

Urinary eosinophil protein X and serum eosinophil cationic protein in infants and young children with atopic dermatitis: Correlation with disease activity

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Background: Eosinophil cationic protein (ECP) and eosinophil protein X (EPX) or eosinophil-derived neurotoxin (EDN) are released by eosinophil granulocytes in allergic diseases. Serum ECP (s-ECP) levels have been correlated with disease activity in atopic dermatitis (AD) in adults and young patients, and high urinary EPX (u-EPX) levels in asthmatic patients seem to reflect active disease. A relationship between AD severity and u-EPX concentration in young children has not been previously studied. **Objective:** This study was performed to evaluate whether the severity of AD in infants and young children was correlated with s-ECP and u-EPX levels.

Methods: Fifty-four infants and children (mean age, 17.7 months; range, 4-48 months) with AD and without other allergic conditions were evaluated. The severity of AD was measured by using the SCORAD index. S-ECP, serum total IgE, serum-specific IgE for common allergens, and peripheral blood eosinophil counts (PBECS) were determined. In forty-two children u-EPX was also measured. Seven age-matched control patients underwent the same determinations.

Results: S-ECP and u-EPX were significantly higher in children with AD than in control children (mean, 23.9 vs 3.5 µg/dL [$P < .001$] and 57.7 vs 6.0 µg/mmol creatinine [$P < .001$]). A significant correlation was found between SCORAD and s-ECP ($P = .002$), u-EPX ($P = .01$), and PBECS ($P = .01$) and between symptom index and uEPX ($P = .0004$). PBECS were strongly correlated to s-ECP and u-EPX ($P < .0001$). However, 5 patients with moderate and severe AD (11.9%) showed low levels of s-ECP, u-EPX, and PBECS.

Conclusion: S-ECP and u-EPX were useful markers of AD activity in infants and young children. When taken together, the two determinations could give more information about the clinical course of the illness. Some patients seemed to have clinical exacerbations without an involvement of eosinophils and their products. (*J Allergy Clin Immunol* 2000;105:353-7.)

Abbreviations used

AD:	Atopic dermatitis
EDN:	Eosinophil-derived neurotoxin
PBEC:	Peripheral blood eosinophil count
s-ECP:	Serum eosinophil cationic protein
SPT:	Skin prick test
u-EPX:	Urinary eosinophil protein X

Key words: Atopic dermatitis, eosinophil cationic protein, eosinophil protein X, eosinophils

Atopic dermatitis (AD) is a chronic inflammatory relapsing skin disorder. Many cell types and their products are involved in its pathogenesis: T_{H2} lymphocytes, skin-homing T lymphocytes, Langerhans' cells, keratinocytes, mast cells, and eosinophils.^{1,2} Eosinophils are important as effector cells in allergic inflammation of AD because they migrate from blood, where they are usually elevated in number, into lesional skin.³ The expansion of IL-4- and IL-5-secreting T_{H2} lymphocytes strongly affects eosinophils in AD. IL-4, inducing the expression of vascular cell adhesion molecule 1, promotes eosinophil transendothelial migration, and IL-5 induces eosinophil differentiation, activation, and survival.¹ On degranulation, activated eosinophils release their toxic products, namely oxygen-free radicals, leukotrienes, platelet-activating factor, cytokines,^{4,5} and especially cationic proteins from specific cytoplasmic granules⁶: eosinophil cationic protein (ECP), eosinophil protein X (EPX) or eosinophil-derived neurotoxin (EDN), major basic protein, and eosinophil peroxidase. Immunohistologic staining shows eosinophil granule protein deposits in the dermis of lesional skin,^{7,8} probably as a result of progressive cytolytic degeneration.⁹ ECP (molecular weight, 21 kd) and EDN/EPX (molecular weight, 18-19 kd) have 67% amino acid sequence identity,¹⁰ and both ECP and EDN genes are in the same region of chromosome 14.¹¹

In many studies of adults and young patients with AD, serum levels of ECP were reported to be elevated and often statistically correlated with disease activity.¹²⁻¹⁶ However, data in infants and young children are lacking,

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although the clinical manifestations of AD are present in the first year of life in 60% of patients,¹⁷ and an earlier onset is associated with a more severe course.¹⁸

Recently, an association between urinary EPX (u-EPX) levels and severity of asthma in children has been reported.¹⁹⁻²¹ The urinary levels of EPX were also elevated in 7 of 14 patients with AD (mean age, 25.7 years),⁷ but no data in infants and young children are reported in the literature of an association between u-EPX levels and severity of AD. The purpose of this study was to evaluate the correlation between AD severity and immunologic parameters, especially serum ECP (s-ECP) and u-EPX levels, in infants and young children.

