

Serum eosinophil cationic protein levels measured during exacerbation of asthma: characteristics of patients with low titres

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Summary

Background Serum eosinophil cationic protein (ECP) levels reflect ongoing eosinophilic airway inflammation and are used as a marker for asthma activity. ECP levels, however, may not be elevated in some asthmatic patients, even when they are symptomatic.

Objective To clarify the characteristics of patients with 'low' ECP titres despite asthma exacerbation.

Methods Serum ECP levels were measured in 113 asthmatic patients during exacerbation. Patients were divided into two groups according to ECP titre: a high ECP group (H; $\text{ECP} \geq 16.0 \mu\text{g/L}$) and a low ECP group (L; $\text{ECP} < 16.0 \mu\text{g/L}$). Twenty-two patients who had recently received systemic steroids were excluded and the clinical features of the remaining patients in H ($n = 54$) and L ($n = 37$) were compared.

Results Gender, atopic or smoking status, disease severity, inhaled steroid or theophylline usage, peak expiratory flow (% personal best) and forced expiratory volume in 1 s (FEV_1) (% predicted) did not significantly differ between the two groups. Patients in L were significantly older and had longer disease duration and lower serum IgE levels than those in H. Multivariate analysis combining age, disease duration and IgE levels showed that age and disease duration were independently associated with ECP level. Airway wall thickness, assessed in a subset of patients using computed tomography, was significantly larger in L.

Conclusion Serum ECP levels in asthmatic patients may not be elevated during exacerbation and thus may not be a useful marker in patients who are older, have longer disease duration or possibly have thicker airway walls. Mechanisms other than eosinophilic inflammation, such as airway remodelling, may be involved in asthma exacerbation in these patients.

Keywords: eosinophil cationic protein, serum, asthma, exacerbation, age, disease duration, airway wall thickening, airway remodelling, IgE

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Introduction

Eosinophilic airway inflammation is a characteristic feature of asthma. Eosinophils in the peripheral blood increase in number and are activated through cytokines released from the airways where inflammation has occurred. Serum levels of

eosinophil cationic protein (ECP), a granular protein derived from activated eosinophils, are a reflection of the degree of activation of the circulating pool of eosinophils [1,2]. Serum ECP is therefore assumed to measure, although indirectly, the intensity of ongoing eosinophilic inflammation in the airways. This hypothesis has been proved by several studies showing a correlation between serum ECP level and the degree of eosinophilic airway inflammation assessed using sputum, bronchoalveolar lavage and bronchial biopsy [3–7].

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A number of studies have indicated that serum ECP may be clinically useful as a marker of asthma activity [6–11]. Serum ECP levels are higher in symptomatic asthmatic patients than in asymptomatic patients or healthy controls [6,8]. Serum ECP levels may also correlate with indices of disease activity such as symptom score, pulmonary function and bronchial responsiveness [6,9–11]. However, the correlation coefficients are not always high and some reports have even failed to reveal such relationships [2,7,10,12,13]. It has been indicated that serum ECP levels may not always rise in parallel with heightened disease activity [7,14]. In fact, in clinical settings, serum ECP levels are very low in some patients even during exacerbation and show little change after anti-inflammatory treatment. In such patients, eosinophilic inflammation may be less involved in the asthma exacerbation than in patients with high ECP levels and serum ECP may not be a useful marker for clinical decision-making.

The present study was conducted to clarify the clinical features of asthmatic patients whose serum ECP titres are low during exacerbation. Clinical indices, such as gender, age, duration and severity of asthma, atopic and smoking status, presence of upper respiratory infection, pulmonary function, serum IgE level and drug usage, were assessed in such patients and the results were compared with those of patients with high ECP titres. Airway wall thickness was also assessed using helical computed tomography (CT) in a subset of patients from both groups when their asthma was stable.

Methods

Subjects

One hundred and thirteen asthma patients (57 males and 56 females) with exacerbation of the disease were enrolled. Asthma was defined according to the American Thoracic Society criteria [15] after assessing reversible airway obstruction and/or bronchial hyperresponsiveness to methacholine. The patients' ages ranged from 16 to 80 years old with a mean of 48.2 ± 16.5 (SD) years and a median of 51 years. Exacerbation was defined according to Wever *et al.* [10]: increase in symptoms such as dyspnoea, wheezing and cough; increased use of inhaled β agonists; need for a short course of oral corticosteroids. Chest X-ray results were normal for all patients without infiltrates suggestive of pulmonary infection or eosinophilic pneumonia.

