

This study was undertaken to investigate the correlation between the serum ECP and the serum eotaxin level, and disease activity as evaluated with pulmonary function in patients with asthma or chronic obstructive pulmonary disease (COPD). 20 patients with stable asthma and 15 patients with COPD, and 15 subjects of the control group took part in this study. The analysis of ECP was performed according to the manufacturer's directions (Pharmacia Diagnostics AB, Uppsala, Sweden). The ELISA test was used to measure eotaxin levels in serum (kits from R&D, USA). The levels of ECP were  $16.9 \pm 6.3 \mu\text{g/L}$  in patients with asthma,  $15.1 \pm 9.3 \mu\text{g/L}$  in patients with COPD and  $11.8 \pm 6.2 \mu\text{g/L}$  in the control group ( $P < 0.05$ ). There was no significant difference in the asthma ECP level compared with the ECP level in COPD. There was a significant difference between the ECP plasma level in asthma compared with the ECP plasma level in the control group ( $p < 0.05$ ). The levels of eotaxin were  $175.8 \pm 49.3 \text{ pg/mL}$  in the control group. There was a correlation of ECP and the eotaxin level in asthma patients ( $r = +0.5$ ,  $p < 0.05$ ). The percentage fall in FEV1 correlated with eotaxin level in asthma ( $r = -0.3$ ,  $p < 0.05$ ) and with the eotaxin level in COPD ( $r = -0.5$ ,  $p < 0.05$ ). Serum outcomes of eotaxin and ECP levels appear to be a useful indicator of atopic asthma, and might provide complementary data disease monitoring. Therefore, further investigations are required to clarify whether serum eotaxin measurements have a role in the clinical evaluation in COPD.

**Key words:** Eotaxin, Eosinophil cationic protein, Asthma, COPD

## Eotaxin in serum of patients with asthma or chronic obstructive pulmonary disease: relationship with eosinophil cationic protein and lung function

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### Introduction

Asthma and chronic obstructive pulmonary diseases are a complex of conditions, which have airflow limitation in common.<sup>1</sup> The most characteristic features of asthma are short-term changes in the airway caliber, reversible airflow limitation, bronchial hyperresponsiveness as well as eosinophilic and lymphocytic airway inflammation. The common definition of COPD implies long-term changes in airway caliber, less or irreversible airflow obstruction, seldom or no bronchial hyperresponsiveness and neutrophilic airway inflammation. Some studies have suggested a pathogenic role for airway inflammation in inducing hypersecretion of mucus, lung destruction and airway obstruction.

Eosinophils are believed to be critical proinflammatory cells in airways mucosal damage in asthma by releasing highly toxic proteins. One of these proteins, eosinophil cationic protein (ECP), can be quantitated in bronchial secretion and peripheral blood. Serum ECP levels have been found to be correlated with the severity of asthma,<sup>2</sup> exercise-

induced bronchoconstriction<sup>3</sup> and allergen exposure.<sup>4</sup> It has been found that ECP can be used to monitor asthma inflammation<sup>5</sup> and may be a useful marker in selecting allergic patients with eosinophil related activation to allergen exposure.<sup>6</sup> Setta *et al.* however, found that the numbers of tissue eosinophils are markedly and significantly increased when there is an exacerbation of bronchitis, and that associated with the exacerbation their numbers are similar to those reported in stable asthma.<sup>7</sup> An increased level of ECP was observed in the sputum and bronchial lavage from patients with COPD.<sup>8,9</sup>

Several chemokines of the CC family, including eotaxin, RANTES, monocyte chemoattractant protein 3 (MCP-3) and MCP-4 have been shown to attract and activate eosinophils via high affinity binding to CCR3.<sup>10,11</sup> However, only eotaxin binds selectively to this receptor, which supposedly explains why eotaxin is relatively selective in eosinophil recruitment.<sup>12</sup>

The CC chemokine eotaxin is a potent and selective chemoattractant, which signals via the CCR3 receptor. It has been found to be a potent chemoattractant for human eosinophils and basophils as well as a

