

Eosinophil Cationic Protein in Nasal Secretions and Blood Serum in Grass-Pollen Allergic Rhinitis

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ABSTRACT

Concentration of the Eosinophil Cationic Protein (ECP) measured in different body fluids has been demonstrated to be a good marker of eosinophilic inflammation. In allergic asthma, ECP levels in both broncho-alveolar-lavage (BAL) fluid and serum can be used to monitor disease activity. In allergic rhinitis, the value of ECP determinations in serum and nasal secretions was not directly compared, so far. In the present study, ECP levels in blood serum (Se) and nasal secretions (NS) of grass pollen allergic and healthy individuals under pollen exposure were analyzed. Forty-three grass-pollen allergic subjects and 19 healthy volunteers were included. Grass-pollen counts were measured using Burkhard traps and a symptom scoring performed. In the allergic subjects, the mean ECP concentration in nasal secretions was 789 ± 515 ng/mL compared with 12.1 ± 7.4 ng/mL in serum; in the healthy volunteers it was 29.6 ± 15.4 ng/mL in NS compared to 9.4 ± 6.9 ng/mL in Se. ECP concentration in NS was significantly higher in allergic than in healthy subjects ($P < 0.001$) and in both significantly higher than in Se ($P < 0.01$). ECP concentration in Se did not differ significantly in patients and healthy volunteers ($P > 0.05$). In the allergic subjects, ECP concentration in NS but not in Se was closely correlated to symptom score and pollen exposure. We conclude that ECP levels in NS are a better marker of eosinophilic inflammation in allergic rhinitis than ECP serum levels. (American Journal of Rhinology 10, 319–322, 1996)

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Allergic rhinitis is an inflammatory condition of nasal mucosa. Following the early allergic response, a late phase reaction with mainly cellular involvement may occur. During the late phase reaction, an allergen-induced nasal hyperreactivity has been observed. This follows the influx of various inflammatory cells, especially eosinophils.^{1,3,7,10,12,15,25}

Activated eosinophils are capable of releasing granular proteins, such as major basic protein (MBP), eosinophil peroxidase (EPO), eosinophil-derived neurotoxin (EDN), and eosinophil cationic protein (ECP). ECP is a particularly good and specific indicator of eosinophil activity, since the content of this protein in other cells is negligible.²⁵

In asthmatic patients, ECP-levels in both broncho-alveolar-lavage (BAL) fluid and serum are closely correlated with disease activity.^{1,17,20,21} Asthma activity can thus be monitored with routinely obtained blood samples. In allergic rhinitis, data directly comparing ECP levels in blood serum (Se) and nasal secretions (NS) are missing, so far. In the present study, we compared ECP levels in Se and NS of grass pollen allergic and healthy individuals. It was of interest whether severity of nasal disease can be equally assessed measuring ECP in Se or NS.

MATERIALS AND METHODS

A total of 43 grass-pollen allergic subjects (23 females, 20 males; mean age 30.2 years, range 18–49 years) with history of at least 2 years of allergic rhinitis requiring medical treatment and positive skin test and/or RAST ($\geq$ class II) to grass-pollen were included.

Exclusion criteria were the coexistence of nonallergic nasal disease, nasal allergy to perennial allergens, chronic disease other than atopic allergy, pregnancy, immunotherapy for allergy during the last 3 years, antiallergic therapy at study entry, refused consent, age under 18, or over 60 years.

Patients were recruited in January and February 1993 and

were asked to contact the study coordinator at the onset of allergic symptoms. At this contact, a date for the sampling procedure 3 weeks later was scheduled. Symptom scoring was performed daily in a diary card from the first day of symptoms. All patients were willing not to take antiallergic drugs during a period from the onset of symptoms until the sampling date. The control group consisted of 19 healthy volunteers (9 females, 10 males; mean age 26.9 years, range 19–43 years) without history or clinical findings of allergic rhinitis.

Nonallergic nasal disease was excluded in every participant by nasal endoscopy,¹⁴ and A-scan-sonography of the paranasal sinuses.¹³ Informed consent was obtained from each patient before the study. The study was approved by the regional ethics committee.

