

Serum eosinophil cationic protein (ECP) as a marker of disease activity and treatment efficacy in seasonal asthma

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This study was carried out to determine whether serum eosinophil cationic protein (ECP) represents a sensitive marker for disease activity in atopic asthmatic patients during the pollen season. The study, in double-blind fashion, was performed between February and June 1994. Two groups of 10 seasonal asthmatic patients randomly received two different treatments. The first group was treated with inhaled beclomethasone dipropionate (BDP) 500 µg bid; the second received a matched placebo (P). At the beginning and every month, blood samples for determination of ECP and eosinophil count were collected and lung function (FEV₁) and methacholine responsiveness (PD₂₀) were performed. Subjects recorded daily symptoms of asthma, salbutamol consumption, and peak expiratory flow (PEF) values. In the P group, all indices, except FEV₁, showed significant changes during the pollen season ($P < 0.001$). In the BDP group, significant changes were detected for symptom score ($P < 0.01$), salbutamol consumption ($P < 0.01$), and eosinophil number ($P < 0.05$). Between the two groups, significant differences for symptom score ($P < 0.001$), salbutamol consumption ($P < 0.001$), ECP levels ($P < 0.05$), eosinophil count ($P < 0.02$), PD₂₀ methacholine ($P < 0.02$), and PEF values ($P < 0.01$) were detected. Changes in serum ECP significantly correlated with changes in other parameters ($P < 0.001$), except FEV₁. Our results provide evidence that serum ECP is a sensitive marker for monitoring of the disease activity in seasonal asthma. Furthermore, it may offer a useful tool for estimating treatment efficacy.

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Airway inflammation is now regarded as a major feature of bronchial asthma (1). Inflammatory changes have been found even in the airways of patients with mild or newly diagnosed asthma (2, 3). Furthermore, there is good evidence that the striking increase in clinical manifestations and bronchial hyperresponsiveness (BHR) observed in asthmatic subjects during the pollen season is a consequence of worsening airway inflammation (1). Evaluating the severity of asthma and the requirement for anti-inflammatory treatment relies, in large measure, on indirect indices to suggest that airway inflammation has worsened. Particularly useful are those indices that are non-invasive and easily obtainable and therefore may

be used routinely in outpatients. The most commonly used measures of asthma severity include subjective evaluation of daily symptoms, as needed (prn) inhaled β_2 -agonist requirement, and peak expiratory flow (PEF). Objective measures of pulmonary function, such as airway responsiveness and spirometry, are also used. However, these measures may not accurately reflect the extent of the inflammatory process in the airways and thereby do not always indicate the best choice of therapy.

A role for the eosinophil granulocyte in asthma has been suggested by recent studies (4–6). Raised concentrations of the secretory proteins of eosinophils, e.g., eosinophil cationic protein (ECP),

Table 1. Subject characteristics

Subject	Sex	Age (years)	Height (cm)	FEV ₁ (l)	FEV ₁ (% pred)	PD ₂₀ MCh (μg)	Atopy
BDP							
1	M	24	180	4.01	88	250	P
2	F	26	148	2.12	81	138	P
3	M	47	167	2.66	80	650	P, G
4	M	33	171	3.51	89	750	P
5	F	44	154	2.10	89	250	P
6	F	36	158	2.64	96	100	P, G
7	F	47	148	2.05	99	166	P
8	F	43	160	2.38	89	209	P
9	F	32	161	2.41	81	410	P
10	F	46	159	2.23	89	250	P, G
Mean		37.8	160.5	2.61	88.1	258 ^a	
±SD		8.7	10	0.65	6.2		
Placebo							
1	F	20	160	2.59	80	230	P
2	F	33	161	3.17	108	155	P
3	F	45	160	2.38	91	102	P
4	M	25	181	4.95	108	680	P, G
5	M	21	166	3.37	83	135	P
6	F	20	174	3.33	88	500	P
7	M	34	173	3.19	80	188	P, G
8	M	23	170	3.81	91	1000	P
9	M	23	180	4.94	108	185	P, G
10	F	47	165	2.38	86	450	P
Mean		29.1	169	3.41	92.3	269 ^a	
±SD		10.1	7.8	0.92	11.5		

P = *Parietaria*; G = grass.
^a Geometric mean.

have been found in the bronchoalveolar fluid in asthmatic patients (4, 7–9). Serum ECP levels are also increased in asthma (9–12), and they have been thought to reflect the degree of bronchial inflammation (6, 7, 13). Thus, it has been proposed that serum ECP could be used as a marker for monitoring the inflammatory component and the efficacy of anti-inflammatory therapy in asthma (14).

This study was carried out to determine whether serum ECP could be a sensitive marker for clinical monitoring of the disease activity and for estimation of treatment efficacy during natural allergen exposure in atopic asthmatic patients. With this aim, we monitored serum ECP values, together with other measures of disease activity, before and during the pollen season in asthmatic patients treated with inhaled steroid or placebo.

Material and methods

Subjects

Twenty adult subjects with atopic mild asthma (12 female and eight male) aged 20–47 years (mean age 33.4 years) gave their informed consent to partici-

pate in the study (Table 1). All patients had positive skin prick tests to *Parietaria*, and six subjects (three in each group) were also positive to grass pollen. They reported seasonal increase or onset of asthma symptoms during pollen exposure. Outside the pollen season, the subjects had usually no or minimal asthma symptoms. They used inhaled β₂-agonists occasionally, mainly during the pollen season. None of the patients were smokers, and none were being treated with inhaled corticosteroids or cromones. Forced expiratory volume in 1 s (FEV₁) was greater than 80% of predicted. All subjects had a positive methacholine test; the provocative dose that caused a 20% fall in FEV₁ (PD₂₀) was less than 1600 μg. None reported recent evidence of respiratory tract infection, or increase in asthma symptoms or increased need of medication in the 4 weeks before the study.

