

Evaluation of serum eosinophil cationic protein as a predictive marker for asthma exacerbation in patients with persistent disease

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Summary

Background Eosinophilic inflammation is a feature of asthma. However, serological markers to indicate eosinophil activation in this process are not fully defined.

Objective To evaluate the relationship of serum eosinophil cationic protein (ECP) to asthma worsening and a marker for treatment effectiveness, 26 adult patients with an asthma exacerbation were identified.

Methods Identified asthma subjects were treated with oral corticosteroids (prednisone) for 14 days. The lung function variables, forced expiratory volume in one second (FEV₁) and peak expiratory flow (PEF), were determined as percentage of predicted and the blood total eosinophil count and serum ECP levels were measured. Patients were re-evaluated after 14 days of corticosteroid treatment and then every 3 months thereafter during a 12-month period.

Results Eighteen patients responded to prednisone treatment, whereas eight did not, assessed as improvement of their lung function parameters. Different serum ECP patterns could be seen in the responders compared with the non-responders. All 18 responders had considerably increased serum ECP at the time of exacerbation, whereas the non-responders had lower serum ECP levels. The serum ECP levels decreased to a greater extent in the responder patient group than in the non-responder patients following prednisone treatment. This difference in patterns was not seen with total blood eosinophil counts.

Conclusion Our findings suggest that serum ECP may be used to predict a response to corticosteroid therapy in adult patients with asthma.

Keywords asthma exacerbation, asthma, corticosteroid, eosinophil cationic protein, eosinophil

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Introduction

Airway inflammation in bronchial asthma is characterized by infiltration with eosinophils and epithelial desquamation [1, 2]. The contribution of eosinophils to this process may occur through a number of mechanisms. Granule-derived mediators, such as eosinophil cationic protein (ECP), eosinophil protein X (EPX), eosinophil peroxidase (EPO) and major basic protein (MBP), are released and can be identified in biopsy specimens from the bronchial mucosa [1, 3], in sputum [4–6], in bronchial alveolar lavage (BAL) [1, 7] and peripheral blood [7–10]. These mediators can contribute to alter airway function in asthma. These findings suggest that eosinophil activation is a component of inflammation in asthma, and the release of eosinophil granular proteins may reflect an active asthmatic process.

A relationship between levels of ECP and exposure to inhaled allergens and asthma activity has been suggested [11–14]. Furthermore, therapy with anti-inflammatory agents, such as corticosteroids, decreases levels of ECP [7, 15–17]. Some studies have indicated that serum ECP levels increase prior to an asthma exacerbation [18, 19]. These observations suggest that ECP may serve as a biomarker for an increase in asthma severity and disease activation. Given this information, we hypothesized that an increase in ECP reflected active inflammation and may predict patients who will benefit from anti-inflammatory treatment with corticosteroids.

The objective of the present study was to investigate whether serum ECP levels or blood eosinophil differential counts were predictive of the asthmatic patient's response to systemic corticosteroids. To accomplish this goal, subjects with an exacerbation of asthma were identified, treated with oral prednisone, and pulmonary functions evaluated in relationship to serum ECP values and peripheral blood eosinophil counts. The response to therapy during a 12-month period was assessed measuring serial FEV₁ and symptoms in a prospective cohort of adults with moderate-severe persistent asthma.

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Materials and methods

Patients

Twenty-six adult patients with moderate-severe persistent asthma as defined by the American Thoracic Society were recruited from a university-based asthma clinic. The patients were new to the clinic and met the overall study inclusion criteria when they presented with an asthma exacerbation that required systemic corticosteroid therapy. All of the subjects, aged 18 to 70 years, were non-smokers at the time of the study. Pre-bronchodilator FEV₁ was required to be 40 to 70% of predicted. The presence of atopy was assessed by an immediate skin test (> 3 mm wheal) response to one or more common aeroallergen extracts at enrolment. The patients were eligible for enrolment with any type of asthma medications except oral or parenteral corticosteroids. Pregnant or lactating women were not invited to participate. All patients provided written informed consent, and the university's institutional review board approved the study.