Sampling of Serum and Nasal Secretions

Blood was collected by venipuncture from a brachial vein and allowed to clot at room temperature for 60 minutes. Serum was separated by centrifugation at 1350 g for 10 minutes twice. Nasal secretions were collected using standard size (19 mm × 19 mm × 4 mm) absorbent rubber-foam samplers. They were placed under rhinoscopic control between septum and head of the inferior turbinate on both sides. After 10 minutes they were put into laboratory tubes on a platform and centrifuged at 2000 g for 15 minutes. The nasal secretions were squeezed out, and the clear sol phase was separated from mucus and cells and pipetted into another tube.⁶ Serum and nasal secretions were immediately frozen and stored at -20°C until laboratory investigations.

ECP Measurement

Eosinophil cationic protein (ECP) was measured in nasal secretions and serum with a double-antibody radioimmunoassay (Pharmacia, Freiburg, Germany) with polyclonal rabbit antibody as originally described by Venge et al.²⁴ This assay has a sensitivity of 2 ng/dL, and the intra-assay variation coefficient is approximately 5% in the employed range.¹⁸ All samples were subject to duplicated measurements. Laboratory quality control was assured by control measurements at the Pharmacia reference laboratory at Freiburg, Germany.

Samples containing over 200 ng/mL were diluted with zero standard, because 200 ng/mL is the highest standard available in the test assay.

Symptom Scores and Grass-Pollen Count

All patients scored their daily symptoms of nasal obstruction, itching, sneezing, secretion, and eye symptoms on a 4-point scale (zero = no symptoms; 1 = mild symptoms; 2 = moderate symptoms; 3 = severe symptoms).

For grass-pollen count, a Burkhard trap was used by courtesy of the German Pollen Information service. Pollen counts were expressed as the mean daily number of pollen grains per cubic meter of filtered air. A modified classifi-

cation system according to Kersten⁹ was used to express the mean pollen concentration in the 3 days before the sampling procedure (class 1 = 0–3 pollen grains/m³; class 2 = 4–20 pollen grains/m³; class 3 = 21–50 pollen grains/m³; class 4 = 50–100 pollen grains/m³; class 5 > 100 pollen grains/m³).

Statistics

Correlation of ECP levels in NS and Se with symptom score and pollen count, respectively, was assessed using Spearman's R. Probability of correlation was evaluated using Spearman's rank correlation test.

RESULTS

A total of 47 patients and 19 volunteers were recruited for the study. Four patients were excluded from the study due to the use of antiallergic medication. Thus, a total of 43 patients and 19 volunteers were evaluated. From these 43 patients, 40 did contact the study coordinator spontaneously between May 10th and May 20th, 1993 because of onset of allergic symptoms. The remaining three patients were contacted by the study coordinator up to May 25th. All sampling procedures took place approximately 3 weeks after this contact, between May 31 and June 14, 1993. At the day of sampling, all patients suffered from at least 3 weeks of documented symptoms of allergic rhinitis-like rhinorrhea, nasal itching, sneezing, and nasal congestion.

In the allergic subjects, the mean ECP concentration in NS was 789 ± 515 ng/mL compared to 12.1 ± 7.4 ng/mL in Se; in the healthy volunteers it was 29.6 ± 15.4 ng/mL in NS compared to 9.4 ± 6.9 ng/mL in Se.

Thus, ECP concentrations in nasal secretions were significantly higher in allergic than in healthy subjects ($P < 0.001$). In the allergic subjects, it was significantly higher in NS than in Se ($P < 0.01$). ECP concentration in serum did not differ significantly in patients and healthy volunteers ($P > 0.05$).

In the allergic subjects, there was no correlation between the ECP levels in NS and Se. ECP concentration in NS was closely correlated with symptom scores (Spearman's, $r = 0.77$, $P < 0.001$) whereas ECP in Se revealed a weak correlation (Spearman's, $r = 0.42$, $P < 0.001$) (Figs. 1 and 2).

