

# Serum Eosinophil Cationic Protein Level and Disease Activity in Childhood Rhinitis

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Rhinitis, a significant cause of morbidity worldwide, is an inflammatory condition of the mucosal linings of the nose that may be mediated by allergic or non-allergic mechanisms. In a genetically predisposed person, exposure to certain substances can lead to the generation of IgE antibodies against a specific allergen. On re-exposure, the allergen binds to IgE antibodies causing mast cell degranulation, which releases a number of mediators, such as histamines, prostaglandins, and leukotrienes.<sup>1</sup> During the subsequent late phase, the eosinophils are the most characteristic cells of the inflammatory reaction.<sup>2</sup> Patients with rhinitis develop symptoms of rhinorrhea, sneezing, nasal obstruction, as well as itchy nose and eyes. Allergic rhinitis is diagnosed by a positive skin prick test or positive specific IgE tests *in vitro*, which are relevant to aeroallergens, such as house dust mites, moulds, cockroaches, domestic animal dander, and so on. In clinical practice, however, approximately fifty percent

**SUMMARY** In order to investigate clinical markers of disease activity in childhood rhinitis, we compared various laboratory parameters to symptom scores of children with allergic rhinitis and non-allergic rhinitis. We measured the levels of serum total IgE, aeroallergen specific IgE, serum eosinophil cationic protein (ECP), and blood eosinophil counts in 71 children with allergic rhinitis and in 62 children with non-allergic rhinitis. We found a statistically significant difference in peripheral blood eosinophil counts between the allergic rhinitis and non-allergic rhinitis groups. Serum ECP levels were positively related to symptom scores in both groups. Peripheral blood eosinophil counts correlated with symptom scores only in the group with non-allergic rhinitis. The results demonstrated that serum ECP levels are of value in assessing disease activity in both allergic and non-allergic rhinitis, and peripheral blood eosinophil counts may play a role in the evaluation of symptom severity in non-allergic rhinitis.

of patients with symptoms of rhinitis are diagnosed with non-allergic rhinitis,<sup>3</sup> which is characterized by nasal congestion of unknown cause where IgE-mediated mechanisms cannot be demonstrated.<sup>4,5</sup> Patients with non-allergic rhinitis have a negative skin prick test or negative specific IgE *in vitro* tests that are relevant to aeroallergens.<sup>3</sup>

Although the pathogenesis of rhinitis, allergic and non-allergic, has been well studied,<sup>6-8</sup> the literature contains only few studies that focus on the relationship between

disease severity and relevant parameters, and furthermore some of these studies are conflicting.<sup>9,10</sup> In order to determine a disease-specific marker, we investigated serum total IgE, aeroallergen specific IgE, peripheral blood eosinophil counts, and serum eosinophil cationic protein (ECP), which is a mediator released from eosinophil granules, in

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children with allergic and non-allergic rhinitis. The aim of this study was to analyze the relationship between these parameters and the symptom scores.

## MATERIALS AND METHODS

### Subjects

We evaluated one hundred and thirty-three children with rhinitis (83 males and 50 females) with a mean of age of 6.54 years (range: 2-18 years) prospectively from July 2000 to November 2001. Each subject presented with at least three of the following symptoms: sneezing, rhinorrhea, nasal obstruction, and itching nose and eyes. Based on the results of the serum total IgE and aeroallergen specific IgE tests, the subjects were classified into two groups: Group I, subjects with allergic rhinitis, and Group II, subjects with non-allergic rhinitis. Subjects with a history of rhinitis whose testing showed a high level of total IgE ( $\geq 100$  kU/l) and positive specific IgE to at least one of the relevant aeroallergens ( $\geq$  class 2 in CAP system) were classified into Group I, allergic rhinitis. Those subjects, who did not meet the criteria for classification into Group I, were classified into Group II, non-allergic rhinitis.

