

Serum eosinophil cationic protein in relation to bronchial asthma in a young Swedish population

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How are the serum concentration of eosinophil cationic protein (S-ECP) and the blood eosinophil count (B-Eos) related to symptoms of asthma, allergy, and bronchial hyperresponsiveness (BHR)? We measured S-ECP, B-Eos, and total and specific IgE in serum in blood samples from 699 randomly selected persons 20–44 years old. They also underwent a structured interview, spirometry, a methacholine provocation test, and skin prick tests as part of the European Community Respiratory Health Survey. B-Eos and S-ECP were found to be closely related to asthma symptom score ($P < 0.001$), total IgE ($P < 0.001$), and BHR ($P < 0.001$). On the basis of the results, the subjects were divided into four groups: healthy controls, patients with allergic rhinitis, patients with nonallergic asthma, and patients with allergic asthma. There were significant differences in both B-Eos and S-ECP among the groups ($P < 0.001$), the highest values being found in the allergic asthma group. B-Eos and S-ECP each had an additive value in predicting the occurrence of asthma. Among persons with high concentrations of both variables, asthma was eight times more common than in those with low concentrations. Allergy and BHR were also found to be independently related to B-Eos and S-ECP levels. Furthermore, both B-Eos and S-ECP showed good correlation to subjective and objective measures of asthma activity. We conclude that both B-Eos and S-ECP and their interrelationship may be of value in assessing the activity of asthma. However, their role in disease management was not established in this cross-sectional study.

E. Björnsson^{1,3}, C. Janson^{1,3},
L. Håkansson^{2,3}, I. Enander⁴,
P. Venge^{2,3}, G. Boman^{1,3}

¹ Department of Lung Medicine and Asthma Research Center, Akademiska sjukhuset, Uppsala; ² Department of Clinical Chemistry, Akademiska sjukhuset, Uppsala; ³ Asthma Research Center, Uppsala University, Uppsala; ⁴ Pharmacia Diagnostics, Uppsala, Sweden

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Dr Eythor Björnsson
Department of Lung Medicine
Akademiska sjukhuset
S-751 85 Uppsala
Sweden

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The participation of the eosinophilic granulocyte in the pathologic process in asthma has been extensively studied (11, 30). Nevertheless, the effect of its influence has been a matter of debate. Eosinophilia is included in the definition of this disease (18), and the eosinophil probably causes many of the histopathologic changes seen in asthma. A high blood eosinophil count (B-Eos) has been shown to be a risk factor for relapse in acute asthma (19), and in several studies the number of eosinophils in peripheral blood has been found to correlate with lung function (15, 27) and the clinical severity of asthma (3, 17). More recently, attempts have been made to establish a link between the extent of activity of this granulocyte and these factors (14). One measure of eosinophil activity is the amount of substances released by the cell from its granules. The granule

proteins can, in turn, induce lethal changes in airway epithelial cells, mimicking the pathologic findings in asthma (13). Among these proteins, the eosinophil cationic protein (ECP) has become widely accepted as an indicator of eosinophil activity (1, 25, 26). This protein has also been shown to correlate negatively to lung function, when measured both in serum (26) and in sputum (32).

Because definite criteria for the diagnosis of asthma have yet to be defined, studies on the relation between asthma and any measurable factor meet with obvious difficulties. In this work, we have defined asthma as the coexistence of bronchial hyperresponsiveness (BHR) and one or more asthma symptoms. This is comparable to the asthma definition recently proposed for epidemiologic use (29). The aim of this population study was to establish the value of S-ECP

and B-Eos and their interrelation as markers of asthma activity.

Material and methods

Study population

An extensive study of the prevalence of allergies and asthma is now being conducted in 48 centers in 23 countries throughout Europe and in other parts of the world as part of the European Community Respiratory Health Survey. Each center has clear geographic and administrative boundaries and a total population of at least 150 000. Sweden contributes data from three such centers, one of which is Uppsala.

In December 1990, a questionnaire including seven questions on asthma-related symptoms was mailed to 3600 men and women, aged 20–44 years, randomly selected from the population register in the municipality of Uppsala. From this cohort, a random sample of 800 persons was selected for further investigation. Additionally, all persons from the main sample who reported use of asthma medication, attacks of asthma, or awakening because of shortness of breath were invited to participate ($n = 216$). This was done to enrich the sample with symptomatic subjects. A total of 1016 persons were thus invited, but the full protocol was applied to 699. The response rates from the symptomatic and the randomly chosen samples were 83% and 68%, respectively. These 699 subjects underwent skin prick tests for 10 common allergens and a bronchial provocation test with methacholine. They were all interviewed and examined by specially trained nurses. This part of the study was conducted from April 1991 to February 1992. The investigations took place from 0800 to 1700 each day. The procedure took about 2 h for each person to complete. The same investigation sequence was applied to all persons, and the blood was drawn just before the methacholine provocation test.

