

The relationship of serum-eosinophil cationic protein and eosinophil count to disease activity in children with bronchial asthma

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Background. The serum-eosinophil cationic protein level (S-ECP) has been promoted as a biomarker of asthma that reflects the degree of bronchial eosinophilic inflammation.

Patients and methods. To investigate whether S-ECP is indeed a clinically useful objective parameter, especially in mild or moderate chronic childhood asthma, we studied 100 outpatient children with chronic asthma symptoms (63 boys and 37 girls, aged three to 15 years, median of age eight) and 25 controls (12 boys and 13 girls aged three to 15 years, median of age eight). Symptom scores, lung function parameters and atopy were compared with S-ECP determined by commercially available tests and eosinophils measured by an autoanalyser.

Results. Asthma symptom scores in the patient group ranged between one and 13 (median of 8), S-ECP between 2.1 and 75.6 µg/l (median of 13.3 µg/l), and eosinophils between 30/µl and 2002/µl (median of 314). Symptom scores and S-ECP were correlated significantly ($P < 0.001$) as were symptom scores and eosinophils ($P = 0.001$). S-ECPs were significantly higher in children with chronic asthma symptoms compared with non-asthmatic, non-atopic children ($P = 0.005$ for non-atopic chronic asthmatics and $P < 0.001$ for atopic asthmatics); similar results were found comparing eosinophils in these groups.

There was no difference in S-ECP between atopic and non-atopic asthmatic children, but the 25 polysensitised asthmatic children especially with sensitisations to mite, pollen and pet allergens were found to have significantly higher S-ECP compared to 15 monosensitised children ($P = 0.002$). Similar results were found when correlating eosinophil numbers with atopy.

Polysensitised (mite, pollen, pet) asthmatics had significantly higher eosinophil counts compared with monosensitised (pollen) asthmatics ($P = 0.01$); there was, however, a better discrimination between atopic and non-atopic asthmatics ($P = 0.001$). Non-asthmatic, non-atopic controls had significantly lower eosinophil counts compared with asthmatics ($P < 0.001$ for both non-atopic and atopic asthmatics). No correlation between S-ECP or eosinophils and any of the lung function parameters measured (FEV₁, FEV₁/FVC, MEF50, airway resistance and ITGV) was found.

Summary. Our data thus indicate that 1) S-ECP is higher than normal in children with asthma symptoms and correlates with asthma symptom score. 2) S-ECP is better correlated to symptom score than to lung function parameters especially in children with mild and moderate asthma symptoms. 3) Raised S-ECP appears to reflect the extent of allergen sensitivity and may also reflect current allergen exposure. 4) Similar correlations were seen when measuring eosinophil number by an autoanalyser instead of S-ECP.

Conclusions. Although S-ECP and eosinophils are not diagnostic of asthma they are useful inflammation markers especially in the context of clinical studies. However, both methods are not yet suitable for use in daily practice because they require extensive procedures and special equipment.

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Asthma has been characterised as eosinophilic desquamative bronchitis (1). Even in mild asthmatics, increased numbers of activated eosinophils were found in biopsies from the airway mucosa (2, 3), in bronchoalveolar lavage (BAL) (4–7) and in sputum (8). Eosinophils from patients with asthma release significantly more ECP after incubation than cells from non-asthmatic controls (9).

Serum-eosinophil cationic protein-level (S-ECP), a biomarker, has been shown to correlate with the number of eosinophils in BAL and lung biopsy as well as with the ECP level in BAL (7), thus reflecting the degree of eosinophilic inflammation. Allergen challenge of such patients has been shown to result in late asthmatic reactions and subsequent rise in S-ECP (10). Recent studies have suggested that the levels of S-ECP in asthmatic children are sensitive indices of allergen exposure as shown after natural allergen exposure (11).

Since we know clinically mild asthmatics are also at risk for severe exacerbation (12), objective parameters that reflect the degree of airway inflammation are needed. This is especially so since diagnosis and scoring of asthma in younger children is often difficult: objective measurements (e.g. lung function parameters) are usually not available and the symptom perception of parents and children is often insufficient (13).

