

Eosinophil cationic protein in serum and nasal washes from wheezing infants and children

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Objective: To compare eosinophil counts and concentrations of eosinophil cationic protein (ECP) in serum and nasal wash fluid from wheezing infants and children with those from age-matched children without respiratory tract symptoms.

Design: A case-control study of 71 children treated for wheezing and 59 control subjects in the University of Virginia Pediatric Emergency Department. The patients ranged from 2 months to 16 years of age. Eosinophil numbers and ECP concentrations were assessed in serum and nasal washes. Total serum IgE was measured and the radioallergosorbent test was used to measure IgE antibody to common inhalant allergens.

Results: Among children less than the age of 2 years, markedly elevated levels of ECP (>200 ng/ml) were measured in nasal washes from 9 (41%) of 22 wheezing patients and 1 (6%) of 17 control subjects ($p < 0.03$). None of these children had a positive radioallergosorbent test result for IgE antibody to common aeroallergens or a nasal smear containing 10% eosinophils. Few of the wheezing children under 2 years of age had either increased concentrations of total IgE or ECP in their serum or an elevated total blood eosinophil count. After the age of 2 years, the percentage of patients with nasal ECP levels greater than 200 ng/ml was also significantly higher in wheezing children than in control subjects ($p < 0.001$), and a positive correlation was observed between ECP concentrations in their nasal washes and other eosinophil responses (total blood eosinophil counts, serum ECP levels, and nasal eosinophil counts).

Conclusion: Increased concentrations of ECP were detected in nasal washes from wheezing infants and children, indicating that eosinophils may contribute to the pathogenesis of airway inflammation in some children who wheeze early in life. (J PEDIATR 1995;127:558-64)

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The strong association between immediate hypersensitivity to inhaled allergens and asthma in school-aged children and

ECP	Eosinophilic cationic protein
RAST	Radioallergosorbent test
RSV	Respiratory syncytial virus

in adults is well recognized.^{1,2} During the first 2 years of life, however, viral infections, especially those caused by respiratory syncytial virus, are the dominant risk factor for

Table I. Patient demographic information

	Children aged <2 yr		Children aged 2 to 4 yr		Children aged 4 to 16 yr	
	Wheezing (n = 22)	Control (n = 17)	Wheezing (n = 18)	Control (n = 12)	Wheezing (n = 31)	Control (n = 30)
Mean age (yr)	0.9	0.7	2.8	3.2	8.7	10.2
Male (%)	68	47	56	50	42	57
Black (%)	50	29	83	25	68	40
Payment requirement*						
100%	9	6	6	8	19	21
10% to 75%	14	0	12	8	16	17
0%	77	94	82	84	65	62

*Payment requirement categories were those used by the hospital to judge each patient's income status. The categories shown reflect the proportion of the emergency department bill charged to the patient's family based on annual family income. Shown for each patient group are the percentage of families required to pay 100%, 10 to 75%, or 0% of their child's bill.

Table II. Characteristics of wheezing and control patients seen in the emergency department

	Children aged <2 yr		Children aged 2 to 4 yr		Children aged 4 to 16 yr	
	Wheezing (n = 22)	Control (n = 17)	Wheezing (n = 18)	Control (n = 12)	Wheezing (n = 31)	Control (n = 30)
Nasal eosinophils (% positive)†	0	0	28	8	52*	23
Blood eosinophils (cells/mm ³)‡	120 (43-585)	83 (0-580)	191 (26-2205)	191 (66-719)	384*** (44-1649)	153 (26-910)
Total IgE (IU/ml)‡	7.0* (2-66)	3.7 (2-12)	121** (4-912)	17 (3-165)	274** (17-1894)	105 (7-2102)
RAST (% positive results)§	0	0	22	17	65***	23
Family history (% positive results)	59	59	39	58	87**	47

Values that are significantly different from those of control subjects are indicated by *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.

†Nasal smears containing $\geq 10\%$ eosinophils were regarded as showing positive results.¹³

‡Total blood eosinophil counts and total IgE levels are reported as geometric means. The range of values measured in each group is shown in parentheses.