All persons gave their informed consent. The protocol of the study was approved by the ethics committee of the Medical Faculty of Uppsala University.

Skin prick tests

Skin prick tests were done in a carefully standardized way with allergen-coated lancets (Phazets®, Pharmacia Diagnostics, Uppsala, Sweden). The following allergens were used: *Dermatophagoides pteronyssinus*, cat, birch, dog, *Cladosporium*, olive, ragweed, mugwort, timothy, and *Alternaria*, with histamine as a positive control. Subjects with dermatographism were excluded.

Lung function and bronchial hyperresponsiveness

Forced expiratory volume in 1 s (FEV_1) was measured in 720 persons by the Spiro Medics computerized dry-rolling seal spirometer system 2130 (Sensor Medics, Anaheim, CA, USA). The predicted values were calculated for each patient (10).

Methacholine challenge was done on 667 subjects with a Mefar dosimeter (Mefar, Brescia, Italy). Patients with a decrease in FEV_1 of 20% with an accumulated methacholine dose of 2 mg or less were defined as having BHR (22).

Peak expiratory flow (PEF) (best of three measurements) was recorded twice daily during 1 week with a Mini-Wright peak-flow meter (Clement Clarke, London, UK). The number of patients who managed to do PEF measurements twice a day on at least 4 of 7 days was 552. Peak-flow variability was calculated by dividing the difference between the highest and lowest daily PEF reading by the daily mean PEF value (16).

Blood samples

From each volunteer the following venous blood samples were collected:

- 1) five milliliters supplemented with EDTA (0.34 mol/l), which was used for analysis of blood eosinophil count on a Technicon H*1 (Technicon Chemicals Company, Tournai, Belgium).
- 2) twenty milliliters from which serum was collected and thereafter kept frozen at -70°C until analyzed for ECP. The concentration of S-ECP was assayed with a double antibody radioimmunoassay (Pharmacia Diagnostics, Uppsala, Sweden). The ECP in the samples competes with a fixed amount of ^{125}I -labeled ECP for the binding sites of specific antibodies. The intra- and inter-assay coefficients of variation were less than 11%, and the detection limit was less than $2\text{ }\mu\text{g/l}$ (24).
- 3) ten milliliters from which serum was collected and analyzed for total serum immunoglobulin E (S-IgE) and specific IgE for *D. pteronyssinus*, cat, birch, *Cladosporium*, and timothy (Pharmacia CAP systems).

Questionnaire

The postal questionnaire used was a modified version of the IUATLD questionnaire (4, 5). It included seven questions related to the diagnosis of asthma. All questions were translated into Swedish and then back into English to minimize translation bias. In Sweden only, the questionnaire included five additional questions related to chronic bronchitis and smoking. The questionnaire was mailed to the participants identified, with a letter of explanation and

a reply-paid envelope. A reminder was mailed after 10 days, and additional questionnaires were sent after 1 month and again after 2 months if no reply had been received. The structure of the questionnaire reflected the period prevalence of airway symptoms (symptoms in the last 12 months) as opposed to point, or cumulative, prevalence.

The interview was conducted by a specially trained nurse. The interview questionnaire was also based on the IUATLD questionnaire. It contained a total of 71 questions on airway symptoms, housing, social situation, education, environmental exposure during childhood, etc. No measures were taken to control the validity of the answers on smoking habits.

Classifications

On the basis of bronchial provocation tests, skin prick tests, and symptom analysis, the patients were classified into four diagnostic categories:

- H. healthy controls, exhibiting no symptoms of asthma or allergic rhinitis, no BHR, and negative prick tests
- AR. patients with allergic rhinitis but no asthma
- NA. patients with nonallergic asthma
- AA. patients with allergic asthma.

For this classification, the following definitions were used.

- 1) BHR was defined as a positive methacholine test; i.e., a reduction in FEV₁ by at least 20% after inhalation of 2 mg or less of methacholine (2).
- 2) Atopy was defined as:
 - a) a prick test reaction to at least one of the above mentioned allergens with a mean of horizontal and vertical diameters ≥ 3 mm
 - b) no dermatographism (9).
- 3) Allergic rhinitis was defined as the presence of atopy, as well as a positive answer to the question "Do you have hay fever or other types of allergic rhinitis?" and a negative answer to the asthma-related questions given below.
- 4) Symptoms of asthma were defined as a positive answer to one of the following questions. "Have you at any time during the last 12 months had:
 - a) wheezing in the chest?
 - b) breathlessness while wheezing and wheezing without a cold?
 - c) a day-time attack of breathlessness during exercise or while resting?
 - d) a nighttime awakening because of breathlessness or tightness in the chest?"