S-ECP measurements, however, could be helpful for assessing the extent of airway inflammation. Asthma patients with elevated S-ECP levels have been shown to be susceptible to inflammatory exacerbation (14). In addition, other studies have demonstrated that effective anti-inflammatory treatment results in a decrease of S-ECP during treatment (15, 16).

The aim of this study was to determine whether S-ECP is higher than normal in children with chronic asthma symptoms, whether S-ECP correlates with measurements of asthma control such as lung function parameters, symptom scores and atopy and whether there is similar information from the determination of eosinophil numbers.

Subjects and methods

Patients

One hundred patients (63 boys and 37 girls) aged three to 15 years (median age of eight), all with chronic asthma symptoms (chronic cough and/or wheezing and/or dyspnea, after exclusion of other diseases) for at least three months as defined by the American Thoracic Society (17), were studied in the Children's Hospitals of St. Gallen and Zurich, Switzerland, from August 1994 to June 1996. Children

with acute febrile respiratory infection, atopic dermatitis (except for xerosis cutis less than 10%, which was shown not to affect S-ECP (18)), urticaria, parasitic disease or any other chronic illness were excluded. Each patient was subjected to all tests the morning of a single day. Sixty-six of the 100 children suffered, in addition to their asthma, chronic nasal obstruction due to rhinitis and/or sinusitis (as reported by symptoms). Thirty-six of the 100 patients had pathological sinus X-rays (mucosal swelling) and 21 were found to have nasal eosinophilia (defined as > 8% in nasal smear). Fifty-nine children had at least one pet at home and 44 were chronically exposed to tobacco smoke (as reported by the parents). Sixty-two patients were without any anti-inflammatory treatment at the time of their first visit and 41 without any medication. Details of treatments given during the last three months are summarised in Table 1.

Control group

Twenty-five children (12 boys and 13 girls) aged three to 15 years (median of age eight) collected by our Department of Anaesthesiology without acute infections or allergic diseases and without any sensitisation, as measured by negative Pharmacia Cap RAST FEIA, served as controls for S-ECP.

Symptom score

Published scores (19) were not suitable for our population, since 41% of our patients had no previous medication and peak expiratory flow rate measurement had not been performed before or at study entry. Besides the lack of good definition of asthma and the lack of "gold standard" objective tests for it, there is in our opinion no score based on symptoms only, which is useful for general practitioners. Because of this, we established a new asthma symptom score in accordance with the clinical criteria defined by the international paediatric asthma consensus group (20) (Table 2). Our score does not take any medication and lung function parameters into ac-

Table 1. Patients' previous medication (n = 100)

Without any medication:	41	(41.0%)
Beta2 on demand per inhalation:	17	(17.0%)
Beta2 per os:	4	(4.0%)
Beta2 + SCG per inhalation:	8	(8.0%)
Beta2 + topical steroids per inhalation:	26	(26.0%)
Beta2 + topical steroids + SCG per inhalation:	2	(2.0%)
Topical steroids alone per inhalation:	2	(2.0%)

Table 2. Asthma symptom score

Asthma symptoms	points			
	0	1	2	3
cough	absent	dry	productive	
wheezing	absent	at infection	at exercise	at rest
asthma symptoms during sport exercise	no		yes	
absence from school	no			yes
sleep	not affected		mildly affected	hardly any sleep
frequency of cough and/or wheezing	< 1x/month	monthly, < 1x/week	1x/week < 1x/day	daily

Severity:
 Points 1–5 : mild
 Points 6–10 : moderate
 Points 11–16 : severe

count and was refined upon a previously standardised and validated questionnaire used in an international study (21). The score ranged from 0 (no symptoms) to 16 (severe symptoms), symptom scores 0 to 5 were defined as mild asthma symptoms, asthma symptom scores 6 to 10 as moderate asthma symptoms and greater than 10 points as severe asthma symptoms.

S-ECP

Blood was collected by venipuncture. Two SST tubes were filled with 4 ml venous blood each and gently inverted five times. In accordance with the instructions of Pharmacia, temperature and time were strictly controlled during serum preparation. The blood was clotted in a waterbath of 24°C for exactly 90 minutes immediately after collection. After the standardised clotting procedure the blood was centrifuged at 1300 × g for ten minutes at room temperature, and the serum decanted into a new tube. It was assayed by Pharmacia CAP system ECP FEIA on the same day. Reserve serum was stored at –70°C. S-ECP levels greater than 15 µg/l were considered above normal (22, 23).

