

ORIGINAL ARTICLE

Correlation between Asthma Severity and Serum IgE in Asthmatic Children Sensitized to *Dermatophagoides pteronyssinus*

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Background. We undertook this study to test the possible correlation between serum concentration of total and specific IgE antibodies and asthma severity in asthmatic children sensitized to the house dust mite *Dermatophagoides pteronyssinus*.

Methods. The study included 157 asthmatic children aged 5–15 years (8 ± 3 years). Clinical diagnosis was based on personal and family history, physical examination, pulmonary function testing and skin tests. Asthma severity was determined according to GINA guidelines. *In vitro* tests included serum concentration of total and specific IgE.

Results. All asthmatic children had elevated serum concentration of total IgE. The children with elevated serum concentration of total IgE also showed an increased serum concentration of specific IgE. Asthma of higher higher severity was present in patients with total IgE concentration >288.0 kIU/L (AUC = 0.736) and specific IgE to *Dermatophagoides pteronyssinus* >44.1 kIU/L (AUC = 0.843). Intermittent asthma was present in 76.9% of children with specific IgE concentration <44.1 kIU/L. The positive predictive value suggested with 79.2% probability that a child with a concentration of specific IgE to *Dermatophagoides pteronyssinus* >44.1 kIU/L would have a more severe form of asthma.

Conclusions. Asthmatic children with higher asthma severity have a higher serum concentration of both total IgE (>288.0 kIU/L) and specific IgE to *Dermatophagoides pteronyssinus* (>44.1 kIU/L), respectively. © 2007 IMSS. Published by Elsevier Inc.

Key Words: Asthma severity, Child, House dust mite, Total IgE, Specific IgE.

Introduction

The manifestation of asthma can vary according to the severity and frequency of symptom exacerbations. At present, classification of asthma into four grades has been accepted: intermittent asthma, mild permanent asthma, moderate permanent asthma, and severe permanent asthma (1). The clinical picture of asthma, i.e., the disease severity, results from a series of complex pathophysiological processes determined by numerous known and unknown factors, from genetic to environmental. Identification of the disease severity is a precondition for appropriate therapeutic approach in

asthma patients. Daily practice has shown that airway obstruction can also be confirmed by spirometry in patients with seemingly normal physical finding of the respiratory system. Thus, measurement of pulmonary function is necessary to evaluate the severity of airway obstruction. According to GINA guidelines (Global Initiative in Asthma), repeated pulmonary function testing is required to determine the severity and frequency of acute asthma attacks.

Recent studies have shown that adult patients with higher asthma severity had a more severe form of the disease in childhood (2). This observation suggests the importance of the earliest possible evaluation of asthma severity in each individual patient to enable an early introduction of appropriate and efficacious therapy and eventually the control of asthma, i.e., minimal chronic symptoms including nocturnal symptoms, minimal number of attacks (rarely) that do

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not require medical intervention, minimal need of β_2 -agonists, no limitation of daily activities including exercise, <20% peak expiratory flow (PEF) variability, nearly normal PEF, and absence of medication side effects (1).

Determination of serum concentrations of total and specific IgE antibodies plays a major role in the diagnosis of childhood asthma. Although IgE are generally circulating intravascularly, they may also be found extravascularly, bound to the high-affinity receptors on mastocytes and basophilic granulocytes (3). Serum concentration of IgE depends on the genetic predisposition to an increased IgE synthesis and exposure to a broad array of environmental allergens (4–6). Elevated serum concentration of IgE can be found in patients with parasitic diseases (7). Both animal studies (8) and human studies in adults (9) have shown that the concentration of IgE antibodies could vary with the degree of immune stimulation, i.e., with the frequency, dose and route of allergen exposure, respectively (8). Very few adult individuals diagnosed with asthma have low concentration of IgE (10). Simpson et al. have also shown that probability of wheeze was less in children with low specific IgE to inhalant allergens (11).

The aim of the present study was to assess the possible correlation between serum concentration of total and specific IgE antibodies and asthma severity in children sensitized to the house dust mite *Dermatophagoides pteronyssinus*.