A positive answer to any of these questions was given a score of 1 point; thus, the range of possible symptom scores was 0–4.

- 5) Subjects who had both BHR and at least one of the above mentioned asthma symptoms were considered to be asthmatic (29).

- 6) Persons fulfilling the above asthma definition and who also had atopy were considered to have allergic asthma.

Furthermore, a classification of the subjects based on S-ECP and B-Eos was applied. S-ECP levels were arbitrarily divided into four levels: $< 10 \mu\text{g/l}$, $10\text{--}20 \mu\text{g/l}$, $20\text{--}30 \mu\text{g/l}$, and $> 30 \mu\text{g/l}$. B-Eos was also divided into four levels: $< 150 \times 10^6/\text{l}$, $150\text{--}300 \times 10^6/\text{l}$, $300\text{--}450 \times 10^6/\text{l}$, and $> 450 \times 10^6/\text{l}$.

Statistical analysis

The correlations between B-Eos and S-ECP levels and continuous variables were determined by single linear regression analysis. Comparisons of B-Eos and S-ECP between subgroups of the population were made by the unpaired *t*-test and ANOVA. Multiple logistic and linear regression analyses were used to investigate the correlation of more than one variable with B-Eos, S-ECP, or asthma prevalence. For all statistical tests, B-Eos, S-ECP, and S-IgE were log transformed to achieve an approximately normal distribution. After log-transformation, none of the three variables had a distribution that was significantly different from a normal distribution when the Kolmogorov-Smirnov test was applied. A *P* value of < 0.05 (two-tailed test) was regarded as statistically significant. Results are presented as mean $\pm$ SD.

Results

The study comprised 699 persons. Results of interview, skin prick tests, and bronchial provocation were available for all; measurements of B-Eos for 684, and measurements of S-ECP and S-IgE for 595

Table 1. Characteristics of study population ($n=699$)

	Mean $\pm$ SD	Range
Age (years)	33 $\pm$ 7	20–45
FEV ₁ (% of predicted)	107 $\pm$ 13	46–144
Total S-IgE (kU/l)	93 $\pm$ 215	2–2000
B-Eos ($\times 10^6/\text{l}$)	194 $\pm$ 144	1–1010
S-ECP ($\mu\text{g/l}$)	14.7 $\pm$ 9.0	2.5–58.2
	<i>n</i>	%
Men	343	49.1
Atopy	281	41.5
BHR	138	21.7
Asthmatic symptoms	327	47.5
Smokers	188	26.8
Inhalation medication	139	20.1
Inhaled steroids	38	5.4
Daily inhaled steroids	12	1.7
Oral steroids	2	0.2
Daily oral steroids	0	

B-Eos=blood eosinophils; S-ECP=serum eosinophil cationic protein; BHR=bronchial hyperresponsiveness.

persons. The characteristics of the study population are shown in Table 1. Inhaled steroids were taken occasionally or daily by 38 patients.

Relationship between eosinophil count and ECP

A significant correlation was found between B-Eos and S-ECP. The correlation coefficient of 0.49 corresponds to a predictive value of 24%. Mean B-Eos and S-ECP were higher in persons who inhaled steroids than in those who did not (B-Eos $371 \pm 217 \times 10^6/l$ and $184 \pm 132 \times 10^6/l$, S-ECP $24.4 \pm 13.6 \mu g/l$ and $14.2 \pm 8.4 \mu g/l$, respectively, $P < 0.001$). However, when the comparison included only patients with the highest symptom score, no difference in mean B-Eos and S-ECP levels was found between those using inhaled steroids ($n = 19$) and those not taking this medication ($n = 29$).

Effect of age, sex, and smoking

Age did not significantly affect B-Eos or S-ECP. Men had significantly higher eosinophil counts than women ($207 \pm 152 \times 10^6/l$ and $182 \pm 137 \times 10^6/l$, respectively; $P < 0.05$), whereas S-ECP was not related to sex. Smokers had significantly higher eosinophil counts than nonsmokers ($213 \pm 145 \times 10^6/l$ and $187 \pm 144 \times 10^6/l$, respectively; $P < 0.05$). A low but significant correlation was found between number of cigarettes smoked per day and B-Eos ($r = 0.17$; $P < 0.05$). S-ECP was not significantly affected by smoking.

Relationship to lung function, atopy, S-IgE, BHR, and asthma prevalence

Both B-Eos and S-ECP showed a significant correlation to lung function as measured by FEV₁ (B-Eos: $r = 0.13$; $P < 0.001$ and S-ECP: $r = 0.12$; $P < 0.01$).