METHODS

Subjects

We evaluated 54 consecutive infants and young children admitted to the Pediatric Allergy Center of the University of Florence, Italy. All children met the diagnostic criteria for AD, as defined by Hanifin and Rajka.²² None of them had other atopic conditions (asthma, rhinitis, and conjunctivitis) nor did they receive antihistamines or systemic or topical corticosteroids during the 3 weeks before clinical evaluation. Seven healthy nonatopic age-matched donors were also evaluated for total serum IgE, s-ECP, peripheral blood eosinophil counts (PBECs; blood sample), and u-EPX (urine sample).

Clinical scoring system

The severity of AD was measured by using the SCORAD index.²³ Briefly, it defines a score for 3 parameters: extent, intensity, and subjective symptoms. Extent is calculated with the rule of nines and expresses the body surface involved. Intensity items are erythema, edema/papulation, oozing/crusts, excoriations, lichenification, and dryness of noninvolved skin (0 to 3 points for each item). Subjective symptoms are pruritus and sleep loss (0 to 10 points for each item). The final score is then calculated by using the following equation: $A/5 + 7B/2 + C$, where A represents extent, B represents intensity items, and C represents subjective symptoms. We considered mild, moderate, and severe AD forms, in which the SCORAD index was less than 25, between 25 and 50, and 50, respectively. When using a statistical method (mild AD, first quintile of the study group; severe AD, fifth quintile of the study group), we obtained SCORAD cut-off points very similar to those established by clinical criteria.

Immunologic parameters

All patients underwent skin prick tests (SPTs) for common inhalants and food allergens (*Dermatophagoides pteronyssinus*, cat dander, *Alternaria alternata*, grass pollen, cow milk, egg, tomato, peanut, cod fish, wheat, rice, and soybean). Wheal responses of 3 mm in diameter or more were considered positive.²⁴ Serum-specific IgE was determined for the same allergens (UniCAP Specific IgE; Pharmacia Upjohn, Uppsala, Sweden), and a result of greater than 0.35 kU/L was considered positive.

Blood samples were collected at the time of the admission for determination of serum total IgE (UniCAP Total IgE; Pharmacia, Uppsala, Sweden), serum-specific IgE, white blood cells, peripheral blood eosinophil counts, and s-ECP. The latter was determined by an immunoenzymatic method (UniCAP ECP; Pharmacia, Uppsala, Sweden).²⁵

Urine samples and measurements of u-EPX

Urine was collected in the morning at the time of clinical evaluation, during micturition, or (for most children under 2 years of age)

with a pediatric urine collector. The sample was immediately frozen at -20°C . EPX was measured by using an RIA (EPX RIA; Pharmacia, Uppsala, Sweden).¹⁹ Briefly, before assessment, urine was diluted 11-fold in a phosphate buffer [pH 7.4] containing 0.15 mmol/L NaCl, 1% BSA, 0.1% Tween-20, 10 mmol/L EDTA, and 0.2% N-cetyltrimethylammonium bromide. Urinary creatinine concentration was measured by using the alkaline picrate reaction (HiCo Creatinine; Boehringer Mannheim GmbH, Mannheim, Germany). Urine-EPX results are expressed as micrograms per millimole of creatinine. All measurements were done in duplicate. The detection limit in the assay was less than 3 $\mu\text{g/L}$, and the coefficient of variation was less than 6% within the assay and less than 11% between the assays.

Statistical analysis

All data were elaborated by using a commercially available statistical computer software package (SPSS, Chicago, Ill). Student *t* tests for independent samples were used for the comparison of means. To assess the linear correlation between variables, the Pearson correlation coefficient was calculated. Probability (*P*) values of less than .05 were considered statistically significant.