§Percentage of sera containing IgE antibody to one or more allergens.

||History of allergic disorders in the immediate family.

wheezing.^{3,4} Infants who are more likely to wheeze with infection are those who (1) have decreased total airway conductance at birth, (2) are exposed to maternal tobacco smoke at home, and (3) are more frequently exposed to infections at day care.⁵⁻⁷ In other respects, children who wheeze early in life have been more difficult to characterize, and the relationship between wheezing illness and allergic risk factors in this age group is not clear. In a previous study we found a low prevalence of sensitization to inhalant and food allergens in children less than 2 years of age who were treated for wheezing in an emergency department.^{3,8} Similarly, another study found that wheezing attacks with infection were not associated with atopy during the first 3 years of life.⁹ Nevertheless, young wheezing children most likely represent a heterogeneous group; some wheeze with infection only during the first year or two of life, and some may have early childhood asthma. The identification of those who may have asthma and who are at risk of having subsequent episodes of wheezing remains a challenge.¹⁰

Studies of eosinophils and the toxic mediators they release have enhanced our understanding of allergic inflammation and the late-phase events of asthma. Among these studies are those indicating that measurements of eosinophilic cationic protein in serum may be a useful way to monitor both eosinophil activation and inflammation in the airways of children with asthma.^{11,12} There is little information, however, concerning the role of eosinophils and their mediators in wheezing illnesses during infancy. The purpose of this study was to compare eosinophil counts and ECP concentrations in serum and nasal wash fluid from wheezing children and from children without respiratory tract symptoms when they were brought to a pediatric emergency department.

METHODS

Study population. Study participants included 130 children who were treated between January 1993 and April 1994 in the pediatric emergency department at the University of Virginia. They ranged in age from 2 months to 16 years.

