

Inflammation markers and symptom activity in children with bronchial asthma. Influence of atopy and eczema

Carlsen K-H, Halvorsen R, Pettersen M, Lødrup Carlsen KC.
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Pediatr Allergy Immunol 1997; 8: 112–120. © Munksgaard 1997.

Background. Eosinophil cationic protein (ECP) has been reported to reflect the eosinophil inflammatory activity in asthma. However, the relative impact of asthma symptoms and atopic eczema upon serum (s)-ECP in asthmatic children has not been established.

Objectives. To examine s-ECP levels and s-myeloperoxidase (MPO) in relation to asthma symptoms and atopic eczema in asthmatic children.

Methods. S-ECP and s-MPO were assessed in relation to symptom activity, lung function, exercise-induced bronchoconstriction and bronchial responsiveness in 101 children; median age 9 years, range 1–16 years; with moderate to severe asthma, admitted to Voksentoppen Center.

Results. S-ECP was significantly higher in children with persistent compared to episodic or no asthma symptoms in the past four weeks. S-ECP was also higher in children with atopic compared to non-atopic asthma, as well as in those with active compared to past history or no history of atopic eczema. S-MPO was higher in children with persistent asthma symptoms, but did not differ in relation to atopy or eczema state.

Persistent asthma symptoms had the greatest impact upon s-ECP levels, followed by atopy and active eczema.

Conclusion. S-ECP may be used in assessing symptom activity in asthmatic children, but with the realisation that active eczema and the presence of atopy may also influence levels.

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Key words: ECP; MPO; asthma; children; clinical symptoms; atopic eczema; bronchial responsiveness; lung function.

Received June 18, 1997

Accepted July 28, 1997

Bronchial asthma is presently considered to be due to airways inflammation with several different cells actively participating (1). Eosinophils, in particular, are implicated, and eosinophilic inflammation of the airways has been related to asthma severity (2). Eosinophil cationic protein (ECP) is considered to reflect activation of eosinophils (3), and several studies report increased levels of serum (s)-ECP in bronchial asthma, particularly in children (4–7). However, conflicting results have been reported as regards levels of s-ECP and symptom activity in childhood asthma. Kristjánsson and coworkers found elevated s-ECP levels in children with acute asthma, and in asymptomatic asthmatic children not taking inhaled steroids, but not in asymptomatic children using regular inhaled steroids (5). Ferguson et al. did not find significantly different s-ECP levels in children with chronic asthma according to symptoms (8), but did not control for use of inhaled

steroids. On the other hand, Koller and coworkers reported elevated s-ECP levels in asthmatic children with present asthma symptoms both in children with and without inhaled steroids (6). Rao and coworkers did not find different s-ECP levels in symptomatic asthmatic children, five to ten years of age, with or without regular treatment with inhaled steroids. They reported a significant though moderate correlation between both s-ECP and serum eosinophil protein X (EPX) and histamine induced bronchial responsiveness as well as between s-EPX and lung function, suggesting a relationship between disease activity and eosinophil inflammation markers (9).

Myeloperoxidase (MPO) is a mediator released mainly from activated neutrophils (but also monocytes), and increased s-MPO levels have been reported in a number of inflammatory respiratory tract disorders, including bronchial asthma (5, 10).

Table 1. Demographic variables and asthma treatment in 101 asthmatic children according to activity of asthmatic symptoms

	Symptom-free <i>n</i> =51	Episodic* <i>n</i> =28	Persistent** <i>n</i> =22	<i>p</i> -value
Age (years)				
Mean (SD)	7.8 (3.98)	8.4 (4.34)	11.1 (4.01)	<i>p</i> <0.01
Median (Range)	8.0 (1–16)	8.5 (1–15)	12.5 (2–16)	
Gender (♂/♀) (<i>n</i>)	30/21	16/12	14/8	<i>n.s.</i>
Present atopy (%)	62.7	67.9	86.4	<i>n.s.</i>
Atopic eczema (<i>n</i>)				
no/non-active/active	30/12/6	16/7/2	15/5/2	<i>n.s.</i>
Regular treatment (<i>n</i>)				
No	4	1	0	
DSCG***	6	1	0	
Inhaled steroids	41	26	21	
Systemic steroids	0	0	1	
Inhaled steroids, dosage (µg/day)	1121	1348	1694	<i>p</i> <0.01
Mean (± 95% C.I.)	(826–1415)	(990–1706)	(1211–2176)	

* Episodic = episodic asthmatic symptoms for less than seven consecutive days and less than 14 days in total during the last four weeks before admission and during the stay in hospital.