### Symptom scores

Disease severity was assessed using symptom scores, which were determined at the same time as blood sampling and by the same investigator who conducted the blood sampling. We evaluated five symptoms of rhinitis including sneezing, rhinorrhea, nasal obstruction, as well as itching nose and eyes. Each symptom was graded on a four-point

scale as follows: 0 = no symptoms; 1 = slight symptoms (symptoms definitely present, but minimal); 2 = moderate symptoms (symptoms present only part of the day, but bothersome); 3 = severe symptoms (symptoms present most of the day, causing significant interference in the patient's life).

### Peripheral blood eosinophil counts

Peripheral blood eosinophil counts were determined using Sysmex SE 9000, an automated hematology analyzer. The total white blood cell counts (per  $\mu$ l) were then multiplied by the percentage of eosinophils.

### Serum total IgE and specific IgE

Venous blood was collected and centrifuged at  $800 \times g$  for 10 minutes. Serum samples were stored at  $4^{\circ}\text{C}$  before testing. Total IgE, and aeroallergen specific IgE to *Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*, Bermuda grass, cockroach, cat dander, dog dander, ragweed, Eucalyptus, and moulds were measured using the Pharmacia CAP system (Pharmacia & Upjohn Diagnostics AB, Uppsala, Sweden). IgE levels were described in kU/l. The upper total IgE limit was 1000 kU/l. The specific IgE cutoff value was 0.35 kU/l and the upper limit 100 kU/l. Aeroallergen specific IgE levels equal to or greater than 0.70 kU/l (class 2) were considered positive. Samples exceeding the upper range of total IgE or specific IgE were retested in 1:4 dilutions.

### Eosinophil cationic protein (ECP)

Because of limitations regarding blood sampling from some

of the patients, ECP levels were determined only in 74 patients (38 patients in Group I and 36 patients in Group II). Venous blood was allowed to clot at room temperature for a half hour after blood sampling and then centrifuged at 1,500 rpm for 10 minutes. The serum was immediately frozen at  $-20^{\circ}\text{C}$  and stored until ECP analysis, which was done by radioimmunoassay (RIA), according to the manufacturer's instructions (Pharmacia, Uppsala, Sweden). The manufacturer suggested a mean value of 4.4  $\mu\text{g/l}$  for apparently healthy adults with a normal upper limit of 16  $\mu\text{g/l}$ . The detection limit of this assay was 2.0  $\mu\text{g/l}$ .

### Statistical analysis

The values of the parameters were presented as mean  $\pm$  SD and 95% confidence interval (CI). SPSS<sup>®</sup> computer software was used for statistical evaluation. The statistical significance of the differences was assessed by unpaired t-test analysis. The relationship between the various parameters and the symptom scores in both groups was described by Pearson's correlation coefficient. The  $p$  values below 0.05 were indicative of a statistical significance.

## RESULTS

Seventy-one children were enrolled in Group I (mean age:  $7.38 \pm 3.90$  years; males = 46, females = 25), and 62 children were enrolled in Group II (mean age:  $5.58 \pm 2.81$  years; males = 37, females = 25). Disease severity, which was presented in symptom scores, was  $7.63 \pm 2.94$ , 95% CI 6.95 to 8.32 in Group I and  $6.26 \pm 3.17$ , 95% CI 5.47 to 7.05 in Group II. Peripheral

blood eosinophil counts, serum levels of total IgE, and serum ECP levels were measured in the two groups, as shown in Table 1. Serum levels of total IgE were  $553.76 \pm 559.32$  kU/l, 95% CI 423.67 to 683.86 kU/l in Group I and  $42.97 \pm 26.65$  kU/l, 95% CI 36.34 to 49.60 kU/l in Group II. Peripheral eosinophil counts and eosinophil percentage were higher in Group I than in Group II ( $484.13 \pm 293.58$  cells/ $\mu$ l, 95% CI 415.84 to 552.41 cells/ $\mu$ l, *versus*  $226.65 \pm 195.85$  cells/ $\mu$ l, 95% CI 177.89 to 275.40 cells/ $\mu$ l,  $p < 0.001$ ;  $6.50 \pm 4.20$  % 95% CI 5.52 to 7.48% *versus*  $3.13 \pm 2.87$ %, 95% CI 2.42 to 3.84%,  $p < 0.001$ , respectively). Serum ECP

