at the end of the run in period of 4–12 weeks. During this period, they were treated with fluticasone dipropionate 200 μg/d (n = 102) or 500 μg/d (n = 78). All parents and children if > 12 years gave their written informed consent. The study was approved by the medical ethics committees of all participating hospitals.

2.2. Symptom scores

Two weeks before visiting the hospital, patients kept a diary in which symptoms (shortness of breath, wheeze, and cough) were scored twice a day each on a 4-point (0–3) scale. Cumulative symptom scores were calculated over 14 days (maximum score 252).

2.3. Fractional exhaled nitric oxide

The fractional concentration of exhaled nitric oxide (FE\textsubscript{NO}) was measured with the online single breath method, using the NIOX NO-analyzer (Aerocrine, Stockholm, Sweden) according to ERS/ATS guidelines [15].

As FE\textsubscript{NO} could only be measured in 1 participating university centre, only part of the children underwent FE\textsubscript{NO} measurements.

2.4. Flow-volume curves

Flow-volume curves and forced expiratory volume in 1 second (FE\textsubscript{V}1) were measured on a dry rolling seal spirometer according to recommendations [16]. Results are expressed as percentage of predicted values [17].

2.5. Bronchial challenge test

Bronchial responsiveness was determined by a methacholine challenge [18]. PD\textsubscript{20} methacholine (provocative dose of methacholine causing FE\textsubscript{V}1 fall 20% from baseline) was assessed by linear interpolation of the last two points of the log dose-response curve where FE\textsubscript{V}1 had fallen below 20% of baseline value.

2.6. Sputum induction and processing

Sputum induction was performed by 5 university centres and 3 paediatric clinics in general hospitals. Sputum was induced according to a standardized method by inhaling an aerosol prepared from hypertonic sodium chloride 4.5% w/v [19, 20]. Differential cell counts of the cytospins were performed by counting 500 cells. Sputum samples containing more than 80% squamous cells were excluded from the analysis [20].

In sputum supernatant, ECP was measured by fluoroenzyme immunoassay (Pharmacia, Uppsala, Sweden)

2.7. Urinary eosinophil protein X

A spot sample urine was collected from each individual at the clinic visit and immediately stored at −20°C. uEPX was determined using a commercial enzyme-linked immunosorbent assay (ELISA) for human EPX in 50-fold diluted samples according to the manufacturers recommendations (Medical and Biological Laboratories, Naka-Ku Nagoya, Japan). The sensitivity of the assay was 0.62ng/mL. Urinary creatinine levels were measured by using the alkaline picrate method (Jaffé reaction) (Roche, Mannheim, Germany). Urinary EPX concentrations were expressed as μg per mmol creatinine (uEPX/c).

2.8. Data analysis

All variables with a non-Gaussian distribution (symptom score, PD\textsubscript{20} methacholine, FE\textsubscript{NO}, % eosinophils in sputum, ECP in sputum, and uEPX) could be normalized by log-transformation. The significance of the relation between uEPX and lung function variables or other markers of inflammation was calculated using Spearman’s rank correlation coefficients. A two-sided P value of <.05 was considered statistically significant.

3. RESULTS

One hundred and eighty subjects (105 boys (58.3%)) participated. Asthma was controlled by fluticasone dipropionate 200 μg/day (n = 102) or 500 μg/day (n = 78). All subjects performed spirometry and recorded symptoms in a diary. Six children inhaled short-acting β-agonist prior to the visit, their results were excluded from analysis. One hundred and seventy eight children performed a bronchial challenge test; two had FE\textsubscript{V}1 < 80% of personal best and were therefore not tested. Children who had used β-agonist within 8 hours before the test (n = 6) were again excluded. For logistic reasons, sputum induction was done in part of the subjects. Forty nine of the 98 sputum inductions yielded adequate sputum samples (50%). At randomization, only one university centre had the facility to measure FE\textsubscript{NO} (n = 24 subjects).