** Persistent = persistent symptoms for at least two of the last four weeks before admission and daily symptoms during the stay in hospital.

***DSCG = Disodium cromoglycate.

Other types of allergic inflammatory disorders such as atopic eczema have been associated with increased s-ECP (5, 11–13). Asthmatic children often have concomitant atopic eczema, but to our knowledge the interaction between bronchial asthma and atopic eczema upon s-ECP levels has not previously been reported.

The primary aim of the present study was to assess levels of s-ECP and s-MPO in children with moderate to severe bronchial asthma in relation to the presence and activity of asthmatic symptoms. Secondary aims were to study how symptom activity of atopic eczema influenced the serum levels of ECP and MPO in asthmatic children, and whether different types of asthma could be reflected in different patterns of serum mediator levels.

Patients and methods

Patients

One hundred and one patients (♂/♀=60/41) from one to 16 years of age (mean: 8.7 years; median 9 years) with moderate to severe bronchial asthma, admitted to Voksentoppen National Center of Asthma, Allergy and Chronic Lung Diseases in Children, were consecutively included into the study. The patients were referred to Voksentoppen Center from other hospitals and paediatric allergologists all over Norway for further assessment and treatment, according to the waiting list, and not due to acute asthma. Demographic variables and regular use of asthma drugs according to asthma symptom activity are shown in Table 1.

The patients were classified into three groups according to asthma symptoms during the four weeks prior to admission and during their stay in hospital. The classification was based upon history obtained from the parents and the patients, and by daily physical examination during their stay in hospital as:

- 1) Symptom-free,
- 2) episodic symptoms and
- 3) persistent symptoms.

The classification was based upon the following symptoms and signs: wheeze, chest wall recession, prolonged expiratory phase of respiration, rapid respiratory rate, and audible rales and rhonchi upon lung auscultation. The symptom-free group had no symptoms of asthma during the four weeks prior to and during hospital stay. The group with persistent symptoms had daily asthma symptoms for at least two of the weeks prior to admission and daily symptoms during their stay in hospital. The group with episodic symptoms had symptoms for less than seven consecutive days and less than 14 days totally.

The patients were considered to be atopic with at least one positive skin prick test or one positive RAST analysis to a panel of seven common Norwegian inhalant allergens.

The patients were also classified into three groups in relation to eczema:

- 1) No atopic eczema ever,
- 2) Past history of atopic eczema (a history of eczema, but no present skin manifestations) and
- 3) Active atopic eczema (present skin manifestations of atopic eczema including erythema, vesiculation, crusting, excoriation and/or li-

chenification, particularly at the common flexure sites).

In patients with active atopic eczema, the extent and severity of the eczema constituted a major problem and was one of the causes for admittance to the hospital. However, none of the patients with active atopic eczema had signs of acute infection in their eczema, due to the elective timing of the admission. Some patients with atopic eczema did not demonstrate present atopy, but were verified as food-allergic during the first two years of life.

Inflammation markers

Venepuncture was performed between 8.00 a.m. and 10.00 a.m. in all patients, and blood was drawn into SST vacutainer tubes and left to clot at stable room temperature for one hour before centrifugation for ECP and MPO assays. The pipetted serum was frozen and stored at -21°C . The majority of s-ECP analyses were performed within one to two weeks after sampling. All handling of the blood tests were performed by only two nurses using a standard procedure. As all blood samples were drawn between 8.00 a.m. and 9.00 a.m., diurnal variation should not have affected the results (14).

S-ECP was measured by the Pharmacia CAP system ECP fluoro enzyme immunoassay (FEIA) method, according to the instructions of the manufacturer (15). Briefly, monoclonal anti-ECP antibodies covalently coupled to immunoCAP (Pharmacia) were allowed to react with patient serum samples and subsequently, after washing, with monoclonal anti-ECP antibodies containing fluorogenic labeling. The level of fluorescence after adding fluorogenic substrate was measured by a Fluorocount 96 (Pharmacia). The measuring range was 2–200 $\mu\text{g/l}$ in undiluted serum. The cross-reactivity with other granulocyte proteins is less than 0.1%, according to the specifications of the manufacturer. The upper reference range for healthy subjects is 16 $\mu\text{g/l}$ (15).