Patients and Methods

Patients

Study subjects were a cross-sectional cohort of 157 children aged 5 to 15 years (8 ± 3 years), stratified by symptoms, and referred by primary care physicians to the Children's Hospital Srebrnjak in Zagreb between August 2004 and July 2005 for evaluation of lower respiratory tract symptoms of >4-weeks duration. Sensitization to the house dust mite *Dermatophagoides pteronyssinus* was verified after recruiting the children for the study, using *in vivo* (skin prick) and *in vitro* (serum specific IgE) tests. Diagnosis of asthma was made on the basis of a standardized diagnostic work-up, whereas asthma severity was determined according to GINA guidelines (1). Briefly, asthma was classified at the degree to which airflow has been obstructed, frequency and duration of symptoms, and the extent symptoms have interfered with daily activities. The following parameters were evaluated: family history of atopic diseases, symptoms and symptom structure, precipitating and/or aggravating factors, and course of disease. On physical examination, attention was paid to the presence of rhinitis, sinusitis, hyperinflation, eczematous skin lesions, and respiratory sounds on auscultation to identify airway obstruction.

Methods

Respiratory Function Testing

Respiratory function was tested by a portable Jaeger spirometer, determining the parameters of FEV₁ and FEV₁/VC ratio (Tiffeneau's index). The measured values of respiratory function were compared with the expected values, determined at Department of Clinical Laboratory Diagnosis, Children's Hospital Srebrnjak, Zagreb (12,13).

Allergy Skin Testing

Allergy skin testing (14) was performed to 13 inhalation allergens manufactured by the Institute of Immunology, Zagreb. Standard inhalation allergens were used: bacterial vaccine, house dust, *Dermatophagoides pteronyssinus*, feather, mold, *Monilia albicans*, bedding of plant and animal origin, animal hair, tree, grass and weed pollen, and tobacco. Testing for *Dermatophagoides pteronyssinus* was performed by the standard national preparation (15).

Laboratory Methods

Blood samples were obtained after clinical examination at the allergology clinic between 8:00 a.m. and 3:00 p.m. Serum concentrations of total IgE and specific IgE to particular allergens chosen according to the skin test result were determined in each study subject.

Determination of Total IgE Concentration

Serum concentration of total IgE was determined by the fluoroimmunochemistry method (Abbott, Abbott Park, IL) on an IMx autoanalyzer from the same manufacturer. The analysis is based on the sandwich technique and enzyme labeling of the antigen–antibody complex (16). The method sensitivity is 0.048 kIU/L (data from the manufacturer's instructions). The 95th centile reference values were used for result comparison (17).

Determination of Specific IgE Concentration

Serum concentration of specific IgE to *Dermatophagoides pteronyssinus* allergen was determined by the UniCAP method (Pharmacia, Uppsala, Sweden), where high sensitivity is achieved by use of a three-dimensional cellulose carrier (flexible hydrophilic polymer) (18). The World Health Organization secondary standard, WHO 75/502, was applied. The range of confidence was 0.35–100 kIU_A/L.

Statistics

Data storage and preparation for statistical analysis were done by use of the Microsoft Office Excel 2000 software (Microsoft, Redmond, WA). Quantitative variables were expressed as arithmetic mean ($\bar{x}$), standard deviation (SD),

median (M), and interval. Because the distribution of IgE is not normal (17), Wilcoxon test was used for comparison of IgE values (19). The receiver operating characteristic (ROC) curve was used to calculate diagnostic validity of particular test results (20).

Results

The study group of 157 asthmatic children showed a male predominance; there were 107 (68.2%) male and 50 (31.8%) female children. Positive family history of atopic diseases was recorded in 49.7% (78/157) of study subjects, with no sex difference. In most patients, the initial symptoms occurred between 3 and 4 years of age. By the end of the fourth year of life, the initial symptoms of wheezing were present in 79/157 (50.3%), and by the end of the eighth year of life in 109/157 (69.4%) study patients. Viral infection of the airways in the first four years of life was recorded in 92/157 (58.6%) patients.

Currently, intermittent asthma was present in 76/157 (48.4%), mild permanent asthma in 65/157 (41.4%), and moderate permanent asthma in 16/157 (10.2%) patients. None of the study patients had severe permanent asthma. According to FEV1 and FEV1/VC, 56.1% (88/157) children had normal finding (FEV1 >80%; FEV1/VC >75%), 37.6% (59/157) and were classified to have mild bronchoobstruction (FEV1 = 60–80%; FEV1/VC = 60–75%), 3.8% (6/157) moderate bronchoobstruction (FEV1 = 40–59%; FEV1/VC = 40–59%), and 2.5% (4/157) severe bronchoobstruction (FEV1 <40%; FEV1/VC <40%), respectively.

