

High levels of eosinophil cationic protein in wheezing infants predict the development of asthma

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Background: In association with respiratory tract infections, infants may have episodes of wheezing, which represent the onset of asthma in some of them. Activated eosinophils play a central part in asthmatic inflammation.

Objective: We investigated whether, in infants experiencing their first episode of wheezing, eosinophil activation is present and can predict the development of asthma.

Methods: In a prospective trial, eosinophil activation was measured by eosinophil cationic protein (ECP) concentrations in serum from 33 nonatopic infants with their first episode of wheezing, 15 nonatopic infants with upper respiratory tract infection without wheezing, and 18 healthy nonatopic infants. One year later, the children were re-evaluated for a diagnosis of infantile asthma.

Results: Wheezing infants had higher median serum ECP levels (13.4 µg/L) than children with nonwheezing respiratory tract infection (7.6 µg/L, $p < 0.005$) or healthy subjects (7.1 µg/L, $p < 0.005$). In addition, wheezing infants ($n = 13$) with serum ECP concentrations greater than 20 µg/L were more likely to have asthma within 1 year than patients with ECP levels less than 20 µg/L (odds ratio = 12.4; confidence interval, 4.6-33.5).

Conclusion: Eosinophil activation measured by serum ECP is present in infants with their first episode of wheezing illness, especially in those infants in whom asthma subsequently develops within 1 year. These data may indicate a predictive value of serum ECP measurements in children with wheezing to identify those patients in whom infantile asthma is developing. These findings probably also indicate that serum ECP may be used to identify the children who need early anti-inflammatory treatment. (*J Allergy Clin Immunol* 1997;99:752-6.)

Key words: Eosinophil cationic protein, wheezing, infants, asthma

Many infants have episodes of wheezing associated with viral respiratory illness.¹ The so-called "wheezy baby syndrome"² is a disease entity, which may precede the onset of childhood asthma in certain patients. Neither the pathogenesis of these episodes nor their rela-

Abbreviation used

ECP: Eosinophil cationic protein

tion to asthma has been completely elucidated. In both wheezy bronchitis and bronchial asthma, reversible airway obstruction and pulmonary hyperinflation are common.³ However, some infants will outgrow the condition in early childhood, but some of them (about 40%) will become asthmatic.⁴⁻⁶ Unfortunately, no variable that could predict whether the wheezing infant is subsequently likely to experience bronchial asthma exists.

Childhood asthma is an inflammatory disease in which activated eosinophil granulocytes play a central role.^{7,8} The assessment of eosinophil activation by measurement of serum eosinophil cationic protein (ECP) is well accepted.^{9,10} The measurement of ECP in serum is assumed to reflect pulmonary inflammation in asthma.⁹

To determine whether eosinophil inflammation is present in wheezing illness, infants with their first episode of wheezing were investigated for serum ECP levels, which were then related to the development of infantile bronchial asthma within a 1-year follow-up period.

METHODS

Patients and control subjects

Thirty-three infants (mean age, 7 months; range, 4 to 9 months; 23 boys and 10 girls), who were treated in the pediatric outpatient department at the University Children's Hospital of Vienna, were included in this prospective trial. Of these infants, none were born before 39 weeks of gestation, and none were mechanically ventilated. At the time of entry into the study, the patients had their first episode of wheezing characterized by cough, wheeze documented by auscultation, and response to inhaled β_2 -agonists in the emergency department. The presence of viral infection of the upper respiratory tract (lower respiratory tract infection was excluded by roentgenogram) was suggested by the lack of neutrophilia and the lack of a marked increase of C-reactive protein. Infants with atopic dermatitis, sensitization to inhalant or nutritional allergens, and increased total serum IgE levels were excluded because in those patients numbers of eosinophils and ECP levels were reported to be increased.¹¹ In addition, none of the patients were treated with topical or systemic steroids.