Study design

The study was double-blind, parallel, and placebo-controlled. The design of the study is schematically represented in Fig. 1. After a 4-week run-in period, the subjects were randomly allocated to two treatment groups of 10 asthmatic patients, to receive

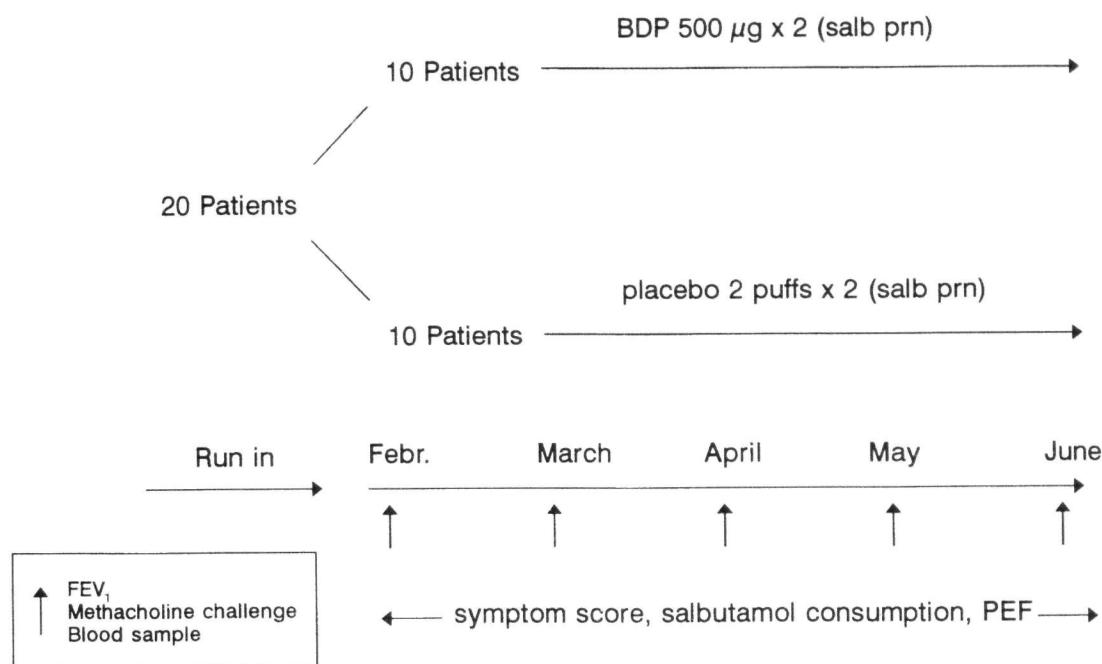


Fig. 1. Design of study. Preseasonal testing was performed in February.

inhaled beclomethasone dipropionate (BDP) (500 µg, twice a day) or a matched placebo (two puffs, twice a day). The patients were permitted to use inhaled salbutamol as needed. The study was carried out for a period of 5 months, between February and June 1994. Tests were performed in the morning, between 9:00 and 11:00, at entry and every month throughout the study. The tests included FEV₁, methacholine provocation, and blood sampling for measurement of eosinophil count and serum levels of ECP. Any medication was interrupted at least 12 h before the tests. All subjects recorded on diary cards daily asthma symptoms, salbutamol consumption, and peak expiratory flow (PEF). Pollen count was performed as described below.

Measurements

FEV₁. FEV₁ was measured on flow-volume equipment (Spirostar FG 90, Werner Gut AG, Basel, Switzerland); the best of three consecutive measurements was taken and expressed as percentage of predicted.

Bronchial reactivity. Methacholine responsiveness was measured with a breath-activated dosimeter (Mefar MB3, Brescia, Italy) connected to a mouth-piece. The dosimeter, with a time of 0.6 s, gave an output of 9.9 ± 0.2 ml/puff. Every inhalation, while the nose was clipped, was performed with a slow submaximal maneuver, beginning at functional residual capacity. Subjects inhaled five puffs of saline, followed by increasing cumulative doses of methacholine (Lofarma, Milan, Italy) from 20 to 3200 µg. FEV₁ was measured 1 min after each dose.

The test was discontinued when FEV₁ had fallen by 20% from the postsaline value. PD₂₀ was recorded after linear interpolation of the last two points on a logarithmic scale. Values of PD₂₀ above 1600 µg were regarded as positive.

Diary cards. Asthma symptoms were graded daily by each patient and recorded in a diary format. The symptoms were then converted to a numeric scale ranging from 0 (no symptoms) to 4 (severe symptoms). Rescue salbutamol consumption was recorded as the number of daily puffs. Morning and evening PEF was measured with a Mini-Wright peak-flow meter. The highest value of three attempts was recorded. Values were expressed as means of recorded data in a period of 28 ± 2 days.

