

# Serum levels of eosinophil cationic protein and eosinophil protein x in pollen atopic patients with stable asthma and its relation with bronchial hyperresponsiveness

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## ABSTRACT

Eosinophils are important effector cells in allergic inflammation described in allergic rhinitis (AR) and allergic bronchial asthma (BA). During the pollen season serum levels of eosinophil cationic protein (ECP) and eosinophil X protein/eosinophil-derived neurotoxin (EPX/EDN) are increased in BA.

The aim of the present study was to evaluate the serum levels of ECP and EPX in pollen atopic patients with AR and BA during the winter.

92 patients were studied. They were divided into three groups: I 29 patients with AR, II 51 patients with BA and III 12 healthy subjects. Allergic rhinitis and bronchial asthma were diagnosed by routine clinical tests: clinical history, skin tests, total IgE and specific IgE. In addition ECP and EPX were determined in serum. All patients were asymptomatic, stable and without medical treatment.

Methacholine challenge test (MCT) was performed in all patients.

MCT were positive in 4 patients of group I and 45 patients of group II.

ECP levels ( $\mu\text{g/l}$ ) were: 21 (I), 24 (II) and 7 (III). EPX levels ( $\mu\text{g/l}$ ) were 35 (I), 45 (II) and 21 (III). Statistical differences ( $p < 0.01$ ) were observed both in ECP and EPX levels in patients with MCT positive in relation to patients with MCT negative, and in allergic patients (I and II) in comparison with the healthy subjects (III) ( $p < 0.01$ ).

ECP and EPX serum levels are increased in patients with a positive MCT in the winter, out of the pollen season, when patients are asymptomatic, stable and without treatment. This fact suggests that eosinophils play an important role in the pathogenesis of bronchial asthma.

**Key words:** Eosinophils. Eosinophil cationic protein (ECP). Eosinophil X protein (EPX). Asthma. Bronchial hyperresponsiveness.

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## INTRODUCTION

The prevalence of allergic rhinitis and bronchial asthma are increasing and are the most frequent allergic diseases in the western world. Both rhinitis and asthma are chronic inflammatory diseases and inflammatory cells play a role in the pathogenesis (1).

Eosinophils are important effector cells described in allergic rhinitis and allergic bronchial asthma (2). The eosinophil granules contain several highly cationic and cytotoxic proteins (ECP, EPX, MBP and EPO) (3). During the pollen season, ECP and EPX serum levels are increased in asthma (4).

An important feature of bronchial asthma is the bronchi hyperreactivity. This bronchial hyperresponsiveness is increased during the pollen season (5), but is also present out of this season, that is to say, in winter.

The aims of this study were:

- To quantify ECP and EPX serum levels in pollinic allergic rhinitis and bronchial asthma during the winter.
- To evaluate the bronchial hyperresponsiveness in pollinic allergic rhinitis and bronchial asthma also during the winter.

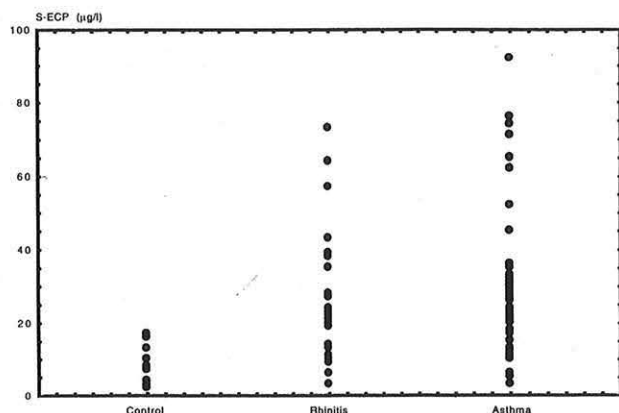


Fig. 1.—Serum levels of ECP in different diagnostic groups.

- To study the possible correlation between ECP and EPX serum levels and bronchial hyperresponsiveness.

## MATERIAL AND METHODS

### Patients

Ninety two patients, divided into three groups, were studied:

- Group I: allergic rhinitis, 29 patients.
- Group II: bronchial asthma, 51 patients.
- Group III: Control group, 12 healthy subjects.

Allergic rhinitis and bronchial asthma were diagnosed by routine clinical test, clinical history and skin tests.

The study was performed in Madrid from October 1992 to February 1993. All patients were asymptomatic, stable and without medical treatment when the serum samples were collected.

