

Short communication

Serum eosinophil cationic protein in the evaluation of asthma severity in children

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To assess the sensitivity and specificity of serum eosinophil cationic protein (ECP) in the diagnosis of asthma and evaluation of asthma severity, we conducted a prospective study to compare parameters of asthma severity, peripheral blood eosinophilia, and serum ECP concentrations in 88 children presenting to a university hospital outpatient clinic with suspected ($n=59$) or recently diagnosed asthma ($n=29$). Serum ECP correlated significantly ($r_s=0.676$, $P=0.0001$) with peripheral eosinophil counts, but only weakly with asthma severity ($r_s=0.21$, $P=0.046$). Serum ECP was significantly higher in atopic children (25 ± 11 $\mu\text{g/l}$) than in nonatopic children (16 ± 15 $\mu\text{g/l}$) ($P=0.01$). Bronchial hyperresponsiveness had no significant correlation ($r_s=-0.21$, $P=0.30$) with serum ECP. Lung function test results had no (peak flow) or only a weak (FEV_1) correlation with serum ECP.

In distinguishing between children with and without asthma or in assessing asthma severity, serum ECP is not superior to the peripheral blood eosinophil count. The diagnostic sensitivity and specificity of ECP in serum for detecting symptomatically active asthma, evaluated against the cutoff level of ECP in serum of 16 mg/l, were 54% and 71%, respectively.

Key words: atopy; asthma; bronchial hyperresponsiveness; eosinophil; eosinophil cationic protein (ECP).

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Asthma severity and the need for treatment interventions may sometimes be difficult to assess, especially in small children, where routine lung function measurement is impossible. Evaluating the inflammatory component of asthma might improve the assessment of asthma severity. Increased concentrations of eosinophil cationic protein (ECP) in serum have been described in subjects with asthma (1, 2), and it has been shown to be a sensitive marker of the disease activity in seasonal asthma (3). Even in young infants, high levels of serum ECP have been shown to predict asthma (4, 5) and to correlate with bronchopulmonary obstruction (6). We therefore conducted a prospective study to compare the different parameters of asthma severity with peripheral blood eosinophilia and serum ECP concentrations in an unhomogeneous group of children with suspected or recently diagnosed asthma.

Material and methods

Patients

Eighty-eight consecutive children, aged 1–16 years (7.4 ± 4 years; $\bar{x} \pm \text{SD}$), who were referred to a university outpatient clinic for suspected asthma ($n=59$) or for evaluation of the severity of previously diagnosed asthma ($n=29$), were included in the study.

The severity of asthma was evaluated by a self-administered questionnaire and by reviewing patient diaries on a scale from 0 to 4: 0=no asthma symptoms during the last year; 1=some but not disturbing symptoms of asthma during the last year; 2=disturbing asthma symptoms for 1–10 days during the last year; 3=asthma symptoms for 11–100 days during the last year; 4=asthma symptoms for more than 100 days during the last year (7). Symptoms were considered disturbing if they interfered with daily activities or sleep. The severity of

cough was evaluated by a similar scale from 0 to 4. The severity of exercise-induced asthma was evaluated as follows: 0=no exercise-induced asthma; 1=some but not disturbing symptoms during exercise; 2=disturbing exercise-induced asthma; 3=exercise-induced asthma symptoms, prevented by β_2 -agonists; 4=disturbing exercise-induced asthma, not prevented by β_2 -agonists.

Pulmonary function was evaluated by peak flow measurement (PEF; Wright peak flow meter) and spirometry (Vitalograph), when proper for age.

When clinically justified, an intervention to decrease asthma symptoms was planned (allergy control measures, anti-inflammatory treatment, and/or increase in inhaled glucocorticoid dose). In 32 subjects, a follow-up visit to check the efficacy of the intervention was scheduled after 1–6 months (3 ± 2 months; $\bar{x} \pm \text{SD}$), and ECP in serum was measured again. Improvement or deterioration of asthma was assessed by a pediatric allergist (T.V.), who was unaware of the ECP results. The decision was based on asthma symptoms, need for bronchodilating drugs, severity of cough, and exercise tolerance.