**Table 1.** FEV1 (% predicted, mean±SD)

	FEV1 before salbutamol	FEV1 after salbutamol
Asthma (n=20)	67.9±6.3 (57–80)	90.2±6.6 (55–70)
COPD (n=15)	63.9±5.1 (55–70)	68.9±3.6 (62–75)
Normal controls (n=15)	90±6.3 (78–100)	Not done
Statistical significance	Asthma vs. COPD, $p=0.05$ Asthma vs. control, $p<0.0001$ COPD vs. control, $p<0.0001$	Asthma vs. COPD, $p<0.0001$

stimulator of oxygen radical production,  $Ca^{2+}$  mobilization, actin reorganization, and CD11b up-regulation for human eosinophils.<sup>13,14</sup> The human eotaxin receptor, CCR3, is expressed on eosinophils, basophils, and TH2 cells.<sup>15</sup> Eotaxin, in association with TH1-derived cytokine IL-2 and TH2-derived cytokine IL-4, is an important T lymphocyte activator, stimulating the directional migration, adhesion, accumulation, and recruitment of T lymphocytes, in parallel with the accumulation of eosinophils and basophils during the process of certain types of inflammation such as allergy. The synergism between remotely acting IL-5 and locally acting eotaxin will induce a rapid recruitment of eosinophils in the allergen-stimulated tissue.<sup>16</sup> To our knowledge, there is no known role of this chemokine in the pathomechanism of asthma and COPD.

The aim of this study was to investigate the correlation between the serum ECP, serum eotaxin level and disease activity as evaluated with pulmonary function in patients with asthma or COPD.

## Material and methods

20 patients (11 women and 9 men; mean age  $27.7 \pm 4.8$ ) with asthma, 15 (6 women and 11 men, mean age  $60.9 \pm 8.1$ ) with COPD and 15 subjects (8 women and 7 men, mean age  $36.8 \pm 10.5$ ) from the control group took part in this study.

The patients with asthma showed the following characteristics: one or more positive skin weal-and-flare response to skin prick testing with a range of local allergens, history indicating several episodes of bronchoconstriction over a period of at least 2 years, and in relation to the exposure to one or more of those allergens, reversible airflow obstruction with a > 20% increase in the forced expiratory volume 1 s (FEV1) after inhaled  $\beta_2$ -receptor agonists (400  $\mu$ g salbutamol) and airway hyperresponsiveness, as demonstrated by a cumulative histamine provocation dose causing a 100% increase in expiratory resistance (PD100Raw) on more than one occasion during the previous 30 days. None of the tested patients was a current smoker. They were stable at the time of testing, requiring only intermittent use of short-acting  $\beta_2$  receptor agonists. The subjects with COPD had

not been hospitalized for the exacerbation of the illness and met the following criteria: chronic sputum production, cough, low FEV1/VC ratio, increase in FEV1 following inhaled  $\beta_2$ -agonist of <12%, no history of seasonal or episodic dyspnea, wheezing or atopy, current cigarette smoking without occupational or other exposure known to cause chronic bronchitis, and no therapy with oral or inhaled glucocorticosteroids within 3 months of entry into the study. All of the control subjects were lifelong non-smokers, with normal spirometry and with no history of atopy.

Each study subject had pulmonary function studies including vital capacity, forced expiratory volume in 1 s (FEV1), forced expiratory flow at 50% (FEF50) and expiratory resistance (Raw). Spirometry was performed with Pneumoscreen equipment between 08:00 and 09:00 am.

Blood drawing, handling and storage were performed by the same researcher in the same department. No significant variation in temperature was observed between the room where blood was drawn and the laboratory where the preparation and storage of the serum took place. The blood samples were centrifuged at  $1300 \times g$  at  $4^\circ C$  for 10 min, stored at  $-70^\circ C$  until measurements of the ECP and eotaxin were taken. The analysis of ECP was performed according to the manufacturer's directions (Pharmacia Diagnostics AB, Uppsala, Sweden). The detection limit of this kit is 2.0  $\mu$ g/L. The analysis was performed blindly and each sample measurement was performed in duplicate.