Serum ECP levels and blood eosinophil count. Venous blood was drawn from a cubital vein directly into a plain glass tube for serum ECP analysis and into a tube containing EDTA for eosinophil counts. The serum tube was allowed to clot at room temperature (20°C) for $1 \text{ h} \pm 10 \text{ min}$. After centrifugation (1350 g for 10 min), the serum was withdrawn and aliquots were stored deep-frozen (-20°C) until analysis. Serum ECP was determined by a double-antibody radioimmunoassay with an ECP RIA kit (Pharmacia, Milan, Italy), according to the manufacturer's instructions, and expressed as µg/l. All of the samples were determined in the same assay. The intra- and interassay coefficients of variation were less than 11%. The detection limit was less than 2 µg/l and the normal range in healthy adults was 2.3–16 µg/l (15). Eosinophils were counted in an automatic

counter (Dasit NE 8000; TOA Medical Electronics, Kobe, Japan), giving normal eosinophil counts between 0 and 400×10^6 cells/l.

Pollen sampling

Registrations were performed daily with a Burkard volumetric pollen trap (Burkard Manufacturing, Rickmansworth, UK), placed on the roof of the hospital. The number of pollen grains was counted and expressed as daily mean value per cubic meter of air.

Data analysis

Results were expressed as means $\pm$ standard error of the mean (SEM). The comparisons between the means of the subject groups were made by Student's unpaired *t*-test. The comparisons within the two treatment groups were made by Student's paired *t*-test. The correlations were calculated with Spearman's rank correlation test. Values of $P < 0.05$ were considered significant. PD_{20} values were analyzed after logarithmic transformation, and data were expressed as geometric means.

Results

Pollen season

The *Parietaria* pollen count in the study area in 1994 reached its peak between the end of May and the start of June. Only a small amount of the *Parietaria* pollen was noted at the beginning and at the end of the season. The grass pollen count was rather low and showed only a small rise in mid-June, toward the end of the season (Fig. 2).

Clinical data

Asthma symptom scores were very low before the start of the season, 0.3 ± 0.02 in the placebo group and 0.27 ± 0.01 in the BDP group. Fig. 3A shows the mean values of the two groups during the season. In the placebo group, the symptoms increased during the pollen season, and the highest values were measured in May (2.37 ± 0.14); the differences from baseline mean value were significant in April, May, and June ($P < 0.001$). A significant difference from baseline was detected in the BDP group in May only ($P < 0.01$). The difference between the two groups was significant from April to June ($P < 0.001$).

Mean salbutamol consumption (Fig. 3B) was very low before the start of the season, 0.33 ± 0.02 (placebo group) and 0.30 ± 0.02 (BDP group). A significant difference from the baseline value was detected in the placebo group in April, May, and

June ($P < 0.001$). The BDP group showed a significant difference from baseline in May only ($P < 0.01$). The difference between the groups was significant from April to June ($P < 0.01$).

PEF mean values (Fig. 3C), expressed in percent of predicted, at baseline, were 97.3 ± 3.4 in the placebo group and 101 ± 3.1 in patients treated with BDP. In the placebo group, PEF values dropped during the season and they were significantly lower than baseline in March ($P < 0.05$), April ($P < 0.01$), and May and June ($P < 0.001$). PEF significantly improved after the first month in BDP-treated patients. No significant changes from baseline were observed in the other months. Between the groups, a significant difference was detected in March, April, and June ($P < 0.02$) and May ($P < 0.01$).

Baseline FEV₁

Mean baseline FEV₁ values (% of predicted) were 92.3 ± 3.6 and 88.1 ± 1.9 in the placebo group and in the BDP group, respectively. FEV₁ slightly changed during the study (Fig. 4). No significant difference was observed within or between the groups at any time.

Methacholine challenge

BHR to methacholine was expressed in PD_{20} (μ g). In the placebo group, mean PD_{20} decreased during the season, and the difference was statistically significant ($P < 0.001$) in April, May, and June in comparison with the preseasonal value. No significant changes were observed in the BDP group. Between the groups, a significant difference was detected in May and June ($P < 0.02$) (Fig. 5).

Serum ECP

Serum ECP levels were also measured in six non-atopic healthy adults (four men, two women; aged 21–39 years). The mean value was 7.6μ g/l (range 3.1–13.4). Before the pollen season, ECP levels varied between 4.1 and 23.3μ g/l in the placebo group and between 6.8 and 25.3μ g/l in the BDP group. The mean ECP values (μ g/l) for the two groups during the study period are presented in Fig. 6. In the placebo group, a significant increase was observed in March ($P < 0.01$), April and May ($P < 0.001$), and June ($P < 0.02$). In the group treated with BDP, there was no significant increase in ECP levels. A significant difference between the groups was detected in May ($P < 0.05$) only.

Blood eosinophil counts

Before the season, blood eosinophil number in the placebo group and in the BDP group ranged from 180