S-MPO was measured by the Pharmacia MPO Radioimmune assay, according to the instructions of the manufacturers (16). Briefly, diluted patient serum and ^{125}I -MPO was incubated with polyclonal anti-MPO antibodies on sepharose. After adding decanting suspension, incubation and decanting, radioactivity was measured in a gamma-counter and concentrations determined by comparison to a standard curve. The cross-reactivity with other granulocyte proteins is less than 0.1%, according to the specifications of the manufacturer.

Total IgE and specific IgE determination

Total IgE was determined by radioimmunosorbent test (Phadebas PRIST, Pharmacia, Lund, Sweden),

whereas specific IgE was determined against the same allergens as in the skin prick test by the radio-allergosorbent test (Phadebas RAST, Pharmacia, Lund, Sweden).

Specific IgE in the skin was assessed by skin prick test performed according to the Nordic guidelines (17) with the following prevalent inhalant allergens in Norway: Mould (*Cladosporium herbarum*), house dust mites (*Dermatophagoides pteronyssimus*) dog dander, cat dander, birch pollen, grass pollen (timothy) and mugwort pollen (Soluprick, ALK, Copenhagen).

To be considered allergic to an allergen, a positive skin prick test of at least ++ (half of the reaction to histamine 10 mg/ml) or RAST class 2 to the allergen was required.

Lung function and measurement of exercise-induced bronchoconstriction

Lung function was measured by maximally forced expiratory flow volume curves (Masterscreen Pneumo, Erich Jaeger GmbH & Co KG, Würzburg, Germany). Predicted lung function values, when used, were according to Zapletal et al. (18).

Exercise-induced bronchoconstriction (EIB) was determined by running on a motordriven treadmill for six minutes with submaximal exercise load (19). The inclination of the treadmill was 5.5%, and the speed adjusted to submaximal load to achieve a steady state heart rate of 170–180 beats per minute. The heart rate was recorded electronically (Sport-Tester PE 3000 with memory function). FEV₁ was measured before run, immediately after, and three, six, ten and 15 minutes after cessation of running.

Maximum percentage fall in FEV₁ after the exercise test was calculated by:

$(\text{Pre-exercise FEV}_1 - \text{minimum post-exercise FEV}_1) / \text{Pre-exercise FEV}_1 \times 100\%$

Minimum post-exercise FEV₁ was taken from the recordings at 0, 3, 6, 10 and 15 minutes after the exercise test.

Bronchial responsiveness was measured by tidal inhalation for two minutes of each concentration of nebulised metacholine, using doubling concentrations from 0.03 mg/ml to 32 mg/ml. A Pari nebuliser with output between 0.13 and 0.16 ml per minute was used, as described by Hargreave and coworkers (20), and in accordance with the European guidelines (21). PC₂₀ was determined as the concentration of metacholine required for a 20 per cent reduction in FEV₁ and determined by linear interpolation on semi-logarithmic plots. The test was considered to be positive with PC₂₀-metacholine of 4 mg/ml or less.

Lung function, EIB and bronchial responsiveness were measured in children six years and older.

The patients did not inhale short-acting β_2 -agonist or disodium cromoglycate the last 12 hours before the treadmill test or measurement of BHR. Long-acting β_2 -agonists or theophyllines were not used by any patient. Antihistamines were discontinued the last week prior to testing. No child used aztemisole as this is not a registered drug in Norway. The use of inhaled steroids was not changed, but the patients did not receive their regular dose on the morning of the treadmill test or the metacholine provocation.

Statistical methods

Results are given as mean values with 95 per cent confidence intervals (C. I.) unless otherwise stated. Demographic data are given as mean values $\pm$ standard deviation (SD).

Differences between two groups were analysed by *t*-tests, or by ANOVA-test for more than two samples, if data were normally distributed, otherwise by the non-parametric Mann-Whitney U-test for two independent samples, alternatively by the Kruskal-Wallis ANOVA-test for more than two samples. Differences in categorical variables were compared by the χ^2 -test. All tests were two-tailed with a significance level of 5 per cent.

Positive likelihood ratios (LR+) and negative likelihood ratios (LR-) for having asthma and persistent asthma were calculated according to a level above or below s-ECP of 20 $\mu\text{g/l}$ (22). The s-ECP level of 20 $\mu\text{g/l}$ which is above the upper reference limit of 16 $\mu\text{g/l}$, was chosen arbitrarily for calculation of the likelihood ratios because the study included asthmatic patients only. Asthmatic patients may have ongoing bronchial inflammation even in the absence of symptoms (23), with the possible slight elevation of inflammation markers as s-ECP.

