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Serum eosinophil cationic protein (sECP) in subjects with a history of asthma symptoms with or without rhinitis

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Key words: asthma; bronchial hyperresponsiveness; eosinophil cationic protein (ECP); eosinophils; peak expiratory flow rate (PEFR); spirometry; symptoms.

Background: Serum eosinophil cationic protein (sECP) has been proposed as a marker of disease activity in bronchial asthma. The study aimed to evaluate the role of sECP in screening asthmatics in a group of subjects with asthma and rhinitis symptoms, and the relationship between sECP and clinical and functional parameters of asthma.

Methods: A total of 185 subjects with asthma symptoms, 149 of them with rhinitis as well, underwent skin tests, spirometry, methacholine (MCH) test, blood sampling for eosinophil percentage (bEOS%) and sECP determination, and nasal secretions smear for eosinophil percentage (nEOS%) determination; PEF values, symptoms, and medication over a period of 4 weeks after sampling for sECP quantitation were recorded on a diary.

Results: A total of 99 (53%) subjects received a diagnosis of asthma (asthmatics), and 86 did not (nonasthmatics). In asthmatics, neither sECP nor bEOS% was significantly different from nonasthmatics. In asthmatics, sECP was higher in subjects with increased than in those with normal daily PEF variability (16.4, 6.8–24.4 vs 5.3, 3.9–8.4 $\mu\text{g/l}$; $P<0.001$). sECP was higher in moderate persistent asthma than in intermittent asthma (24.8, 10.6–53 vs 8.4, 5.6–14.1; $P<0.05$). In nonasthmatics (73 with a history of rhinitis), both sECP and bEOS% correlated with nEOS% ($\rho=0.35$; $P<0.01$ and $\rho=0.53$; $P<0.001$).

Conclusions: In adults with asthma symptoms with or without rhinitis, sECP did not distinguish asthmatics from nonasthmatics. In asthmatics, sECP was associated with PEF variability and symptom severity. In subjects with asthma and rhinitis, as well as in subjects with only rhinitis, sECP levels are possibly influenced by nasal inflammation.

Date:

Accepted for publication 6 April 1999

To cite this article:

Perfetti L., Galdi E., Bramé B., Speciale L. & Moscato G. Serum eosinophil cationic protein (sECP) in subjects with a history of asthma symptoms with or without rhinitis. *Allergy* 1999, **54**, 962–967.

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ISSN 0105-4538

In bronchial asthma, eosinophils play a major role in producing bronchial mucosa injury by releasing highly toxic proteins. One of these proteins, eosinophil cationic protein (ECP), can be quantitated in bronchial secretion and peripheral blood.

In asthmatic children, serum ECP (sECP) was higher than in healthy controls (1, 2), but similar to that found in allergic rhinitis (3), and was correlated with spirometry parameters (4), bronchial hyperresponsiveness (BH) (5), and severity of symptoms (6–8) although this last correlation was not confirmed (3).

In adult asthmatics, sECP was higher than in healthy controls (9–11) and correlated with symptom score (9), frequency of symptom exacerbation, FEV₁/FVC (12), and BH (9, 12, 13).

In allergic rhinitis, which is frequently associated with asthma in atopics, sECP was increased both in children (3) and in adults (14), although other authors did not find any difference (15). A relationship between ECP in nasal secretion and in serum has been shown in pollen-allergic children (16).

In asthmatics, the influence on sECP of the concomitant presence of rhinitis has not yet been elucidated.

The aim of our study was to measure sECP in subjects with asthma symptoms with or without rhinitis in order to determine whether sECP can screen out asthmatics. We also investigated whether there was a relationship between sECP and clinical and functional parameters of asthma and nasal inflammation.

Material and methods

Subjects

We enrolled 185 outpatients referred to our allergy department with a history of asthma symptoms with or without rhinitis symptoms, excluding subjects with conditions known to affect sECP (i.e., extensive atopic dermatitis [17], acute infection in the 4 weeks before the study [18], and treatment with topical or oral steroids and/or chromones in the 4 weeks before or during the study [19]). Ages ranged from 14 to 65 years; 107 subjects were female. Fifty subjects were smokers, and 31 ex-smokers. A total of 115 subjects had a history of perennial asthma symptoms and 63 of seasonal asthma symptoms (39 evaluated in the symptomatic period), while seven reported only occasional episodes. Ninety-seven subjects had also a history of rhinoconjunctivitis and 52 of rhinitis. The duration of

asthma symptoms (median) was 4 years (interquartile range: 2–9).

Design of the study

Subjects were monitored over a 4-week period. All investigations took place in the first week. On the first day, patients underwent blood sampling for sECP, white blood cells, and eosinophil count, and they were instructed in PEF monitoring. In subjects with a history of rhinitis, nasal secretions were also collected. Subjects then underwent skin prick test, spirometry, bronchodilator test, and methacholine (MCH) challenge test. The diagnosis of asthma was made according to GINA (20). During the study, patients were allowed to take short-acting β_2 -agonist as needed.