An ELISA system sensitive enough to detect 5 pg/ml of human eotaxin was used to determine eotaxin serum level (kits from R&D, USA). The variation in the measured eotaxin levels was always less than 3% when samples were analyzed in duplicate on an ELISA plate.

The study was approved by the ethics committee of our hospital, and written informed consent was obtained from all participating subjects.

## Statistical analysis

Statistical analysis of the data was performed by the SPSS Window 5.0 statistics program. Results were

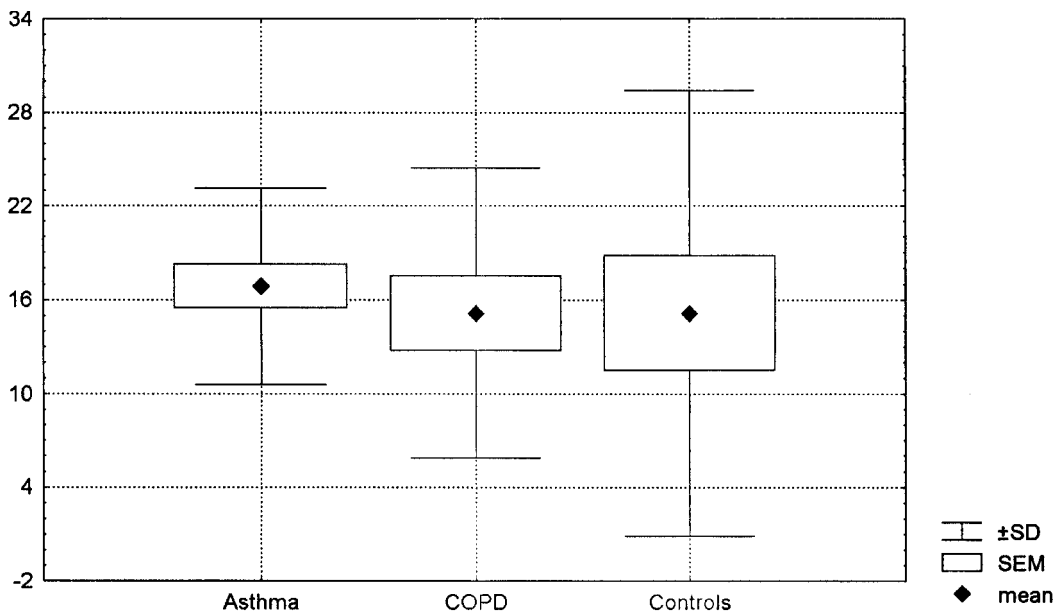


FIG. 1. Serum ECP ( $\mu\text{g/L}$ ) in asthma, COPD, and controls.

expressed as mean  $\pm$  SD. Correlations among variables were determined with an analysis to give the Pearson's rank correlation coefficient. A  $p$  value of less than 0.05 was considered significant.

## Results

The mean levels of expired volumes after 1 s (FEV1) are shown in Table 1. FEV1 was significantly reduced in patients with asthma and COPD compared to the control group ( $p < 0.05$ ). All of the patients studied had detectable levels of plasma ECP and eotaxin.

The levels of ECP were  $16.9 \pm 6.3 \mu\text{g/L}$  in patients with asthma,  $15.1 \pm 9.3 \mu\text{g/L}$  in patients with COPD and  $11.8 \pm 6.2 \mu\text{g/L}$  in the control group ( $p < 0.05$ ) (Fig. 1). There was no significant difference in the asthma ECP level compared with the ECP level in COPD. There was a significant difference between the ECP plasma level in asthma compared to the ECP plasma level in the control group ( $p < 0.05$ ).