Bronchial provocation tests

For further evaluation of their exercise tolerance, 17 subjects were investigated by free-running exercise challenge test. The running speed was adjusted by monitoring the heart rate with a Polar Edge Heart Rate Monitor™ (Polar Electro Ky, Kempele, Finland). Subjects raised their heart rate to 85% of the predicted maximal rate during a 2-min warm-up and maintained this rate for 6 min. Peak expiratory flow (PEF) values were measured before the run and 5, 10, 20, and 30 min after the test. The result was expressed as percentage change in PEF (the difference between the pre-exercise and the lowest postexercise PEF).

Bronchial hyperresponsiveness was assessed in 27 children by the methacholine challenge test. Increasing doses of methacholine were inhaled to obtain the PD_{20} value (the provocative dose of methacholine resulting in a 20% reduction in FEV_1). A dosimeter, the jet nebulizer Spira Elektro 2 (Respiratory Care Center, Hämeenlinna, Finland), was used for the inhalation test.

Skin prick tests

Skin prick tests were performed with birch, grass, and mugwort pollen; horse, cat, and dog dander; a mold mixture; and house-dust mite (*Dermatophagoides pteronyssinus*) extracts (ALK, Denmark). Additional allergens were included if they were considered clinically important. The diluent was

used as a negative control, and histamine hydrochloride 10 g/l was used as a positive control. Subjects with wheal reactions to any of the allergens of at least half of the size of the histamine wheal were considered atopic (8).

Serum ECP levels and blood eosinophil count

ECP in serum was measured with a radioimmunoassay (ECP RIA, Pharmacia & Upjohn Diagnostics, Uppsala, Sweden). Blood collection, serum preparation, and the RIA were done according to the instructions of the manufacturer. Blood was collected in Vacutainer® SST blood-collection tubes (Becton Dickinson Vacutainer Systems Europe, Meylan Cedex, France). The samples were allowed to clot at room temperature for 60–120 min before centrifugation at 1300 g for 10 min at room temperature. The separated sera were stored at -70°C before analysis. The eosinophil count in blood was analyzed with the Technicon H₂ automatic analyzer (Technicon Ltd, Swords Co, Dublin, Ireland).

Statistical methods

Spearman rank correlation coefficients were used in correlation analyses between the levels of ECP in serum and the various measures of asthma severity. Student's paired *t*-test was used to evaluate the significance of the changes in the levels of ECP in serum before and after the intervention. Student's unpaired *t*-test or one-way ANOVA was used to evaluate the differences of means between subgroups.

Results

Peripheral eosinophil counts had a statistically significant correlation ($r_s=0.676$, $n=76$, $P=0.0001$) with serum ECP (Fig. 1). Of subjects with eosinophilia of $\geq 0.35 \times 10^9/\text{l}$, 82% had ECP levels of $\geq 16 \mu\text{g/l}$, and those without peripheral eosinophilia ($< 0.35 \times 10^9/\text{l}$) mostly (77%) had ECP levels less than $16 \mu\text{g/l}$.

Clinical asthma severity correlated weakly with the levels of ECP in serum ($r_s=0.21$, $n=88$, $P=0.046$) (Fig. 1). The correlation was not higher even if subjects with previously diagnosed asthma, who already were on inhaled glucocorticosteroids ($n=19$), were excluded from the calculations. Weak correlation was also found between the levels of ECP in serum and the severity of cough ($r_s=0.28$, $n=88$, $P=0.009$) and the clinical assessment of exercise-induced asthma ($r_s=0.22$, $n=83$, $P=0.044$) (Fig. 1).

The levels of ECP in serum were significantly higher in atopic children ($25 \pm 11 \mu\text{g/l}$) than in non-atopic children ($16 \pm 15 \mu\text{g/l}$, $P=0.01$).