The relations between s-ECP and the determinants of interest were estimated in linear regression analysis adjusted for potential confounders. Indicator variables, coded 1/0, were constructed to represent polytomous covariates in the model. The following core covariates were fitted in all the models: activity of asthma symptoms, activity of atopic eczema, and the presence of atopy. Age and gender were fitted as optional covariates and retained in case they would change the estimates of interest. An analysis including the core and the optional variables was performed including all patients.

Results

Fifty-one of the patients were classified as symptom-free, 28 children as suffering from episodic asthma symptoms, and 22 children as having persistent symptoms during the four weeks prior to admission and during their stay in hospital. The chil-

dren with persistent asthma symptoms were older than the other children ($p < 0.01$), but the children did not differ significantly as regards gender, the presence of atopy or atopic eczema according to asthma symptom activity (Table 1). The non-atopic asthmatic children (mean age 5.7 years, median 4 years) were significantly younger ($p < 0.001$) than the atopic asthma children (mean age 10.0 years, median 10 years).

The use of regular asthma drugs is shown in Table 1. Most children used inhaled steroids on a regular basis (89 out of 101 patients), but significantly fewer in the symptom-free group ($p = 0.04$). The dose of inhaled steroids was significantly higher among the children with persistent asthma symptoms than among the two other groups ($p < 0.01$) (Table 1).

Inflammation markers

S-ECP levels differed significantly according to asthma symptom activity ($p < 0.0001$) with highest levels in children with persistent symptoms (mean 29.2 $\mu\text{g/l}$), intermediate levels in children with episodic symptoms (mean 21.4 $\mu\text{g/l}$), and lowest in the symptom-free children (mean 13.5 $\mu\text{g/l}$), as shown in Table 2. Individual levels of s-ECP according to symptom activity are shown in Fig. 1.

Fig. 2 shows the levels of s-ECP according to symptoms for the atopic and non-atopic patients. S-ECP levels were significantly higher in the atopic group than in the non-atopic group ($p < 0.002$). S-ECP levels differed significantly according to symptom activity both within the atopic group ($p = 0.001$) and within the non-atopic group ($p = 0.05$) (Fig. 2). Due to few patients with persistent symptoms in the non-atopic group, episodic symptoms and persistent symptoms were joined into one group and compared with the symptom-free group for the non-atopic children.

The positive likelihood ratio (LR+) for having asthma symptoms compared to not having with s-ECP above 20 $\mu\text{g/l}$ was 2.47, and LR- was 0.55. LR+ for having persistent asthma with s-ECP above 20 $\mu\text{g/l}$ was 3.29, and LR- was 0.30.

S-ECP was significantly higher in children with active atopic eczema compared to children with a past history of atopic eczema ($p = 0.02$), and no history of atopic eczema ($p = 0.03$). S-ECP did not differ significantly between the two latter groups (Fig. 3, Table 3).

With the core covariates included into a linear regression model (Table 4), it was found that having persistent asthma symptoms corresponded to an increase of s-ECP of 14.9 $\mu\text{g/l}$ ($p = 0.0001$), whereas having episodic symptoms corresponded to an increase in s-ECP of 6.7 $\mu\text{g/ml}$ ($p = 0.02$). The presence

Table 2. Serum-eosinophilic cationic protein (s-ECP), s-myeloperoxidase (s-MPO), eosinophil counts, leukocyte-counts, total IgE, lung function (FEV₁ and MEF₅₀), maximum reduction of FEV₁ after standardized exercise test (EIB-test), and PC₂₀-metacholine according to symptoms of asthma. Results are shown as mean values with 95% confidence intervals in parentheses unless otherwise stated