RESULTS

The ages of the 54 children evaluated (34 male and 20 female subjects) were from 4 to 48 months (mean age $\pm$ SD = 17.7 ± 11.88 months). The mean of SCORAD index values was 36.7 (7-65): 10 (18.5%) patients had an index value of less than 25 (mild form); 34 (63%) patients had moderate AD (SCORAD index 25-50); and 10 (18.5%) patients had severe AD (SCORAD index >50).

The mean ECP values in the AD group was higher than that in the nonatopic control subjects (23.9 $\mu\text{g/dL}$ and 3.5 $\mu\text{g/mL}$, respectively; $P < .0001$). A significant relationship was found between serum ECP levels and severity of AD expressed with the total SCORAD index ($P = .002$, $r = 0.40$; Fig 1, A) or with each clinical parameter: skin surface area ($P = .003$, $r = 0.37$), intensity ($P = .004$, $r = 0.37$), and subjective symptoms ($P = .024$, $r = 0.30$).

U-EPX levels were higher in the AD group than in the control group (mean 57.7 vs 6.0 $\mu\text{g/mmol}$ creatinine, $P < .0001$). The values were correlated to the degree of severity in AD (SCORAD) ($P = .01$, $r = 0.37$; Fig 1, B) and to the items of the SCORAD index as follows: skin surface area ($P = .005$, $r = 0.42$), intensity ($P = .13$, $r = 0.22$), and subjective symptoms ($P = .0004$, $r = 0.50$). A significant correlation between s-ECP and u-EPX was found ($P = .027$, $r = 0.33$).

PBECs showed a strong linear correlation with s-ECP ($P < .0001$, $r = 0.65$) and u-EPX ($P < .0001$, $r = 0.61$) levels and, to a lesser extent, with disease activity (SCORAD; $P = .01$, $r = 0.33$). No correlation was found between circulating neutrophil counts and s-ECP ($P = .33$) or u-EPX ($P = .31$).

Total serum IgE levels were elevated (>2 SD for age) in 49 (87.4%) of the 54 children (mean 398.8 kU/L), and positive SPT responses or serum-specific IgE (CAP) for common inhalant and food allergens were present in 36 (66.8%) of the 54 patients (atopic children). However, no

significant relationship was found between total or specific IgE levels and the other clinical and immunologic parameters evaluated (SCORAD, s-ECP, and u-EPX). No significant difference in mean total serum IgE levels was observed between atopic and nonatopic children with AD. The relationship between SCORAD index value and eosinophil products did not change when repeating the analysis separately for atopic and nonatopic children.

Mean s-ECP values were significantly higher in moderate than in mild forms of AD, but no significant difference was found between moderate and severe AD (Fig 2, A). Mean u-EPX values were higher in severe than in moderate forms, but no difference was found between moderate and mild forms (Fig 2, B).

Twenty-three (67.6%) of 34 patients with moderate-to-severe AD who underwent both s-ECP and u-EPX measurements had s-ECP levels greater than 15 mg/mL. Six more children had u-EPX levels of 20 μ g/mmol or greater creatinine in spite of low levels of s-ECP. A total of 29 (85%) of 34 subjects had high levels of at least one of the two parameters.

Five patients (11.9% of the 42 children with both s-ECP and u-EPX measurements) showed, in spite of an elevated SCORAD index (>35), low levels of s-ECP (<15 mg/mL, mean 12.5), u-EPX (<20 μ g/mmol creatinine, mean 13.3), and PBECs (<500/mm³, mean 360). This condition was not statistically associated with positive or negative SPT or RAST results.

DISCUSSION

In the past 10 years, serum levels of ECPs were studied with respect to the clinical parameters of AD and other allergic conditions.^{7,11-15,26,27} In AD a significant relationship was found between serum major basic protein and body surface involvement ($P < .02$).⁷ Other authors evaluated the levels of s-ECP in AD groups by using different methods of grading the severity of skin involvement. Three studies on adults and adolescents^{13,15,16} showed s-ECP levels correlated to AD activity ($P < .01$, $P < .05$, and $P < .001$, respectively). One study evaluated 24 children with AD (median age 6 years) and failed to demonstrate a significant relationship between s-ECP and disease activity.¹² In a group of 20 patients (mean 13.7 years), AD severity showed a positive correlation with s-ECP levels ($P < .004$).¹⁴