Atopic persons had higher mean values for both B-Eos ($225 \pm 153 \times 10^6/l$ and $169 \pm 132 \times 10^6/l$, respectively) and S-ECP ($16.9 \mu g/l \pm 10.4$ and $13.0 \pm 7.3 \mu g/l$, respectively; $P < 0.001$) than healthy subjects.

Total S-IgE was significantly correlated to both B-Eos ($r = 0.27$) and S-ECP ($r = 0.24$) ($P < 0.001$).

B-Eos and S-ECP were significantly higher in persons with BHR than in those without (B-Eos, $271 \pm 179 \times 10^6/l$ and $171 \pm 119 \times 10^6/l$, respectively; S-ECP, $19.2 \pm 12.2 \mu g/l$ and $13.6 \pm 7.7 \mu g/l$, respectively; $P < 0.001$). When multiple linear regression analysis was applied, B-Eos and S-ECP were found to correlate significantly with both total S-IgE and BHR, but not with age, sex, smoking, or FEV₁ (Table 2).

The relationship between prevalence of asthma and B-Eos and S-ECP levels was analyzed by di-

Table 2. Relationship among blood eosinophil count, level of serum eosinophil cationic protein (S-ECP), total S-IgE, and bronchial hyperresponsiveness (BHR), analyzed by multiple linear regression

Blood eosinophil count ($r = 0.34$; $P < 0.001$)			
	Partial correlation coefficient	F-value	P
Total S-IgE	0.12	32.3	< 0.001
BHR	0.15	21.0	< 0.001
S-ECP ($r = 0.27$; $P < 0.001$)			
	Coefficient	F-value	P
Total S-IgE	0.09	20.7	< 0.001
BHR	0.15	12.2	< 0.001

viding the patients into four arbitrarily defined B-Eos and S-ECP groups. The prevalence of asthma (allergic and nonallergic) was 4–5 times as high in the groups with the highest B-Eos ($> 450 \times 10^6/l$ and S-ECP ($> 30 \mu g/l$) than in the low-level groups (B-Eos $< 150 \times 10^6/l$ and S-ECP $< 10 \mu g/l$) (Table 3). Multiple logistic regression analysis showed that B-Eos and S-ECP were independently correlated with asthma ($\beta = 0.004$; $P < 0.001$ and $\beta = 0.04$; $P < 0.01$). When the B-Eos and S-ECP values were combined, the group of patients with B-Eos $> 450 \times 10^6/l$ and S-ECP $> 30 \mu g/l$ had a prevalence of asthma that was eight times higher than in patients with either Eos $< 150 \times 10^6/l$ or ECP $< 10 \mu g/l$ (Fig. 1).

Relationship to symptoms

Persons who reported at least one asthma-related symptom had higher mean levels of both B-Eos ($223 \pm 161 \times 10^6/l$) and S-ECP ($16.4 \pm 10.0 \mu g/l$) than those without symptoms ($172 \pm 125 \times 10^6/l$ and $13.3 \pm 7.9 \mu g/l$, respectively; $P < 0.001$). To quantify the severity of symptoms, we used the symptom score described above. Its relationships to S-ECP and B-Eos are shown in Fig. 2. Both variables show a significant correlation ($P < 0.001$). The proportion of persons with BHR likewise shows a significant relationship to the symptom score (Fig. 2).

Table 3. Prevalence of asthma (allergic and nonallergic) in groups with different blood eosinophil counts (B-Eos) and levels of eosinophil cationic protein (S-ECP) (n = number of persons in each group)

B-Eos ($\times 10^6/l$)	<i>n</i>	%	S-ECP ($\mu g/l$)	<i>n</i>	%
< 150	326	10	< 10	204	12
150–300	237	18	10–20	263	12
300–450	79	34	20–30	87	25
> 450	42	53	> 30	41	49