Patients were divided into two groups according to serum ECP levels measured during exacerbation. The 'high ECP group' consisted of patients with serum ECP levels of ≥ 16.0 $\mu\text{g/L}$ ($n = 61$) and the 'low ECP group' consisted of patients with serum ECP levels < 16.0 $\mu\text{g/L}$ ($n = 52$). The threshold of 16.0 was chosen because 15.9 $\mu\text{g/L}$ has

been proposed as the upper limit of the normal range [16]. Since systemic steroid usage is known to potentially decrease serum ECP titre [9,17,18], 22 patients (seven from the high ECP group and 15 from the low ECP group) who had taken systemic steroids within the previous month were excluded and the remaining 91 patients (54 from the high ECP group and 37 from the low ECP group) were analysed.

The severity of asthma was classified based on the clinical features before treatment and daily medication required to maintain control, according to the Global Initiative for Asthma (GINA) [19]: Step 1 (intermittent), Step 2 (mild persistent), Step 3 (moderate persistent), Step 4 (severe persistent). Since many of the asthmatic patients entered the study during their first visit to our hospital, severity was determined after the medication required to maintain control was identified. The presence of an upper respiratory infection was defined as sneezing, nasal obstruction and discharge, sore throat and sputum production [20] and was often accompanied by fever and an increase in C-reactive protein.

In addition to the measurement taken during exacerbation, baseline ECP levels were examined in 22 patients when they were stable.

Inter-individual variation of serum ECP levels during several episodes of exacerbation was also assessed in a subset of patients. Each patient showed ECP levels that were always categorized as either 'high' (≥ 16 $\mu\text{g/L}$) or 'low' (< 16 $\mu\text{g/L}$) during exacerbations.

The study was approved by the ethics committee at our Institution and written informed consent was obtained from all participating subjects.

Blood samples

Numbers of blood eosinophils and serum ECP levels were determined as reported previously [6]. Briefly, the blood eosinophil number was counted using a Coulter Maxm (Coulter Corp., Hialeah, FL, USA) and determined by multiplying the total leucocyte count ($\times 10^6/\text{L}$) by the percentage of eosinophils (%). The blood samples for ECP measurement were collected into an SST tube (Becton Dickinson, Mountain View, CA, USA), stored for 60 ± 5 min at 25°C , and subsequently centrifuged at $1300 g$ at 4°C for 10 min. The sera were then stored at -20°C until measurement of ECP concentration with an ECP radioimmunoassay (RIA) kit (Pharmacia Diagnostics, Uppsala, Sweden). The detection limit of this kit is 2.0 $\mu\text{g/L}$.

Total and specific serum IgE antibody titres were measured by radioimmunosorbent testing using commercially available kits (Pharmacia & Upjohn, Tokyo, Japan). Patients were considered atopic when one or more specific IgE antibodies against house-dust mites, cat dander, dog dander, weed pollen, grass pollen or molds were positive.

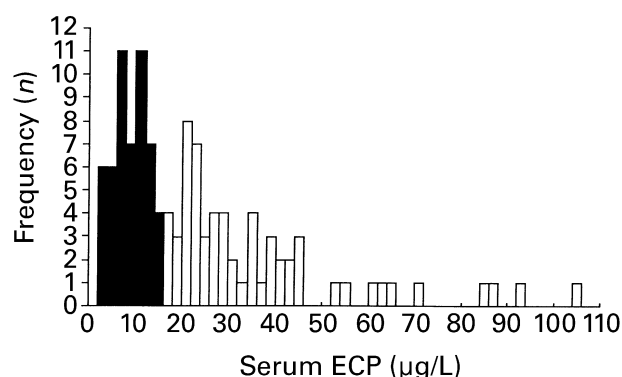


Fig. 1. The frequency distribution of serum eosinophil cationic protein (ECP) levels of the original 113 patients. Closed and open bars represent the patients with low ($<16.0 \mu\text{g/L}$) and high ($\geq 16.0 \mu\text{g/L}$) ECP levels, respectively.

To examine the influence of seasonal variation on serum ECP level, the season of ECP sampling was checked in patients sensitized to seasonal allergens.

Pulmonary function

Patients were instructed to record their peak expiratory

flow (PEF) values using a Miniwright peak flow meter (Clement Clarke Int. Ltd, Harlow, Essex, UK) twice daily. The ratio (%) of the PEF rate during exacerbation to the maximum PEF rate obtained from diary data (patient's personal best value) was determined. For patients measuring PEF for the first time upon enrolment in the study, the personal best value was obtained later.

Forced expiratory volume in 1 s (FEV_1) values were obtained using a spirometer (Chestac-65 V; Chest, Tokyo, Japan) during the visit when the patient had exacerbation.