Methods

Skin prick tests were performed for the most common aeroallergens (Lofarma S.p.A., Milan, Italy) (21). Atopy was defined as the presence of a positive skin reaction, as previously defined (22), to one or more allergens.

White blood cells were counted by an automated hematology analyzer (Coulter S 880). A blood smear was stained with May-Grünwald-Giemsa for the manual count of eosinophils, expressed as the percentage of the total white cell number (bEOS%).

To evaluate nasal eosinophilia, patients blew their nose into a piece of wax paper (12×12 cm). A glass microscope slide was then placed on top of the secretions. After drying, the smear was stained with May-Grünwald-Giemsa. With exclusion of epithelial cells, cells were counted in units of 500, and nEOS% was expressed as the ratio of eosinophils to PMNs.

For sECP determination, sera were handled according to the manufacturer's instructions and stored at –20°C. sECP was measured with a double antibody radioimmunoassay (Pharmacia Diagnostics, Uppsala, Sweden).

Spirometry was performed by means of a computerized, water-sealed spirometer (Biomedin, Padova, Italy).

The MCH test was performed by means of a nebulizer (MEFAR, Brescia, Italy) connected to a dosimeter, as previously reported (21). The final datum obtained was the dose of MCH[®] causing a 20% fall in FEV₁ (PD₂₀FEV_{1mch}).

Patients were instructed in the use of a portable peak flow meter (Mini-Wright). PEF was measured four times daily, and recorded on a diary together with symptoms and use of β_2 -agonist p.r.n. Daily PEF variability was calculated as the

difference between evening and morning values, expressed as the percentage of the mean daily PEF value (20).

According to the daily PEF variability in the period of observation, asthmatics were divided into three subgroups: normal variability ($\Delta\text{PEF}\%$ always $<20\%$), occasional variability ($\Delta\text{PEF}\% \geq 20\%$ on 1 day only), and increased variability ($\Delta\text{PEF}\% \geq 20\%$ on 2 or more days).

On the basis of symptoms and β_2 -agonist p.r.n. use, subjects were subgrouped according to the clinical features of the four levels of the classification of asthma severity (20). A level 0 (asymptomatic during the 4-week period of observation) was added.

Statistical analysis

Results are presented as median and interquartile ranges (1st–3rd quartiles), as variables did not approach a normal distribution even after log-transformation. The Mann–Whitney U-test and the Kruskal–Wallis test were used where appropriate. When the Kruskal–Wallis test indicated a significant difference, the subgroups were independently compared with the Mann–Whitney U-test, and the Bonferroni correction was applied. Spearman's rank correlation coefficient (ρ) was calculated for correlations between variables. A P value of <0.05 was regarded as statistically significant.

Results

A total of 130 subjects were found to be atopic. Only 99 (53%) fulfilled the criteria for asthma (20). No differences in bEOS% and sECP were found in the different subgroups classified with respect to atopy, smoking, and history (asthma or asthma associated with rhinitis).

sECP was not significantly different between asthmatics and nonasthmatics (9.9, 5.7–19.3 vs 8.2, 4.7–13.7, respectively), while $\text{PD}_{20}\text{FEV}_{1\text{mch}}$ was significantly different (520, 162–1013 vs >2600 , >2600 – >2600 , respectively).

In the overall group, bEOS% significantly correlated with sECP and nEOS%, while sECP significantly correlated with $\text{PD}_{20}\text{FEV}_{1\text{mch}}$ and with nEOS% (Table 1).

In nonasthmatics, sECP significantly correlated with bEOS% (Table 1); both parameters correlated with nEOS% (Table 1).

In asthmatics, sECP correlated with $\text{PD}_{20}\text{FEV}_{1\text{mch}}$ and showed a tendency to correlate with nEOS% (Table 1), while no correlations between sex, smoking, and atopy and sECP or bEOS% were found.

Table 1. Correlations between eosinophils (percentage), sECP, and different parameters in overall group and in two subgroups defined according to final diagnosis

	All subjects (n=185)		Nonasthmatics (n=86)		Asthmatics (n=99)	
	bEOS%	sECP	bEOS%	sECP	bEOS%	sECP
FEV ₁ /VC %pred	–0.15	–0.12	–0.16	0.03	–0.08	–0.14
PD ₂₀ -FEV ₁ (μg)	–0.14	–0.22***	0.001	0.08	–0.01	–0.22*
Blood eosinophils (%)	—	0.30****	—	0.48****	—	0.12
sECP (μg/l)	0.30****	—	0.48****	—	0.12	—
Nasal eosinophils (%)	0.29***	0.31****	0.53****	0.35**	0.01	0.24#

Values expressed as Spearman rho coefficient. #0.05<P<0.1 *P<0.05 **P<0.01 ***P<0.005 ****P<0.001.