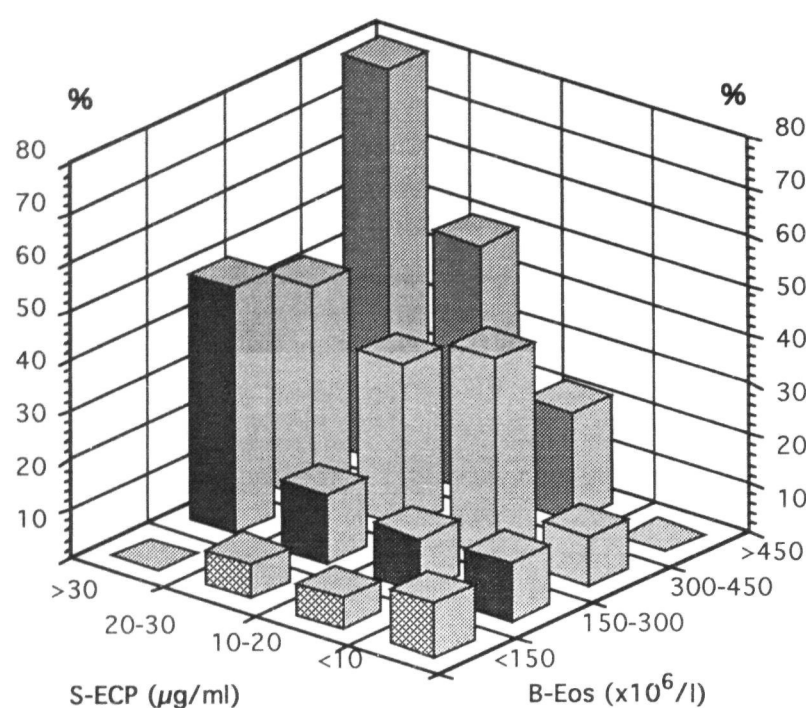


Fig. 1. Prevalence of asthma (bronchial hyperresponsiveness and asthmatic symptoms) in relation to blood eosinophil count (B-Eos) and level of serum eosinophil cationic protein (S-ECP).

Relationship to different diagnostic categories

The B-Eos and S-ECP values in the four diagnostic categories described above are presented in Fig. 3. The highest mean levels were found in patients with allergic asthma and the lowest in the healthy controls ($P < 0.001$).

Discussion

Because the inflammatory nature of asthma has become clearer and potent anti-inflammatory drugs

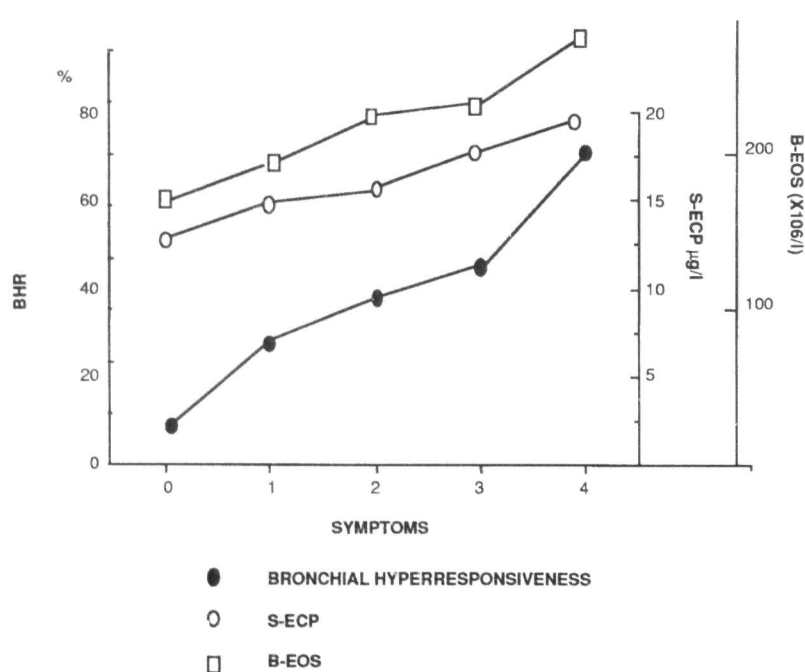


Fig. 2. Relationship between asthma symptom score, proportion of persons with bronchial hyperresponsiveness (BHR %), and blood eosinophil count (B-Eos) and level of serum eosinophil cationic protein (S-ECP). Values given are mean values.