The levels of eotaxin were  $175.8 \pm 49.3 \text{ pg/mL}$  in asthma patients,  $286.0 \pm 101.4 \text{ pg/mL}$  in COPD patients and  $109.6 \pm 56.1 \text{ pg/mL}$  in the control group (Fig. 2). There were significant differences in the asthma eotaxin level as compared to COPD

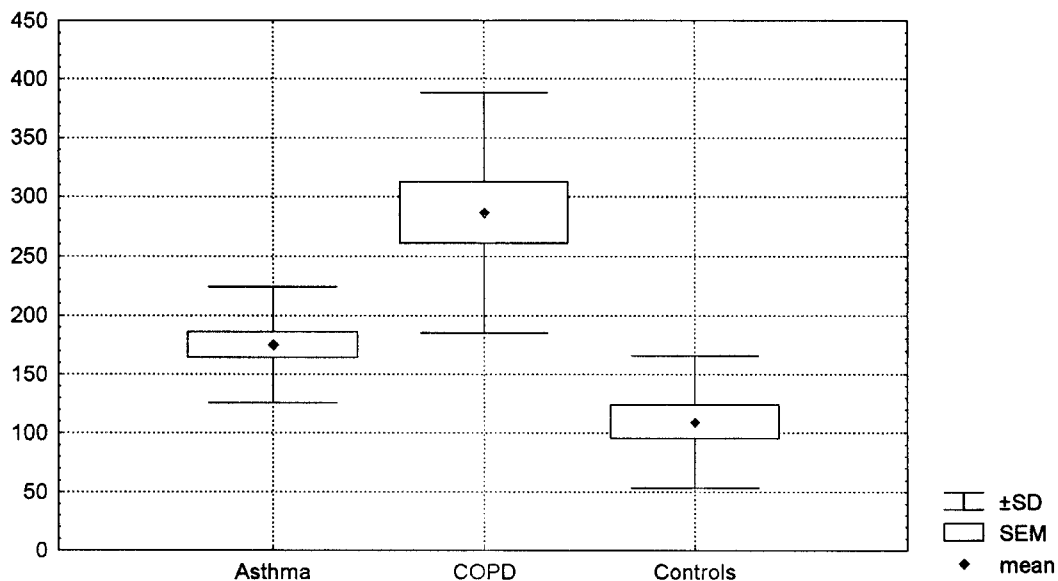


FIG. 2. Serum eotaxin ( $\text{pg/mL}$ ) in asthma, COPD and controls.

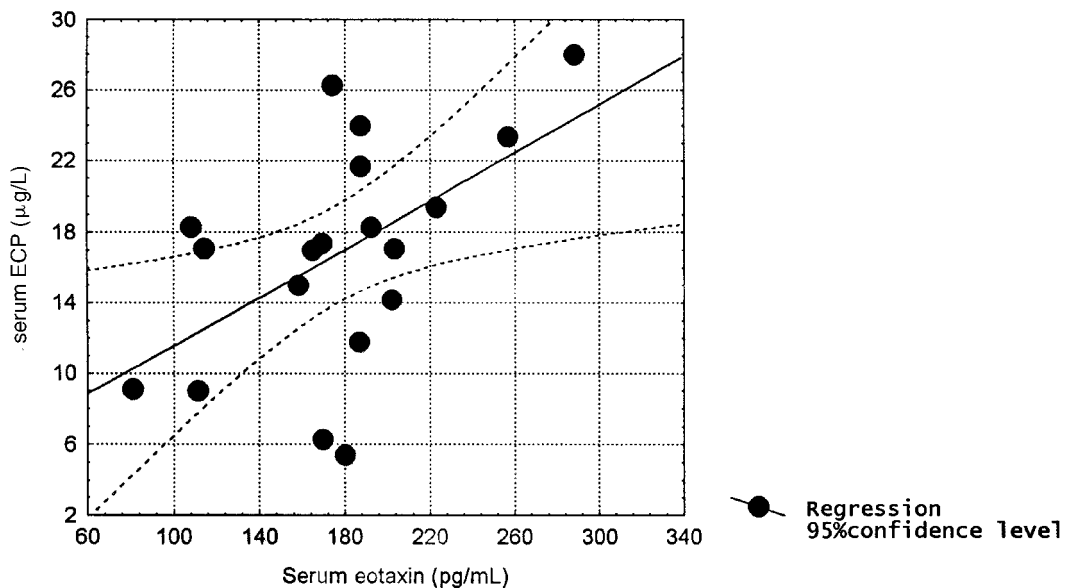


FIG. 3. Relationship between ECP and eotaxin levels in asthma patients. Corr. coeff. = +0.5.