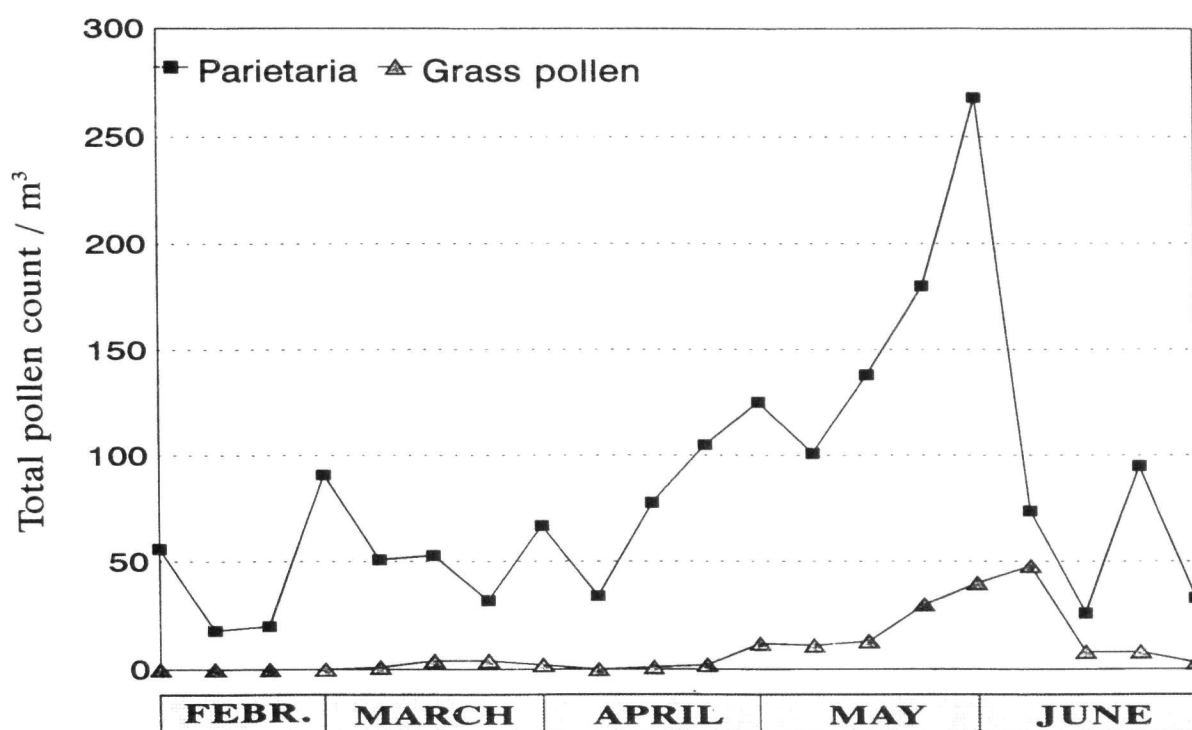


Fig. 2. Total pollen count in 1994 in study area.

to $380 \text{ cells} \times 10^6/\text{l}$ and $150 \text{ to } 470 \text{ cells} \times 10^6/\text{l}$, respectively. Mean eosinophil numbers in the two groups at different times are reported in Fig. 7. Compared to baseline, significant changes were detected in March ($P < 0.01$) and April, May, and June ($P < 0.001$) in the placebo group. A slight but significant ($P < 0.05$) increase was observed in May in the BDP group. There was a significant difference between the two groups in May ($P < 0.02$) and June ($P < 0.01$).

Correlation between ECP and other parameters

In the placebo group, ECP showed significant negative correlations with PD_{20} ($r = -0.328$, $P < 0.05$) and PEF ($r = -0.538$, $P < 0.001$). Significant positive correlations were found between serum ECP levels and blood eosinophil count ($r = 0.648$, $P < 0.001$), salbutamol consumption ($r = 0.488$, $P < 0.001$), and symptom score ($r = 0.469$, $P < 0.001$). No correlation was found between serum ECP and FEV_1 .

Correlation between eosinophil counts and other parameters

In placebo-treated patients, the number of blood eosinophils had a significant negative correlation with PD_{20} ($r = -0.300$, $P < 0.05$) and PEF ($r = -0.437$, $P < 0.01$) and a positive correlation with salbutamol consumption ($r = 0.599$, $P < 0.001$) and symptom score ($r = 0.633$, $P < 0.001$). No correlation with FEV_1 was detected.

Discussion

Airway inflammation is viewed as an important contributor to mechanisms of asthma, and its eval-

uation may represent a helpful tool for monitoring disease activity. Useful information about the state of airway inflammation may be provided by the number of eosinophils in peripheral blood. However, evaluation of the extent of eosinophil activation has been recently suggested as a more sensitive marker (7, 12, 16). Serum ECP is currently accepted as an indicator of eosinophil activity (7, 16, 17). In our study, serum ECP increased significantly during the pollen season in placebo-treated patients, but not in the beclomethasone group. Furthermore, the increase in ECP levels significantly correlated with the changes of the other parameters of asthma activity, except FEV_1 . In fact, the increase in serum ECP coincided with an increase in eosinophil number and symptom score and a decrease in PD_{20} and PEF values. The correlation between serum ECP and PD_{20} in this study points to the role of inflammation in driving airway hyperresponsiveness. However, in view of a similar study by Rak et al. (17) which did not demonstrate such a close relation, we cannot conclude that bronchial hyperresponsiveness is only a reflection of airway inflammation. Other non-inflammatory mechanisms are probably involved in enhancing airway responsiveness.

The increase in serum ECP completely followed the *Parietaria* pollen curve; indeed, the increase in ECP was already significant at the beginning of the season (March) and reached the maximum in May, when the *Parietaria* pollen load was the highest. Therefore, serum ECP appears to be the earliest indicator of allergen-induced bronchial inflammation when changes in asthmatic symptoms or in bronchial responsiveness are not yet evident.