Study design

The study was a prospective trial over 12 months. After baseline assessments and the diagnosis of an asthma exacerbation were established, subjects were treated with a standardized course of oral prednisone (10 mg t.i.d. $\times$ 7 days, then 10 mg q.d. $\times$ 7 days). The actual prednisone supply was provided to subjects to enhance adherence. Revisits were re-evaluated at 14 days post-prednisone treatment and thereafter at 3-month intervals. Spirometry was performed at each visit. Blood samples were obtained at study entry and at each revisit for measurements of serum ECP and white blood cell counts and differential. Patients recorded asthma symptoms, peak expiratory flow measurements (morning and evening) and asthma medication usage.

Assignment of responder status

A global asthma assessment score was used. Thus 'excellent' (0) was defined as no change of asthma medication was required or reduction of medication was planned. 'Good' (1) was defined if the inhaled corticosteroids were continued at the same dose. 'Fair' (2) was defined if the patient required continued treatment with oral prednisone as a maintenance dose, or if there was a need for increased dose of prednisone or inhaled corticosteroids, or if therapy with long-acting bronchodilator was required. 'Poor' (3) was defined if the subject needed a burst of prednisone at the time of visit to the clinic. Asthma symptom score was defined as 'None' (0), no reported wheezing, nocturnal awakenings or use of rescue bronchodilator; 'Mild' (1), wheezing, bronchodilator use, nocturnal awakenings reported 1 to 3 days in the previous 4 weeks; 'Moderate' (2), wheezing, bronchodilator use, nocturnal awakenings reported on 4 to 10 days in the previous 4 weeks; 'Severe' (3), wheezing, bronchodilator use, nocturnal awakenings reported on 11 to 28 days in the previous 4 weeks (adapted from Ferguson et al. [20]).

Based on clinical asthma global assessment and asthma symptom scores after the 2 weeks of prednisone therapy, each subject was assigned 'responder status' if the following criteria were met: improvement in global assessment score from 3 (poor) to 2, 1 or 0; improvement in asthma score from 3 (severe) to 2, 1 or 0; improvement in FEV₁ from visit 1 baseline; and

ability to discontinue prednisone at visit 2 (post-prednisone treatment of 14 days). A study investigator blinded to laboratory and serum ECP data assigned these scores.

Sample collection and analysis

Venous blood samples for serum ECP and cell counts were collected and processed immediately after venipuncture. Becton Dickinson 10 mL serum separating tubes (SST-tubes) were used for all serum ECP collections. The filled tubes were gently inverted for 5 min and allowed to clot for 60 to 120 min at room temperature (20 °C). The tubes were centrifuged at 1000 to 1350 *g* for 10 min; 1 mL aliquots were decanted and stored in a -70 °C freezer until analysed. Serum ECP was analysed blindly utilizing Pharmacia ECP RIA at the research centre and at Pharmacia & Upjohn Diagnostics AB Laboratory, who were also blinded as to the study design, by utilizing UniCAP ECP in parallel according to the manufacturer's instructions. A blinded, certified laboratory technician, counted the white blood cell counts with a haemocytometer using Turk's solution. A whole blood smear was prepared and stained with Giemsa-based Diff-Quick stain (Baxter Scientific Products, McGaw Park, IL, USA) for a differential cell count from which eosinophil values were determined.

Statistical analysis

Estimation of the sample size required for the study was based on the serum ECP level between an 'asthma exacerbation' occasion and a follow-up visit. To detect a decrease of 15 µg/L of serum ECP, 22 subjects would be required for a two-sided significance level of 0.05 and a power of 0.80. For each of the efficacy variables, a Student's *t*-test was applied to test changes of the variables for the subjects between visits. Pearson's product moment correlation coefficient was used to estimate the relationship between variables at the first visit and changes in variables between visits during the 12-month follow-up period.

A descriptive analysis of the subject's characteristics and the efficacy variable collected was performed. An arithmetic mean with 95% confidence interval was calculated for pulmonary function measures; a geometric mean with 95% confidence interval was calculated for serum ECP levels and blood eosinophil counts. The values of serum ECP and eosinophil counts were log₁₀ transformed to achieve approximate normality of the values. Exploratory analyses were made for two predefined subgroups, responders and non-responders. Mean values for the efficacy variables at each visit were calculated. The analysis of covariance described above was repeated for the responders.

Results

Subject characteristics

Thirty subjects were enrolled. One subject withdrew after the first visit and a second individual dropped out due to a family emergency. A third subject left the country for studies abroad and a fourth person was discovered to have vocal cord dysfunction and not asthma. Twenty-six subjects completed the study and were fully evaluated (Table 1).