levels had a tendency to be higher in allergic children than in non-allergic children, but the differences were not statistically significant ( $13.64 \pm 9.47$   $\mu$ g/l, 95% CI 10.63 to 16.65  $\mu$ g/l *versus*  $9.47 \pm 10.67$   $\mu$ g/l, 95% CI 5.99 to 12.96  $\mu$ g/l,  $p = 0.079$ ). The relationships between the parameters and symptom scores are shown in Table 2. The levels of total IgE had no significant relationship with the symptom scores in the allergic or the non-allergic group ( $r = 0.105$ ,  $p = 0.125$  in Group I and  $r = -0.020$ ,  $p = 0.194$  in Group II). Peripheral blood eosinophil counts were associated with symptom scores only in Group II ( $r = -0.083$ ,  $p = 0.476$  [Group I]

*versus*  $r = 0.266$ ,  $p = 0.041$  [Group II] as shown in Fig. 1). Serum ECP levels correlated with symptom scores in both groups ( $r = 0.393$ ,  $p = 0.019$  and  $r = 0.347$ ,  $p = 0.043$ , respectively, shown in Fig. 2). In the study of aeroallergen specific IgE, 98.6 percent of Group I patients had a positive specific IgE response to *Dermatophagoides pteronyssinus* and 89 percent of Group II patients had a positive specific IgE response to *Dermatophagoides farinae*. In these patients, the serum specific IgE levels to these aeroallergens did not significantly correlate with the symptom scores ( $p > 0.05$ , as shown in Table 2.).

**Table 1** Background and outcomes of Groups I and II patients

|  | Allergic rhinitis                             | Non-allergic rhinitis                        | <i>p</i> |
|--|---|--|----------|
| <b>Number of subjects</b>                    | 71  | 62   |          |
| <b>IgE (kU/l)</b>                            | $553.76 \pm 559.32$<br>(423.66 - 683.86)      | $42.97 \pm 26.65$<br>(36.34 - 49.60)         |          |
| <b>Eosinophils (%)</b>                       | $6.50 \pm 4.20$<br>(5.52 - 7.48)              | $3.13 \pm 2.87$<br>(2.42 - 3.84)             | < 0.001  |
| <b>Eosinophils (cells/<math>\mu</math>l)</b> | $484.13 \pm 293.58$<br>(415.84 - 552.41)      | $226.65 \pm 195.85$<br>(177.89 - 275.40)     | < 0.001  |
| <b>ECP (<math>\mu</math>g/l)</b>             | $13.64 \pm 9.47$ (N = 38)*<br>(10.63 - 16.65) | $9.47 \pm 10.67$ (N = 36)*<br>(5.99 - 12.96) | 0.079    |

Values are given as means  $\pm$  SD (95% confidence interval).

\*Serum ECP levels were measured in 38 children with allergic rhinitis (Group I) and 36 children with non-allergic rhinitis (Group II).

**Table 2** The correlations between various parameters and symptom scores in allergic rhinitis and non-allergic rhinitis patients

|                                       | Allergic rhinitis |                | Non-allergic rhinitis |                |
|---------------------------------------|-------------------|----------------|-----------------------|----------------|
|                                       | <i>r</i>          | <i>p</i> value | <i>r</i>              | <i>p</i> value |
| <b>IgE vs. Score</b>                  | 0.105             | 0.125          | -0.020                | 0.194          |
| <b>Specific IgE to D.p. vs. Score</b> | 0.005 (N = 54)    | 0.314          |                       |                |
| <b>Specific IgE to D.f. vs. Score</b> | -0.180 (N = 48)   | 0.574          |                       |                |

A *p*-value < 0.05 is considered statistically significant.