In comparison with in-house reference values (17), all study subjects had elevated serum concentration of total IgE. The mean IgE concentration (Figure 1) was lowest in children with intermittent asthma ($\bar{x}$ = 290.4 kIU/L; median = 191.8 kIU/L), higher in children with mild permanent asthma ($\bar{x}$ = 559.7 kIU/L; median = 328.4 kIU/L), and highest in children with moderate permanent asthma ($\bar{x}$ = 706.5 kIU/L; median = 542.6 kIU/L). Medians were consistently lower than arithmetic means, indicating a shift towards higher values in the distribution of total IgE in each group of subjects. There was no statistically significant gender difference in IgE concentration: 537.0 ± 587.3 kIU/L (median 405.1 kIU/L) in male and 511.5 ± 656.4 kIU/L (median 472.3 kIU/L) in female ($p > 0.05$).

Comparison of the concentrations of total IgE and specific IgE to *Dermatophagoides pteronyssinus* showed the children with a higher concentration of specific IgE to also have an increased serum concentration of total IgE. The children with class 1–3 concentration (0.36–17.4 kIU_A/L) had lowest serum concentration of total IgE ($\bar{x}$ = 4.4 kIU_A/L; median 2.1 kIU_A/L). The children with classes >3 concentration had higher concentration of total IgE; those with class 4 concentration of specific IgE (17.5–50.0 kIU_A/L) had average serum concentration of to-

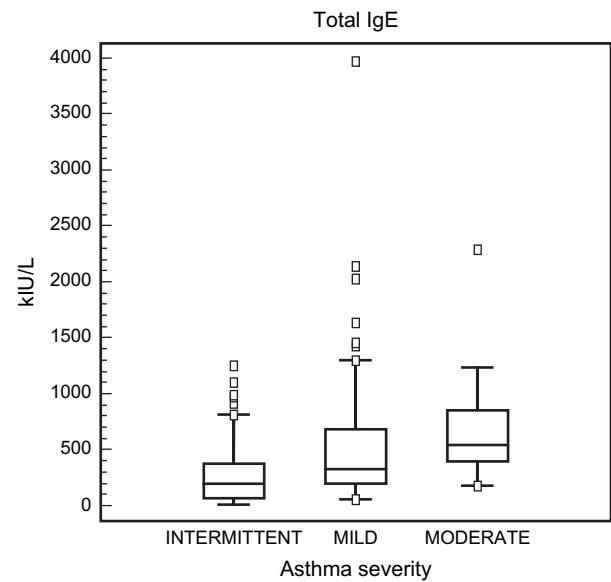


Figure 1. Concentration of total serum IgE in children according to asthma severity.

tal IgE, $\bar{x}$ = 209.4 kIU/L (median 169.8 kIU/L); those with class 5 concentration of specific IgE (50.0–100.0 kIU_A/L) had higher serum concentration of total IgE ($\bar{x}$ = 481.2 kIU/L; median 358.8 kIU/L); and those with class 6 concentration of specific IgE (>100.0 kIU_A/L) had highest serum concentration of total IgE ($\bar{x}$ = 1058.6 kIU/L; median 913.1 kIU/L), the difference being statistically significant ($p < 0.001$). The severity of asthma was not taken in consideration.

Patient distribution according to asthma severity (Table 1) revealed the patients with a higher concentration of specific IgE to *Dermatophagoides pteronyssinus* to have a higher grade of asthma severity (χ^2 = 56.635; df = 6; p = 0.000). The lowest concentration of specific IgE ($\bar{x}$ = 4.4 kIU/L; median 2.1 kIU/L) was most frequently recorded in patients with intermittent asthma, those with specific IgE 0.35–17.4 kIU_A/L (n = 31), higher concentration of specific IgE ($\bar{x}$ = 69.9 kIU/L; median 70.1 kIU/L) in patients with mild asthma (n = 28), and highest concentration of specific IgE (>100 kIU/L all) in patients with moderate asthma (n = 12). These findings were additionally supported by the coefficient of rank correlation values (r = 0.791; p = 0.000; 95% confidence interval for r = 0.7245–0.8434), pointing to a correlation between the concentration of specific IgE and asthma severity. Quantitative values of the concentration of specific IgE to *Dermatophagoides pteronyssinus* were not submitted to statistical analysis because the concentration of specific IgE >100 kIU_A/L was not exactly determined in diluted serum samples.