All of the subjects enrolled in this prospective study had asthma symptoms and airflow obstruction with decreased FEV₁ values ($60.5 \pm 10.7\%$ of predicted, mean $\pm$ SD). The

Table 1. Demographic data

Sex: <i>n</i> (%)	
Females	18 (69%)
Males	8 (31%)
FEV ₁ , % predicted (at baseline)	60.5 ± 10.7 (mean ± SD)
Atopy: <i>n</i> (%)	
Non-atopics	2 (8%)
Atopics	24 (92%)
Age: yr, mean (range)	40.0 (18–69)
Asthma duration: <i>n</i> (%)	
< 2 years	3 (12%)
2–10 years	4 (15%)
> 10 years	19 (73%)
Severity: <i>n</i> (%)	
Moderate	13 (50%)
Severe	13 (50%)
Smoking habits: <i>n</i> (%)	
Never smoked	18 (69%)
Ex-smokers	8 (31%)
Smoking: yr, mean (range)	17.5 (7–28)
Pack-years: mean (range)	16.6 (2.7–33)

nature of the subjects' exacerbation was not known, although eight of the 26 individuals presented with symptoms of a respiratory infection at the initial visit.

Results of subjects separated as responders and non-responders to systemic corticosteroid treatment

There were 18 responders and eight non-responders according to definitions established in the 'Material and methods' section. When the responders were compared with the non-responders, the lung function values showed different patterns of response to the oral prednisone treatment between the groups. The responders demonstrated a trend towards higher mean lung function values at the initial visit, FEV₁ of 62.6 and PEF 80.4% of predicted, respectively, compared with 55.8 and 72.6% of predicted, respectively, for the non-responders (Fig. 1a). The responders also demonstrated a greater improvement in lung function after the 14 days on oral steroids: increases to FEV₁ 87.6 and PEF 110.4% of predicted, compared with 58.8 and 74.2% for the non-responders (Fig. 1b). The severity of the exacerbation was not apparent at the pre-treatment evaluation as each subject was new to the clinic and previous values of lung function were not available.