Measurement of airway wall thickness

Airway wall thickness was quantitatively measured using CT according to a validated method [21–23] in 41 patients (18 from the low ECP group and 23 from the high ECP group) from whom consent was obtained. Briefly, with a Toshiba X-Vigor CT scanner (Toshiba, Tokyo, Japan), helical CT images at the trunk of the apical bronchus of the right upper lobe were obtained and analysed on a workstation (X-tension; Toshiba). The region of interest (ROI) was traced manually along the internal perimeter (the luminal border of the airway) and the outer perimeter (the parenchymal border). Airway wall area (mm^2) was

Table 1. Background and outcomes of the two asthmatic groups

	Low ECP group	High ECP group	Mean difference (95% CI)	P
Number of subjects	37	54		
Male:female	15:22	33:21	−0.2 (−0.4, 0.0)	0.058
Age, years	53 (23–80)	39 (16–73)*		
	54.0 ± 14.7	40.5 ± 15.7	13.5 (7.0, 20.0)	<0.0001
Age at disease onset, years	47 (2–71)	30 (2–73)*		
	40.7 ± 21.0	35.0 ± 19.1	5.6 (−2.8, 14.0)	0.189
Disease duration, years	3.0 (0.05–65)	2.0 (0.02–34)*		
	13.5 ± 19.9	5.6 ± 8.4	7.9 (1.8, 13.9)	0.011
Atopic:non-atopic	23:14	38:16	−0.08 (−0.2, 0.1)	0.497
Smoker:non-smoker	3:34	9:45	−0.08 (−0.2, 0.04)	0.347
Disease severity (step), 1:2:3:4	0:14:15:8	1:12:30:11		0.311
	3 (2–4)	3 (1–4)*		0.414†
Upper respiratory infection (present:absent)	5:32	5:49	0.04 (−0.09, 0.1)	0.734
Usage of inhaled steroid (yes:no)	9:28	13:41	0.002 (−0.1, 0.1)	>0.99
Dose of inhaled steroid ($\mu\text{g/day}$)	154 ± 301	115 ± 241	38.9 (−74.9, 152.8)	0.498
Usage of sustained-release theophylline (yes:no)	16:21	24:30	−0.01 (−0.2, 0.1)	>0.99
PEF (% of personal best)	65.6 ± 18.7	61.8 ± 25.5	3.8 (−13.2, 20.8)	0.650
FEV_1 (% predicted)	78.8 ± 23.7	74.7 ± 23.3	4.1 (−6.5, 14.9)	0.519
Log IgE (U/mL)	2.1 ± 0.6	2.5 ± 0.6	−0.3 (−0.6, −0.1)	0.004
Blood eosinophils ($10^6/\text{L}$)	435 ± 358	797 ± 620	−362.5 (−587.2, −137.8)	0.001
Airway wall area/body surface area (mm^2/m^2)‡	$15.2 (6.4–25.8)$	$12.6 (7.6–21.9)^*$		0.035†

Values are given as mean $\pm$ SD except where noted.*Median (range).†Mann–Whitney *U*-test.‡Examined using computed tomography in 18 patients from the low eosinophil cationic protein (ECP) group and 23 patients from the high ECP group.

Table 2. Explanatory variables associated with low eosinophil cationic protein (ECP) level (logistic regression analysis in a stepwise approach)

Variables	Odds ratio (95% CI)	P-value
Age		
10 years increase	1.51 (1.09–2.08)	0.012
Disease duration		
5 years increase	1.27 (1.01–1.61)	0.039
Log IgE	0.44 (0.21–1.13)	0.095

calculated as total airway area minus luminal area [21–23] and airway wall area corrected by body surface area (mm^2/m^2) was used as the parameter of airway wall thickness. Scans were performed when the patients' asthma was stabilized with appropriate anti-inflammatory medications, including inhaled steroids. The mean $\pm$ SD of FEV₁ with CT scanning was 91.2 ± 16.3 (% predicted).

Statistical analysis

Variables are expressed as mean $\pm$ SD unless otherwise stated. IgE was log transformed. Unpaired *t*-test, Mann–Whitney's *U*-test and χ^2 test were used to analyse differences between the high and low groups. Relationships between data were analysed using Spearman's rank correlation test. Logistic regression analysis (SPSS™; SPSS Inc., Chicago, IL, USA) was performed in a stepwise approach to test for independent effects of age, disease duration and serum IgE level on low serum ECP levels. A *P*-value < 0.05 was considered significant.