In 75 asthmatics, the PEF reading was satisfactory: sECP was higher in subjects with augmented than normal variability (16.4, 6.8–24.4 vs 5.3, 3.9–8.4 μg/l; $P<0.001$); subjects with occasional variability showed a tendency to higher values of sECP than subjects showing normal variability (Fig. 1). sECP was higher in moderate persistent than in intermittent asthma (24.8, 10.6–53 vs 8.4, 5.6–14.1; $P<0.05$), showing, as well, a tendency to higher values than in asymptomatic subjects (Fig. 2). $\text{PD}_{20}\text{FEV}_{1\text{mch}}$ was not different in the different subgroups according to PEF variability and symptom severity.

Discussion

Our study shows that in a group of subjects with a history of asthma symptoms, with or without rhinitis symptoms, sECP does not help in anticipating a diagnosis of asthma. In asthmatics, sECP correlated with $\text{PD}_{20}\text{FEV}_{1\text{mch}}$ and was

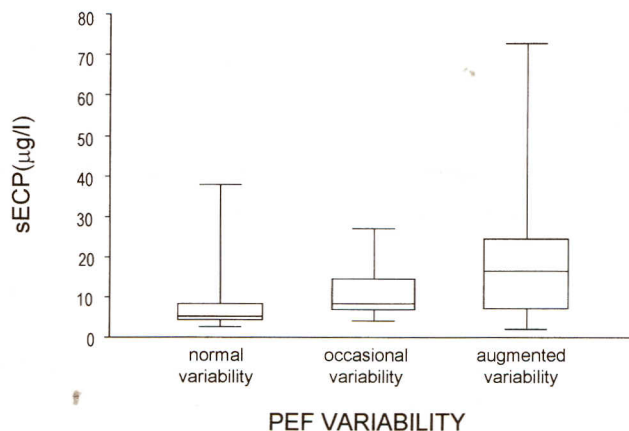


Figure 1. sECP concentration in different subgroups of asthmatics defined according to PEF diurnal variability.

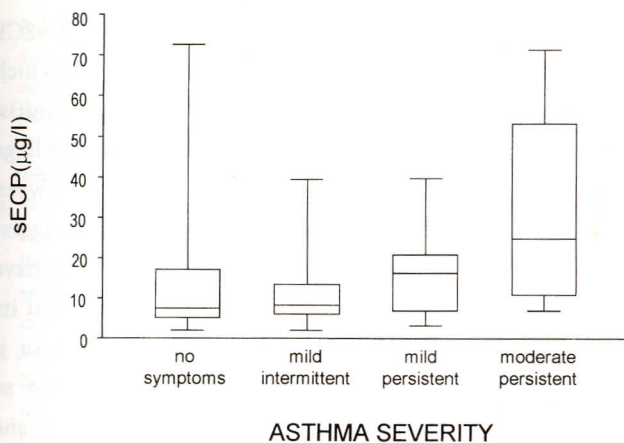


Figure 2. sECP concentration in different subgroups of asthmatics defined according to symptom severity.

associated with PEF daily variability and symptom severity in a period of 4 weeks after sECP determination.

In asthmatics, sECP higher than in healthy controls has already been reported (1, 9–11). In our population, the lack of a difference between asthmatics and nonasthmatics may be due to two reasons. First, our asthmatics had lower sECP than in previous studies (10, 12), and, secondly, our nonasthmatics had sECP higher than healthy controls (23), corresponding to the higher values of the range in a normal population (22). This may be related to the low severity of the disease in our asthmatics, when compared with those of previous studies, and to the fact that our nonasthmatics were not healthy controls: some of them may have been latent asthmatics, being investigated at a time when no objective parameters of asthma were obtained; in addition, most of them were atopic and reported symptoms of rhinitis, which is associated with increased sECP (3, 14, 24).

In our asthmatics, neither bEOS% nor sECP correlated with FEV₁ and FEV₁/VC. In patients with chronic asthma under inhaled steroids, inverse correlations between sECP and FEV₁, PEF, maximal mid-expiratory flow (MMEF), and FEV₁/FVC were found (12). In asthmatic children, Ulrik (25) found an inverse correlation between FEV₁ and bEOS%. Once more, the fact that our patients suffered from less severe asthma than subjects of previous studies (12, 25) could account for the discrepancy between our results and those of previous studies. Furthermore, the presence, in most of our patients, of allergic rhinitis may have confounded possible correlations; i.e., some subjects with mild asthma with normal lung function may have had high sECP due to the presence of nasal inflammation.