Eosinophil counts

Venous blood was placed into EDTA-tubes, a peroxidase coloration was performed, and the stained eosinophils (> 10 000 cells) were counted by a Technikon H1 autoanalyser.

A number more than 500/µl was considered elevated (24). In six of the 100 patients the number of

eosinophils could not be detected for technical reasons.

Nasal eosinophilia

In 38 of our patients nasal smears were stained by May-Grünwald-Giemsa and 100 cells were counted by microscopy. In 62 patients we could not examine nasal eosinophils for technical reasons.

Atopy

In all children skin prick tests (SPTs) to a battery of common aeroallergens (grass and tree pollens, house dust mites (HDM), cat dander, dog dander and moulds) were performed and specific IgE levels determined to the same common inhalant allergens using the Pharmacia CAP RAST FEIA. We defined atopy as at least one or more positive SPT (characterised by a wheal of at least 3 mm diameter with a positive histamine control, and a negative control to normal saline) and/or at least one specific IgE of at least CAP-RAST class 2.

Lung function

In children more than six years of age (81 of our 100 patients) lung function was measured by bodyplethysmography (Jaeger, Würzburg); the parameters FEV₁, FEV₁/FVC, MEF50, airway resistance and ITGV were chosen for further analyses.

X-rays

Conventional X-rays of chest and maxillary sinuses were performed in all children.

Statistical analysis

Logarithms of S-ECP and square roots of eosinophil counts were taken to obtain more symmetric distributions. The Pearson's product moment correlation was used to describe the relationship between parameters. The Wilcoxon rank-sum test was applied to test the differences between groups.

Ethics

This study was approved by the Medical Ethical committees of the two children's hospitals. Informed consent was provided.

Results

Symptom score

The severity of symptoms ranged from one to 13 (median of 8). Severe asthma symptoms, as defined by symptom score levels more than ten were found in 11 patients. Moderate asthma symptoms with symptom score levels between six and ten occurred in 60 patients. Twenty-nine patients had levels less than six as defined for mild asthma symptoms.

S-ECP

The S-ECP ranged between 2.2 and 75.6 $\mu\text{g/l}$ (median of 13.3 $\mu\text{g/l}$). Forty-two of 100 patients were detected with raised S-ECP (defined > 15 $\mu\text{g/l}$), 19 with levels above 30 $\mu\text{g/l}$.

Eosinophils

The number of eosinophils ranged between 30 and 2002/ μl (median of 314/ μl). 33 of 100 patients had eosinophil numbers above 500/ μl .

S-ECP, eosinophils and symptom score

The Pearson correlation between S-ECP and symptom score ($r = 0.51$, 95% confidence interval: $0.35 < r < 0.64$) is statistically significant (with $P < 0.001$) as shown in Fig. 1a.

A significant correlation ($r = 0.33$, 95% confidence interval: $0.14 < r < 0.49$) was also found between eosinophils and symptom scores with $P = 0.001$ as shown in Fig. 1b.

S-ECP, eosinophils and lung function

No significant correlation could be found between S-ECP and the measured lung function parameters. Neither were significant correlations found for eosinophils and lung function parameters (data not shown).

S-ECP, eosinophils and atopy

The S-ECP in the control group was significantly lower than in the asthmatic children ($P = 0.005$ for non-atopic asthmatics and $P < 0.001$ for atopic asthmatics) (Fig. 2a). No differences in median S-ECP were found between atopic and non-atopic asthmatics (Fig. 2a); some non-atopics had raised S-ECP, in