The correlation of total (Table 2) and specific IgE (Table 3) with asthma severity was expressed by use of ROC analysis. Due to the small number of study children with

Table 1. Concentration of specific IgE to *Dermatophagoides pteronyssinus* in children according to asthma severity

Asthma severity	Specific IgE (kIU _A /L)			
Specific IgE (kIU _A /L)	0.35–17.4	17.5–50.0	50.0–100.0	> 100
Intermittent	<i>n</i> = 31	<i>n</i> = 26	<i>n</i> = 13	<i>n</i> = 6
$\bar{x} \pm$ SD	4.4 $\pm$ 4.5	30.3 $\pm$ 7.8	65.2 $\pm$ 9.8	> 100
Median	2.1	30.1	64.1	
Interval	0.47–13.2	17.9–44.1	52.9–89.3	
Mild	<i>n</i> = 0	<i>n</i> = 17	<i>n</i> = 28	<i>n</i> = 20
$\bar{x} \pm$ SD		30.5 $\pm$ 9.9	69.9 $\pm$ 14.3	> 100
Median		33.7	70.1	
Interval		17.6–48.3	53.2–97.7	
Moderate	<i>n</i> = 0	<i>n</i> = 0	<i>n</i> = 4	<i>n</i> = 12
$\bar{x} \pm$ SD			71.6 $\pm$ 15.8	
Median			68.9	
Interval			49.0–99.4	

$\chi^2 = 56.635$; *df* = 6; *p* = 0.000; *r* = 0.791; *p* = 0.000; 95% confidence interval for *r* = 0.7245–0.8434.

moderate asthma, ROC analysis could not be performed for this patient group. The total IgE cut-off concentration of 156.2 kIU/L and 288.0 kIU/L, respectively, distinguishing children with intermittent asthma from those with more severe forms of the disease showed adequate (intermittent: mild asthma, AUC = 0.689) or good (intermittent: mild + moderate asthma, AUC = 0.736) diagnostic efficiency. Cut-off value of 156.2 kIU/L may prove useful in practice, indicating that 87.7% of children with serum concentration of total IgE < 156.2 kIU/L might have intermittent asthma, while 42.1% of children with total IgE > 156.2 kIU/L may have mild asthma. Cut-off value of 288.0 kIU/L has almost the same sensitivity, specificity, positive and negative predictive values (Table 2) indicating the similar number of true positive (67.1%) and true negative (68.3%) results.

The cut-off concentration of specific IgE to *Dermatophagoides pteronyssinus* of 44.1 kIU_A/L (Table 3), discrim-

inating children with intermittent asthma and those with more severe forms of the disease showed very good diagnostic efficiency (AUC = 0.814 for intermittent:mild, and 0.843 for intermittent:mild + moderate, respectively). The sensitivity of 75.0% implied that 75.0% of subjects with mild or moderate asthma had a concentration of specific IgE to *Dermatophagoides pteronyssinus* > 44.1 kIU_A/L. The positive predictive value suggested with 79.2% probability that a child with a concentration of specific IgE to *Dermatophagoides pteronyssinus* > 44.1 kIU_A/L would have a more severe form of asthma or would not have intermittent asthma.

Discussion

The results of the present study showed the asthmatic children with higher asthma severity to have a higher serum

Table 2. ROC analysis of total serum IgE according to asthma severity

ROC analysis	Total IgE	
	Intermittent/mild	Intermittent/ moderate + mild
AUC	0.689	0.736
Cut-off values of total IgE	156.2 kIU/L	288.0 kIU/L
Sensitivity	42.1%	67.1%
Specificity	87.7%	68.3%
Positive predictive value	80.0%	66.2%
Negative predictive value	56.4%	69.1%

AUC, area under ROC curve; sensitivity, probability that IgE concentration is higher than cut-off value when more severe asthma is present (true positive rate); specificity, probability that IgE concentration is lower than cut-off value when more severe asthma is not present (true negative rate); PPV, positive predictive value, i.e., probability that more severe asthma is present when IgE exceeds cut-off value; NPV, negative predictive value, i.e., probability that more severe asthma is not present when IgE is lower than cut-off value.