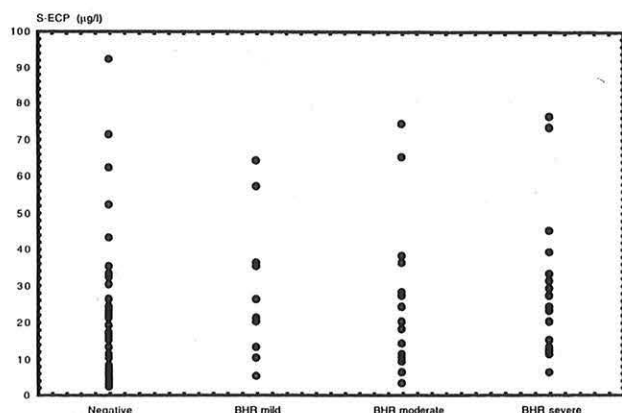


Fig. 2.—Serum levels of ECP in patients with different hyperresponsiveness degree.

### Serum samples

Sera were centrifugated at 1500 x g for ten minutes after being kept stationary at room temperature for 60 minutes, and then stored at -20 °C until the assay was performed.

### Total IgE and specific IgE

Total IgE and specific IgE to *Lolium perenne* were measured by FEIA-CAP (Pharmacia Diagnostics, Uppsala, Sweden).

### Skin tests

Standard prick test was performed with common inhalant allergens: *Lolium perenne*, *Cynodon dactylon*, *Phragmites communis*, *Dactylis glomerata*, *Secale cereale*, *Olea europea*, *Parietaria judaica*, *Artemisia vulgaris*, *Salsola Kali*, *Plantago lanceolata*, *Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*, *Canis familiaris*, *Felix domesticus*, *Mucor racemosus*, *Aspergillus fumigatus*, *Alternaria tenuis* and *Penicillium notatum* (Alergia e Inmunología Abello, Spain).

Histamine was used as a positive control. Patients with dermatographism were excluded.

### Eosinophil counts

Eosinophil counts, after staining with eosin, were performed in peripheral blood using a Fuchs-Rosenthal chamber.

### ECP and EPX

Serum ECP and EPX were measured by specific radioimmunoassays (Pharmacia Diagnostics AB, Uppsala, Sweden). ECP and EPX in the samples competes with a fixed amount of 125I-labeled ECP /EPX for the binding sites of specific antibodies. The coefficients of variation of the RIAs for ECP and EPX were less than 10%, and the detection limit were less than 2 µg/l and 3 µg/l, respectively.

### Methacholine challenge test

Non specific bronchial reactivity was assessed by a methacholine challenge test. Before the methacholine challenge test (MCT) a basal spirometry and flow-volume curves were performed.

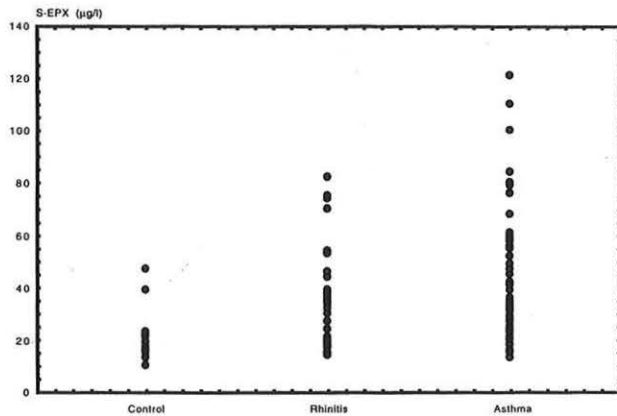


Fig. 3.—Serum levels of EPX in different diagnostic groups.

MCT modified from Cockcroft was performed in all patients. Four different concentrations: one, five, ten and twenty five mg/ml were used.

The bronchial hyperresponsiveness was classified as severe when test was positive (decrease of 20% of FEV<sub>1</sub>) with less than 50 accumulated units (AU) of methacholine, moderate with between 50 and 200 AU, mild with between 200 and 330 AU and negative when it was positive with more than 330 AU.

### Statistic treatment

Kolmogorov-Smirnov test was used to study if the data were normally distributed. The comparison of independent means was used in the case of data groups normally distribute, while the Mann Whitney test was performed for not normally distributed data.

The correlation coefficient between couples of quantitative variables was analysed by two per two matrices for everyone of them.

When the variables were normally distributed the Pearson lineal correlation coefficient was used, while the Spearman correlation coefficient was used in the case of not normally distributed variables.

The level of significance that was accepted in every cases was the corresponding to an alfa error ( $p$ ) of less than 0.05.

