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Serum eosinophil cationic protein measurements in the management of perennial and periodic asthma: a prospective study

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Serum eosinophil cationic protein measurements in the management of perennial and periodic asthma: a prospective study. F. de Blay, A. Purohit, R. Stenger, P. Gries, C. Hamberger, B. David, N. Frossard, G. Pauli. ©ERS Journals Ltd 1998.

ABSTRACT: We performed a prospective study in order: 1) to determine whether a correlation could be found between serum eosinophil cationic protein (ECP) levels and clinical and functional status in perennial asthmatics during a 5 month prospective study; and 2) to evaluate the relationship between allergic exposure and ECP levels in periodic asthmatics.

Two groups of asthmatic patients were selected: a group of acutely ill perennial asthmatics and a group of periodic asthmatics. The acutely ill perennial asthmatics (n=22, mean age=39.4 yrs) were included on the basis of hospitalization for acute asthma. At the end of the hospitalization, there was a 5 month follow-up of clinical, functional and medication scores, as well as eosinophil counts and ECP levels. The periodic asthmatic group was composed of asthmatics sensitized to birch and tree pollens (n=10, mean age=33.8 yrs). The same measurement were performed on this group, before, during and after the pollen season.

Under corticosteroid treatment in the acutely ill patients, there was a significant decrease in serum ECP levels between the first day of hospitalization and the day of discharge (mean: 23.2 $\mu g \cdot L^{-1}$ and 9.5 $\mu g \cdot L^{-1}$, respectively; p=0.006). No correlation was found between the clinical status, functional status and serum ECP levels during the 5 month follow-up. A significant increase in ECP levels was found in periodic asthmatics during the pollen season.

Our results suggest that serum eosinophil cationic protein is a useful marker of allergen exposure and of acute asthma treatment. This could be of importance in the prevention and follow-up of allergic asthma; the value of serum eosinophil cationic protein measurements in the day-to-day management of adult asthmatics needs to be further clarified.

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Lung infiltration by inflammatory cells, and especially eosinophils, is a characteristic pathological feature of bronchial asthma. Activated eosinophils in asthmatics release their granular proteins, supporting the view that they have a pro-inflammatory role in the development of airway narrowing in asthma. One such protein, eosinophil cationic protein (ECP), was detected in bronchial biopsies and measured in bronchoalveolar lavage [1], sputum and peripheral blood [2–4]. Serum ECP levels have been found to be correlated with the severity of asthma [5]; exercise-induced bronchoconstriction [6] and allergen exposure [7]. It has been found that ECP can be used to monitor asthma inflammation [8]. However, it has recently been reported that serum ECP levels were not correlated with the severity of asthma and did not differ between asthmatic and rhinitic patients [9].

We carried out a 5 month prospective study in order: 1) to determine if a correlation could be found between serum ECP levels, and clinical and functional status in perennial adult asthmatics; and 2) to evaluate the relationship between allergen exposure and ECP levels in periodic asthmatics sensitized to birch and tree pollens.

Patients, materials and methods

Trial design

In order to determine whether a relationship between serum ECP and clinical functional status could be found in perennial asthmatics, "acutely ill perennial asthmatics" were included in a prospective study based on hospitalization due to asthma exacerbation. Asthma status was based on the International Consensus (clinical and medication scores and lung function values) [10]. Patients were hospitalized for 5–14 days. Besides emergency treatment, they received intravenous steroids for a few days followed by oral corticosteroids for approximately 1 week as anti-inflammatory treatment. Blood sampling and lung function testing (AS 500; Minato, Paris, France) were performed during hospitalization and at follow-up visits, 2 weeks (V1), 4 weeks (V2), and 2,3,4 and 5 months (V3–V6) following the acute episode. Patients were instructed to make unscheduled visits in case of exacerbation of their asthma.

In such an event, the patients had follow-up visits after 2 and 4 weeks.

In order to evaluate the relationship between allergen exposure and ECP levels, "periodic asthmatics" were selected. They made four visits (inclusion, and after 2, 4 and 6 months).

At each visit, both groups of patients had their clinical and medication scores measured at the same time as blood sampling and lung function tests (AS 500; Minato).

Patients

Acutely ill perennial asthmatics. All patients were aged 18–60 yrs. They were able to perform spirometry and had no contra-indication to the use of oral steroids and/or high doses of inhaled steroids (1–2 mg·day^{-1}). All patients required intravenous administration of corticosteroids and nebulized β_2-agonists. Those who had already received intramuscular, intravenous or oral corticosteroids more than 2 h before hospitalization were not considered eligible for inclusion. Those receiving topical (inhaled) steroids, β_2-agonists, cromoglycate and theophylline were included.