Baseline results of lung function, symptom score, and markers of inflammation are given in Table 1. uEPX/c showed a log-normal distribution, median 185 μg/mmol creatinine (range 2–3114 μg/mmol creatinine). UEPX/c did not correlate with age and was not different between boys and girls.

3.1. Relation between uEPX/c and clinical markers of asthma severity (Table 2)

UEPX/c did not correlate with symptom scores or inhaled steroid dose. There was a significant inverse correlation of uEPX/c with FE\textsubscript{V}1 (r = −.18 , P = .02) (Figure 1). The association between uEPX/c and FE\textsubscript{V}1 did not significantly differ between children using 200 μg fluticasone per day and those using 500 μg (Anova, P = .19). For each 10% points increase
Table 1: Characteristics of study subjects. Values are median (range). FEV\textsubscript{1} is forced expiratory volume in 1 second; PD\textsubscript{20} methacholine is provocative dose of methacholine causing FEV\textsubscript{1} fall 20% from baseline; ECP is eosinophil cationic protein; FE NO is fractional concentration of nitric oxide in exhaled air; uEPX/c is urinary eosinophil protein X per mmol creatinine.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Fluticasone dose</th>
<th>Total</th>
<th>Fluticasone dose</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>200 μg/day</td>
<td>500 μg/day</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>10(96.4 – 16.8)</td>
<td>11.3(6.4 – 16.7)</td>
<td>10.3(6.4 – 16.8)</td>
<td></td>
</tr>
<tr>
<td>Gender (m/f)</td>
<td>60/40</td>
<td>45/33</td>
<td>105/75</td>
<td></td>
</tr>
<tr>
<td>FEV\textsubscript{1} (pred. %)</td>
<td>99(56 – 135)</td>
<td>96(56 – 96)</td>
<td>97(56 – 135)</td>
<td></td>
</tr>
<tr>
<td>Cumulative symptom score</td>
<td>18.5(0 – 113)</td>
<td>14(0 – 152)</td>
<td>17.0(0 – 152)</td>
<td></td>
</tr>
<tr>
<td>PD\textsubscript{20} methacholine (μg)</td>
<td>200(3 – &gt; 1570)</td>
<td>48(1 – &gt; 1570)</td>
<td>68(1 – &gt; 1570)</td>
<td></td>
</tr>
<tr>
<td>Eosinophils sputum (%)</td>
<td>1(0 – 72)</td>
<td>1(0 – 43)</td>
<td>1.0(0 – 72)</td>
<td></td>
</tr>
<tr>
<td>ECP sputum (ng/ml)</td>
<td>17(0 – 2345)</td>
<td>38(0 – 538)</td>
<td>29(0 – 2345)</td>
<td></td>
</tr>
<tr>
<td>FE NO (ppb)</td>
<td>11(5 – 63)</td>
<td>9(1 – 29)</td>
<td>10(1 – 63)</td>
<td></td>
</tr>
<tr>
<td>uEPX/c (μg/mmol)</td>
<td>189(2 – 2828)</td>
<td>180(10 – 3114)</td>
<td>185(2 – 3114)</td>
<td></td>
</tr>
</tbody>
</table>

Table 2: Correlations between uEPX or uEPX-c and clinical markers of asthma severity or markers of asthmatic inflammation. \( r \) values were all analyzed by Spearman’s rank correlation tests. uEPX/c is urinary eosinophil protein X per mmol creatinine; FEV\textsubscript{1} is forced expiratory volume in 1 second; PD\textsubscript{20} methacholine is provocative dose of methacholine causing FEV\textsubscript{1} fall 20% from baseline; ECP is eosinophil cationic protein; FE NO is fractional concentration of nitric oxide in exhaled air.