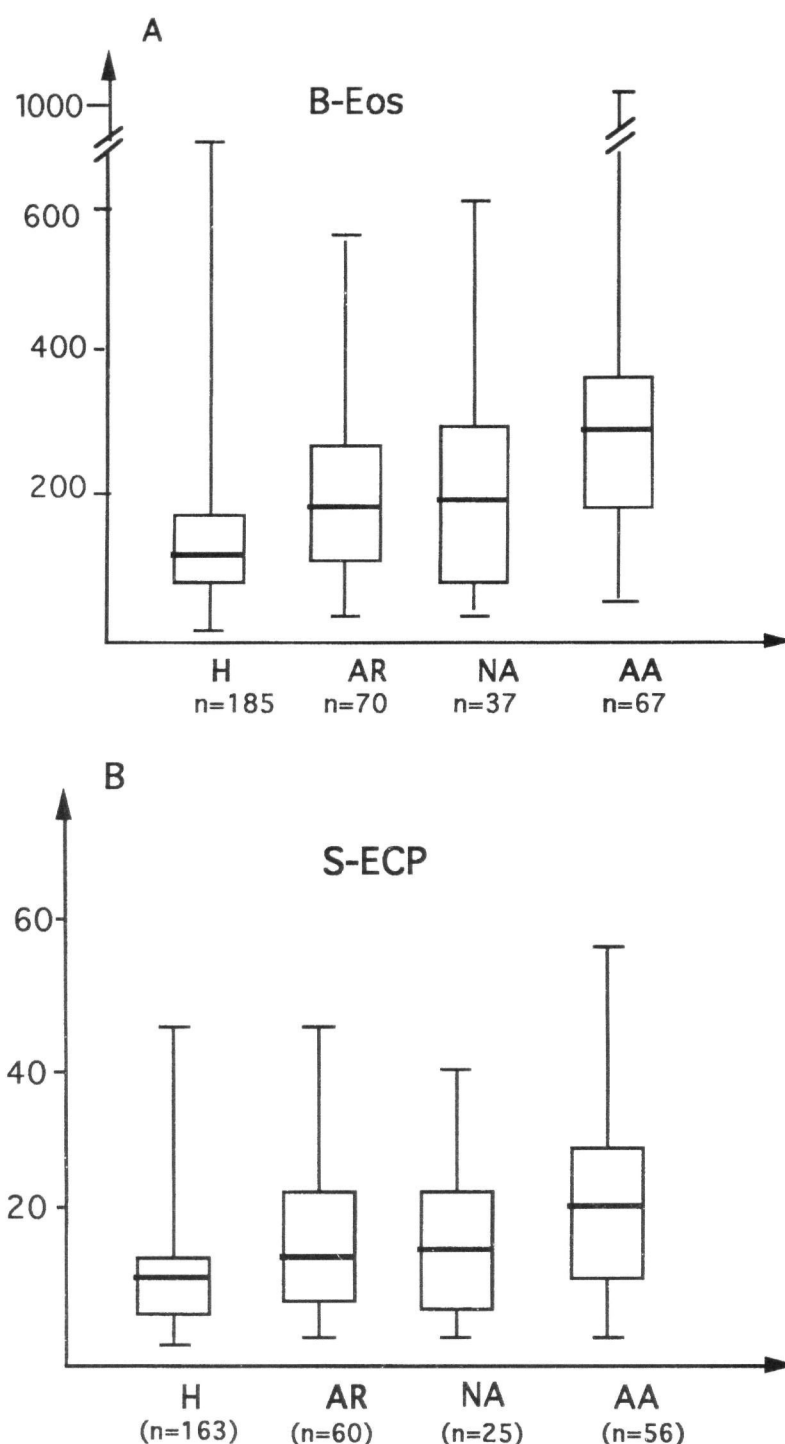


Fig. 3. A) Blood eosinophil count (B-Eos) and B) level of serum eosinophil cationic protein (S-ECP) in four diagnostic groups (median, interquartile range (25–75%) and total range). H = healthy controls, AR = allergic rhinitis, NA = nonallergic asthma, AA = allergic asthma.

have become available, a marker of disease activity is required. Ideally, it should be simple to measure, reliable, and inexpensive. No such marker has yet been found for asthma. We set out to investigate the value of S-ECP as a serologic marker of airway inflammatory activity, expressed in terms of a symptom score, as well as of lung function and BHR. We have studied its relation to the blood eosinophil count (B-Eos) and the level of total S-IgE. We have also compared S-ECP and B-Eos in four different diagnostic groups. We found that S-ECP was, unlike B-Eos, insensitive to smoking habits or sex. S-ECP and B-Eos were closely connected with symptoms and BHR, and had additional value as predictors of asthma.

Relationship to eosinophil count and to smoking

The connection between B-Eos and S-ECP is not straightforward, and a number of factors may influence the two variables differently. Differences between the diurnal profiles of B-Eos and S-ECP have been seen in both normal and asthmatic subjects. Eosinophil levels spike during the night, but S-ECP levels in the evening (8). In one report, the investigators proposed the existence of two subgroups, one with high levels of S-ECP and the other with low levels, irrespective of eosinophilia (31). In our material, the connection between B-Eos and S-ECP was statistically significant.

The mean level of S-ECP in the healthy group was 11.9 µg/l. This is somewhat higher than the reference values (24, 33). The cause of this discrepancy is not clear. Smoking has been said to increase the serum level of ECP (20). However, the difference in S-ECP levels between healthy nonsmokers and healthy smokers in this population was not significant.

On the other hand, the effect of both smoking and sex on the eosinophil count was significant, men and smokers having higher levels. Neither variable affected the S-ECP level.

Relationship to lung function, IgE, and prevalence of asthma

The negative effect of eosinophilia on lung function has been established (11, 12, 23, 28). However, the association of eosinophilia with FEV₁ has mainly been attributed to the presence of asthma (6, 7) and smoking (21). We noted a significant relation of lung function, as expressed by FEV₁, with both S-ECP and B-Eos. However, after correction for BHR and atopy, this connection was eliminated.