At the time of entry into the study, the parents of the infants completed a questionnaire about their own history of respira-

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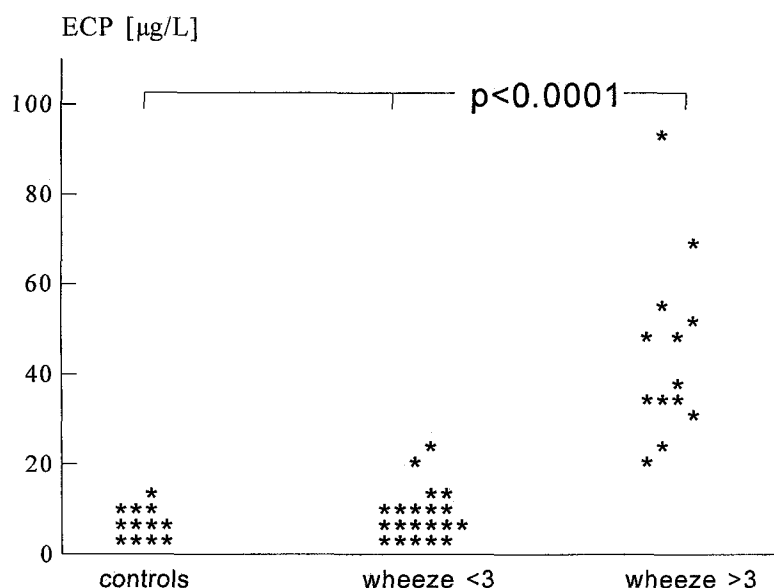


FIG. 1. Serum ECP concentrations in healthy infants, infants subsequently having less than three episodes of wheezy bronchitis, and patients with more than three episodes of wheezing. The latter patients were defined as having early childhood asthma. Median ECP levels in healthy infants were 7.1 µg/L; in children with less than three episodes, 9.3 µg/L; and in children with more than three episodes (i.e., those with infantile asthma), 39.6 µg/L.

tory illness, atopy, and smoking habits (Table I). In addition, they were instructed on the use of a daily diary, which recorded symptoms as wheeze, cough, and breathlessness. Whenever the children had any of these symptoms again, the parents were told to bring them to our hospital to confirm the parents' reports. The treatment of wheezing and cough consisted of inhaled β_2 -agonist (salbutamol) at home when needed according to the guidelines of an international consensus report.¹² One year after the first examination, the parents again answered a questionnaire on the child's respiratory illness. In that questionnaire, asthma was defined as at least three episodes of wheezy bronchitis after other conditions has been excluded, as recommended previously.^{12,13} As in patients with asthma, increased incidence of airway hyperreactivity has also been observed in patients with wheezy bronchitis and in their first-degree relatives.¹⁴ On the basis of this evidence, it has been postulated that a child who has bronchitis and wheezing actually has asthma.¹⁵ Atopic status was re-evaluated by measurement of IgE antibodies. During the entire observation period, none of the patients were treated with antiinflammatory agents but only with β_2 -agonists when needed.

As control subjects, 15 children (mean age, 8 months; range, 3 to 12 months; 9 boys and 6 girls) with upper respiratory tract infection without wheezing and 12 healthy nonatopic infants (mean age, 7 months; range, 2 to 13 months; 7 boys and 5 girls) were enrolled. None of the latter group had a history of atopy or lower respiratory tract illness at the time of assessment or had been treated with steroids.

After parental agreement was obtained, blood was drawn for routine tests in all cases at the time of entering the study and 1 year later.

Definition of atopy

Children were identified as being atopic by increased total serum IgE levels (above age-dependent normal values) and the

TABLE I. Characteristics of infants with wheezing ($n = 33$)

	Number of patients	Percentage of patients
Maternal asthma	2	6.1
Paternal asthma	3	9.1
Maternal smoking during pregnancy	6	18.2
Smoking in household	10	30.3
Had asthma within 1 year	13	39.4
Had atopic disease within 1 year	6	18.2

presence of specific serum IgE antibodies (RAST class 2) against any of seven common allergens (cow's milk, egg, soy, grass and birch pollens, cat, and *Dermatophagoides pteronyssinus*).

Assessment of serum ECP and blood eosinophil counts

Blood samples were drawn and processed according to the manufacturers' recommendations.¹⁶ ECP in serum was determined by means of a specific and sensitive double-antibody radioimmunoassay (Pharmacia AB Diagnostics, Uppsala, Sweden) in duplicate. In brief, ECP in the samples competes with a fixed amount of iodine 125-labeled ECP for the binding sites of specific antibodies.¹⁶ The interassay coefficient of variation of the radioimmunoassay was below 10%. The limit of detection of ECP was 2.0 µg/L. Before the study, repeated measurements of ECP in serum samples obtained within 3 subsequent days did not result in significant differences in ECP levels in either wheezing infants ($n = 4$) or healthy control subjects ($n = 3$).