The inverse correlation between sECP and PD₂₀FEV_{1mch} in our asthmatics is in agreement with the results of previous studies (5, 12, 13, 24). In asthmatics, after exposure to allergens, BH to MCH increases together with eosinophilic activation (26). Thus, it is not surprising to find a correlation between these two parameters, especially in our population where most subjects were atopics.

In our asthmatics, increased PEF daily variability was associated with higher sECP. Airway lability, as expressed by PEF daily variability, is regarded as an index of airway inflammation (27), which could be marked in its turn by sECP. As in our study PEF monitoring followed blood sampling for sECP, the difference of sECP in the subgroups defined according to PEF variability points to a possible role of sECP in predicting PEF daily variability in patients with asthma.

In our asthmatics, sECP was higher in moderate persistent than in mild intermittent asthma. ECP in sputum was higher in symptomatic than in asymptomatic asthmatics (28). sECP was also related to symptom severity, being increased in severe and moderate compared to mild asthma, but not in severe compared to moderate asthma (10). Our results confirm the association between sECP and symptom severity in mild to moderate asthmatics, even if significant differences were found only between mild intermittent and moderate asthma, possibly due to the relatively low number of subjects in the other subgroups. On the other hand, in contrast to Ulrik (25), who reported a correlation between PEF variability, symptom severity, and blood eosinophil count, such a correlation was not found in our population, possibly because of the lower severity of the disease in our asthmatics. Generally, symptom severity is proportional to the magnitude of PEF daily variability, even if a good correspondence is sometimes lacking, as, for instance, in mild intermittent asthma, where PEF variability may be absent (20). In our population, when considering the different statistical significance levels, we may conclude that PEF variability is better reflected by sECP than is symptom severity. We may speculate that this is due to the fact that most of our asthmatic subjects had mild asthma, where dissociation between PEF variability and symptom severity seems most probable (20).

Interestingly, as regards the relation between PEF variability, symptom severity, and BH, PD₂₀FEV_{1mch}, unlike sECP, was not different in the subgroups defined by PEF variability and symptom severity. Both sECP (29, 30) and BH (31) may be considered markers of bronchial inflammation: in our asthmatics, only sECP was associated with PEF

variability and symptom severity. Our results suggest that sECP may be a better predictor of the severity of the disease.

Recently, Wever et al. (32) found that adjusting steroid therapy guided by sECP led to reduced exacerbation of asthma, suggesting the possibility of anticipating worsening of asthma by means of sECP monitoring. Our results support this hypothesis. In fact, in our study, MCH challenge and blood sampling for sECP and bEOS% quantitation were almost concomitant, taking place during the first week of the investigation period. Only sECP was different in the subgroups defined by PEF daily variability and symptom severity.

As concerns the possible influence of nasal inflammation on sECP, sECP in our asthmatics showed a tendency to correlate with nEOS%, while good correlations between nEOS% and both sECP and bEOS% were found in the overall group, and particularly in nonasthmatics. In nonasthmatics, the correlation between nEOS% and both bEOS% and sECP suggests nasal inflammation to be the source of the increased bEOS% and sECP. This may be explained by the fact that these last are the subjects where the presence of asthma was excluded and who presumably had little or no inflammation at the bronchial level. These results are in keeping with the association between rhinitis and increased sECP (14, 24) and the positive correlation between ECP concentration in nasal washes and sECP (16). On the other hand, in our asthmatics, most of whom had a diagnosis of rhinitis as well, sECP may have been determined by nasal and/or bronchial inflammation, which may not necessarily correlate. We may assume that the presence of asthma confounds the correlation between nEOS% and both bEOS% and sECP. The presence of rhinitis

may also have confounded the correlation between sECP and markers of bronchial inflammation, such as BH, which one might expect to be better in asthmatics without rhinitis. Unfortunately, in our population, this group was not large enough to allow statistically significant correlation between the different parameters. Thus, our results indirectly suggest that in asthmatics nasal eosinophil inflammation may have contributed to determine sECP, a fact which may limit its use as a marker of bronchial inflammation. In any case, if this happened, the effect in our asthmatics was not so marked as to abolish the association between sECP and some parameters of asthma.

In conclusion, our results show that sECP determination cannot help in distinguishing subjects with asthma from subjects in whom a suggestive history is not confirmed after thorough investigations, and that in mild to moderate asthma, sECP correlated with the degree of BH, with PEF daily variability, and with symptom severity, while the eosinophil count did not. The fact that both symptom severity score and PEF daily variability were measured during a 4-week period following the day of sampling for sECP quantitation suggests that sECP may serve as a marker of severity.

However, one has to consider that, despite some significant correlations, the dispersion of values of single patients around the mean/median and the nonnegligible overlapping of sECP between the subgroups limit the utilization of universal reference values of sECP. It should be investigated whether defining personalized sECP reference values could enhance the power of sECP as a marker of severity of asthma to be used in follow-up assessments.

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