	Symptom-free n=51	Episodic* n=28	Persistent** n=22	p-value
ECP (µg/l)	13.5 (10.1–16.9)	21.4 (16.5–26.4)	29.2 (22.3–36.2)	p<0.0001 $\alpha\Phi\#$
Eosinophils, 10 ⁹ /l	215 (166–263)	329 (238–419)	360 (216–504)	p=0.02 $\alpha\Phi$
MPO, µg/l	563.5 (506.8–620.2) (n=48)	537.1 (470.1–604.1) (n=28)	767.8 (628.9–906.7) (n=20)	p<0.01 $\Phi\#$
Leukocytes, 10 ⁹ /l	6.3 (5.8–6.8)	6.3 (5.7–6.9)	7.8 (6.7–9.0)	p=0.05 $\Phi\#$
Total IgE, kU/l geometric mean	118.2 (72.2–193.7)	229.7 (123.5–427.0)	299.2 (186.1–483.3)	n.s.
FEV ₁ , % predicted	100.1 (94.7–105.5) (n=31)	98.9 (89.8–107.9) (n=18)	84.5 (77.1–91.8) (n=18)	p<0.005 $\Phi\#$
MEF ₅₀ , % predicted	77.7 (68.4–86.9) (n=31)	69.2 (58.4–79.9) (n=18)	56.4 (44.2–68.6) (n=18)	p<0.01 $\Phi\#$
EIB-test, Fall in FEV ₁ (%)	9.7 (6.3–13.1) (n=32)	21.5 (12.4–30.7) (n=13)	20.9 (13.4–28.4) (n=9)	p=0.005 $\alpha\Phi$
PC ₂₀ -metacholine, mg/ml geometric mean	1.59 (0.95–2.67) n=30	0.80 (0.47–1.35) n=16	0.61 (0.18–2.06) n=10	p=0.07

* Episodic = episodic asthmatic symptoms for less than seven consecutive days and less than 14 days in total during the last four weeks before admission and during the stay in hospital.

** Persistent = persistent symptoms for at least two of the last four weeks before admission and daily symptoms during the stay in hospital.

α = significant differences between the symptom-free group and the episodic group.

Φ = significant differences between the symptom-free and the persistent group.

= significant differences between the episodic and the persistent group.

of active eczema corresponded to an increase of s-ECP of 8.8 µg/l ($p=0.03$), whereas past history of eczema did not significantly influence s-ECP. The presence of atopy corresponded to an increase of s-ECP of 9.9 µg/l ($p<0.01$). Adding the optional covariates age and gender did not add further information to the regression model.

S-MPO was significantly higher in the children with persistent asthma symptoms compared to the symptom-free children and the children with episodic symptoms ($p<0.01$) (Table 2).

S-MPO did not differ significantly between the atopic asthma children (mean value: 603.7 µg/l; 542.0–665.4 (95% C. I.)) and the non-atopic asthma children (586.7; (520.6–652.7)).

S-MPO did not differ significantly between asthma children with active atopic eczema (mean value: 698.3 µg/l; 485.6–911.1 (95% C. I.)), past history of eczema (515.6 µg/l; 449.1–582.1) and children with no history of eczema (620.0 µg/l; 556.7–683.3).

Other laboratory values

S-eosinophils were significantly lower in symptom free asthma children compared to children with symptoms ($p=0.02$) (Table 2), but eosinophil levels were not significantly different between children with episodic and persistent symptoms.

S-eosinophils did not differ significantly in children without atopic eczema (mean: 246; 186–305, 95% C. I.), past history of eczema (319; 213–425) or active atopic eczema (376; 178–575).

S-IgE levels and leukocyte levels are seen from Table 2, and also did not differ significantly between patient groups.

Lung function and bronchial responsiveness

Lung function by maximal expiratory flow volume curves were successfully measured in 67 children older than six years. FEV₁ (% predicted) and MEF₅₀ (% predicted) were significantly lower in children with persistent asthma symptoms than in children with episodic symptoms or symptom free children ($p<0.005$), but did not differ between symptom free children and children with episodic symptoms (Table 2).

Test by treadmill run for exercise induced bronchoconstriction (EIB) was successfully performed in 54 children older than six years. Reduction in FEV₁ (per cent) after standardised run on treadmill was significantly less in symptom-free children than in children with episodic asthma symptoms and children with persistent symptoms ($p=0.005$) (Table 2).