Periodic asthmatics. All patients were aged 18–60 yrs (mean 33.8 yrs). They had a clinical history of exclusive sensitization to birch and/or other tree pollens, and all had a history of chronic reversible lung disease. β_2-agonists, topical steroids, cromoglycate and theophylline were permitted before inclusion in the study.

The atopy status of all patients was assessed by means of prick tests (Stallergènes Pasteur, Antony, France) and specific immunoglobulin E (IgE) determinations (Alastat-Behring, Rueil, Malmaison, France) with respect to grass, tree and weed pollens, mites, animals and molds. A skin-prick test was considered positive when the mean diameter of the wheel was Š75% of the mean diameter obtained with the positive control (codeine 9%). According to our previous studies [11], specific IgEs were considered positive when greater or equal to class 2. Pollen counts were measured by Burkard sampler and expressed as grains per cubic millimetre.

Measurement of ECP and eosinophil counts

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 preparation and storage of serum took place. The analysis of ECP was performed according to the manufacturer's directions (Pharmacia Diagnostics AB, Uppsala, Sweden) [12]. The analysis was performed blindly and each sample measurement was performed in duplicate. The intra-assay coefficient was 4% for 5 μg·L^{-1} and 5.2% for 200 μg·L^{-1}.

Blood eosinophil counts were performed at the time of ECP measurement using an Automate Sysmex WE 9000 (Tokyo, Japan).

Statistical analysis

The significance level was set at 5%. Normality of the distribution for each quantitative variable was tested using the Kolmogorov-Smirnov fitting test. Principal component analysis was performed on the ECP variables in order to assess the existence of several groups according to the time evolution of ECP. Linkage between the quantitative variables was tested using Pearson's linear and fitted coefficient of correlation, after natural log-transformation of the variables. To compare the linkage between ECP and clinical status, a linear regression χ^2 test was used.

Results

Patients

Acutely ill perennial asthmatics. In total, 22 patients were selected for the study during hospitalization. Two of them were withdrawn from the follow-up study, since they had too few follow-up visits.

Patient characteristics are given in table 1. Seventeen out of the 22 were atopic. All atopic patients were sensitized to at least one perennial allergen. The mean age and the sex ratio were the same in the two groups and 15 patients had an asthma duration of more than 10 yrs.

Ten patients were receiving inhaled steroids (1,000–2,000 μg·day^{-1}) during the 7 days prior to the last acute exacerbation. Intravenous corticosteroids were instituted on the day of hospitalization, and given from 1–11 days (mean 6.5 days; median 6 days), with a starting dose of 40–120 mg·day^{-1}, which was subsequently reduced in all cases. After withdrawal of the intravenous steroids, oral steroids were given for 3–20 days, with a starting dose of 15–80 mg·day^{-1} and with a tapering dose schedule in all patients. During follow-up, 16 of the patients received the same dose of inhaled corticosteroids (1,200–2,000 μg·day^{-1}).

Periodic asthmatics. Ten periodic asthmatic patients were enroled. All patients were sensitized to tree pollens. None of these patients had an asthma attack during the pollen season. Hence, none of them needed inhaled, oral or intravenous corticosteroid treatment during the pollen season.

Clinical and functional status, eosinophil counts and ECP values

During hospitalization (table 2). Improvements in clinical status (from stage IV to stage I), and in forced expiratory volume in one second (FEV_1) and peak expiratory flow rate (PEFR) were observed during hospitalization. At the same time, serum ECP levels decreased significantly from a mean of 23.2 μg·L^{-1} to 9.5 μg·L^{-1}. On the day of hospitalization, no significant correlation was found between the ECP level on the one hand and eosinophil counts or any of the lung function variables on the other. The ECP values on Day 0 did not correlate with percentage changes (Day 0–Day of discharge) in eosinophils or lung function