( $p < 0.0001$ ), asthma as compared to the control group ( $p < 0.0001$ ), and COPD as compared to the control group ( $p < 0.0001$ ).

There was correlation of the ECP and the eotaxin level in asthma patients ( $r = +0.5$ ,  $p < 0.05$ ) (Fig. 3). The percentage fall in FEV1 correlated with the eotaxin level in asthma ( $r = -0.3$ ,  $p < 0.05$ ). There was correlation of the percentage fall in FEV1 with the eotaxin level in COPD ( $r = -0.5$ ,  $p < 0.05$ ).

## Discussion

To our knowledge, this is the first time that an increase of eotaxin and ECP levels in serum of patients with asthma and COPD as well has been demonstrated. The study documented that serum eotaxin levels were significantly higher in COPD patients than in asthma patients and control subjects. Moreover, the current investigation has shown that serum eotaxin levels are inversely related to the lung function (FEV1) in patients with asthma or COPD. However, only in asthma patients was significant correlation between ECP and eotaxin levels found.

The ELISA test is an accurate and useful method for quantifying human eotaxin in the blood, but the process of preparing blood samples affects the measurement of eotaxin levels.<sup>17</sup> The previous study (Ref. 17) demonstrated that eotaxin levels in serum samples were significantly higher than those in matched plasma samples separated from the blood collected in tubes containing EDTA.

Since the serum measurement of ECP is relatively easy to perform and non-invasive, the usefulness of serum ECP as a clinical marker in asthma has recently been studied.<sup>6,18</sup> The serum ECP level has been shown to be correlated with all the indices of

eosinophilic inflammation or asthma activity, suggesting that serum ECP might be a very useful marker in the disease.<sup>18</sup> In asthmatic patients, the serum ECP level demonstrated correlation with the number or percentage of eosinophils in BALF, and tissue, whereas the blood eosinophil count correlated only with the percentage of eosinophils in BALF. Our study showed that the serum level of ECP correlated with the elevated serum level of eotaxin. Elevated levels of eotaxin protein and mRNA have been demonstrated in the airways of asthmatic patients at baseline<sup>19</sup> and after allergen inhalation.<sup>20</sup> Moreover, eotaxin level and eosinophils increased at the onset of a viral respiratory infection in guinea pig bronchoalveolar lavage.<sup>21</sup> Lilly *et al.* found higher plasma eotaxin levels in patients with acute asthma.<sup>22</sup> Acutely ill asthmatic subjects who did not respond to an emergency treatment had higher eotaxin levels than did those admitted to hospital or those discharged home. To our knowledge no studies have been conducted to investigate serum eotaxin levels in COPD patients. No study has also been performed on the correlation between the serum ECP level and the serum eotaxin level in COPD. Interestingly, the increase of eosinophils in the exacerbation of bronchitis is reported not to be accompanied by increases in the number of inflammatory cells showing immunoreactivity for IL-5 protein.<sup>23</sup> Zhu *et al.* (unpublished) have, however, recently found that airways resected from smokers' lungs do demonstrate marked gene expression for IL-4 and IL-5, and that is, in particular, associated with the bronchial glands of subjects with chronic hypersecretion. The observation of the occurrence of both IL-4 and IL-5 gene expression in COPD, questions further the distinctions between asthma and COPD and presents us with a new challenge for further

research. In conclusion, we found a significantly higher serum eotaxin level in patients with COPD compared to patients with asthma. However, the outcomes of serum eotaxin and ECP levels appear to be a useful indicator of atopic asthma, and might provide complementary data in disease monitoring. Further longitudinal investigations should establish the role of measuring serum eotaxin in the clinical evaluation and monitoring of asthma, in particular, of COPD patients.

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