Measurement of direct BHR by metacholine inhalation (PC₂₀) was completed in 56 children, but

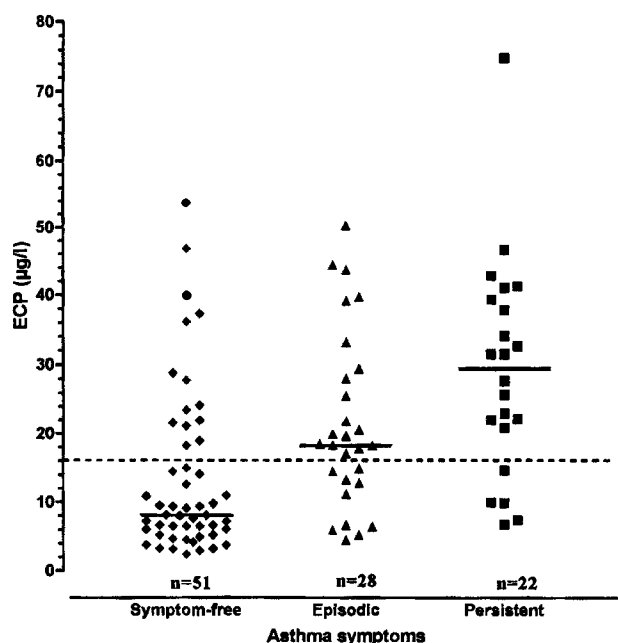


Fig. 1. Individual serum ECP values according to asthma symptoms. Median values are shown with solid lines. Dotted line represents upper reference limit of s-ECP (16 µg/l) (15). Significant differences were found between the three symptom groups ($p < 0.0001$).

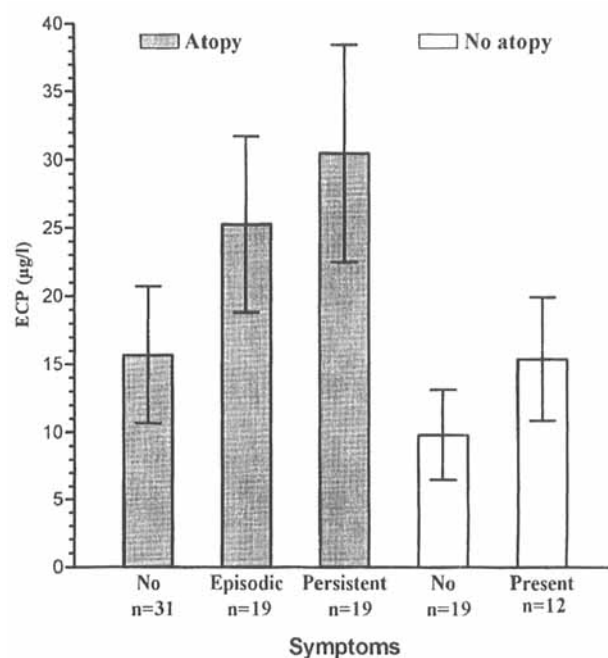


Fig. 2. Serum ECP levels (mean with 95% C.I.) according to asthma symptoms in the atopic and non-atopic group of patients. As only three patients in the non-atopic group had persistent symptoms, episodic and persistent symptoms in the non-atopic group were joined into "Present" symptoms. S-ECP levels differed significantly between the atopic and the non-atopic group ($p = 0.002$). Significant differences according to symptoms were found both within the atopic ($p = 0.001$) and the non-atopic group ($p = 0.05$).

geometric mean of PC_{20} did not differ significantly according to asthma symptoms ($p = 0.07$) (Table 2).

No measure of BHR (EIB or PC_{20}) showed any association with history or signs of atopic eczema.

Discussion

In the present study s-ECP and s-MPO were significantly higher in asthma children with persistent asthma symptoms than in children with episodic symptoms. In children with episodic symptoms s-ECP, but not s-MPO were higher than in symptom-free children. Atopic asthmatic children had significantly higher s-ECP levels than non-atopic asthmatic children for all levels of asthma symptoms. Asthmatic children with signs of active atopic eczema had significantly higher s-ECP, but not significantly higher s-MPO than children with a past history of atopic eczema, or children without eczema. Persistent asthma symptoms were found to have the greatest influence upon s-ECP levels, followed by the presence of atopy and active eczema.