TABLE II. Blood eosinophil counts, serum ECP levels, and total serum IgE levels in infants with wheezing, children with upper respiratory tract infection without wheezing, and healthy nonatopic control subjects at the beginning of the study

	Infants with wheezy bronchitis (<i>n</i> = 33)	Infants with upper respiratory tract infection without wheezing (<i>n</i> = 15)	Healthy control subjects (<i>n</i> = 18)
Eosinophil counts (cells/ μ L)	180 (125-220)	175 (125-220)	180 (110-218)
Serum ECP (μ g/L)	13.4 (8.7-34.9)*	7.6 (6.3-9.5)	7.1 (5.8-9.7)
Total serum IgE (kU/L)	8.0 (4.0-11.0)	7.0 (4.0-10.0)	8.0 (3.0-12.0)

Results are expressed as median (quartile 1 to quartile 3).

Eosinophil counts and total serum IgE levels were not statistically different between the groups.

*Serum ECP concentrations in children with wheezing were significantly increased compared with the other groups of patients ($p < 0.005$ and $p < 0.005$, respectively).

TABLE III. Relationship between serum ECP levels greater than and less than 20 μ g/L and development of asthma within 1 year in infants with their first episode of wheezing

	Asthmatic		Nonasthmatic		Total	
	No.	%	No.	%	No.	%
ECP > 20 μ g/L	12	36.4	1	3	13	39.4
ECP < 20 μ g/L	1	3	19	57.6	20	60.6
Total	13	39.4	20	60.6	33	100

Median ECP levels were: (day 1) 27.8 μ g/L, (day 2) 25.4 μ g/L, (day 3) 28.9 μ g/L versus (day 1) 5.8 μ g/L, (day 2) 6.4 μ g/L, (day 3) 4.9 μ g/L.

ECP was selected as marker of eosinophil activation because it is the most accepted reference value.⁹ In addition, ECP is more specific for eosinophils¹⁷ than another frequently mentioned eosinophil protein, major basic protein, which is also present in basophils.¹⁸ Blood eosinophils were counted automatically (Sysmex NE-5500; Müller, Vienna, Austria), with a coefficient of variation of 7%.¹⁷

Assessment of total serum IgE and specific IgE antibodies

Total serum IgE and specific IgE antibodies were measured by means of enzyme immune assays (Kallestad Diagnostics).

Statistical analysis

Results were expressed as medians (quartile 1 to quartile 3) unless mentioned otherwise. Correlation coefficients were obtained by using the Kendall Tau B test. Nonparametrical statistical tests were used in comparative analysis. The Kruskal-Wallis analysis of variance procedure was applied first to the three groups. When this was significant, each pairing was examined by means of the Mann-Whitney U test. Odds ratio was calculated by the Mantel-Haenszel chi square procedure to determine the influence of ECP levels on the development of asthma. Probabilities of less than 5% were considered statistically significant.

RESULTS

Patient characteristics

The results of evaluation of family and patient history are shown in Table I. Six of 33 wheezing infants had atopic disease within 1 year, which persisted as atopic dermatitis in six cases and cow milk's allergy in two cases. In these atopic patients total serum IgE levels were higher at the time of re-evaluation after 1 year (39.0 kU/L [22.5 to 66.0 kU/L]) as compared with healthy control subjects (9.0 kU/L [4.5 to 11.8 kU/L]; $p < 0.005$). As shown in Table I, 13 infants were identified as having asthma, after they experienced a median of six wheezing episodes (range, 4 to 12 episodes) within 1 year. Four of them had become atopic as well. The remaining 20 patients had a total of one to two episodes of wheezy bronchitis.

Eosinophil counts and serum ECP measurements

In wheezing infants, eosinophil counts were not different from those in children with colds without wheezing or healthy control subjects (Table II). Serum ECP concentrations, however, were significantly increased in wheezing infants compared with the control groups (Table II). Serum ECP concentrations and eosinophil counts were not correlated in patients with wheezy bronchitis ($r = -0.054$, NS). In addition, serum ECP levels at the beginning of the study were significantly higher in the wheezing infants who had bronchial asthma within 1 year ($n = 13$) than in those without repeated wheezing episodes (Fig. 1). Children with serum ECP levels above 20 μ g/L had an increased risk of developing asthma within 1 year compared with children with ECP values less than 20 μ g/L (odds ratio, 12.4; 95% confidence interval, 4.6 to 33.5). ECP levels above 20 μ g/L predicted the development of infantile asthma with a sensitivity of 92.3%. The specificity for ECP measurements with a threshold of 20 μ g/L for asthma prediction was 95% (Table III).