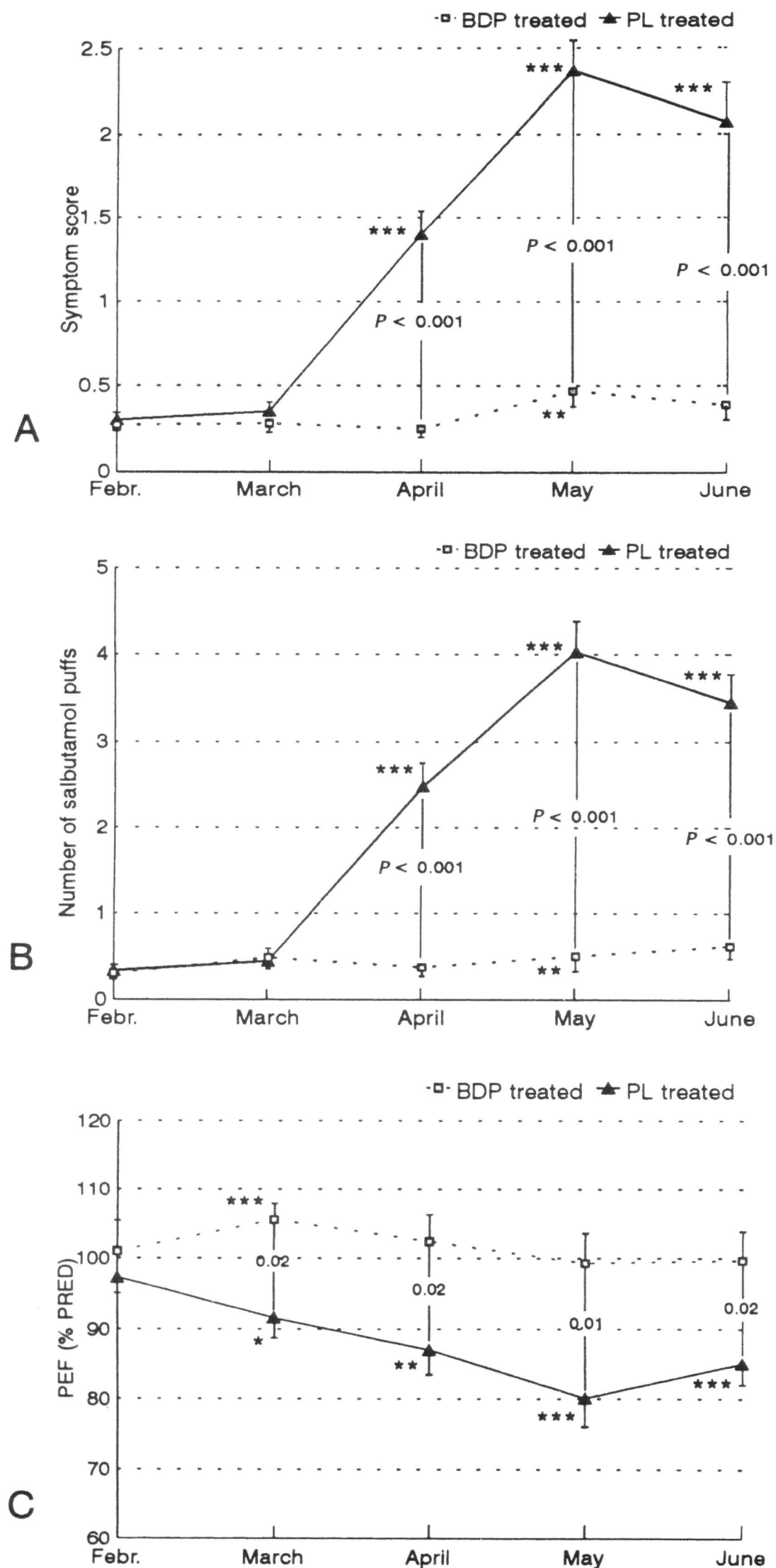


Fig. 3A–C. Mean value (SEM) of clinical data in two treatment groups during study period. A) Symptom score (0–4); B) number of salbutamol puffs; C) PEF values (percentage of predicted). * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$, compared with preseasonal value.