Results

The frequency distribution of serum ECP levels among the original 113 patients is presented in Fig. 1. A skewed distribution with the peak deviated to the left is observed. Geometric mean serum ECP levels in the low ECP group ($n = 37$) and the high ECP group ($n = 54$) are $8.2 \mu\text{g/L}$ (range 2.0 – $15.5 \mu\text{g/L}$) and $32.7 \mu\text{g/L}$ (range 16.2 – $105.0 \mu\text{g/L}$), respectively.

Table 1 shows the background and outcomes of the two asthmatic groups. The ratio of male patients is higher in the high ECP group than in the low ECP group, but this did not reach statistical significance. There was no significant difference between the two groups in age of disease onset, atopic and smoking status, disease severity, the prevalence of upper respiratory infection, use of inhaled steroid or its mean dosage, or use of theophylline. Also, neither PEF (% of personal best) nor FEV₁ (% predicted) on the day of ECP measurement differed.

Most patients were sensitized to house-dust mites, the most common perennial allergen in Japan. Only one out of 91 patients had seasonal asthma. The prevalent season for the seasonal allergens and the season of ECP sampling agreed in four out of 23 patients (17%) for the low ECP group and four out of 38 patients (10%) for the high ECP group. This was not significantly different ($P = 0.461$).

Serum IgE level and blood eosinophil count were both significantly lower in the low ECP group than in the high ECP group. Age was significantly higher and disease duration was significantly longer in the low ECP group compared with the high ECP group. When the two groups of patients were analysed together, there was no relationship between age and disease duration ($\rho = 0.14$, $P = 0.19$). A significant correlation was found, however, between age and serum IgE level ($\rho = -0.4$, $P = 0.0002$) and blood eosinophil count and serum ECP level ($\rho = 0.47$, $P < 0.0001$). Stepwise logistic regression analysis was performed to test for independent effects of age, disease duration and serum IgE level on low serum ECP levels. Low serum ECP levels were significantly associated with ageing or longer disease duration but not with serum IgE level (Table 2). These results did not change when gender was included as an additional independent factor; low serum ECP levels were not associated with gender.

Airway wall area/body surface area was significantly larger in the low ECP group compared with the high ECP group (Table 1). This index of airway wall thickness was not included in the logistic regression analysis because CT scanning was performed in only a small subset of patients.

Baseline ECP level was measured in 22 patients when

Table 3. Serum eosinophil cationic protein (ECP) level during exacerbation and at baseline

	<i>n</i>	ECP level ($\mu\text{g/L}$)		Exacerbation vs. baseline	
		Exacerbation	Baseline	<i>P</i> -value	ΔECP ($\mu\text{g/L}$)
High ECP group	15	37.0 ± 26.2	16.5 ± 11.6	0.004	$20.5 \pm 23.1^*$
Low ECP group	7	7.9 ± 3.5	5.7 ± 3.8	0.095	2.2 ± 2.9

* $P = 0.008$ vs. low ECP group.

they were stable and not receiving systemic steroids. In the high ECP group, ECP levels measured during exacerbation showed a significant increase compared with those measured with a stable condition. Such an increase was not evident in the low ECP group. The absolute increase of serum ECP level was significantly larger in the high ECP group than in the low ECP group (Table 3). When the 22 patients were divided into two arbitrary groups by age, seven patients ≤ 40 years old showed a trend toward a larger increase in ECP level during exacerbation than the 15 patients > 40 years old (26.8 ± 32.0 vs. 9.1 ± 10.3 $\mu\text{g/L}$, $P = 0.062$).

Discussion

In this study we measured serum ECP levels in 113 asthmatic patients during exacerbation. The ECP levels showed a skewed distribution with the peak deviated to the left as reported in healthy individuals [16] or random population samples that included asthmatic subjects [24]. Patients who had been given systemic steroids within 1 month prior to the study were excluded and data for the remaining 91 patients were analysed. Patients showing low ECP levels (≤ 16 $\mu\text{g/L}$; $n = 37$) were older, had longer-standing asthma and had lower levels of serum IgE compared with those with high levels of ECP (≥ 16 $\mu\text{g/L}$; $n = 54$). The intensity of airflow obstruction was similar in the two groups, suggesting a comparable severity of exacerbation. Multivariate analysis combining age, disease duration and serum IgE revealed that ageing and prolonged disease duration were independently associated with lower ECP levels. Airway wall thickness, assessed in a subset of patients through the use of CT, was significantly larger in the low ECP group compared with the high ECP group.