Total serum IgE was closely related to both the eosinophil count and S-ECP. This is perhaps to be expected in view of the interrelating pathways by which these factors are thought to be controlled.

The combination of S-ECP and B-Eos seemed to increase the predictive value of these variables for asthma (Fig. 1). We believe that this illustrates that neither one of these tests makes the other obsolete, but rather that they complement each other.

Relationship to symptoms

In our study, both BHR and all the studied laboratory variables displayed a close relationship to the symptom score (Fig. 2). This clearly illustrates the value of symptom combinations in assessing asthma activity and has implications both for epidemiologic research and the day-to-day clinical management of asthma.

Fig. 2 also shows the association of the S-ECP

and B-Eos levels with the severity of asthma, an association which is relevant to the use of these variables to monitor therapy effects. However, this cross-sectional study did not establish the value of S-ECP as a prospective marker of disease activity in asthma.

No definite normal values for PD₂₀ in the methacholine test by the dosimeter method have been generally agreed upon. Our choice of cutoff value is close to that used by Balzano et al. (2).

Relationship to disease categories

To avoid confusion, we limited our analysis to four diagnostic groups. Subjects with nonallergic rhinitis, or subjects with asthmatic symptoms but without BHR, etc. are therefore not included in this comparison. The values of B-Eos and S-ECP in the four groups are shown in Fig. 3. There was a significant difference between the healthy subjects, on one hand, and patients with allergic rhinitis or nonallergic asthma on the other, as well as between these last two groups and the group with allergic asthma. This may suggest that separate stimuli for eosinophil proliferation and degranulation operate in allergic diseases and in the induction of BHR. Because FEV₁ and the symptom score were comparable in both asthmatic groups, the difference is not merely a reflection of disease activity.

Conclusions

Our study provides data on the relation between eosinophil markers in peripheral blood and asthma activity, and also the effects of age, sex, and smoking. Furthermore, it indicates that allergy and BHR are independently connected with eosinophilia.

It is evident that information on a combination of symptoms is of value in the assessment of airway inflammation as reflected by eosinophil activity and BHR, and also that S-ECP and B-Eos are well correlated to both subjective and objective indices of asthma activity.