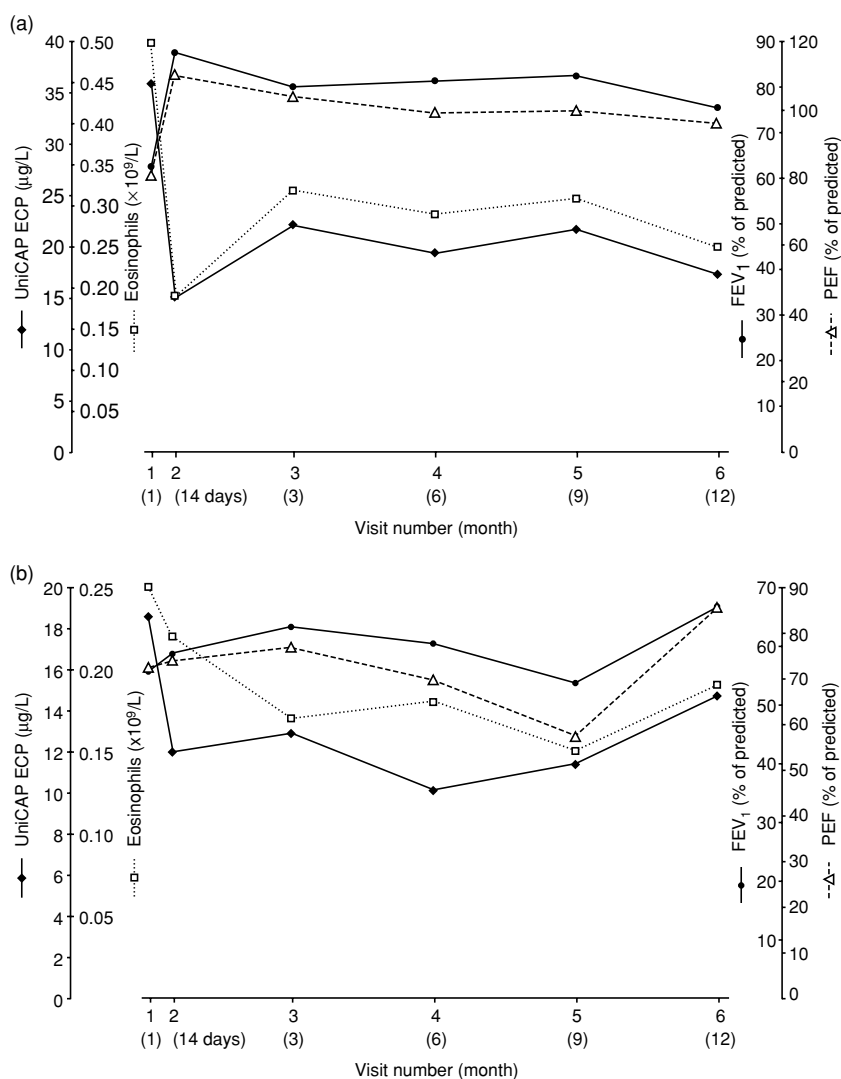


Fig. 1. The patterns of lung function variables, FEV₁ and PEF, peripheral blood total eosinophil counts and serum ECP values (a) in patients responding to corticosteroid treatment and (b) in patients not responding to corticosteroid treatment.

Different peripheral blood eosinophil and serum ECP patterns were seen in the responder (Fig. 1a) and non-responder groups (Fig. 1b). Before treatment, the responders had higher eosinophil counts (0.5×10^9 cells/L [0.36–0.69]) than non-responders (0.25×10^9 cells/L [0.17–0.36]), $P = 0.015$. Similarly, all of the 18 responders had elevated ECP values at the initial visit; the maximum level measured was as high as $105 \mu\text{g/L}$ and eight subjects showed ECP levels above $50 \mu\text{g/L}$. The mean ECP levels in the responder group were almost double those in the non-responder group, 35.9 (26.4 to 48.8) and 18.6 (13.5 to 25.6) $\mu\text{g/L}$, respectively, $P = 0.01$. After the 14-day course on oral corticosteroids, the ECP levels fell in both groups, but the fall was more pronounced in the responder group, $15.0 \mu\text{g/L}$ or by 58%, compared with the non-responder group, $12.0 \mu\text{g/L}$ or 35%. A similar trend was seen for the eosinophil numbers, although the differences were not significant (Table 2). Six of the eight non-responders had ECP levels $< 20 \mu\text{g/L}$ at the initial

visit. The two patients with ECP $> 20 \mu\text{g/L}$ differed from the typical non-responders. One patient had developed asthma after isocyanate exposure and might therefore have had irreversible airway changes. During the course on oral corticosteroids, the ECP levels dropped. The information in Fig. 1 is summarized in Table 3.

The within-patient correlation coefficient between ECP and eosinophils, FEV₁ and PEF after oral steroids (visit 2) and all following visits was also calculated for the responders ($n = 18$). As for the whole study group taken together, a strong correlation was found between ECP and peripheral blood eosinophils ($r = 0.53$, $P = 0.0001$).

Discussion

The present study demonstrated that, as a group, asthma patients experiencing an increase in symptoms and diminished FEV₁ values responded differently to systemic corticosteroid treatment. After 2 weeks of oral corticosteroid treatment, a subgroup of these subjects showed an improvement in FEV₁ values and symptoms, whereas the other group did not. The study demonstrated that the initial FEV₁ values at the time of an exacerbation could not predict the response to oral corticosteroids. The eosinophil counts in the two subgroups were in the normal range, except in four subjects, and also did not provide guidance as to who might respond to treatment and who would not. In contrast, the values of the serum eosinophil granule protein ECP were elevated, primarily in those subjects who eventually demonstrated improvement in FEV₁ with corticosteroid therapy. These data suggest that serum ECP levels may be useful to predict the likelihood for a response to corticosteroid treatment in patients with asthma.

After 2 weeks of prednisone treatment, both serum ECP levels and blood eosinophil counts decreased significantly. This is in agreement with other studies in asthma subjects [7, 21, 22]. The lung function variables improved, altogether indicating a resolution of the acute exacerbation of airflow obstruction. However, there was no absolute correlation between the serum levels of ECP and the lung function

Table 2. Changes in ECP and eosinophil counts for responders and non-responders (geometric mean of the ratio (later visit/earlier visit), 95% confidence level)