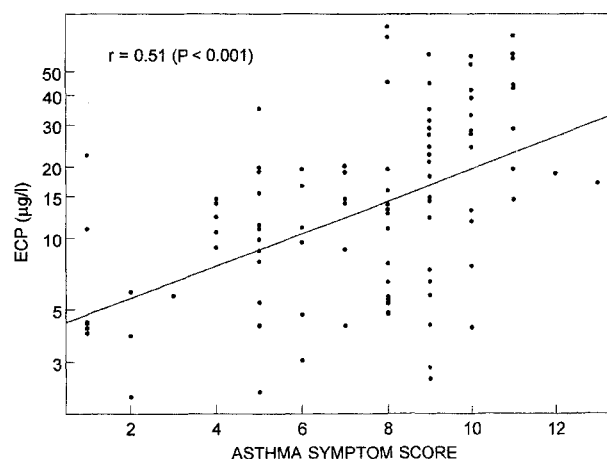


Fig. 1a. Relationship between S-ECP and asthma symptom score

three cases levels were > 60 $\mu\text{g/l}$. Comparable results were found when correlating with eosinophil numbers, however with better discrimination between atopic and non-atopic asthmatics ($P = 0.001$) (Fig. 2b)

A subgroup of patients with polysensitisation (to mite, pollen and pet allergens) had significantly higher S-ECP compared to pollen-only sensitised patients ($P = 0.002$) (Fig. 3a). Sixty-one percent of the pollen-sensitised patients were seen outside the pollen season (between September and January).

Eosinophil numbers were significantly higher in polysensitised asthmatic children compared with monosensitised asthmatic children ($P = 0.01$, Fig. 3b)

S-ECP and eosinophils

S-ECP and eosinophil counts were significantly correlated with P -value < 0.001 and r -value of 0.63 (data not shown).

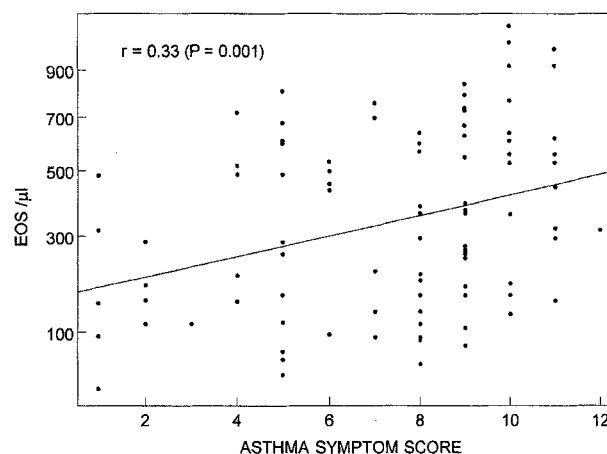


Fig. 1b. Relationship between eosinophil counts and asthma symptom score.

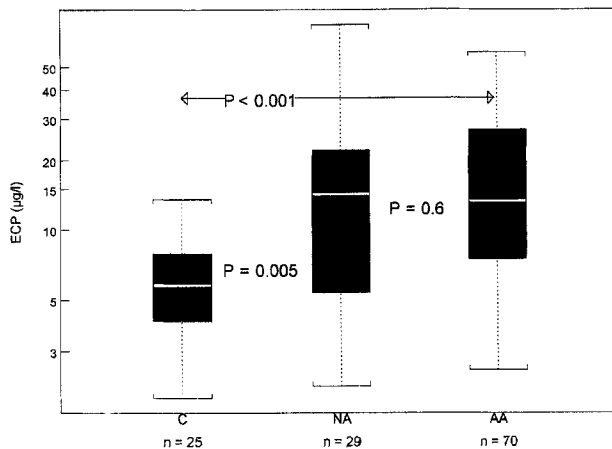


Fig. 2a. S-ECP in non-asthmatic, non-atopic control children compared with S-ECP in non-atopic and atopic asthmatics (median, interquartile range (25–75%) and total range). C = healthy, non-atopic controls, NA = non-atopic asthmatics, AA = atopic asthmatics

Nasal eosinophil count, S-ECP and symptom score

No correlation was found between nasal eosinophils and S-ECP as well as between nasal eosinophils and symptom score (data not shown)

Discussion

The characteristic pathology of asthma is an inflammatory process of the airways, involving different inflammatory cells and mediators that have various pathophysiological effects (25). An objective pa-