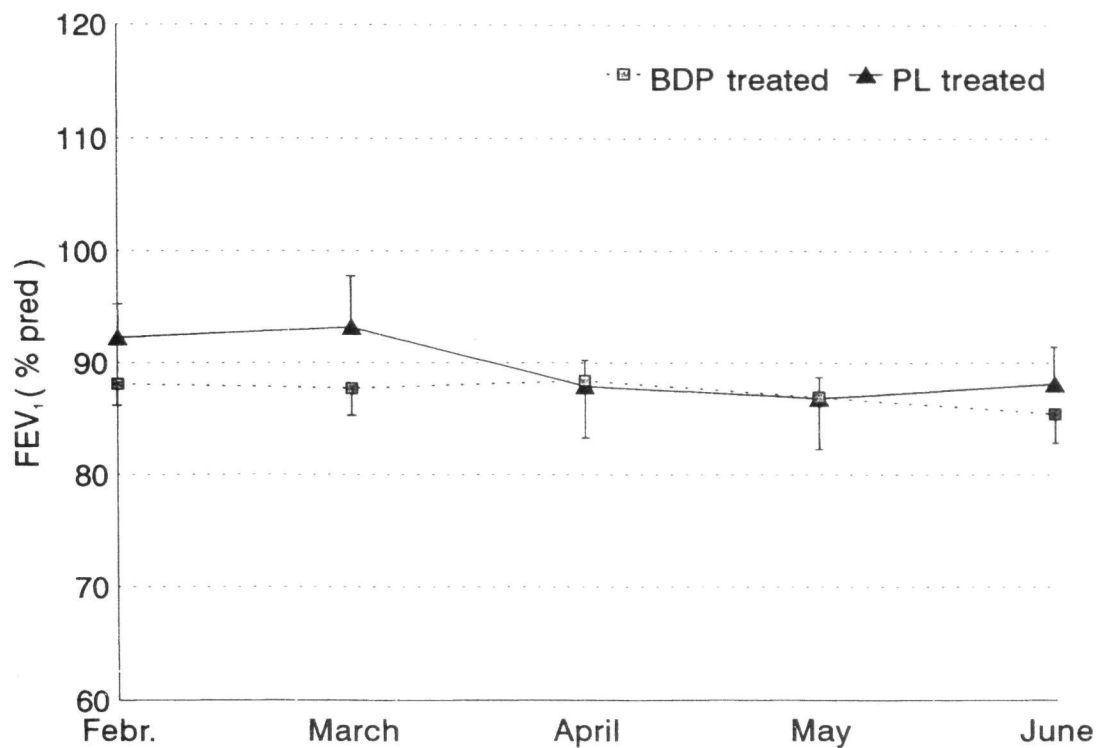


Fig. 4. FEV₁ mean values and SEM (% predicted).

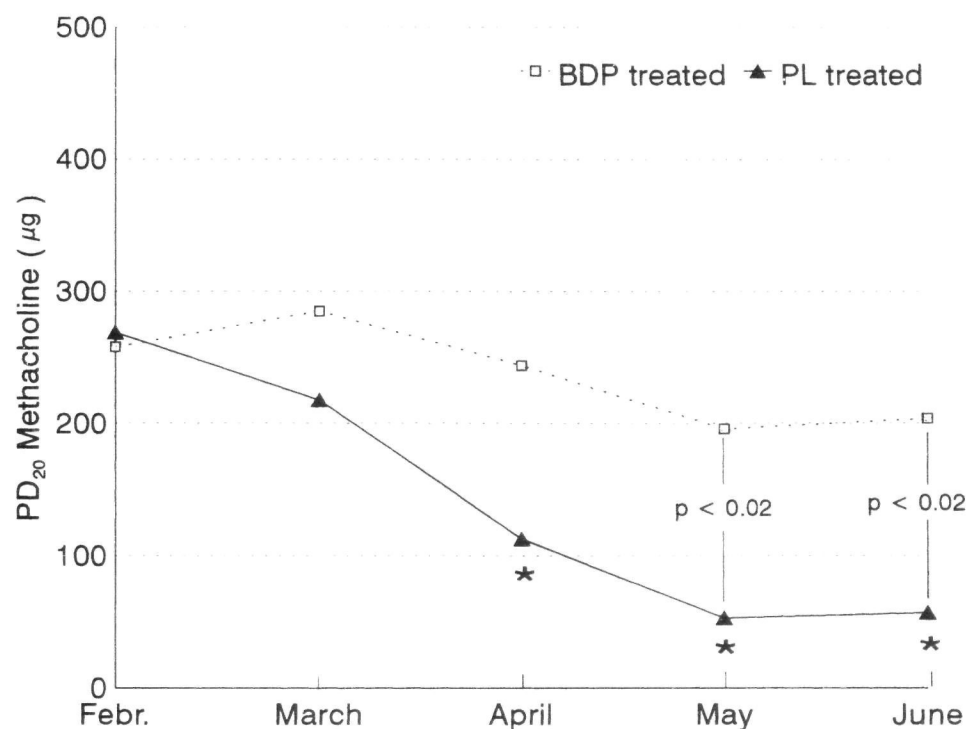


Fig. 5. PD₂₀ methacholine (geometric mean values) in two groups before and during pollen season. * $P < 0.001$, compared with baseline.

The present findings are in keeping with previous studies that showed increased levels of serum ECP during natural exposure to allergen in sensitized asthmatic subjects (17–19). Increased serum ECP levels also occurred after bronchial challenge with allergen (13, 20) or isocyanates (21). Conversely, serum ECP values decline when allergen exposure is reduced (22), or antiasthma treatments are instituted (13, 16, 19, 23). In particular, inhaled steroid treatment has been shown to reduce ECP levels in serum and in the bronchoalveolar lavage of asthmatic subjects (7, 12, 13). In a recent study of asthmatic children, inhaled budesonide reduced

serum ECP levels, and the higher the ECP level at entry, the more it decreased (23). Wempe et al. observed a decrease in serum ECP levels and blood eosinophil numbers, especially at night, after inhaled steroid treatment (24). Other investigators have also shown an inhibition of eosinophil activation as reflected by a reduction in the number of hypodense eosinophils after topical corticosteroid therapy (25). In this study, beclomethasone treatment significantly prevented the worsening of asthma during the pollen season. This preventive effect was probably due to steroid anti-inflammatory action. In fact, in the