Ratio/difference	ECP UniCAP	Eosinophils
Responders		
Day 0–14	0.42 (0.32–0.53)	0.37 (0.25–0.55)
<i>P</i>	< 0.0011	< 0.001
<i>n</i>	18	18
Non-responders		
Day 0–14	0.65 (0.47–0.88)	0.94 (0.71–1.24)
<i>P</i>	0.01	0.61
<i>n</i>	8	8
Responders		
Day 0–Month 12	0.47 (0.32–0.69)	0.49 (0.30–0.79)
<i>P</i>	0.0008	0.006
<i>n</i>	17	17
Non-responders		
Day 0–Month 12	0.79 (0.53–1.17)	0.74 (0.35–1.59)
<i>P</i>	0.20	0.38
<i>n</i>	8	7

Table 3. Comparison of peripheral blood eosinophils, ECP and pulmonary functions in the responder and non-responder groups

Visit no.	Serum ECP	Eosinophils ($\times 10^9/\text{L}$)	FEV ₁ % predicted	PEF % predicted
(a) Responders ($n = 18$)				
Day 0	35.9 (26.4–48.8)	0.50 (0.36–0.69)	62.6 (57.3–67.9)	80.4 (65.9–94.8)
Day 14	15.0 (11.3–19.7)	0.19 (0.14–0.24)	87.6 (81.0–94.1)	110.4 (99.9–120.8)
Month 3	27.6 (15.9–30.6)	0.32 (0.23–0.46)	80.2 (73.4–87.0)	103.7 (90.9–116.4)
Month 6	19.3 (14.4–25.8)	0.29 (0.21–0.39)	81.5 (74.2–88.8)	99.1 (86.4–111.8)
Month 9	21.7 (15.8–29.7)	0.31 (0.23–0.42)	82.7 (75.1–90.2)	99.8 (87.0–112.6)
Month 12	17.2 (12.2–24.1)	0.25 (0.17–0.37)	75.6 (67.8–83.4)	96.1 (82.2–110.0)
(b) Non-responders ($n = 8$)				
Day 0	18.6 (13.5–25.6)	0.25 (0.17–0.36)	55.8 (47.7–63.9)	72.6 (55.9–89.2)
Day 14	12.0 (8.3–17.5)	0.22 (0.15–0.32)	58.8 (42.4–75.1)	74.2 (43.3–105.2)
Month 3	12.9 (9.3–18.1)	0.17 (0.13–0.22)	63.4 (45.8–81.0)	77.0 (50.9–103.1)
Month 6	10.1 (7.2–14.3)	0.18 (0.11–0.28)	60.5 (44.3–76.6)	69.8 (47.0–92.6)
Month 9	11.4 (6.6–19.7)	0.15 (0.10–0.24)	53.7 (40.7–66.6)	54.4 (31.0–77.8)
Month 12	14.7 (7.8–27.6)	0.19 (0.11–0.33)	66.7 (50.2–83.2)	85.7 (57.5–114.0)

Geometric mean (95% confidence interval) for ECP and eosinophils. Arithmetic mean (95% confidence interval) for FEV₁ and PEF.

parameters, and there was no correlation between the decrease of serum ECP and the normalization of the lung function parameters. Other investigators [16, 21, 23] have also demonstrated such lack of correlation. Although being within a normal range, peripheral blood eosinophil counts also seemed to follow the same pattern as ECP during the study and decreased following prednisone treatment. This is consistent with the possibility that serum ECP may reflect a marker of blood eosinophil activation during an asthma exacerbation [3].

In the other subgroup of subjects, little or no response to prednisone treatment occurred in respect to lung function or eosinophil values. These non-responders appeared to have an irreversible component to their airway obstruction despite the apparent acute nature to their illness. In these subjects the contribution of airway inflammation to the obstruction appeared secondary to other structural changes. The different patient profiles observed in this study reflect an ongoing discussion that asthma should be considered a heterogeneous disease in relationship to lung function, inflammation (cells and mediators) and response to therapy such as glucocorticoids. Furthermore, it is unlikely that the difference is related to patient's smoking history as there were five smokers in the responder group and three in the non-responder group.

Our findings suggest that serum ECP may be a biomarker of eosinophilic inflammation and useful to predict a response to corticosteroid therapy. Total peripheral blood eosinophil counts were not elevated in any of these subjects and did not discriminate between responders and non-responders. In contrast, the serum ECP determination appeared to identify a subpopulation of asthmatic patients with a reversible component of airflow obstruction and raises the possibilities that the associated airflow obstruction is eosinophil-associated and responsive to corticosteroids. This corresponds to other studies investigating the blood levels of these parameters [3, 15, 21, 23].

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