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References

1. ÄDELROTH E, ROSENHALL L, JOHANSSON SÅ, LINDEN M, VENGE P. Inflammatory cells and eosinophil activity in asthmatics investigated by bronchoalveolar lavage: the effects of antiasthmatic treatment with budesonide or terbutaline. *Am Rev Respir Dis* 1990; 142: 91–9.
2. BALZANO G, CARRI ID, CALLO C, COCCO G, MELILLO G. Intrasubject between-day variability of PD₂₀ methacholine assessed by the dosimeter inhalation test. *Chest* 1989; 95: 1239–45.
3. BOUSQUET J, CHANEZ P, LACOSTA JY, et al. Eosinophilic inflammation in asthma. *N Engl J Med* 1990; 323: 1033–9.
4. BURNEY PGJ, CHINN S. Developing a new questionnaire for measuring the prevalence and distribution of asthma. *Chest* 1987; 91 (Suppl.): 79–92.
5. BURNEY PGJ, LAITINEN LA, PERDRIZET S, et al. Validity and repeatability of the IUATLD (1984) Bronchial Symptoms Questionnaire: an international comparison. *Eur Respir J* 1989; 2: 940–5.
6. BURROWS B, HASAN FM, BARBEE RA, HALONEN M, LEBOWITZ MD. Epidemiologic observations on eosinophilia and its relation to respiratory disorders. *Am Rev Respir Dis* 1980; 122: 709–19.
7. BURROWS B, KNUDSON RJ, CLINE MG, LEBOWITZ MD. A reexamination of risk factors for ventilatory impairment. *Am Rev Respir Dis* 1988; 138: 829–36.
8. DAHL R, VENGE P, OLSSON I. Blood eosinophil leucocytes and eosinophil cationic protein. *Scand J Respir Dis* 1978; 59: 323–5.
9. DREBORG S, ed. Skin tests used for epidemiological studies. *Allergy* 1989; 44 (Suppl.): 52–9.
10. EUROPEAN COMMUNITY FOR COAL AND STEEL. Standardization of lung function tests. *Clin Respir Phys* 1983; 19 (Suppl. 5): 22–7.
11. FRETTE C, ANNESI I, KOROBAEFF M, NEUKIRCH F, DORE MF, KAUFFMANN F. Blood eosinophilia and FEV₁. *Am Rev Respir Dis* 1991; 143: 987–92.
12. GIBSON PG, DOLOVICH J, DENBURG J, RAMSDALE EH. Chronic cough: eosinophil bronchitis without asthma. *Lancet* 1989; i: 1346–8.
13. GLEICH GJ, FLAVAHAN NA, FUJISAWA T, VANHOUTTE PM. The eosinophil as a mediator of damage to respiratory epithelium: a model for bronchial hyperreactivity. *J Allergy Clin Immunol* 1988; 81: 776–81.
14. GRIFFIN E, HÅKANSSON L, FORMGREN H, JÖRGENSON K, PETERSON C, VENGE P. Blood eosinophil number and activity in relation to lung function in patients with asthma and with eosinophilia. *J Allergy Clin Immunol* 1991; 87: 548–57.
15. HEDLIN G, AHLSTEDT S, ENANDER I, HÅKANSSON L, VENGE P. Eosinophil cationic protein, eosinophil chemotactic activity, neutrophil chemotactic activity and tryptase in serum before and during bronchial challenge in cat-allergic children with asthma. *Pediatr Allergy Immunol* 1992; 3: 144–9.
16. HIGGINS BG, BRITTON JR, CHINN S, COOPER S, BURNEY PGJ, TATTERSFIELD AE. Comparison of bronchial reactivity and peak expiratory flow variability measurements for epidemiological studies. *Am Rev Respir Dis* 1992; 145: 588–93.
17. HORN BR, ROBIN ED, THEODORE J, KESSEL A. Total eosinophil count in the management of bronchial asthma. *N Engl J Med* 1975; 292: 1152–5.
18. International consensus report on diagnosis and management of asthma. *Allergy* 1992; 47 (Suppl. 13): 1–5.
19. JANSON C, HERALA M. Blood eosinophil count as risk factor for relapse in acute asthma. *Respir Med* 1992; 86: 101–4.
20. JENSEN EJ, PEDERSEN B, SCHMIDT E, VENGE P, DAHL R. Serum-ECP and serum-lactoferrin in non-atopic smokers, non-smokers and recent ex-smokers; relation to lung function, airway symptoms and smoking history. *Eur Respir J* (in press).
21. KAUFFMAN F, NEUKIRCH F, KOROBAEFF M, MARNE MJ, CLAUDE JR, LELLOUCH J. Eosinophils, smoking and lung function. *Am Rev Respir Dis* 1986; 134: 1172–5.
22. KNOX AJ, WISNIEWSKI A, COOPER S, TATTERSFIELD AE. A comparison of the Yan and a dosimeter method for methacholine challenge in experienced and inexperienced subjects. *Eur Respir J* 1991; 4: 497–502.
23. MENSINGA T, SCHOUTEN JP, RIJCKEN B, WEISS ST, SPEIZER FE, VAN DERLENDE R. The relationship of eosinophilia and positive skin test reactivity to respiratory symptom prevalence in a community-based population study. *J Allergy Clin Immunol* 1990; 86: 99–107.
24. PETERSON CGB, ENANDER I, NYSTRAND J, ANDERSSON AS, NILSON 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.
25. RAK S, LÖVHAGEN O, VENGE P. The effect of immunotherapy on bronchial hyperresponsiveness and eosinophil cationic protein in pollen allergic patients. *J Allergy Clin Immunol* 1988; 82 (no. 3, Part 1): 470–80.
26. SUGAI T, SAKIYAMA Y, MATUMOTO S. Eosinophil cationic proteins in peripheral blood of pediatric patients with allergic diseases. *Clin Exp Allergy* 1992; 22: 275–81.
27. TAYLOR KJ, LUKSZA AR. Peripheral blood eosinophil counts and bronchial responsiveness. *Thorax* 1987; 42: 452–6.
28. TAYLOR RG, GROSS E, JOYCE H, HOLLAND F, PRIDE NB. Smoking, allergy and the differential white blood cell count. *Thorax* 1985; 40: 17–22.
29. TOELLE BG, PEAT JK, SALOME CM, MELLIS CM, WOOLCOCK AJ. Towards a definition of asthma for epidemiology. *Am Rev Respir Dis* 1992; 146: 633–7.
30. VENGE P, HÅKANSSON L. Current understanding of the role of the eosinophil granulocyte in asthma. *Clin Exp Allergy* 1991; 21 (Suppl.): 31–7.
31. VENGE P, ZETTERSTRÖM O, DAHL R. Low levels of eosinophil cationic protein in patients with asthma. *Lancet* 1977; ii: 373–6.
32. VIRCHOW JC, HÖLSCHER U, VIRCHOW C. Sputum ECP levels correlate with parameters of airflow obstruction. *Am Rev Respir Dis* 1992; 146: 604–6.
33. 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.

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