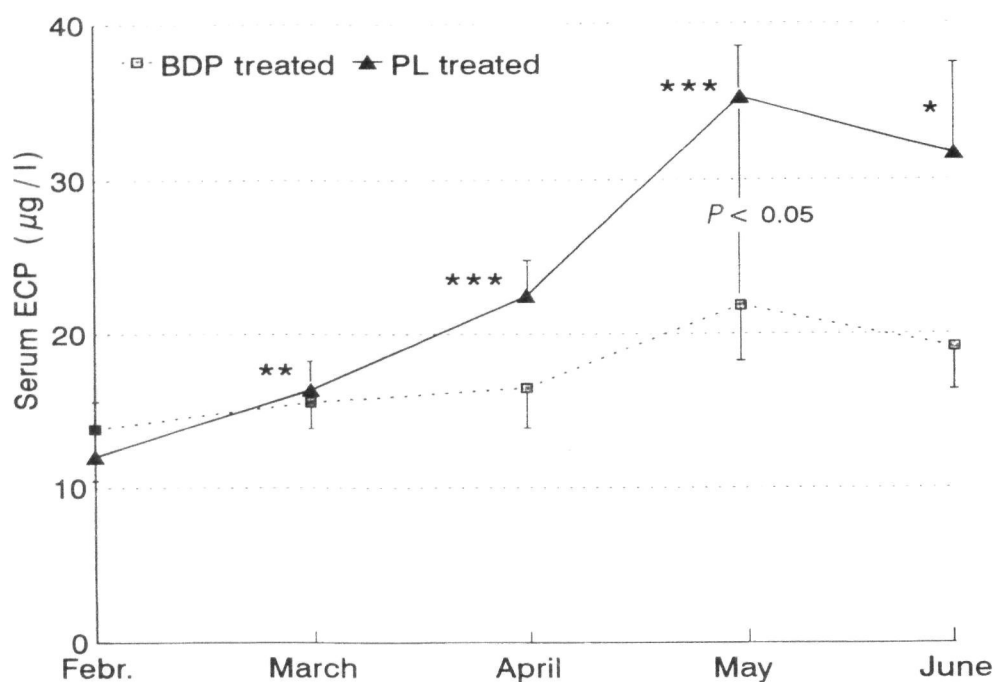


Fig. 6. Serum ECP mean values and SEM in two treatment groups. * $P < 0.02$; ** $P < 0.01$; *** $P < 0.001$, compared with baseline.

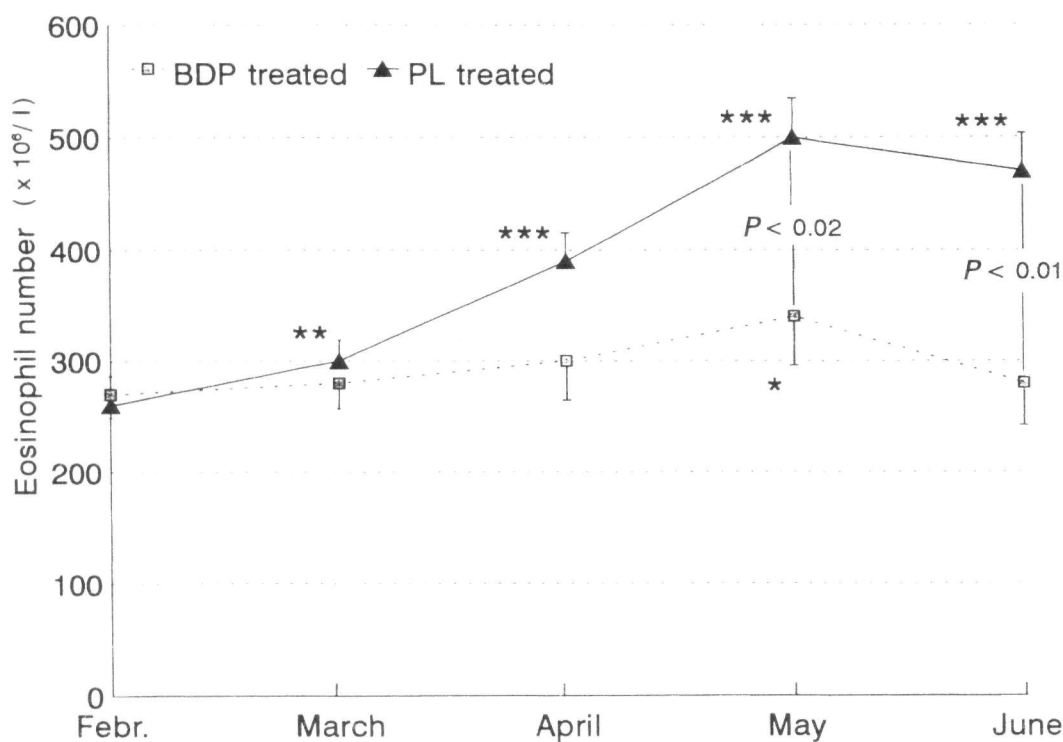


Fig. 7. Mean blood eosinophil number (SEM) during study period. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$, compared with baseline.

beclomethasone-treated group, the increase in eosinophil activation, reflected by serum ECP levels and bronchial responsiveness, was inhibited. The preventive effect of inhaled steroids was presumably due to reduced production in the airways of cytokines such as interleukin-5 and granulocyte/macrophage colony-stimulating factor, leading to a reduction in the total number of circulating eosinophils and the percentage of hypodense eosinophils (25).

In conclusion, our findings provide evidence that serum ECP levels and blood eosinophil numbers are sensitive markers of the inflammatory changes in the airway of asthmatic patients during natural pollen exposure. Therefore, these peripheral meas-

ures, especially serum ECP, may be clinically useful as objective parameters in monitoring disease activity and estimating anti-inflammatory treatment efficacy in patients with seasonal asthma.