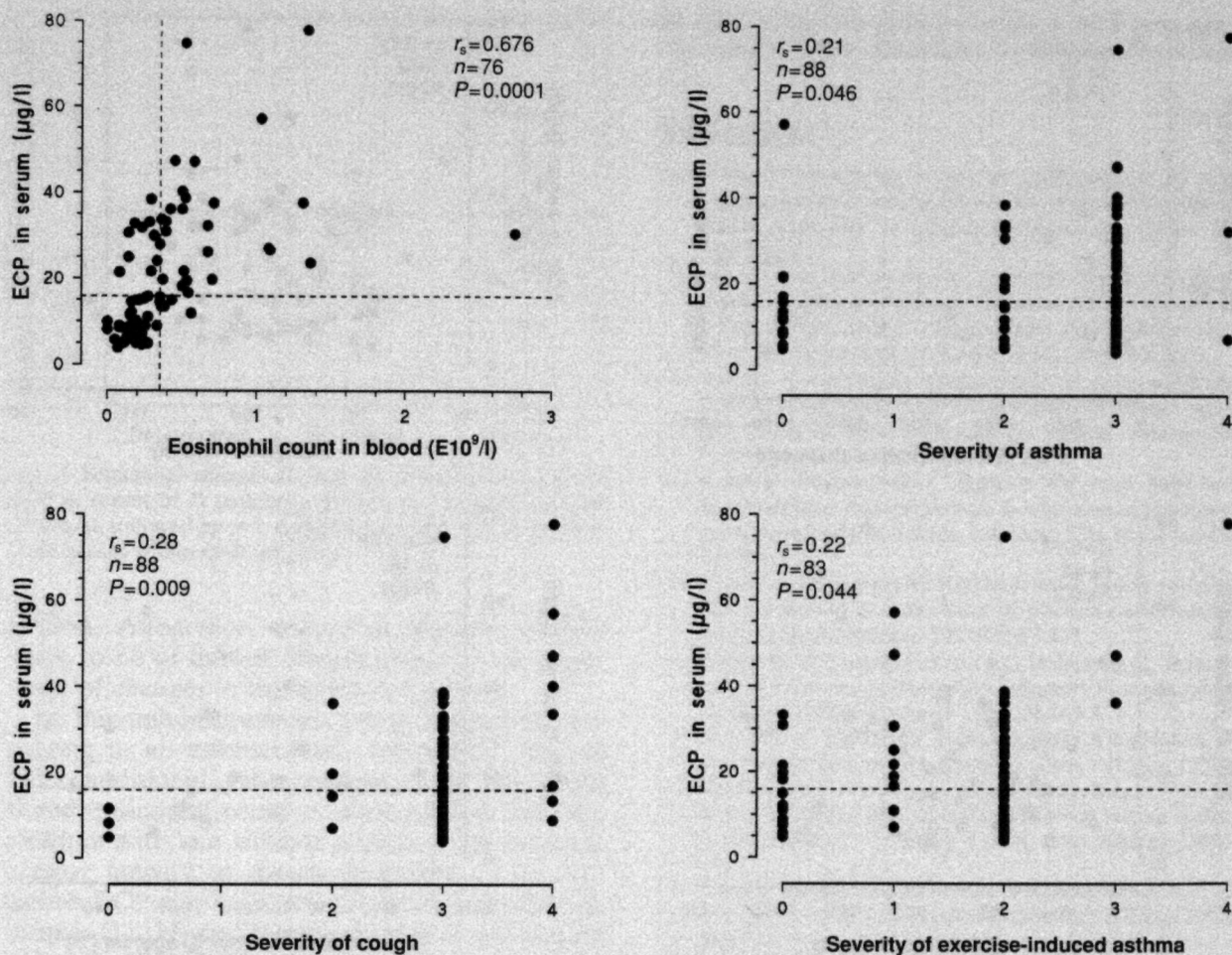


Fig. 1. Correlation of levels of ECP in serum with eosinophil count in blood, severity of asthma, severity of cough, and severity of exercise-induced asthma. Dashed lines show upper limits of ECP in serum ($16 \mu\text{g/l}$) and eosinophil count ($0.35 \times 10^9/\text{l}$).

Bronchial hyperreactivity as measured by PD_{20} of methacholine had no significant correlation ($r_s = -0.21$, $n = 27$, $P = 0.3$) (Fig. 2) with ECP in serum, and ECP levels in subjects with no bronchial hyperreactivity ($\text{PD}_{20} > 2900 \mu\text{g}$) were not significantly ($P = 0.3$) lower than in subjects with a variable degree of bronchial hyperreactivity (17 ± 12 vs $23 \pm 19 \mu\text{g/l}$, respectively).

Serum ECP levels did not correlate with peak flow levels (percentage of predicted normal; $r_s = 0.12$, $n = 62$, $P = 0.3$), and had only a poor ($r_s = 0.17$, $n = 45$, $P = 0.3$) correlation with FEV_1 (percentage of predicted normal; Fig. 2). No correlation was found between serum ECP and the degree of bronchodilation (percentage) after inhaled β_2 -agonist ($r_s = -0.23$, $n = 16$, $P = 0.4$) (Fig. 2).