### RESULTS

All patients from groups I and II showed positive skin tests to grass pollen. The total IgE values were higher in asthma (311 KU/l) in comparison with rhinitis (247 KU/l), without statistical differences. There were statistical differences ( $p < 0.01$ ) between pollinic patients and control group (28.5 KU/l).

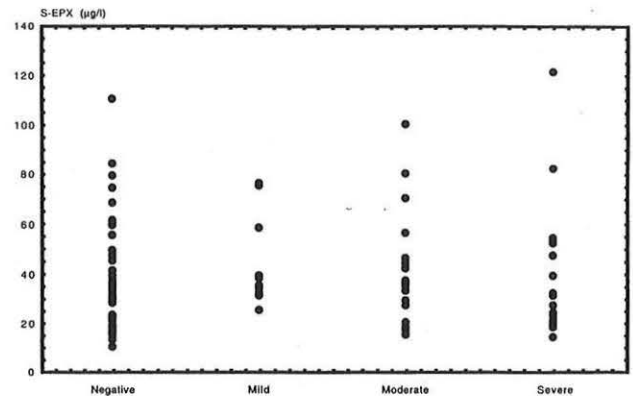


Fig. 4.—Serum levels of EPX in patients with different hyperresponsiveness degree.

The specific IgE to *Lolium perenne* was higher in asthma group (58.1 KU/l) in comparison with rhinitis (51.3 KU/l), without statistical differences. There were statistical differences between pollinic patients and control group, in which specific IgE was negative.

The eosinophil counts were:  $173 \times 10^9/l$  in rhinitis group,  $196 \times 10^9/l$  in the asthma group and  $100^9 \times 10^9/l$  in control group. We observed statistical differences between asthmatics and control group ( $p = 0.004$ ) and between rhinitis and control group ( $p = 0.003$ ).

The MCT was negative in 24 of rhinitis, mild in 3 of rhinitis and moderate and severe in 1 case of rhinitis respectively. In asthmatic patients 6 were negative in MCT, 8 were mild, 19 were moderate and 18 were severe. In control group the MCT was negative in all cases. We observed statistical differences ( $p < 0.001$ ) between asthmatics and rhinitis, and also between asthmatics and control group ( $p < 0.001$ ).

ECP values were 21  $\mu g/l$  in rhinitis, 24  $\mu g/l$  in asthma and 7.5  $\mu g/l$  in control group (fig. 1).

There were statistical differences between asthma & rhinitis in the ECP values,  $p < 0.01$  and  $p = 0.003$  respectively, in comparison with the control group. Statistical differences ( $p < 0.001$ ) were observed when the ECP values were studied in relation with the hyperresponsiveness degree between the patients with MCT positive versus patients with MCT negative. There were not statistical differences between the patients with various degree of severity of bronchial hyperresponsiveness (fig. 2).

EPX values were 35.9  $\mu g/l$  in rhinitis, 45.3  $\mu g/l$  in asthma and 21.4  $\mu g/l$  in control group (fig. 3).

The EPX values were higher in asthma group in comparison with rhinitis, although the differences did not reach significance ( $p < 0.1$ ). Statistical differences between pollinic patients and control group ( $p < 0.001$ ) were observed.

Statistical differences ( $p < 0.001$ ) were observed when the EPX values were studied in relation with the hyperresponsiveness degree between patients with MCT positive versus patients with MCT negative. There were not statistical differences between the patients with various degree of severity of bronchial hyperresponsiveness (fig. 4).

A positive correlation was observed between eosinophil counts and ECP ( $r = 0.42$ ) ( $p < 0.001$ ) and between eosinophil counts and EPX ( $r = 0.38$ ) ( $p < 0.001$ ). A negative correlation was observed between ECP and MCT ( $r = -0.28$ ) ( $p < 0.06$ ). A negative correlation was observed between EPX and MCT ( $r = -0.30$ ) ( $p < 0.003$ ). A strong correlation was observed between ECP and EPX levels ( $r = 0.80$ ) ( $p < 0.001$ ).

## DISCUSSION

In our study the skin tests, total IgE and specific IgE were positives in allergic patients and allow us to perform the correct etiological diagnosis both in allergic rhinitis and bronchial asthma.

The problem is to know the exact severity degree. Asthma is a chronic inflammatory disease in which many cells play a role (6), and there is a relationship between inflammation and hyperreactivity (1). Eosinophils are the cells that characterize the inflammation in asthma (2) and eosinophils are related with bronchial hyperresponsiveness (7-9), basic feature of bronchial asthma. ECP and EPX are proteins secreted by eosinophils with toxic properties for the respiratory epithelium (10). ECP and EPX have been studied in serum (10-12), sputum (13, 14) and bronchoalveolar lavage from asthmatics (15).