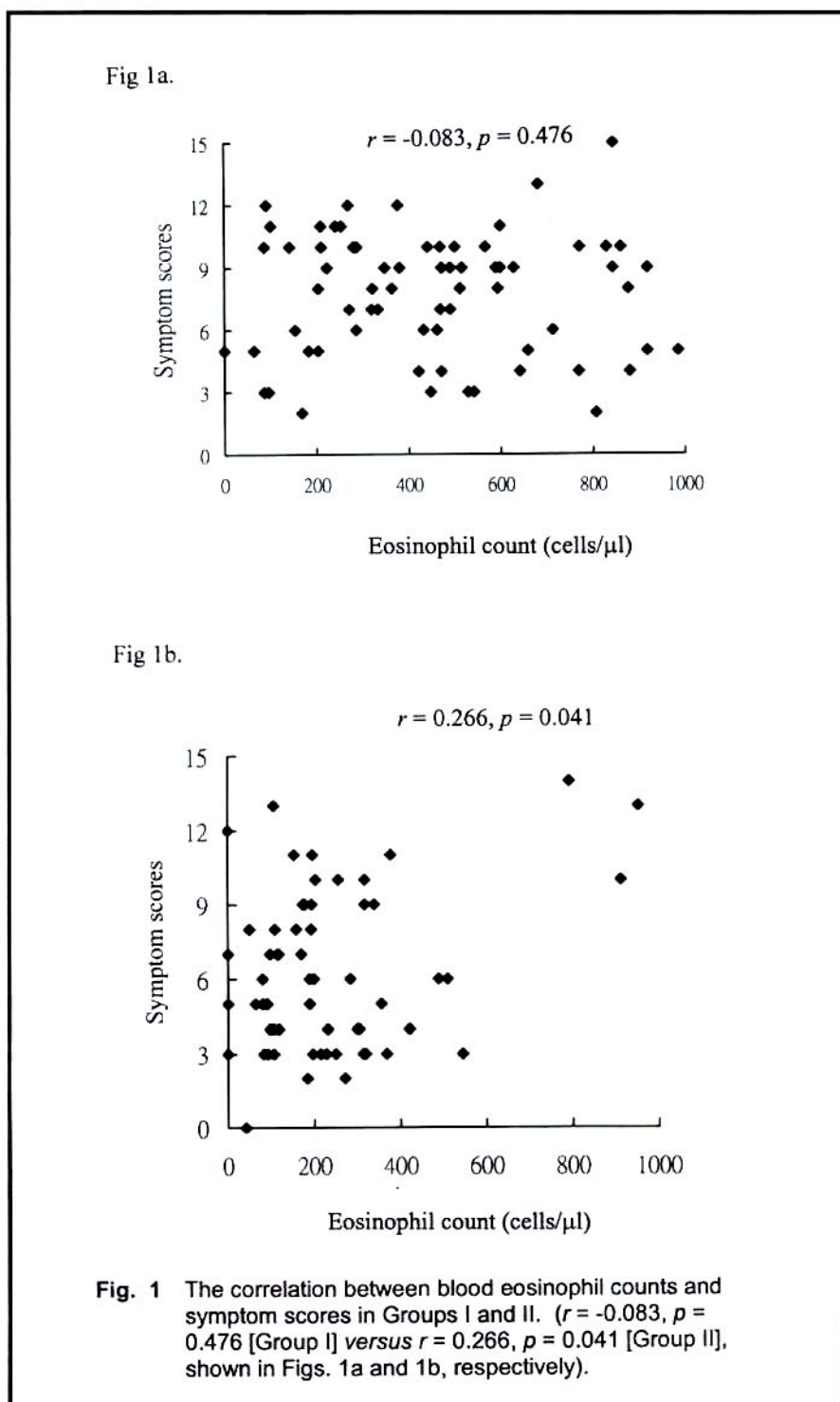
D.p. and D.f. refer to *Dermatophagoides pteronyssinus* and *Dermatophagoides farinae*, respectively.