At the follow-up visit, asthma had deteriorated in only three subjects, in whom serum ECP increased from 21 ± 7 to $30 \pm 13 \mu\text{g/l}$ ($P = 0.55$). Clinically, asthma symptoms were decreased in 17 subjects, all receiving inhaled glucocorticoids. No significant changes were noted in the levels of

serum ECP in any of the three subgroups (Fig. 3).

No significant differences were found in the initial levels of ECP in serum between the three subgroups (21 ± 14 , 19 ± 15 , and $21 \pm 10 \mu\text{g/l}$) in patients in whom asthma symptoms had improved, remained unchanged, and deteriorated, respectively, at the follow-up visit.

The diagnostic sensitivity and specificity of ECP in serum for detecting symptomatically active asthma, evaluated against cutoff levels of ECP in serum of $16 \mu\text{g/l}$ and severity of asthma of 0 vs 2 or more, were 54% and 71%, respectively. The sensitivity and specificity of peripheral blood eosinophilia ($\geq 0.35 \times 10^9/\text{l}$) were 42% and 75%, respectively.

Discussion

Determination of ECP has been advocated as an aid for management of asthma, since ECP is released from eosinophil granulocytes at the

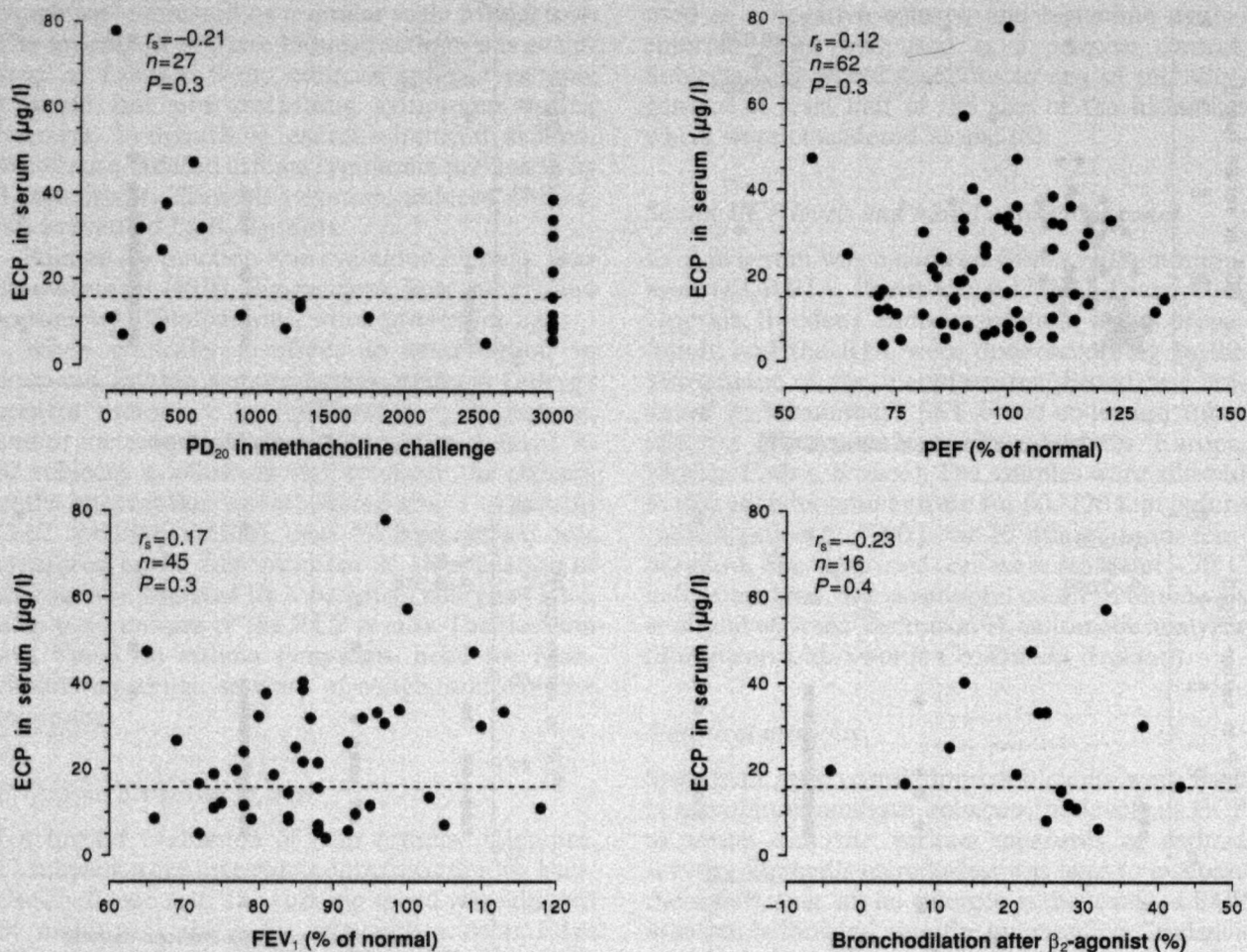


Fig. 2. Correlation of levels of ECP in serum with bronchial hyperreactivity in methacholine challenge, peak expiratory flow (PEF), and bronchodilation after administration of β_2 -agonist. Dashed lines show upper limits of ECP in serum (16 µg/l).

inflamed bronchial mucosa, thereby reflecting the inflammatory activity in asthma. Furthermore, ECP in serum is presumed to parallel more closely the state of eosinophil activity than the eosinophil count in blood, since ECP is released from the eosinophils during the clotting of blood after venipuncture (9).

In the cross-section part of our study, we found weak though statistically significant correlations between the level of ECP in serum and all measures of the severity of symptoms of asthma. The inflammation of asthma is thus reflected in the activation of eosinophils in peripheral blood. However, considerable overlap was found in the levels of ECP in serum and the severity of symptoms; high levels of symptoms were not uniformly associated with elevated levels of ECP, whereas it was mostly within normal range in patients with no symptoms. However, atopy assessed by skin prick test was reflected in significantly higher levels of ECP.