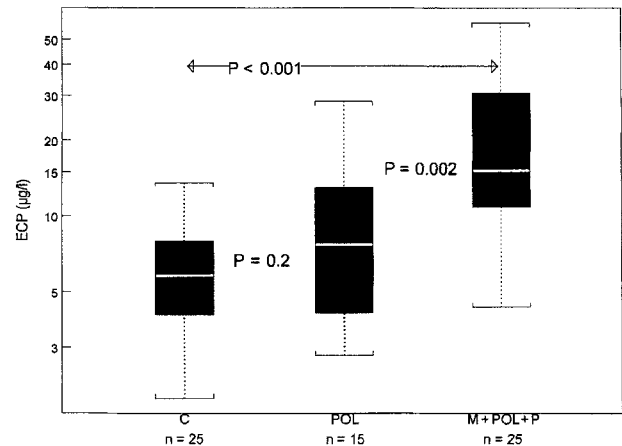


Fig. 3a. S-ECP in the control group compared with pollen-only sensitised asthmatics and with mite, pollen and pet sensitised asthmatics (median, interquartile range (25–75%) and total range). C = healthy, non-atopic controls, Pol = pollen only sensitised asthmatics, M + Pol + P = asthmatics sensitised to mite, pollen and pet allergens

rameter for measuring the extent of bronchial inflammation in childhood asthma is needed and would be most helpful in monitoring the disease activity and in evaluating the efficacy of anti-inflammatory treatments.

The role of eosinophils in the inflammatory pathogenesis of asthma is well established (1, 10). Recent studies could show local induction of allergen-induced airway inflammation with the help of positron emission tomography (26). Eosinophils of asthmatic patients are activated and release significantly more of their cytotoxic granule proteins than eosinophils of healthy controls (9). The isolated

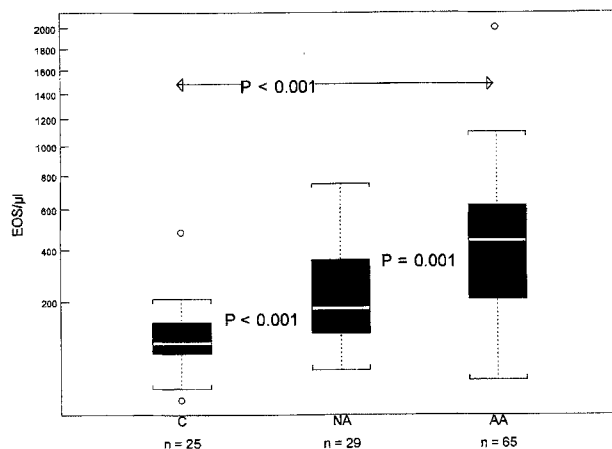


Fig. 2b. Eosinophil numbers in non-atopic, non-asthmatic children compared with eosinophil numbers in non-atopic and in atopic asthmatics (median, interquartile range (25–75%) and total range). C = healthy, non-atopic controls, NA = non-atopic asthmatics, AA = atopic asthmatics

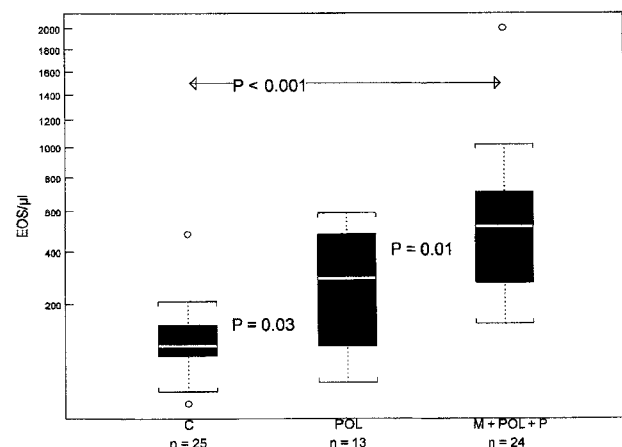


Fig. 3b. Eosinophil numbers in the control group compared with pollen-only sensitised asthmatics and with mite, pollen and pet sensitised asthmatics (median, interquartile range (25–75%) and total range). C = healthy, non-atopic controls, Pol = pollen only sensitised asthmatics, M + Pol + P = asthmatics sensitised to mite, pollen and pet allergens

granule proteins have been demonstrated to induce increased airway hyperresponsiveness (27).