The eosinophil degranulation may be stimulated by different cytokines, as IL-5 or GM-CSF (15).

In our work the degranulation of eosinophils was measured in serum. Primed eosinophils seems to have a higher propensity to release mediators such as ECP and EPX.

ECP normal values are 6 ug/l (2.3-15.9) and EPX normal values are 17.7 ug/l (8.2-38.5) (16). In our patients the ECP values were higher in asthma group in comparison with rhinitis. EPX values were higher in asthmatic patients in comparison in patients with allergic rhinitis.

We observed a positive correlation between eosinophil counts and ECP & EPX serum levels.

It is described that degranulation of eosinophils from pollinic patients is increased during the pollen season (4, 17). In Madrid pollen season takes place mainly between May and June (18). We observed ECP and EPX values higher than normal in pollinic patients out of the pollen season, when they were

stable, asymptomatic and without medical treatment.

Airway hyperresponsiveness is the airway constrictive response to a variety of chemical, physical, or pharmacologic stimuli (5). It is a feature of symptomatic asthma (19), but it can be present in asymptomatic asthmatics and in others diseases (6).

Among our asthmatic patients studied most of them showed bronchial hyperresponsiveness with MCT positive, however among the rhinitis patients studied most of them were MCT negative.

In our patients with MCT positive the ECP and EPX values were higher than patients with MCT negative.

We observed a significative and negative correlation between ECP and EPX serum levels and MCT, theoretically well correlated with the severity of the asthma. ECP and EPX levels were increased, compared to controls, indicating eosinophil activation in peripheral blood in our patients that were studied out of the pollen season.

The strong correlation observed between ECP and EPX allow us to study one of them to know the eosinophil activation.

In conclusion ECP and EPX serum levels are increased in allergic rhinitis and bronchial asthma in the winter, when patients are asymptomatic, stable and without treatment, ECP and EPX are increased in patients with MCT positive. This fact suggests that eosinophils play an important rol in the pathogenesis of bronchial hyperresponsiveness.

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## RESUMEN

El asma bronquial se caracteriza por la existencia de inflamación crónica de las vías aéreas, que es la causa del incremento de la respuesta a estímulos muy diversos, y por cursar con episodios de obstrucción reversibles espontáneamente o con tratamiento.

La hipótesis del presente trabajo ha sido que dado que en el asma bronquial extrínseco por sensibilización a pólenes existe inflamación de las vías aéreas, incluso en épocas extraestacionales, debería ser posible objetivar esta inflamación mediante la cuantificación de marcadores de activación de los eosinófilos en el suero de los pacientes. Los objetivos han sido: 1) valorar el grado de hiperreactividad bronquial (HB); 2) estudiar la presencia de proteína catiónica y

X del eosinófilo (ECP y EPX), y 3) conocer si existe relación entre el grado de HB y los marcadores citados.

Se han estudiado 92 sujetos distribuidos en tres grupos: I (rinitis; 29), II (asma; 52) y III (control; 12 sujetos sanos).

Se han realizado en todos los sujetos: pruebas cutáneas a neuroalergenos habituales, recuento de eosinófilos, IgE total, IgE específica, ECP, EPX y prueba de hiperreactividad bronquial con metacolina. La recogida de muestras se llevó a cabo entre los meses de octubre de 1992 y febrero de 1993, época en la que no existen recuentos significativos de pólenes en Madrid. Se realizó estudio estadístico de los resultados.

La prueba de HB resultó positiva en 5 de los pacientes del grupo I y en 46 de los pacientes del grupo II. Los marcadores de activación del eosinófilo ECP y EPX, presentaron mayor concentración en los pacientes asmáticos que en el resto de los grupos. Las diferencias fueron significativas ( $p < 0,01$ ) entre los pacientes polínicos respecto del grupo control. Igualmente se encontraron diferencias significativas entre los pacientes con prueba HB positiva respecto de HB negativa ( $p < 0,01$ ). Se observó una fuerte correlación (0,80) entre ECP y EPX.

Las principales conclusiones son: 1) los asmáticos polínicos presentan un grado de HB leve-moderado fuera de la primavera; 2) los pacientes asmáticos presentan mayores concentraciones de ECP y EPX; 3) sería suficiente determinar ECP o EPX para evaluar la activación de los eosinófilos.

**Palabras clave:** Eosinófilos. Proteína catiónica del eosinófilo (ECP). Proteína X del eosinófilo (EPX). Asma. Hiperreactividad bronquial.

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