CLINICAL ASPECTS

ASSESSMENT OF INFLAMMATION MARKERS IN ASTHMA

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Abstract: *Background and purpose: The airway inflammation defining asthma is regulated by a network of interacting cytokines. The functional role of each individual cytokine in the immunopathogenesis of the disease remains controversial. Allergic diseases are based on to Th2 immune responses, which are characterized by high levels of proinflammatory interleukin (IL) IL-4, and a defective interferon-gamma (IFNγ) production, the principal Th1 effector cytokine. Materials and methods: I studied serum markers of allergic inflammation in 136 pediatric patients with wheezing and especially asthma (IL-4, IFNγ, serum eosinophils and total IgE), using commercially available ELISA kits. Results: I found an imbalance between serum levels of pro and antiinflamatory cytokines: higher levels of IL-4, in contrast with a lower production of IFNγ. Conclusions: The findings are relevant; the serum level of IL-4 may be elevated in contrast with decreased level of IFNγ in asthma. Determination of serum levels of IL-4 and IFNγ and correlations between them may be helpful for understanding the immunological mechanism in asthma.*

INTRODUCTION

A great part of the researches on allergic diseases have proposed the idea that the immunopathogenesis of allergic diseases is linked to a misbalance between Th1/Th2 immune responses. Allergic diseases are based on Th2 immune responses, which are characterized by high levels of proinflammatory interleukin (IL) IL-4, with development of mucus hypersecretion and airway wall remodelling. In contrast, there is a defective interferon-gamma (IFNγ) production, the principal Th1 effector cytokine (figure no. 1).

Figure no. 1. CD4+ differentiation
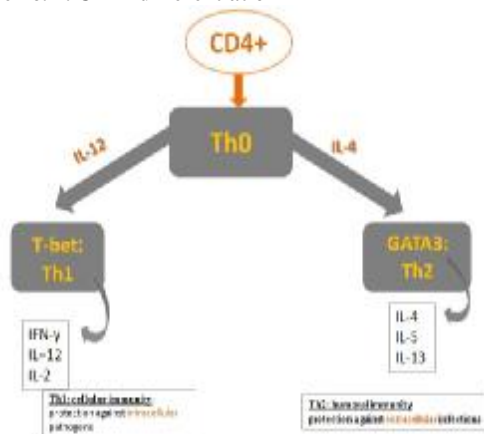
These cytokines orchestrate the recruitment and activation of different effector cells, such as eosinophils and mast cells. Interleukin-4, the main cytokine in the development of allergic inflammation, is associated with increased secretion of immunoglobulin (Ig) E by B lymphocytes, due its ability to upregulate IgE receptors on the cell surface.

Summarizing, high levels of IL-4 are essential for IgE production, responsible for the differentiation, activation and recruitment of eosinophils.

PURPOSE

The main objective of this paper was the study of serum markers of allergic inflammation in patients with recurrent wheezing and especially asthma (IL-4, IFNγ, serum eosinophils and total IgE). Subsequently to this central objective, I aimed at achieving the statistical processing of important parameters in conditioning the diagnosis of asthma.

MATERIALS AND METHODS

To achieve the proposed objectives, there was selected a group of 136 children of both genders diagnosed with occasional wheezing, recurrent and asthma in Sibiu Pediatric Hospital, Clinic of Pediatrics, between 2012-2015. Patient recruitment was conducted under the study protocol approved by the Ethics Committee for Clinical Trials, of the Clinical Hospital of Pediatrics.

Patients met the following inclusion and exclusion criteria to reduce the influences of other phenomena on markers of allergic inflammation.

Criteria for inclusion in the study: below 14 years, informed consent to be admitted in the study, wheezing or asthma in history.

Exclusion criteria of the study: parent's or tutor's refusal; congenital affections that evaluate with expiratory dyspnea (mucoviscidose, α1 antitrypsin deficiency, secretory IgA deficiency, diencephalic epilepsy, tracheobronchial dyskinesia, gastroesophageal reflux), malabsorption, immunological or oncological diseases, birth defects

In these patients, there was made a clinical and para-clinical evaluation to establish the diagnosis of wheezing or asthma. Clinical examination focused on anamnesis, identifying allergising risk factors and physical examination in order to

determine the severity of the disease. Based on detailed anamnesis, there was obtained data from previous medical history of allergic nature, the presence of a family history of respiratory allergy, presence of other manifestations of atopy (respiratory, cutaneous), onset of symptoms.

For the para-clinical evaluation, the blood samples from the asthmatic subjects were collected by veinpuncture method under appropriate conditions. Serum was acquired after coagulation of the blood for 1–2 hours at room temperature. The supernatant was centrifuged for 10 min at 2000g. The serum thus acquired was then aliquoted and stored at -80°C until analysis. The serum of the patients was analyzed by ELISA method (enzyme linked immunosorbent assay), for IgE total, IL-4 and IFNγ.

Data were loaded and processed using SPSS 18.0 statistical functions.