Levels of IgE had no correlation with either blood eosinophil counts or serum ECP levels ( $r = 0.081$ ,  $p = 0.225$  and  $r = -0.121$ ,  $p = 0.387$ , respectively). However, serum ECP levels were positively related to blood eosinophil counts in both groups ( $r = 0.384$ ,  $p = 0.023$  in Group I and  $r = 0.475$ ,  $p < 0.01$  in Group II).

## DISCUSSION

Serum total IgE, allergen specific IgE antibodies, blood eosinophils, and ECP (which is released by activated eosinophils) are associated with the pathogenesis of allergic rhinitis. Patients whose histories contain either a negative skin test or a negative specific IgE test to relevant aeroallergens and normal serum IgE are considered to have non-allergic rhinitis.<sup>3</sup> Non-allergic rhinitis is caused by various etiologies such as vasomotor rhinitis, non-allergic rhinitis with eosinophilia (NARES syndrome),<sup>10</sup> drug-induced rhinitis (such as topical decongestants, clonidine, alcohol, estrogens, and aspirin), nasal polyps, nasal irritants (such as smoke and noxious fumes), atrophic rhinitis, and certain metabolic states (such as pregnancy and hypothyroidism).<sup>3,11</sup>

We attempted to determine whether serum total IgE levels, aeroallergen specific IgE levels, blood eosinophil counts, and serum ECP levels were related to the severity of nasal symptoms in children with allergic rhinitis and non-allergic rhinitis. In our study, serum total IgE levels did not show a close correlation with symptom severity in children with allergic rhinitis or non-allergic rhinitis. Serum specific IgE levels did not correlate



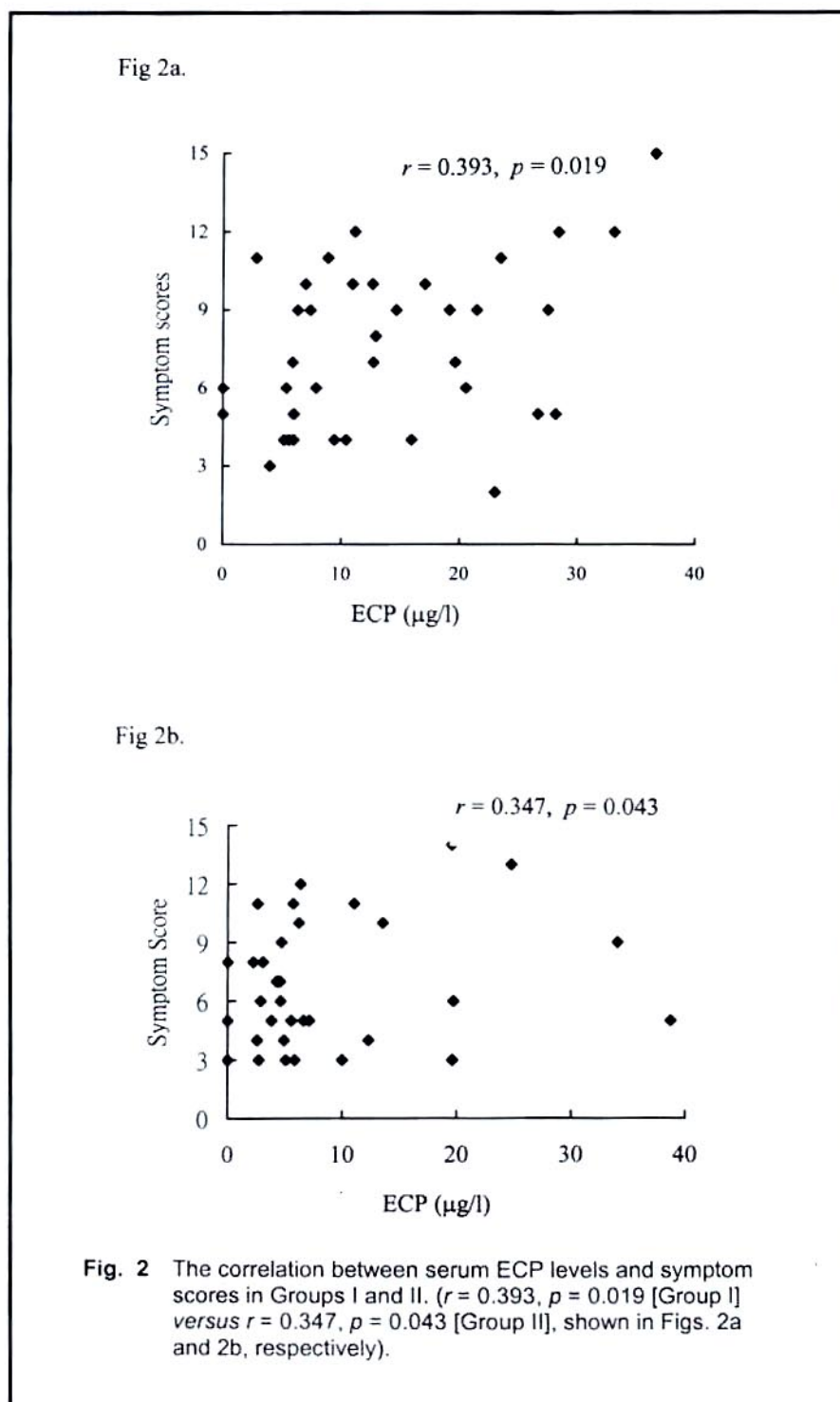
with the symptom scores of patients with allergic rhinitis. However, blood eosinophil counts correlated statistically with symptom scores in patients with non-allergic rhinitis instead of patients with allergic rhinitis.