At the time of re-evaluation after 1 year in these patients, ECP levels remained high (25.6 μ g/L [14.8 to 39.4 μ g/L]) compared with healthy control subjects (6.8

$\mu\text{g/L}$ [4.9 to 10.2 $\mu\text{g/L}$]; $p < 0.001$). Such results were not obtained for eosinophils (median: 180 cells/ μl vs 160 cells/ μl).

DISCUSSION

The perception of the very nature of childhood asthma and other wheezing disorders in early childhood has been altered by the recognition that there are several distinct wheezing disorders masquerading as asthma in preschool children, each with its own cause, natural history, and prognosis.¹⁹ Wheezing-associated respiratory tract illnesses are very common in the first 2 years of life, but most of these children seldom experience wheezing after that age.²⁰ Nonetheless, in infants and toddlers asthma is likely to be a heterogeneous group of conditions, which share the clinical findings of wheezing and some common risk factors such as exposure to passive smoking, difficult living conditions, and increased risk in boys.²¹ In the past, terms such as wheezy bronchitis or asthmatoïd bronchitis were used to describe wheezing in the very young. The term *asthma* was avoided because episodic wheezing triggered by infection in infants and toddlers had a more benign prognosis than the disease in older children.²² The term *asthma* was used only in older children when aeroallergen responsiveness had begun. This distinction was later found to be associated with both underdiagnosis of asthma and its inappropriate treatment.^{23,24} Consequently, pediatric pulmonologists suggested that children who continue to experience wheezing should be diagnosed with bronchial asthma after more than three episodes of wheezing.^{13,15} However, no variable that could predict the development of bronchial asthma in these children exists.

Our findings demonstrate that high serum ECP levels may be a predictor of infantile asthma in children with their first episode of wheezing. In bronchial asthma, eosinophils and their products are well established as markers of bronchial inflammation in both adults and children.^{7,10} In addition, the results of this study indicate 12-fold increased risk for the development of infantile asthma in children with ECP levels greater than 20 $\mu\text{g/L}$.

Previously, we hypothesized that the onset of eosinophilic inflammation occurs in the very young child with asthma.⁷ From this study, it has become evident that the inflammatory process might be ongoing at the first episode of wheezing without knowledge of whether the infant became asthmatic.

Most of the patients with high levels of ECP had normal eosinophil numbers. In a study of cystic fibrosis, eosinophils were also not apparent in peripheral blood, but high ECP concentrations were measured.²⁵ These observations suggest that the enumeration of eosinophils alone may not accurately reflect their involvement in the inflammatory process.

Recently, increased ECP concentrations in serum and in nasal lavage fluid were measured²⁶ in wheezing infants in a cross-sectional study, and ECP levels were in agreement with our results in this longitudinal study.

In epidemiologic investigations it has been demonstrated that various factors (e.g., maternal atopy, asthma, or smoking in the household) might influence the development of childhood asthma.²⁷ But until now, it was not possible to predict the individual outcome of a wheezy patient at an age of less than 12 months. Possibly, serum ECP measurements allow the prediction of infantile asthma. In addition, it could be speculated that, by identification of these patients at high risk for asthma, early antiinflammatory intervention might reduce the severity of infantile asthma, as previously shown for older children,²⁸ or prevent the chronic disease course in these children. Nonetheless, the diagnosis of asthma in this age group of patients is controversial. However, as recommended previously, asthma could be diagnosed after three episodes of wheezing even in infants and toddlers.^{13,15}

In conclusion, our findings demonstrate that eosinophilic activation is present in infants with their first episode of wheezing before signs of a chronic obstructive pulmonary disease are evident. A subgroup of infants (up to 40%) will continue to experience wheezing as they grow older.⁶ Until now, these children were difficult to identify, but serum ECP measurements appear to be useful for identifying those infants with wheezing at high risk for infantile asthma and those who would benefit from early antiinflammatory treatment.

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