<table>
<thead>
<tr>
<th>Variable</th>
<th>( N )</th>
<th>( r )</th>
<th>( \log \text{uEPX/c} )</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>180</td>
<td>-.01</td>
<td>.90</td>
<td></td>
</tr>
<tr>
<td>Symptom score</td>
<td>180</td>
<td>.03</td>
<td>.72</td>
<td></td>
</tr>
<tr>
<td>FEV\textsubscript{1}</td>
<td>174</td>
<td>-.18</td>
<td>.02</td>
<td></td>
</tr>
<tr>
<td>PD\textsubscript{20} methacholine</td>
<td>172</td>
<td>-.14</td>
<td>.08</td>
<td></td>
</tr>
<tr>
<td>% eosinophils in sputum</td>
<td>49</td>
<td>.17</td>
<td>.26</td>
<td></td>
</tr>
<tr>
<td>ECP sputum</td>
<td>43</td>
<td>-.03</td>
<td>.83</td>
<td></td>
</tr>
<tr>
<td>FE NO</td>
<td>24</td>
<td>.16</td>
<td>.46</td>
<td></td>
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</tbody>
</table>

Of FEV\textsubscript{1} (pred. %) the geometric mean EPX/c ratio decreases 18% (95% CI: 5,30%). The correlation between uEPX/c and PD\textsubscript{20} methacholine was borderline significant (\( r = -.14, P = .08 \)).

3.2. Relation between uEPX and markers of asthmatic airway inflammation (Table 2)

uEPX/c did not correlate with the % eosinophils or ECP in induced sputum, or with FE NO. Relations between uEPX and PD\textsubscript{20} methacholine or markers of asthmatic airway inflammation did not significantly differ when analysis was adjusted for fluticasone dose.

Correlations were similar when children with eczema were excluded from the analysis.

4. DISCUSSION

We found a significant correlation of uEPX/c and FEV\textsubscript{1}, and no association between uEPX/c and bronchial responsiveness or symptom scores in a large group of children with moderately severe allergic asthma. In subgroups, no significant correlations between uEPX/c and other markers of eosinophilic airways inflammation (% eosinophils and ECP in induced sputum or FE NO) were found.

This is the first study reporting uEPX/c levels in relation with markers of asthma severity and inflammation in a large population of children with atopic asthma, treated with inhaled steroids. Lugosi et al. have shown that uEPX levels were increased in symptomatic versus nonsymptomatic children with asthma, treated with inhaled steroids or disodium.
significant negative correlation between FEV1 and uEPX/c. Lavage cell counts in adult asthmatic patients [10]. An al-
found no correlation between uEPX/c and bronchoalveolar percentage eosinophils in induced sputum. Others likewise confirmed, as we found no correlation between uEPX/c and the percentage eosinophils or ECP levels in induced sputum. We cannot exclude that heterogeneity of study groups with respect to other atopic disorders than asthma could have affected the correlation between uEPX/c and other markers of eosinophilic airways inflammation.

At the onset of our study, a circadian rhythm of uEPX/c had not been reported. Urine samples were not all obtained at the same time of the day. Since the start of our study, it became evident that a circadian rhythm of uEPX/c with lowest levels at 7 p.m. and highest at 7 a.m. in both asthmatic and healthy controls exists [23, 29–31]. Hence, diurnal variability may have introduced scatter of uEPX, thus weakening a possible correlation.

Two previous studies reported significant positive correlations between uEPX/c and FE NO in corticosteroid-dependent childhood asthma [11, 29]. We found no significant correlation between uEPX/c and FE NO in a small subgroup of the study population. For FE NO, no important circadian variation was found, employing the same measurement technique that we have used [32], but conflicting results have also been published [27, 33]. A possible circadian rhythm might have affected FE NO and weakened any cross-sectional relationship.

In conclusion, the present data show a weak inverse correlation between uEPX/c and FEV1, and a borderline correlation between uEPX/c and PD 20 methacholine. No significant correlation was found between uEPX/c and markers of eosinophilic airway inflammation including % eosinophils or ECP levels in induced sputum or FE NO. The number of children performing FE NO was small, therefore this correlation should be interpreted with caution. Our findings are not encouraging for uEPX/c as a complementary marker of airway inflammation in asthma. As to whether uEPX/c can be useful as a marker for monitoring asthma management in children is worth prospectively looking at.
APPENDIX

This work has been prepared by the authors on behalf of the CATO Study Group.

CATO Study Group Members


ACKNOWLEDGEMENTS

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REFERENCES


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