ECP concentration in NS or Se did not correlate with grass-pollen count on the day of sampling. However, ECP concentrations revealed a strong and statistically significant correlation with the cumulative pollen concentration of the 3 days preceding the sampling procedure when measured in NS (Spearman's, $r = 0.87$, $P < 0.001$), but only a weak, but statistically significant correlation when measured in Se (Spearman's, $r = 0.44$, $P < 0.01$) (Figs. 3 and 4).

DISCUSSION

The aim of the present study was to compare ECP-levels in nasal secretions and blood serum of patients with allergic rhinitis and healthy controls under natural pollen

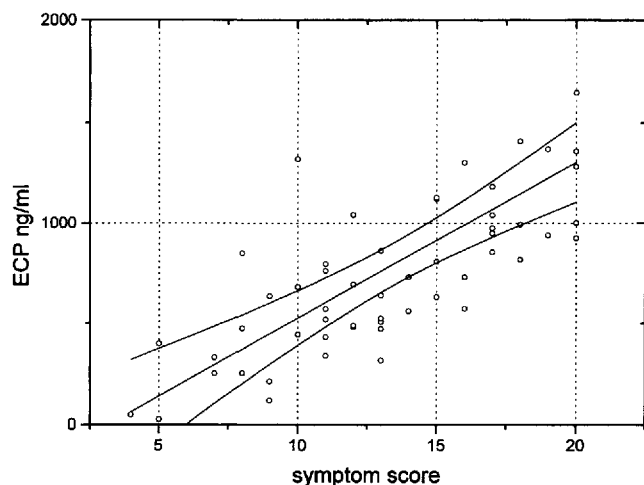


Figure 1. ECP concentration in nasal secretions is closely correlated to symptom score (Spearman's, $r = 0,77$, $P < 0.001$, 95% interval indicated).

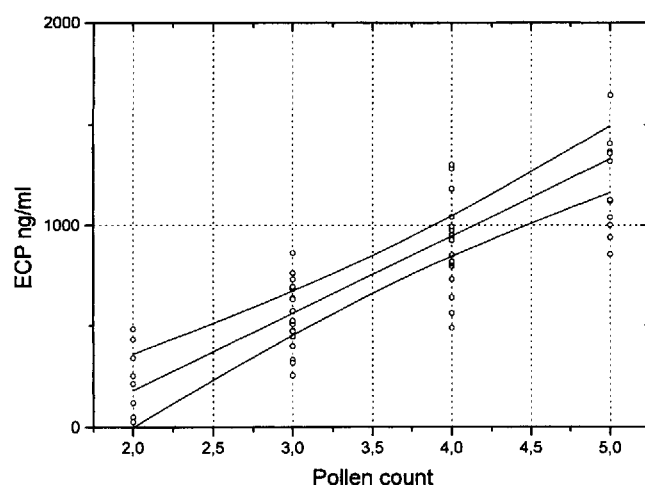


Figure 3. ECP concentration in nasal secretions is correlated to grass pollen concentration in the three preceding days (Spearman's, $r = 0,87$, $P < 0.001$, 95% interval indicated).

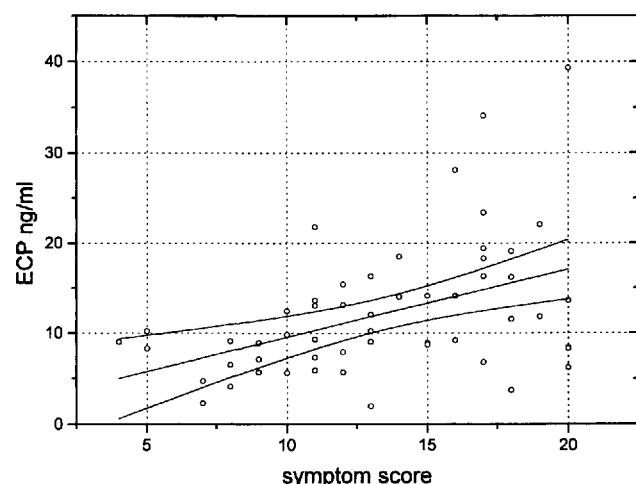


Figure 2. ECP concentration in blood serum is weak correlated to symptom score (Spearman's, $r = 0,42$, $P < 0.001$, 95% interval indicated).