S-ECP measurement has been shown to be a useful parameter in asthmatic adults and children (14, 15). While the usefulness of ECP-determination in more severe forms of asthma has already been evaluated, our study examines outpatient children with mostly mild to moderate chronic asthma symptoms in order to investigate whether S-ECP may aid assessment of disease.

In our survey of children with chronic asthma symptoms using a highly standardised collection of serum and blood samples, we found a statistically significant correlation between S-ECP and asthma symptoms as well as between eosinophil numbers and asthma scores despite some individual variations. As already shown in BAL and sputum studies (4, 7) raised S-ECP thus seems to reflect ongoing eosinophilic airway inflammation even in milder forms of asthma.

Although a correlation between eosinophil counts and S-ECP is present, it is not perfect, since not every eosinophil is activated in the blood and S-ECP assesses only the activated eosinophil subpopulation released from the bone marrow upon inflammatory stimulation (9). In other studies, S-ECP has been shown to be closer correlated to a subgroup of eosinophils which is activated and hypodense (9, 30).

Two thirds (66%) of our asthmatic children had clinical signs of chronic rhinitis/sinusitis, as previously reported by other investigators (28). Eosinophilic inflammation in asthma frequently involves both upper and lower airways (29). Since primed, e.g. pre-activated, eosinophils theoretically may be on their way from the bone marrow to both sites, increased S-ECP may indicate inflammation at both localisations. As we found no correlation between nasal eosinophil counts and S-ECP as well as symptom score, nasal inflammation at least in our study does not seem to influence S-ECP and asthmatic symptoms significantly.

S-ECP in our study was higher in polysensitised patients especially in those having pollen, mite and pet sensitisations when compared with pollen-only sensitised asthmatics. This may reflect the extent of allergen exposure (11). Exclusively pollen-sensitised children were in some cases investigated outside the pollen season, so that in the absence of allergen exposure residual eosinophil inflammation could be searched for, but was not found. Future studies measuring the degree of exposure of patients to pollen, HDM and pet allergens may reveal even a quantitative relationship between allergen exposure and the extent of eosinophil inflammation as reflected by S-ECP as well as eosinophils.

In some patients with high S-ECP, we were unable to detect underlying atopy by a battery of SPTs

and circulating specific IgE-determinations to ubiquitous allergens. We cannot, however, exclude the possibility that these patients are sensitised to rarer allergens not tested and thus have also to be considered atopic.

After natural allergen exposure, S-ECP levels were shown to be more sensitive indices of inflammation than bronchial hyperreactivity (11). The lack of correlation between lung function parameters and S-ECP levels or eosinophils in our study may therefore be surprising only at first sight and has already been shown by other investigators (31). The majority of our patients even with elevated S-ECP had only slight changes in lung function: only 11 out of 100 were found to have $FEV_1/FVC < 80\%$, and 36 out of 100 were found with $MEF50 < 80\%$. Bronchial inflammation must likely be massive before it causes any significant degree of bronchial obstruction. This supports the hypothesis that S-ECP or eosinophils could be a more sensitive objective parameter concerning airway inflammation than baseline lung function. On the other hand, S-ECP reflects the activity of only one cell in a complex inflammatory process involving multiple inflammatory cell types with different mediators as well as non-inflammatory mechanisms. In addition, S-ECP cannot reflect late chronic repair or remodelling processes, which by themselves cause bronchial hyperactivity.

In conclusion S-ECP reflected symptom score and the degree of allergen sensitisation. It may reveal active inflammatory disease better than lung function parameters (14). Comparable information on inflammatory disease activity may also be obtained by eosinophil numbers if assessed by an auto-analyser method (counting $> 100\ 000$ cells).

Both S-ECP and blood eosinophils may indicate the degree of clinical as well as subclinical inflammation in asthmatic children and therefore may be used to optimise the anti-inflammatory treatment during clinical studies. For daily practice, however, both parameters are until now not practicable enough, because procedures are time consuming and need special equipment. Long term follow-up of the patients with raised S-ECP will determine whether it is a useful predictor of the course of disease. We hope to report on this in the future.

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