The effect of age on serum ECP levels in normal adults has been investigated in previous reports. Peterson *et al.* [16] studied 101 subjects (mean age 36 years; range 20–63 years) and found no age-dependent distribution in serum ECP level. In contrast, Marks *et al.* [24] demonstrated an inverse relationship between age and serum ECP level. Although the subjects investigated in the latter study were relatively young (i.e. samples from a population aged 20–44 years), this suggests that blood eosinophils of the elderly are less activated in physiological conditions than those of younger people. Our study, conducted in asthmatic patients with a wider age range and higher mean age has demonstrated for the first time that aged asthmatic patients might show lower ECP levels during exacerbation. In a rat model of asthma sensitized by ovalbumin, aged rats failed to accumulate eosinophils in bronchial lavage fluid 2 days after exposure to ovalbumin, while younger rats showed a marked increase of eosinophils [25]. This suggests that eosinophils are involved to a different degree in allergic

airway inflammation in aged and younger rats. However, there have been no human studies examining the intensity of eosinophilic airway inflammation in older asthmatic patients in comparison with that of younger patients with comparable disease activity or severity. It is possible that factors contributing to eosinophil production and activation (e.g. cytokines, such as IL-5 or granulocyte-macrophage colony-stimulating factor) are produced less in elderly asthmatic patients [25]. There has been no evidence, however, of an age-related decrease in the production of such eosinophilopoietic cytokines in humans. Involvement of 'non-eosinophilic' inflammation in aged asthmatic patients may also be a possible mechanism, but this is only speculation.

Longer duration of asthma was found to be another independent predictor of low ECP level during exacerbation of the disease. In addition, airway wall thickness assessed using CT, although performed in only a small subset of patients, was greater in the low ECP group than in the high ECP group. James *et al.* [26] analysed autopsied lungs using a computer model. They showed that airway wall thickening observed in asthmatic lungs, which may progress with prolonged disease duration [23,27], can profoundly affect airway narrowing caused by smooth muscle shortening [26]. This suggests that patients with longer duration of asthma have thicker airways and thus their condition is easily exacerbated by even minimal airway inflammation. Irreversible airflow obstruction, which develops in some patients with long-standing disease [28], may also lead to exacerbation of asthma in the absence of intense eosinophilic inflammation.

Smoking status was not related to serum ECP levels in our study. Two studies [24,29] that surveyed serum ECP levels in normal subjects showed higher ECP levels in smokers than in non-smokers. In contrast, Björnsson *et al.* [30] reported that serum ECP levels in a young asthmatic population were not related to smoking status, which was consistent with our result. As for atopic status, studies conducted in asthmatic children invariably show that atopic patients have significantly higher serum ECP levels than non-atopic patients, while adult asthmatics do not show such a tendency [6,7,18,31]. In our study, atopy was not related to serum ECP levels. The influence of seasonal variation on the serum ECP level seemed negligible in this study. Serum IgE levels were significantly higher in the high ECP group compared with the low ECP group. Logistic regression analysis revealed, however, that serum IgE levels were not related to serum ECP. Since an ageing-related decrease in serum IgE level has been suggested [32], higher IgE levels in the high ECP group may have been affected by the younger age in this group. Wever *et al.* [10] reported that patients with suspected 'infectious exacerbations' showed significantly lower serum ECP

levels than those without symptoms of respiratory infection. In our study, the prevalence of upper respiratory infection was not different between the two groups. The association between serum ECP level and gender in asthmatic patients is unknown. In our study, the high ECP group showed a trend toward a higher ratio of male patients than the low ECP group. Stepwise regression analysis revealed, however, that gender was not related to serum ECP levels.

Treatment with inhaled steroids decreases serum ECP levels in asthmatic patients [9,31,33]. As most patients included in our study were first-time visitors who were inhaled steroid-naïve, the mean dose of inhaled steroid was very low in both groups. The dose or prevalence of inhaled steroid usage was consequently not significantly different between the two groups. Long-term treatment with sustained-release theophylline may also reduce serum ECP levels in asthmatic patients [33]. In our study, the prevalence of theophylline usage did not differ between the two groups.

Compared with baseline values, serum ECP levels markedly and significantly increased during exacerbation in the high ECP group, while such an increase was not seen in the low ECP group. These results suggest that serum ECP levels changed in parallel with asthma activity in patients in the high ECP group, whereas it remained rather constantly low in the low ECP group.

In conclusion, the present study demonstrates for the first time that serum ECP levels have a tendency to remain low during exacerbation of asthma in patients who are aged, have longer disease duration and possibly have thicker airway walls. Mechanisms other than eosinophilic inflammation, such as airway wall remodelling, may be involved in asthma exacerbation in these patients, although further clarification of this issue is necessary. From a clinical standpoint, we should be aware that serum ECP may not be useful as a marker of disease activity when managing asthmatic patients who are aged or have longer disease duration.

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