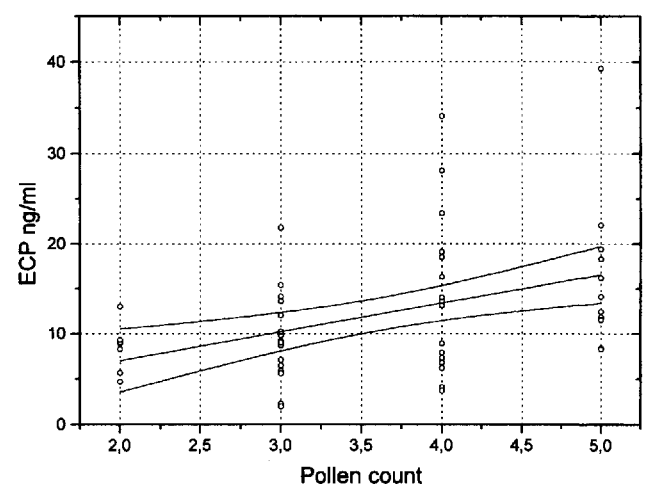


Figure 4. ECP concentration in blood serum is only weak correlated to the cumulated grass pollen concentration (Spearman's, $r = 0,44$, $P < 0.01$, 95% interval indicated).

exposure. The correlation between ECP levels, symptoms, and pollen count was investigated.

The inflammatory stimulus was assessed by measuring grass pollen counts with a Burkhard trap, the clinical disease severity by using a symptom scoring system based upon diary cards. These data were correlated to ECP levels in both nasal secretions and serum.

The mean ECP concentration was 27 times higher in patients than in controls when measured in nasal secretions, but equal when measured in serum. In the patients with allergic rhinitis examined for this study, no correlation between the levels in nasal secretions and serum was found, in contrast to similar studies examining correlation of local body fluid (broncho-alveolar-lavage) and serum in asthmatic patients.^{1,5,17,20,21}

No correlation between ECP nasal secretion levels and grass-pollen count on the day of sampling was found. However, there was a strong correlation between cumulated pollen concentration in the 3 days before the sampling and ECP levels in nasal secretions. This might be explained by the time delay that is needed until eosinophil immigration and activation after antigen exposition takes place.¹⁹ Different authors found a delayed increase of ECP levels in nasal secretions following nasal allergen challenge and a correlation with allergic nasal late phase reaction.^{2,3,4,8,16,22,23}

In the allergic subjects, ECP concentration in nasal secretions revealed a strong and statistically significant correlation to the clinical disease severity measured by symptom scores, whereas the ECP concentration in serum revealed only a weak, but statistically significant correla-

tion. These results are consistent with the results of Pipkorn et al.,¹⁹ who demonstrated an increase of eosinophilic granulocytes on the surface of the nasal mucosa under natural pollen exposure with a relationship to nasal symptoms.

Our data indicate that ECP is a valid marker to assess inflammatory disease activity in allergic rhinitis. It should be measured in nasal secretions rather than serum.