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References

1. Kay AB. Asthma and inflammation. *J Allergy Clin Immunol* 1991;87:893-910.
2. Beasley R, Roche WR, Roberts JA, Holgate ST. Cellular

- events in the bronchi in mild asthma and after bronchial provocation. *Am Rev Respir Dis* 1989;139:806-17.
3. Laitinen LA, Laitinen AM, Haahtela T. Airway mucosal inflammation even in patients with newly diagnosed asthma. *Am Rev Respir Dis* 1993;147:697-704.
4. Bousquet J, Chané P, Lacoste JY, et al. Eosinophilic inflammation in asthma. *N Engl J Med* 1990;323:1033-9.
5. Laitinen LA, Laitinen A, Heino M, Haahtela T. Eosinophilic airway inflammation during exacerbation of asthma and its treatment with inhaled corticosteroid. *Am Rev Respir Dis* 1991;143:423-7.
6. Venge P, Henriksen J, Dahl R. Eosinophils in exercise-induced asthma. *J Allergy Clin Immunol* 1991;88:699-704.
7. Adelroth E, Rosenhall L, Johansson S-A, Linden M, Venge P. Inflammatory cells and eosinophilic activity in asthmatics investigated by bronchoalveolar lavage: the effects of antiasthmatic treatment with budesonide or terbutaline. *Am Rev Respir Dis* 1990;142:91-9.
8. Bousquet J, Chané P, Lacoste JY, et al. Indirect evidence of bronchial inflammation assessed by titration of inflammatory mediators in BAL fluid of patients with asthma. *J Allergy Clin Immunol* 1991;88:649-60.
9. Durham SR, Loegering DA, Dunnette S, Gleich GJ, Kay AB. Blood eosinophil-derived proteins in allergic asthma. *J Allergy Clin Immunol* 1989;84:931-6.
10. Carlson M, Håkansson L, Peterson C, Stålenheim G, Venge P. Secretion of granule proteins from eosinophils and neutrophils is increased in asthma. *J Allergy Clin Immunol* 1991;87:27-33.
11. Wever A, Wever-Hess J, Hensgens H, Hermans J. Serum eosinophil cationic protein (ECP) in chronic asthma. Relationship to spirometry, flow-volume curves, PC₂₀, and exacerbations. *Respir Med* 1994;88:613-21.
12. Zimmerman B, Lanner A, Enander I, Zimmerman RS, Peterson CGB, Ahlstedt S. Total blood eosinophils, serum eosinophil protein x in childhood asthma: relation to disease status and therapy. *Clin Exp Allergy* 1993;23:564-70.
13. Venge P, Dahl R, Peterson CGB. Eosinophil granule proteins in serum after allergen challenge of asthmatic patients and the effects of anti-asthmatic medication. *Int Arch Allergy Appl Immunol* 1988;87:306-12.
14. Ahlstedt S, Enander I, Peterson CGB, Lanner A. The clinical assessment of the inflammatory component in asthma with emphasis on the eosinophils. *Pract Allergy Immunol* 1993;8:149-54.
15. Peterson CGB, Enander I, Nystrand J, Anderson AS, Nilsson L, Venge P. Radioimmunoassay of human eosinophil cationic protein (ECP) by an improved method. Establishment of normal levels in serum and turnover *in vivo*. *Clin Exp Allergy* 1991;21:561-7.
16. Sugay T, Sakiyama Y, Matumoto S. Eosinophil cationic protein in peripheral blood of pediatric patients with allergic diseases. *Clin Exp Allergy* 1992;22:275-81.
17. Rak S, Lowhagen O, Venge P. The effect of immunotherapy on bronchial hyperresponsiveness and eosinophil cationic protein in pollen-allergic patients. *J Allergy Clin Immunol* 1988;82:470-80.
18. Carlson M, Håkansson L, Kampe M, Stålenheim G, Peterson C, Venge P. Degranulation of eosinophils from pollen atopic patients with asthma is increased during pollen season. *J Allergy Clin Immunol* 1992;89:131-9.
19. Rak S, Bjornson A, Håkansson L, Sorenson S, Venge P. The effect of immunotherapy on eosinophil chemotactic activity in the lung of subjects with asthma during natural pollen exposure. *J Allergy Clin Immunol* 1991;88:878-88.
20. Hedlin G, Ahlstedt S, Enander I, Håkansson L, Venge P. Eosinophil cationic protein (ECP), eosinophil chemotactic activity (ECA), neutrophil chemotactic activity (NCA) and tryptase in serum before and during bronchial challenge in cat-allergic children with asthma. *Pediatr Allergy Immunol* 1992;3:144-9.
21. Mapp CE, Plebani M, Faggian D, et al. Eosinophil cationic protein (ECP), histamine and tryptase in peripheral blood before and during inhalation challenge with toluene diisocyanate (TDI) in sensitized subjects. *Clin Exp Allergy* 1994;24:730-6.
22. Boner AL, Peroni D, Sette L, Valletta EA, Piacentini G. Effects of allergen exposure-avoidance on inflammation in asthmatic children. *Allergy* 1993;48:119-24.
23. Juntunen-Backman K, Jarvinen P, Sorva R. Serum eosinophil cationic protein during treatment of asthma in children. *J Allergy Clin Immunol* 1993;92:34-8.
24. Wempe JB, Tammeling EP, Koeter GH, Håkansson L, Venge P, Postma DS. Blood eosinophil numbers and activity during 24 hours: effects of treatment with budesonide and bambuterol. *J Allergy Clin Immunol* 1992;90:757-65.
25. Evans PM, O'Connor BJ, Fuller RW, Barnes PJ, Chung KF. Effect of inhaled corticosteroids on peripheral eosinophil counts and density profile in asthma. *J Allergy Clin Immunol* 1993;91:643-50.

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