Our results show that elevated levels of ECP in serum indicate symptomatically active disease, but

the value of the test is limited by low sensitivity; i.e., not all patients with active disease have elevated levels of ECP. Furthermore, we found no significant correlation between the levels of ECP and measures of airways obstruction or bronchial reactivity. One source of the low sensitivity of the test may be that the inflammation process in asthma is primarily localized in the airways; thus, the correlation between disease activity and eosinophil activation may be higher in sputum formed at the inflammation site than in peripheral blood used as a specimen in our study.

In the 31 patients who were followed up during a second visit, no significant changes in the level of ECP in serum were found. The finding partly reflects the low sensitivity of the test. In most patients, the clinical status either improved or was unaltered. Since ECP in serum was not uniformly high in patients with high disease activity, ECP can be expected to decline markedly in some patients only. Nevertheless, in several patients, the levels of ECP increased despite clinical improvement of

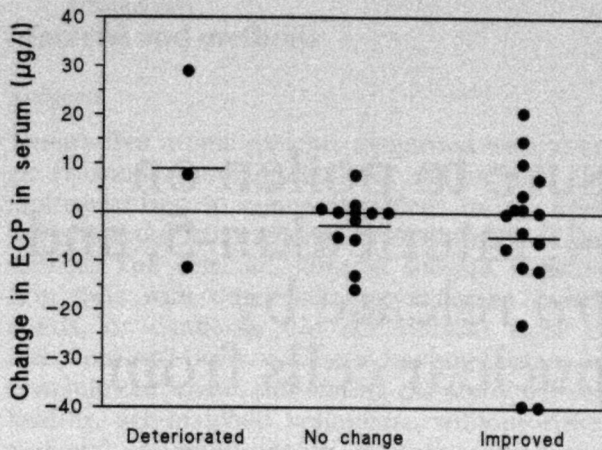


Fig. 3. Sequential changes (follow-up minus initial levels) in ECP in serum in 31 patients according to changes in activity of asthma followed up at 1–6-month intervals. Horizontal lines show means within each subgroup.

asthma. Altogether, sequential changes of ECP seem to be of limited clinical utility in the evaluation of changes in the activity of asthma.

In this unhomogeneous group of children presenting to an asthma clinic, serum ECP did not offer additional value compared to peripheral blood eosinophil count in distinguishing between children with and without asthma, or in assessing disease activity, as shown previously (3, 10, 11). Serum ECP may be increased also in nonasthmatic atops (11, 12) and in subjects with atopic eczema (13, 14). High levels of serum ECP may predict asthma, but they are not specific for asthma, and patients with mild or moderate asthma may have normal levels of serum ECP. Although weak correlations were found at the study group level between ECP level and clinical activity of asthma, the utility of ECP measurement in an individual patient is limited.

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