Table 3. ROC analysis of specific serum IgE to *Dermatophagoides pteronyssinus* according to asthma severity

ROC analysis	Specific IgE	
	Intermittent/mild	Intermittent/ moderate + mild
AUC	0.814	0.843
Cut-off values of specific IgE	44.1 kIU _A /L	44.1 kIU _A /L
Sensitivity	75.0%	75.0%
Specificity	76.9%	81.7%
Positive predictive value	79.2%	79.2%
Negative predictive value	72.5%	77.9%

AUC, area under ROC curve; sensitivity, probability that IgE concentration is higher than cut-off value when more severe asthma is present (true positive rate); specificity, probability that IgE concentration is lower than cut-off value when more severe asthma is not present (true negative rate); PPV, positive predictive value, i.e., probability that more severe asthma is present when IgE exceeds cut-off value; NPV, negative predictive value, i.e., probability that more severe asthma is not present when IgE is lower than cut-off value.

concentration of both total IgE and specific IgE to *Dermaphagoides pteronyssinus*, respectively.

Identification of asthma severity is a precondition for an appropriate therapeutic approach in asthma patients. As adults with higher asthma severity have been found to have suffered from a more severe form of the disease in childhood (2), determination of asthma severity followed by timely and appropriate treatment in childhood is of utmost importance to modify the course of the disease. According to Wright, the diagnosis of asthma can be confirmed at the age of 6 years in children already suffering from wheezing (21). These children usually have elevated IgE concentration and positive skin tests. In the study by Martinez et al. (22), 50 to 80% of children with asthma showed initial symptoms before age 5. In our study, the initial symptoms of wheezing manifested by the end of the fourth year of life in 50.3% and by the end of the eighth year of life in 69.4% of subjects. In the majority of subjects, the initial symptoms occurred between 3 and 4 years of age. The risk of asthma is higher in atopic subjects (23) and in those whose one or both parents suffer from asthma (24).

Wheezing in the first year of life is not a prognostic indicator for asthma; however, later occurrence of wheezing in children with atopy clearly points to the potential development of asthma (25). To predict the development of asthma in children with acceptable accuracy, Castro-Rodriguez et al. (26) have introduced an index for asthma—a simple index obtained by using clinical data. A stringent index for asthma implies either frequent wheezing during the first 3 years of life accompanied by one major risk factor (parental history of asthma or eczema) or two minor risk factors (wheezing without cold, blood eosinophilia and allergic rhinitis). On the other hand, a loose index for asthma implies the presence of any wheezing during the first 3 years of life accompanied by the same risk factors mentioned previously.

Viral infections are known to play a major role in the exacerbation of asthma in children with atopy and vice versa, the occurrence of asthma can be provoked by viral infection (27). In the present study, 92/157 (58.6%) children had viral infection of the airways in the first four years of life. Von Mutius et al. (28) describe a group of children with asthmatic symptoms caused by recurrent infections in early childhood, whereas Bueving et al. (29) point to the association between influenza and asthma exacerbation. Viral infections in early childhood, especially those caused by respiratory syncytial virus (RSV), are considered to be “asthmagenic,” suggesting that these children might be predisposed to relapsing bronchospasm and lower airway infections (30–33). Viruses appear to play a more important role than inhalation allergens in the occurrence of airway obstruction in infancy. Recurrent respiratory infections in children up to 2 years are one of the risk factors for early-onset persistent wheeze (34). Estimation of the effects of recall bias (when participants in a study are asked about their risk factors) can be made from case-control or epi-

miologic studies (35). However, some children (or their parents) may forget that they (their children) had viral infections in early childhood (36).

With time, however, while approaching school age, the role of exposure to environmental allergens in the occurrence of asthma is on an increase (37,38). According to Wilson et al. (25), the majority of small children have a mild form of asthma, whereas some authors consider that young children under age 2 are prone to the more severe form of the disease (39). The grade of acute airway obstruction is, among others, influenced by seasonal alteration in environmental allergen concentration. The highest concentration of *Dermatophagoides pteronyssinus* in house dust was recorded in August and September and lowest in November and December (40). Therefore, bronchial hyperresponsiveness increases in asthma patients sensitized to *Dermatophagoides pteronyssinus* during summer. This seasonal variation may correlate with changes in skin testing and specific IgE concentration.