The finding of increasing s-ECP levels with increasing asthma symptoms is in agreement with findings of Koller et al. (6), who reported that s-ECP was increased in children who had present ac-

tive asthma symptoms whether or not the children received inhaled steroids. Similar findings were also reported by Zimmerman et al. (4). Kristjánsson and co-workers found significantly increased s-ECP levels in children with acute asthma. They also found increased s-ECP levels in asymptomatic asthmatic children without inhaled steroid treatment compared to the asymptomatic children with inhaled steroids (5). The positive LR+ of 2.47 for symptomatic asthma versus symptom-free, and LR- of 0.55 with s-ECP above 20 µg/l and LR+ of 3.29 and LR- of 0.30 for persistent asthma, suggest that s-ECP may contribute moderately, but not conclusively to the assessment of disease control obtained by implemented therapeutic measures, including anti-inflammatory treatment (22). On the other hand Ferguson et al. could not find that symptom activity influenced s-ECP levels. In their symptomatic group, however, 14 out of 24 children received inhaled steroids, whereas none of the non-symptomatic children received inhaled steroids (8). This important difference between the two groups makes interpretation of their results uncertain. However, in the present study, the patients with persistent asthma symptoms also used significantly higher doses of inhaled steroids (Table 1), and had higher s-ECP and

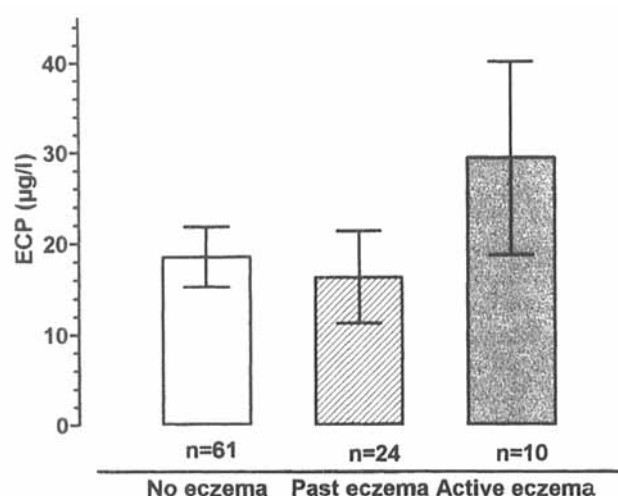


Fig. 3. Serum ECP levels (mean with 95% C.I.) according to history and symptoms of atopic eczema. S-ECP differed significantly between patients with a history of no eczema and active eczema ($p=0.03$), and between patients with history of past eczema and active eczema ($p=0.02$).

s-MPO levels. Thus, the influence exerted upon s-ECP levels by present asthma symptoms supports the concept that the activity of asthma symptoms is the main determining factor for raised s-ECP. This is supported by the study of Rao et al., reporting a significant correlation between both s-ECP and s-EPX and bronchial responsiveness in a group of symptomatic asthma children, 23 without and 25 with inhaled steroids, suggesting a relationship between disease activity and eosinophil inflammation markers such as s-ECP (9).

Asthma is a complex disorder, and a number of methods including symptom score, lung function, bronchial responsiveness and inflammation markers, may be used to assess disease activity. In order

to assess the usefulness of the different methods, there is a need of objective assessment of the methods. One such way of assessing the usefulness is by use of likelihood ratios (22). In the present study we chose a level of s-ECP of 20 µg/l as a basis for calculation of likelihood ratios for symptom activity. The level of 20 µg/l, moderately higher than the upper normal limit of 16 µg/l, was chosen because all patients included in the study were asthmatics, and ongoing bronchial inflammation had been reported in the absence of active symptoms (23).

Our finding of increased s-ECP levels in atopic asthmatic children compared with non-atopic children agrees with a previous report (6). However, the higher s-ECP levels in symptomatic compared to symptom-free non-atopic asthmatic children suggest that eosinophil inflammation also plays a role in bronchial asthma without recognised atopy.

As increased s-ECP levels have been reported in children with atopic eczema (5, 12, 24), and atopic eczema is often a concomitant finding in children with bronchial asthma, it is important to assess the relative contribution of eczema and airways inflammation upon s-ECP levels in asthmatic children. Our finding that asthmatic children with active symptoms of atopic eczema had higher s-ECP levels than children with a past history or no history of atopic eczema (Table 3) is important when employing s-ECP in assessment of asthma activity. The significant influence of active atopic eczema upon s-ECP levels (Table 4) must consequently be taken into account.