Children with AD usually show lower mean ECP levels than adults^{12,14,28} but higher than asthmatic patients.²⁹ However, infants and young children have not been studied. EPX, but not ECP, is found in detectable amounts in urine of healthy subjects³⁰; the low urinary levels of ECP are probably related to tubular reabsorption.^{31,32} U-EPX was elevated in 7 of 14 patients with AD (mean age, 25.7 years),⁷ but no positive relationship was reported with extent of skin involvement or serum, urine, or skin cationic protein levels. Recently, u-EPX was better studied in asthmatic patients: higher levels than in control subjects and higher levels in symptomatic than in asymptomatic patients were reported.^{19-21,33}

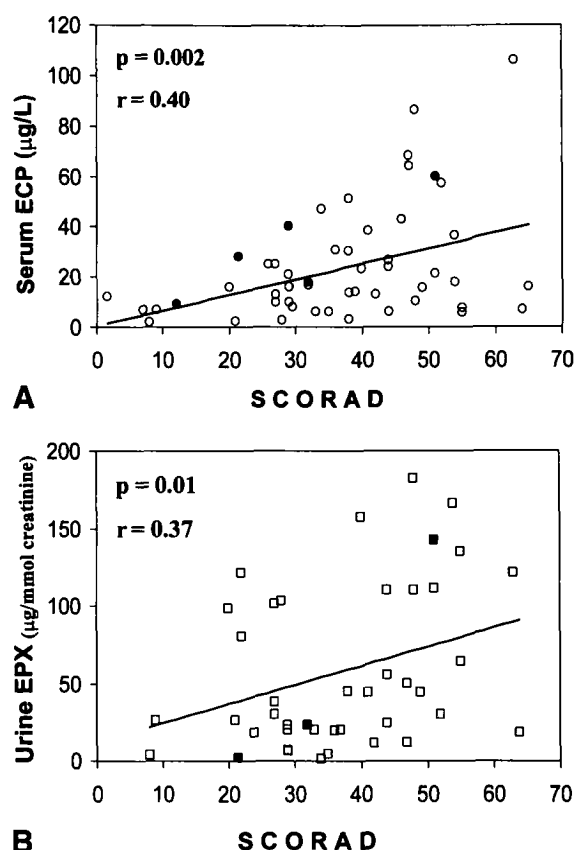


FIG 1. A, Scatter plot of s-ECP levels against SCORAD index values ($n = 54$ children). Filled circles, Patients with a normal serum IgE level. B, Scatter plot of u-EPX levels against SCORAD index values ($n = 42$ children). Filled squares, Patients with a normal serum IgE level.

In the present study the usefulness of u-EPX, s-ECP, PBECs, total serum IgE determination, and SPT results for common allergens in monitoring disease activity were evaluated. We chose infants and young children to evaluate patients with early-onset AD in the first years of illness. The mean age of the 54 patients was 17.7 months, and the male/female ratio was 1.7, as reported for infantile AD.³⁴ None of these patients ever had other atopic conditions. A positive relationship was found between u-EPX and the SCORAD index, although the extent of skin lesions and especially the score for subjective symptoms (pruritus and sleep loss) were more strongly correlated with the levels of the urinary protein. This observation may support the hypothesis that eosinophils and their products may have a pathogenetic role in the pruritus of AD.

We found that a significant rise in s-ECP exists until the AD clinical score reaches a certain level of severity (Fig 2, A). On the other hand, u-EPX levels rose significantly only from moderate-to-severe forms of AD (Fig 2, B). To explain the different behavior of the two proteins, we may speculate that, in some cases, ECP reaches maximum serum levels, and then it is deposited in skin and other tissue. It is possible that EPX is partially reab-