Table 1. – Characteristics of the "acutely ill" asthmatic patients

Patient No.	Sex	Age yrs	Smoking	Atopy	Inhaled steroids+	PB eosinophil count* cells·mm^{-3}	Serum ECP values* µg·L^{-1}	Inhaled steroids† mg·day^{-1}
1	F	20	Y	N	N	250	8.0	2000 S
2	F	37	Y	Y	Y	230	9.1	1000 S
3	M	57	N	Y	Y	410	27.7	2000 S
4	M	60	Y	Y	N	290	13.3	1600 P
5	M	21	Y	Y	N	770	41.4	1500 S
6	M	27	N	Y	Y	250	6.2	1500 S
7	M	34	N	Y	Y	30	5.1	1600 (1) P 1200 (3.5) P
8	F	45	N	Y	Y	40	10.8	1200 (2.0) P 1600 (2.5) P
9	M	60	Y	N	Y	180	7.5	1600 P
10	F	36	N	N	N	180	15.1	1500 (1.5) S 2000 (3.0) S
11	M	57	Y	Y	Y	10	7.1	2000 S
12	F	43	N	Y	N	250	27.4	2000 S
13	F	56	N	Y	N	1240	25.2	2000 S
14	M	28	N	Y	N	450	26.5	2000 S
15	M	26	N	N	Y	130	9.8	2000 S
16	F	26	N	Y	Y	20	9.3	1500 (1.5) S 2000 (3.0) S
17	M	26	N	Y	Y	450	38.4	1500 (1.5) S 2000 (3.0) S
18	M	56	N	N	N	1370	33.3	1500 S
19	M	42	Y	Y	N	300	10.9	1600 P
20	F	37	N	Y	Y	350	13.4	1600 P
21	M	23	N	Y	Y	40	14.0	ND
22	F	24	Y	Y	N	20	8.3	ND

Patients 21 and 22 were excluded during the follow-up, at visit 5 and visit 1, respectively. The data from the follow-up of these two patients were not used for statistical analysis. +: prior to admission. *: on the day of admission. †: during follow-up (values in parentheses are the treatment periods using inhaled corticosteroids, in months). PB: peripheral blood; ECP: eosinophil cationic protein; F: female; M: male; Y: yes; N: no; S: spray without air chamber; P: dry powder; ND: not determined.

Table 2. – Variation of clinical status, peak expiratory flow rate (PEFR), forced expiratory volume in one second (FEV_1), eosinophil count and eosinophil cationic protein (ECP) at first day of hospitalization (H1) and day of discharge (H4)

	H1 (n=22)	H4 (n=22)
Clinical status	IV	I
PEFR % pred	56.8±5.0 (46.6–67.2)	81.7±3.3 (74.5–88.9)#
FEV_1 % pred	58.4±5.8 (46.1–70.2)	78.5±4.5 (66.9–88)#
Eosinophils cells·mm^{-3}	330±0.07 (40–1370)	160±0.03 (40–700)#
ECP µg·L^{-1}	23.2±6.5 (8.0–41.4)	9.5±1.3 (4.0–26.4)+

Values are presented as mean±SEM, and range in parenthesis. Clinical status: on a scale from I (least serious) to IV (most serious). #: $p<0.00005$; +: $p<0.006$ (Wilcoxon test). % pred: percentage of predicted value.

Table 3. – Correlation between serum eosinophil cationic protein (ECP) values, clinical status, forced expiratory volume in one second (FEV_1) and eosinophil counts during the follow-up of the "acutely ill patients" group

	Clinical status	FEV_1	Log of eosinophil counts
Serum ECP	χ^2 LR=0.357 NS	df=153 r=0.005 NS	df=129 r=0.36 p<0.00001
FEV_1	χ^2 LR=6.65 p=0.04	-	df=125 r=0.06 NS
Clinical status	-	-	χ^2 LR=0.65 NS

χ^2 LR: linear regression Chi squared; df: degrees of freedom. NS: nonsignificant.

variables. Furthermore, the percentage change in ECP (Day 0–Day of discharge) did not correlate with percentage changes in eosinophils or lung function variables. Eosinophils decreased sharply following institution of corticosteroids.

During follow-up in the acute asthmatic group (table 3). Twenty patients were studied during the follow-up (*i.e.*, from V1 to V6 after the hospitalization). No correlation was found between the clinical status, serum ECP levels and eosinophils during follow-up. No correlation was found between the FEV_1, serum ECP levels (r= -0.29, p=0.19) and eosinophils during follow-up.

Serum ECP levels in the periodic asthmatics (fig. 1)

ECP measurements before, during and at the end of the tree pollen season were 17.4, 25.6 ($p<0.05$) and 19.8 µg·L^{-1}, respectively. Concomitantly, there were no differences between preseasonal FEV_1 values and those during and after the tree pollen season: 99.7, 104.1 and 107.5%, respectively. Also, no difference was observed between eosinophil counts before, during and after the tree pollen season (mean 300, 240 and 260 cells·mm^{-3}, respectively).