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REFERENCES

1. Ådelroth E, Rosenhall L, Johansson SA, et al. Inflammatory cells and eosinophil activity in asthmatics investigated by bronchoalveolar lavage. *Am Rev Resp Dis* 142:91-99, 1990.
2. Andersson M, Andersson P, Venge P, Pipkorn U. Eosinophils and eosinophil cationic protein in nasal lavages in allergen-induced hyperresponsiveness: Effects of topical glucocorticosteroid treatment. *Allergy* 44:342-348, 1989.
3. Bascom R, Pipkorn U, Gleich G, et al. The influx of inflammatory cells into nasal washings during the late response to antigen challenge. Effect of systemic corticosteroids. *Am Rev Resp Dis* 138:406-412, 1988.
4. Bisgaard H, Gronborg H, Mygind N, et al. Allergen-induced increase of eosinophil cationic protein in nasal lavage fluid: Effect of the glucocorticoid budesonide. *J Allergy Clin Immunol* 85:891-895, 1990.
5. Bonini S, Tomassini M, Adriani E, et al. Markers of eosinophilic inflammation in allergic diseases. *Allergy* 48:133-137, 1993.
6. Cohen AB, Goldberg S, London RL. Immunoglobulins in nasal secretions of infants. *Clin Exp Immunol* 6:753-757, 1970.
7. Dahl R. Eosinophil and eosinophil products. In: N Mygind, U Pipkorn (Eds.) *Allergic and vasomotor rhinitis: Pathophysiological aspects*. Munksgaard, Copenhagen, Denmark, pp 136-139, 1987.
8. Fujieda S, Noda I, Sugimoto C, et al. Effect of pretreatment with Ketotifen on pollinosis: Correlation with eosinophil cationic protein. *Am J Rhinol* 8:49-53, 1994.
9. Kersten W, Puls KE. Pollenflug-Vorhersage. *Allergologie* 7:81-114, 1984.
10. Klementsson H. Time relation between allergen-induced changes in nasal responsiveness and eosinophil granulocytes. *Rhinology* 32:90-91, 1994.
11. Linder A, Ronquist G, Deuschl H. Random distribution of exogenous lithium in nasal secretion and its application in substance determination. *Acta Otolaryngol* 96:287-293, 1983.
12. Linder A, Venge P, Deuschl H. Eosinophil cationic protein and myeloperoxidase in nasal secretion as markers of inflammation in allergic rhinitis. *Allergy* 42:583-590, 1987.
13. Mann W. Ultraschalldiagnostik. *Arch Oto-Rhino-Laryngol* (Suppl. I) 71-98, 1989.
14. Messerklinger W. Technik und Möglichkeiten der Nasenendoskopie. *HNO* 20:133-135, 1972.
15. Naclerio RM, Proud D, Peters SP, et al. Inflammatory mediators in nasal secretions during induced rhinitis. *Clin Allergy* 16:101-110, 1986.
16. Nishioka K, Saito C, Nagano T, et al. Eosinophil cationic protein in the nasal secretions of patients with mite allergic rhinitis. *Laryngoscope* 103:189-192, 1993.
17. Pedersen B, Dahl R, Larsen BB, Venge P. The effect of salmeterol on the early- and late-phase reaction to bronchial allergen and postchallenge variation in bronchial reactivity, blood eosinophils, serum eosinophil cationic protein, and serum eosinophil protein X. *Allergy* 48:377-382, 1993.
18. Pharmacia: ECP-RIA-Directions for use. Kabi Pharmacia Diagnostics AB, Uppsala, Sweden, Rev. Ed. 1992.
19. Pipkorn U, Karlsen G, Enerbäck L. Cellular response of the human allergic nasal mucosa to natural allergen exposure. *J Allergy Clin Immunol* 82:1046-1054, 1989.
20. Rak S, Björnson A, Hakansson L, et al. The effect of immunotherapy on eosinophil accumulation and production of eosinophil chemotactic activity in the lung of subjects with asthma during natural pollen exposure. *J Allergy Clin Immunol* 88:878-888, 1991.
21. Schmekel B, Venge P. Markers for eosinophils and T-lymphocytes as predictors of late asthmatic response. *Allergy* 48:94-97, 1993.
22. Svensson C, Andersson M, Persson CGA, et al. Albumin, bradykinins, and eosinophil cationic protein on the nasal mucosal surface in patients with hay fever during natural allergen exposure. *J Allergy Clin Immunol* 5:828-833, 1990.
23. Toorerebergen van AW, Wijk van RG, Vermeulen AM, Zijlstra FJ. Increase of albumin, eosinophil cationic protein, histamine, leukotriens, and mast cell tryptase in nasal lavage fluid after challenge with inhalant allergen extract. *Agents Actions* 8:421-424, 1992.
24. Venge P, Roxin LE, Olsson I. Radioimmunoassay of human eosinophil cationic protein. *Br J Haematol* 37:331-335, 1977.
25. Venge P. Soluble markers of allergic inflammation. *Allergy* 49:1-8, 1994. □

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