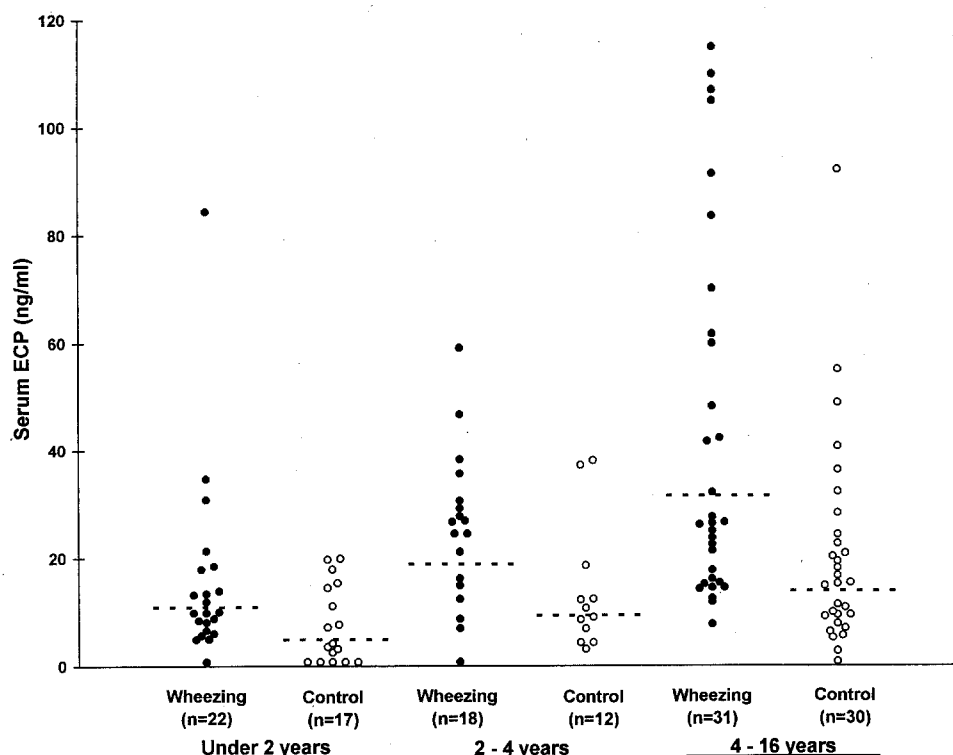


Fig. 1. Concentrations of serum ECP. Geometric mean levels of serum ECP are indicated by dashed lines. For children younger than 2 years of age the geometric means were 11.2 and 5.5 ng/ml for wheezing and control children, respectively ($p < 0.02$); for children 2 to 4 years of age the values were 20.2 and 10.2 ng/ml, respectively ($p < 0.04$); and for children 4 to 16 years of age the values were 32.7 and 14.6 ng/ml, respectively ($p < 0.001$). Symbols on the abscissa represent sera that tested below the detection limits of the assay (ECP < 2 ng/ml).

Seventy-one children had wheezing documented by auscultation and were treated with at least a β_2 -agonist by inhalation. Patients with a history suggestive of bronchopulmonary dysplasia or who had taken a course of systemic corticosteroids within a week of their visit to the emergency department were not included in the study. Fifty-nine children without respiratory symptoms or a previous history of wheezing were enrolled as control subjects; most were seen for minor trauma, rashes, and gastrointestinal complaints. Patients were further characterized through information obtained from their parents about allergic disease in the immediate family (parents and siblings). Additional demographic information is presented in Table I. The study was approved by the human investigation committee at the University of Virginia, and informed written consent was obtained from parents, patients, or both.

Nasal washes and blood sampling. Nasal washes were obtained by instilling 2 ml of physiologic saline solution into each nostril with the patient lying supine. Wash fluid and secretions were immediately aspirated into a sterile 70 ml specimen trap (Sherwood Medical, St. Louis, Mo.) attached to wall suction by inserting the tip of a flexible 18F Tri-Flo

suction catheter (Baxter Healthcare Corp., Valencia, Calif.) into the anterior nares. For children older than 4 years of age, a Yankauer suction tube (Sherwood Medical) was used. Each nostril was washed, after which 1 ml of saline solution was aspirated through the catheter to rinse secretions into the trap. The combined washes resulted in volumes of 2.5 to 3 ml of fluid and secretions.

After the wash, a smear of nasal secretions was prepared on a glass slide and a Hansel stain was done to assess eosinophil numbers. Smears containing more than 10 eosinophils per 100 cells counted were judged to show positive results.^{3,13} Each remaining specimen of wash fluid and secretions was stored on ice for up to 30 minutes and then transferred to a conical tube, agitated in a vortex mixer, and centrifuged at 1000g for 5 minutes. The supernatant was stored at -70°C for subsequent measurements of ECP.

Five milliliters of blood was obtained by venipuncture from each patient. For an assessment of total blood eosinophils, 0.5 ml was sent to the University of Virginia Hospital clinical laboratory, and a complete blood cell count and differential cell analysis were done in an H* 1 Blood Cell Analyzer (Technicon Instruments Corp., Tarrytown, N.Y.). The