Definitive grading of asthma requires repeated measurement of respiratory function in order to determine both the severity and frequency of acute attacks (1). In children, assessment of asthma severity can be done by use of a questionnaire filled out by parents (25,41), evaluating the frequency and severity of wheezing episodes as the main criterion. According to the French EGEA study (42), not all factors responsible for the prevalence of asthma have a comparable impact on asthma severity in childhood; some have major (serum concentration of total IgE) and the others minor impact (sensitization to allergens and eosinophilic granulocyte count in the blood). Determination of serum concentration of total IgE plays a role in screening of children with inherited susceptibility to an increased IgE synthesis. Patients with asthma symptoms may have higher IgE concentrations (mean: 465.0 kIU/L) than symptom-free patients (mean: 143.0 kIU/L) (43). Literature data on the correlation between IgE concentration and asthma severity are rather inconsistent. Some authors believe that the level of total IgE determines allergic disease but not its severity (44–47), whereas others report on positive correlation between total IgE (42) and higher asthma severity in children under age 16, or between specific IgE to house dust mite allergens and pulmonary function (48,49). The results of the present study also showed the children with higher asthma severity to have a higher serum concentration of total IgE than the children with lower asthma severity (Figure 1). The children with moderate permanent asthma had a statistically significantly higher serum concentration of total IgE than those with intermittent or mild permanent asthma ($p < 0.05$). The difference in IgE concentration between the children with intermittent asthma and those with mild permanent asthma did not reach statistical significance ($p > 0.05$). As the means exceeded the medians, it was concluded that the distribution of IgE concentration in asthma children was asymmetrical. An asymmetrical

distribution of IgE concentration has already been observed in both healthy children and those with elevated IgE concentration (17).

Analysis of the mean concentrations of total IgE and specific IgE to *Dermatophagoides pteronyssinus* revealed the patients with higher mean concentration of total IgE also have an increased concentration of specific IgE to *Dermatophagoides pteronyssinus*. Differences in the concentration of total IgE were statistically significant ($p < 0.001$).

Differences in patient distribution according to asthma severity and specific IgE concentration (Table 1) were statistically significant, suggesting a trend of the subjects with higher concentration of specific IgE to have a higher grade of asthma severity. The rank correlation coefficient also pointed to an association between the concentration of specific IgE and asthma severity.

Yet, the presence of specific antibodies to a particular allergen in a patient does not imply that the patient will develop clinically significant symptoms upon exposure to the allergen. Pastorello et al. (50) considered the cut-off value of specific IgE antibodies to be of major importance in discriminating symptomatic and asymptomatic subjects. According to these authors, the cut-off value varies with different allergens. The mean cut-off value was 11.7 kIU_A/L for specific IgE, irrespective of the causative allergen, 10.7 kIU_A/L for seasonal allergens, and 8.4 kIU_A/L for perennial allergens. These authors consider the concentration of specific IgE determined by the CAP method to have higher sensitivity and specificity and thus greater diagnostic value than skin tests (50).

The cut-off values (obtained by ROC analysis) for specific IgE (Table 3) may prove better diagnostic efficiency in practice than cut-off values for total IgE (Table 2), indicating that 75.0% of children with serum concentration of specific IgE to *Dermatophagoides pteronyssinus* >44.1 kIU_A/L might have mild permanent or moderate permanent asthma. The positive predictive value suggested with 79.2% probability that a child with a concentration of specific IgE to *Dermatophagoides pteronyssinus* >44.1 kIU_A/L would have a more severe form of asthma (would not have intermittent asthma). These results should be confirmed in a larger group of subjects.

Our results showed the asthmatic children with a higher serum concentration of total IgE to also have a higher serum concentration of specific IgE. In addition, children with more severe asthma had a concentration of total IgE >288.0 kIU/L and of specific IgE to *Dermatophagoides pteronyssinus* >44.1 kIU_A/L. Ultimately, such information may allow for the severity of the disease to predict in children with extremely high serum concentrations of total and specific IgE to mite allergens, thus to modify the course of the disease by a comprehensive therapeutic approach, to reduce the number of hospitalizations, and to prevent the disease persistence to adulthood.

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