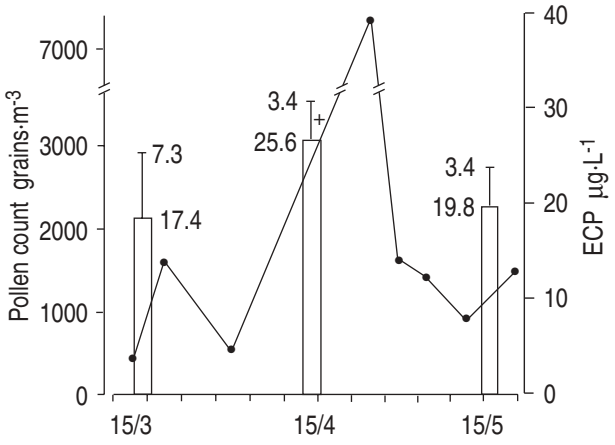

Fig. 1. – Pollen counts and serum eosinophil cationic protein (ECP) levels before, during and after tree pollen season. Bars represent ECP values (mean±SEM) and the solid line represents the pollen count. 15/3: 15th March; 15/4: 15th April; 15/5: 15th May. +: p=0.016, *versus* before pollen season.

Discussion

The present study showed that in perennial asthmatics, serum ECP levels did not correlate with clinical and functional status, during a 5 month follow-up, although a significant decrease in serum ECP was associated with clinical improvement during the hospitalization period. In contrast, in periodic asthmatics, a significant increase in ECP levels was found during the pollen season, in the absence of asthma symptoms, as compared with before pollen season, indicating that there was a relationship between ECP and allergen exposure.

Serum ECP measurements have been recommended in the management of asthma [13–15]. In order to determine if a correlation could be found between serum ECP levels, clinical status and functional values we performed a prospective follow-up of 20 asthmatic patients over 5 months, with strictly controlled maximum doses of corticosteroids after a hospitalization period due to asthma exacerbation. Our results showed that there was no correlation between serum ECP values and clinical and functional status on the day of hospitalization. This could be due to inhaled corticosteroid treatment prior to hospitalization in 10 patients. ADELROTH *et al.* [16] did not find any significant differ-ence in serum ECP levels between a group of asthmatic patients receiving regular treatment with inhaled corticosteroids and a control group. In order to assess the influence of inhaled corticosteroids on eosinophils, we separated the 22 acutely ill patients who were hospitalized into two groups: one composed of patients using inhaled corticosteroids prior to admission and a second one without. We did not find any correlation between serum ECP levels, FEV1 and the clinical state in the two groups (data not shown). In the present study, we have shown that during an acute asthma attack a significant decrease in serum ECP values was obtained following the use of intravenous corticosteroids. These results are in accordance with previous studies showing that inhaled and oral corticosteroids reduce blood eosinophil and serum ECP levels [13, 17, 18].

During the follow-up period, we did not find any relationship between serum ECP levels and clinical and functional values. Our results are in accordance with those of JUNTUNEN-BACKMAN *et al.* [18] and FERGUSSON *et al.* [9], who did not find a correlation between asthma activity and ECP levels. One can suppose that the absence of a correlation is a consequence of the low values of ECP found in our patients. Such values have often been found in other studies of symptomatic asthmatic patients [9]. DURHAM *et al.* [19] found a mean value less than 3 $\mu g \cdot L^{-1}$ in 12 asthmatic patients. Our results differed from those of WEVER *et al.* [15] who found a correlation between FEV1 and serum ECP values in a group of adult asthmatic patients with inflammatory exacerbation and high serum ECP values.

It has been underlined that ECP measurements vary widely, depending on ambient temperature, excessive time allowed for clotting, insufficient centrifugation and diurnal variation [20, 21]. We avoided such biases by having the same researcher in the same building drawing blood, centrifuging and storing the serum. No significant variation in temperature between the laboratory and the outpatient clinic was observed.

The absence of a significant difference between FEV1 measured before, during and after the pollen season is explained by the absence of asthma symptoms in the 10 periodic asthmatic patients. This is the first report of an increase in ECP values during the pollen season in non-symptomatic patients. Our results are in accordance with previous studies which found that in symptomatic patients, an increase in ECP occurs after natural allergen exposure during pollen season [22] or during alternate stays at high and low altitudes with subsequent natural allergen exposure [7].

In conclusion, the results of our prospective study suggest that:

1) Even in the absence of asthma symptoms, an increase in serum eosinophil cationic protein levels can be found in periodic allergic asthmatics. This suggest that allergen-induced inflammation in the bronchi is capable to provoke an increase in the release of ECP from activated eosinophils in clotted peripheral blood.

2) Serum eosinophil cationic protein levels could be a useful marker for selecting allergic patients with eosinophil related activation to allergen exposure, and could be of great importance in the prevention of allergic disease.

3) The use of serum ECP measurements in the day-to-day management of adult periodic asthmatics still needs to be clarified. Further studies are required to understand the relationship between ECP levels, eosinophil activation in the bronchi and nonspecific bronchial hyperresponsiveness.

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