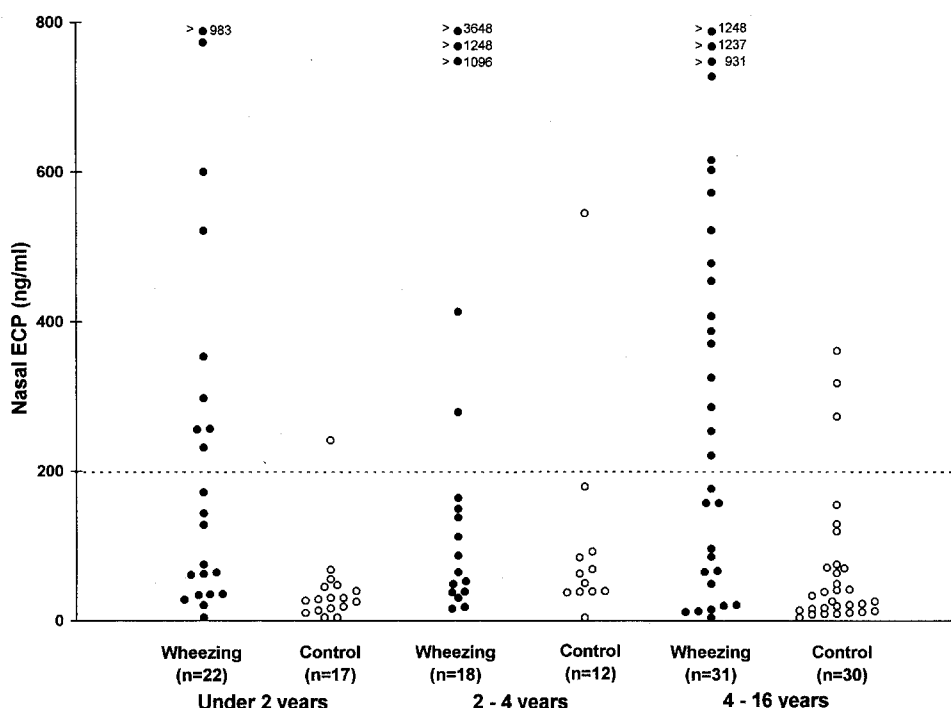


Fig. 2. ECP levels in nasal washes. Before the age of 2 years, the proportion of children with ECP levels >200 ng/ml in their nasal wash fluid was significantly higher in wheezing children than in control children (41% and 6%, respectively; $p < 0.03$). This was also true for wheezing and control patients older than 2 years (45% and 10%, respectively; $p < 0.001$). In addition, the geometric mean levels of nasal ECP were significantly higher for wheezing children than for control children less than 2 years of age (115 and 24 ng/ml, respectively; $p < 0.001$) and more than 2 years of age (143 and 40 ng/ml, respectively; $p < 0.001$). Symbols on the abscissa represent nasal washes that tested below the detection limits of the assay (ECP < 2 ng/ml).

remaining blood sample was allowed to clot in Vacutainer SST tubes (Becton Dickinson, Rutherford, N.J.) at room temperature for 60 minutes as previously described.¹⁴ After centrifugation at 1000g for 10 minutes, the serum was removed and stored at -20°C .

Immunoassays. Measurements of ECP were done in serum and nasal lavage fluid with a monoclonal antibody-based fluorometric assay (Pharmacia CAP System) kindly provided by Pharmacia Diagnostics, Uppsala, Sweden.¹⁴ Undiluted serum from each patient was analyzed in duplicate. Nasal lavage fluid was analyzed after being diluted one half and one fourth in 1% bovine serum albumin in phosphate-buffered saline solution (pH 7.4), and the results were expressed in nanograms per milliliter. Allergen-specific IgE antibody was measured by quantitative radioallergosorbent test with cyanogen bromide-activated disks coated with allergen extracts (Miles Inc., Hollister Stier Division, Spokane, Wash.) as previously described.¹⁵ Each serum was tested for IgE antibody to dust mite (*Dermatophagoides pteronyssinus* and *Dermatophagoides farinae* were tested separately), cat epithelium, short ragweed, rye grass, and cockroach by use of an extract containing allergens from

German, American, and Oriental species. Sera containing 40 RAST units or more per milliliter (equivalent to ~ 4 ng of allergen-specific IgE antibody per milliliter) to one or more allergens were defined as showing RAST positive results.³ Total serum IgE assessments were done with the monoclonal antibody-based CAP system assay developed by Pharmacia Diagnostics. The detection of RSV antigen in nasal wash fluid was done with a membrane enzyme immunoassay (Abbott Diagnostics Division, Chicago, Ill.).

Statistical analysis. Data from wheezing and control patients were compared in three different age groups—children younger than 2 years of age, children 2 years through 3 years 11 months of age, and children 4 through 16 years of age. Chi-square analyses were used to compare differences in the percentages of wheezing and control patients who were sensitized to inhaled allergens or who had elevated numbers of eosinophils or ECP concentrations in their nasal washes. When samples were small, we relied on the Fisher Exact Test.¹⁶ Two-sample t tests were used to compare continuous variables between wheezing and control patients in the various age groups. All tests of significance were two sided.