This correlation suggests that eosinophils could play an important role in assessing disease activity in non-allergic rhinitis, as eosinophils not only play a critical role in the pathogenesis of allergic rhinitis, but

they may also be involved in the inflammatory process of non-allergic rhinitis. Another possible reason for the correlation is that out of all patients with non-allergic rhinitis a large proportion of patients might have the non-allergic rhinitis with eosinophilia syndrome (NARES syndrome), which is diagnosed by eosinophil counts higher than 10 percent of the overall granulocyte counts in the nasal secretion.

Serum eosinophil cationic protein (ECP) levels reflect ongoing eosinophilic airway inflammation.<sup>12</sup> It has been reported that ECP causes damage to the respiratory epithelium<sup>13</sup> and enhances responsiveness to histamines in the nasal mucosa.<sup>14</sup> A number of studies have indicated that serum ECP levels correlate with the asthma symptom score, pulmonary function, and bronchial hyper-responsiveness; thereafter, the ECP level has been used as a marker for asthmatic activity.<sup>15-20</sup> However, some reports have failed to reveal such relationships.<sup>12,21-23</sup> Studies concerning the relationship between serum ECP levels and rhinitis severity have been rare and controversial.<sup>8,9</sup> A positive relationship between serum ECP levels and rhinitis severity was found in both allergic rhinitis and non-allergic rhinitis in our study. Beppu *et al.*<sup>24</sup> reported that ECP levels in serum and nasal secretions were significantly higher in patients with allergic rhinitis than in healthy controls. Our study also showed that serum ECP levels were higher in children with allergic rhinitis than in those with non-allergic rhinitis; however, due to the small sample size, this difference was not statistically significant.

In previous reports, a positive correlation was noticed between the



serum ECP concentration and peripheral eosinophil counts.<sup>24-27</sup> In our study, we also found that in patients with allergic rhinitis and non-allergic rhinitis, serum ECP levels correlated with blood eosinophil counts.

However, other reports did not find such a correlation.

In summary, we conclude that serum ECP levels might be of value in assessing the disease activity of

allergic rhinitis and non-allergic rhinitis. Blood eosinophil counts may play a role in the evaluation of symptom severity in childhood non-allergic rhinitis. We also found a positive correlation between serum ECP levels and blood eosinophil counts in patients with allergic as well as non-allergic rhinitis.

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