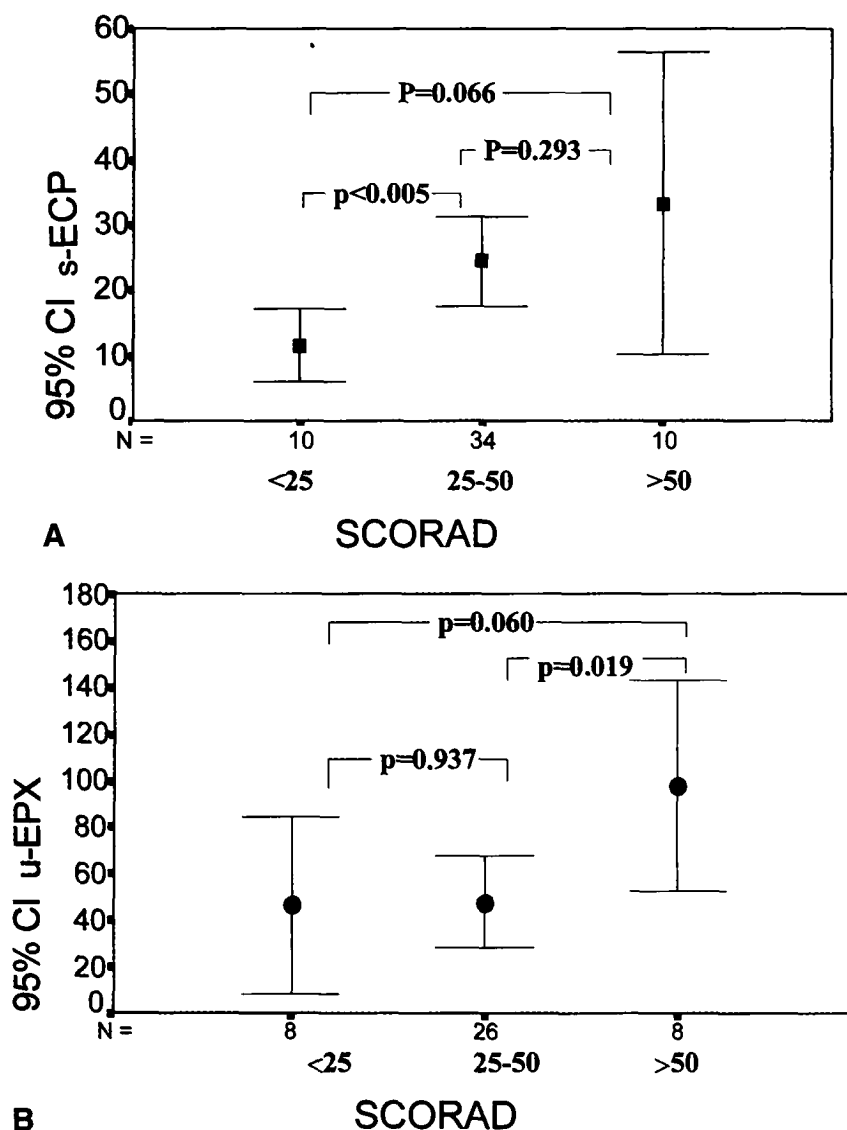


FIG 2. A, Levels of s-ECP (in micrograms per milliliter; mean and 95% confidence interval [CI]) by AD severity groups. B, Levels of u-EPX (micrograms per millimole creatinine; mean and 95% confidence interval) by AD severity groups.

sorbed in the renal tubular system, and, when a renal threshold is exceeded, its concentration begins to increase in urine.

Both ECP and EPX can be found in eosinophil and neutrophil granulocytes,³⁵ but our results show that the high levels of these proteins were due to eosinophil activation. Indeed, we found a strong correlation between s-ECP and u-EPX with circulating eosinophil counts but not with circulating neutrophil counts.

Patients who showed low levels of eosinophil products in spite of an elevated SCORAD index value (about 12%) may represent, as occasionally reported,¹³ a subset of patients with AD in whom eosinophils play a minor role in skin damage.

In conclusion, our study shows the importance of

eosinophils and their cationic proteins in monitoring the severity of AD in infants and young children. By using the SCORAD index, disease activity is better related to s-ECP than to u-EPX. U-EPX, however, shows some unique characteristics. Its levels are, differently from s-ECP, better related to clinical severity in moderate-to-severe forms of AD than in mild-to-moderate ones. A very positive relationship exists between u-EPX levels and intensity of subjective symptoms, and some patients with moderate-to-severe AD have high levels of u-EPX in spite of low levels of s-ECP. Moreover, urine samples are easier to collect than blood samples, especially for the young patients with AD, in whom many determinations are probably needed. Thus taken together, the two parameters can give more information about the ongoing

skin damage in AD. Some patients with high SCORAD index values present low or very low levels of s-ECP, u-EPX, and PBECs. In this group other mechanisms than eosinophil involvement are probably responsible for the relapsing of AD.

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