Table III. Nasal ECP, other eosinophil responses, and assessments of IgE (wheezing patients)

Nasal ECP (ng/ml)	Patients (No.)	Nasal eosinophils* (% positive)	Blood eosinophils† (cells/mm ³)	Serum ECP† (ng/ml)	Total IgE† (ng/ml)	RAST‡ (% positive)
Children aged <2 yr						
>200§	9	0	172 (54-585)	14.6 (5-84)	9.3 (2-66)	0
<200	13	0	111 (43-226)	9.3 (2-31)	5.8 (2-23)	0
Children aged >2 yr						
>200§	22	64	449 (150-2205)	41.5 (14-200)	240 (17-1894)	50
<200	27	26	212 (26-1028)	19.5 (2-60)	177 (4-1395)	52

*Percentage of nasal smears containing 10% or more eosinophils.

†Total IgE levels, total blood eosinophil counts, and serum ECP concentrations are reported as geometric means.

‡Percentage of RAST-positive sera containing IgE antibody to one or more allergens.

§Among children less than 2 years of age with nasal ECP concentrations greater than 200 ng/ml, three had elevated serum ECP levels (84, 35, and 21 ng/ml). One of them had elevated total IgE concentrations and blood eosinophil counts (66 IU/ml and 585 cells/mm³, respectively); one had a total IgE concentration of 48 IU/ml; and one had a blood eosinophil count of 482 cells/mm³. After the age of 2 years, wheezing children with nasal ECP levels greater than 200 ng/ml had a significantly greater percentage of nasal smears with 10% or more eosinophils ($p < 0.02$), and significantly higher geometric means for total blood eosinophil counts ($p < 0.005$) and serum ECP levels ($p < 0.025$).

RESULTS

Patient characteristics. The results of assessments of family history, nasal and peripheral blood eosinophils, total serum IgE concentrations, and IgE antibody to inhalant allergens are shown in Table II. Of these variables, the only significant difference between wheezing and control patients less than 2 years of age was the geometric mean for total IgE. However, total IgE levels were generally low in this age group, and only three wheezing children had levels greater than 20 IU/ml (66, 48, and 23 IU/ml). Three wheezing patients had a history of eczema, but only one had evidence of eczema on physical examination and required topical corticosteroids. Among children aged 2 to 4 years, the geometric mean of total IgE was significantly higher in wheezing patients than in control subjects; wheezing patients also had positive RAST results and nasal eosinophilia more frequently than did control subjects. After the age of 4 years, all characteristics examined were significantly associated with wheezing.

Serum ECP concentration. Geometric mean levels of ECP in serum were significantly higher in wheezing children than in control children in all three age groups (Fig. 1). A positive correlation between serum ECP concentrations and total blood eosinophil counts was observed for all wheezing patients ($r = 0.63$; $p < 0.01$). This correlation was similar in the three age groups. For children aged 4 to 16 years, the geometric mean of serum ECP concentrations in wheezing patients who had positive RAST results for inhaled allergens was not significantly different from the geometric mean in wheezing patients with negative RAST results (31 and 36 ng/ml, respectively).

Nasal ECP. The geometric mean of ECP concentrations

in nasal wash fluid in wheezing children was significantly higher than in control children in each age group (Fig. 2). In addition, a large proportion of wheezing children at all ages, had nasal wash ECP levels greater than 200 ng/ml, whereas 85% of the control patients had levels less than 100 ng/ml (mean concentration, 68 ng/ml.) Among nine children less than age 2 years with nasal ECP levels greater than 200 ng/ml, none had a positive RAST result or a nasal smear with 10% or more eosinophils (Table III). Three of these children had elevated serum ECP levels and an elevated total IgE or blood eosinophil count; one of the three had eczema. The other six children had only high levels of nasal ECP. Seven (78%) of the nine wheezing infants with nasal ECP greater than 200 ng/ml, compared with 8 (61%) of 13 wheezing infants with nasal ECP less than 200 ng/ml, had RSV antigen ($p =$ not significant). None of the control patients had RSV antigen. After the age of 2 years, levels of nasal ECP greater than 200 ng/ml had a significant positive correlation with other eosinophil responses (nasal eosinophils, blood eosinophils, and serum ECP levels), but not with measurements of total IgE or specific IgE antibody by RAST (Table III).