S-ECP levels increased with increasing asthma symptoms, but other measures of disease activity increased as well: lung function as measured by FEV₁ and MEF₅₀, direct bronchial responsiveness (PC₂₀-metacholine) (on the border of significance), indirect bronchial responsiveness as assessed by EIB, and s-eosinophils. Thus, all these measures reflect

Table 3. S-ECP (µg/l), s-MPO (µg/l) and asthma symptoms (n) according to history and symptoms of atopic eczema. Values are given as mean with 95% C.I. in parenthesis. S-ECP differed significantly between patients with no eczema and active eczema ($p=0.03$) and between patients with past eczema and active eczema ($p=0.02$)

	No eczema n=61	Past eczema n=24	Active eczema n=10	p-value
ECP (µg/l)	18.6 (14.8–22.4)	16.4 (11.3–21.37)	29.4 (18.6–40.3)	$p=0.05\Phi\#$
MPO (µg/l)	620 (556.8–683.3)	515.6 (449.1–582.1)	698.3 (485.6–911.1)	n.s.
Asthma symptoms (n)				n.s.
Persistent	15	5	2	
Episodic	16	7	2	
Symptomfree	30	12	6	
Present atopy (%)	59	87.5	90	$p=0.01\alpha\Phi$

α = significant differences between the group with no eczema and the group with past history of eczema.

Φ = significant differences between the group with no eczema and the group with active eczema.

= significant differences between the group with past eczema and the group with active eczema.

Table 4. Influence of persistent asthma symptoms, episodic asthma symptoms, active atopic eczema, and atopy including the optional covariates age and gender, upon s-ECP levels assessed by multiple linear regression

	Effect upon s-ECP, µg/ml mean	95 percent confidence intervals
<i>Core covariates:</i>		
Asthma symptoms		
Persistent	14.9	8.4–21.4
Episodic	6.7	0.8–12.7
Active eczema	8.8	0.3–17.3
Past history of eczema	–5.3	–11.4–0.9
Atopy	9.9	3.4–16.3
<i>Optional covariates:</i>		
Age (years)	–0.4	–1.1–0.3
Gender ($\sigma^2=1$, $\eta^2=0$)	–4.5	–9.6–0.5

disease activity in childhood asthma. This finding is in agreement with our previous report of the relation between s-ECP and change in lung function after salbutamol inhalation in early wheezy infants (25). In accordance with previous reports, the present study confirms that s-ECP is a better indicator of symptom activity in asthmatic children than eosinophils and s-IgE (5–7, 24, 26).

Bronchial asthma is a multifactorial disease with several clinical modes of expression (27). It has been suggested that the airways inflammation may differ dependent on the type of asthma, and that various etiologic factors may activate different pathways in the inflammation cascade (28). Several cell types may be activated to varying degrees in different forms of asthma, and this may be reflected in mediator levels. In the present study, we found that active atopic eczema, compared to past or no eczema, was characterised by increased s-ECP levels, but not increased s-MPO levels, thus suggesting activation of eosinophils. Whereas s-ECP was significantly higher among atopic subjects with asthma compared to non-atopic subjects, this was not the case for s-MPO. Thus, symptomatic atopic asthma was characterised by high s-ECP levels and high s-MPO levels, whereas active non-atopic asthma was characterised by high s-MPO but not high s-ECP levels. This suggests different patterns of inflammation in atopic and non atopic asthma.

Active atopic eczema becomes easily infected, which may be reflected by increased s-MPO levels. In such cases the s-ECP and s-MPO levels may possibly both be elevated as it was found in active atopic asthma. This ought to be considered in the assessment of asthmatic patients with atopic eczema.

Non-atopic asthmatic children had elevated levels of s-MPO, but not s-ECP, suggesting an important part played by the neutrophils in the airways inflammation in these patients. As the non-atopic children

with asthma were significantly younger than the atopic asthma children, this may suggest different immunopathologic mechanisms involved at different ages.

The s-ECP level in an individual asthma patient may provide additional information in the assessment of asthma activity, as demonstrated by the likelihood ratios based upon an s-ECP level of 20 µg/l. However, the spread of individual s-ECP levels within groups of asthma symptom activity (Fig. 1) demonstrates that s-ECP level in an individual patient is not sufficient on its own as the only parameter employed in the assessment of asthma activity, but must be seen in relation to symptoms and clinical signs. However, it is not surprising that s-ECP levels may be increased above normal levels in symptom free asthmatics, as it has been reported from studies with bronchial biopsies that airways inflammation may continue in the absence of symptoms (23).

In conclusion, s-ECP may contribute moderately to the clinical assessment of disease activity in asthmatic children with the realisation that active eczema and the presence of atopy influence s-ECP levels.

Acknowledgements

We thank Professor Staffan Ahlstedt and Pharmacia Upjohn for supplying analysing kits for s-ECP and s-MPO.

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