DISCUSSION

Recent longitudinal studies^{11, 12} have suggested that measurements of ECP in serum may be valuable in monitoring symptom exacerbations in school-aged children with asthma. The advantage of measuring ECP concentrations rather than total blood eosinophil counts is based on information indicating that ECP release reflects eosinophil activation.¹⁷ Among the wheezing children seen in our emergency department, the positive correlation between serum ECP concentrations and total blood eosinophil counts was simi-

lar to that reported in a previous study.¹¹ For children in each age group, the mean serum levels of ECP were significantly higher in wheezing patients than in control patients, although considerable overlap in values was observed among wheezing and control patients younger than the age of 4 years. In contrast, striking levels of ECP were detected in the nasal wash fluid of a significant percentage of wheezing children at all ages. The concentrations of ECP were consistent with those detected by others in respiratory tract secretions (e.g., in bronchoalveolar lavage fluid, sputum, and nasal washes from patients with rhinitis).¹⁸⁻²⁰ In many cases, nasal ECP levels were greater than 20-fold higher than parallel measurements of ECP in serum. These results suggest that assessments of eosinophils and their mediators in peripheral blood may underestimate the contribution of these cells to the inflammatory process at the mucosal surface in the respiratory tract. This observation is in agreement with other studies of eosinophils and their by-products in bronchoalveolar lavage fluid from adult patients.²¹

In two previous studies, Garofalo et al.^{22,23} observed elevated nasal ECP levels in wheezing infants with RSV infection. Levels greater than 50 ng/ml appeared to distinguish those with RSV infection and bronchiolitis from those who had RSV infection and symptoms of rhinitis or pneumonia. This observation was noted to be consistent with previous reports from the same group of IgE antibody to RSV antigen in nasal washes from wheezing infants²⁴; however, IgE antibody to RSV was not assessed in their study of nasal ECP. In our study the relationship between elevated levels of nasal ECP and a positive finding for RSV antigen was not significant.

The concentrations of ECP in the nasal washes from our study were not standardized for albumin or total protein. Problems standardizing the measurements of mediators in nasal wash fluid based on these proteins have been discussed elsewhere.^{22,25} Using concentrations of these proteins to make adjustments in the analysis of ECP may falsely diminish the high levels of this mediator in nasal wash fluid, because the levels of albumin and total protein usually increase with vascular leakage resulting from mucosal inflammation.^{25,26} The dilutional effects of lavage fluid and secretions on ECP levels have been examined in patients with allergic rhinitis, and the effects of dilution on mean ECP concentrations appeared to be minimal.²⁰ In our study, two thirds of the wheezing patients with ECP levels greater than 200 ng/ml had levels higher than 400 ng/ml. By comparison, most control patients had values less than 100 ng/ml. These large differences between wheezing and control patients are unlikely to have been strongly influenced by the effects of dilution.

Several patients, especially the infants, had high levels of ECP in their wash fluid, but their nasal smears did not con-

tain many eosinophils. In a study of atopic dermatitis,²⁷ eosinophils were also not apparent in biopsy specimens of inflamed skin, which nevertheless stained heavily for eosinophil major basic protein. These observations suggest that the enumeration of eosinophils alone may not accurately reflect their involvement at the site of inflammation.

In prospective studies, many infants who wheeze have been shown to outgrow their symptoms, and asthma is not likely to develop.^{28,29} Furthermore, wheezing during infancy is not generally associated with atopy.^{3,9} This has led to the speculation that the pathogenesis of wheezing in many infants may not be the same as that in older children with asthma.^{9,30} Nevertheless, a subgroup of infants (possibly up to 40%) will continue to wheeze as they grow older.^{29,31} At present, these children are difficult to identify. However, the results from our study suggest that, in prospective studies of wheezing infants, it may be useful to monitor ECP in nasal washes to learn whether those with high levels have an increased risk of having asthma.

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