RESULTS AND DISCUSSIONS

Demographic data presented in the personal study showed that the age of the patients included in the study ranged from 1 to 14 years, 77.9% of patients with the age between 1 - 4 years. The results are similar to those found in the specialty literature where is reported the beginning of asthma in the age of 5 years. Despite the clinical exploration and difficulties that may be criminalized in diagnosing asthma in young children, over 80% of the asthma cases are diagnosed at a preschool age. Increased prevalence of early age was recorded in ISAAC study Phase 3, where there was observed an increased incidence of respiratory allergies in the age group 6-7 years.(1)

In the present study, the processed data showed that wheezing and asthma diagnosis was more common in male children (55.9%), the sex ratio being of 1.27: 1. Taking into account the age of the population included in the present study, the obtained results coincide with the data available in the specialty literature. The prevalence of asthma is almost two times higher in boys than in girls up to 14 years. As children mature, the gender gap is reversed in adulthood asthma prevalence is higher in women than in men. The age at which changes appear seem to be the age of 12 due to the rising incidence of respiratory allergic diseases in girls in the 2ndage decade. Several explanations have been proposed: the small diameter of airways compared with the lung volume in boys and the immunological differences between the genders could be the cause of this report.(2)

Number of eosinophils, considered as a marker of allergy, or parameter risk for developing allergic pathology, exceeded the reference limit in 41,2% of total group and were observed significantly higher values of eosinophils in children from rural areas (p = 0.036), resulting in inconsistency with literature data that consider the rural protection in the development of allergies.(3) A significantly higher average value of the percentage of eosinophils was recorded 4,38% in females, while in meales, it was 3,74% (p =0.047) values, in accordance with the literature, which considers eosinophils as a sex-dependent biomarker.(4) Depending on the diagnosis, there has been a significant difference in the percentages of eosinophils between the recurrent and occasional wheezing (4 times higher), the observation being consistent with the literature data that, beyond the controversy, consider increasing the number of eosinophils as a marker of allergy, or parameter risk for developing allergic pathology.(5)

After assessing the relationship between eosinophils and total IgE resulted that the values of the two markers were poorly correlated (r= +0.194; R^2=0.0375; p=0.042), this correlation being sometimes used in diagnosing of asthma. The two markers have registered higher values in the case of asthma, and for recurrent wheezing, values consistent with the observations in the literature, which relate to the connection of diagnostic severity, and which are influenced not only by one, but by a number of risk factors.(6)

Eosinophils number and IL-4 values, showed a moderate direct correlation (r= +0.670; R^2=0.4485; p=0.001) having increased values in asthmatic children. The data are consistent with the findings of other researchers.(7)

In contrast with the literature data, the values of IFNγ were weekly correlated indirectly with the values of eosinophils (r = -0.226; R2=0.0513; p=0.048). Atopic asthma is characterized in addition by a correlation between the intensity of eosinophilic inflammation and increased of IL-4 concentration with a decrease in parallel, the production of IFNγ.(8)

Regarding total IgE level in this personal study, a 36% percent of patients recorded values above the maximum limit of reference. Were noted significant medium values, increased in the age group over 4 years (70.46 *vs.* 48,97 kU/L; p=0,001) and in children from urban areas (59.57 *vs.* 28,81 kU/L; p=0,001).

The results did not show any statistically significant correlation with the patients' gender, family history, or type of food in the first 6 months of life, similar to the opinions of the specialty literature.(9) Total IgE are increased in patients with respiratory allergies, but can be influenced by age, genetic predisposition, ethnicity, immunity and some stages of the disease. Thus, measurement of total IgE levels may have limited value as a screening test for allergic diseases.(10)

We evaluated the relationship between total IgE levels and IL-4, which correlates moderately positive, but statistically significant (r= +0.415; R^2=0.1725; p=0.025). The conclusions are similar to those obtained in previous studies in the literature, which reported increased levels of both IgE and IL-4 in patients with asthma.(6)

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From the total of patients, 78.4% had IL-4 statically significantly increased. (p=0,004) mean value the greater being detected in patients with asthma (16.85pg/ml). In the studied cases are noted medium values that are slightly increased at the age group over 5 years (18.57 *vs.* 16.12 pg/ml), and on genders the medium values of IL-4 were approximately equal (16.97 *vs.* 16.66 pg/ml).

Figure no. 2. IL-4 levels
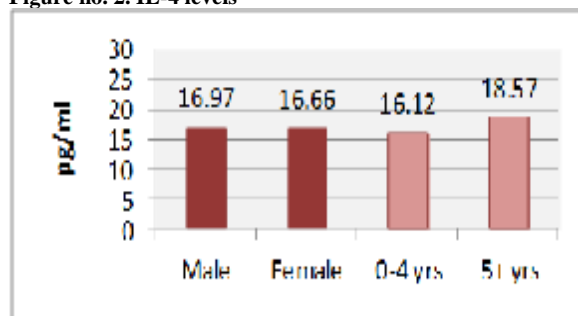
Individual values of IL-4 have underlined direct moderate correlation correlations with eosinophils levels (r= +0.670; R^2=0.4485; p=0.001).

The direct correlation between the values of IL-4 and eosinophils values although moderated is statistically significant, relation that corresponds to general observations in

the literature.(12)

Analysing the relationship between the levels of total IgE and IL-4, Pearson correlation shows a moderate direct correlation (r= +0.415; R^2=0.1725; p=0.025), statistically significant, correlation that is in accordance with data of specialized literature.(13)

Figure no. 3. IFNγ levels
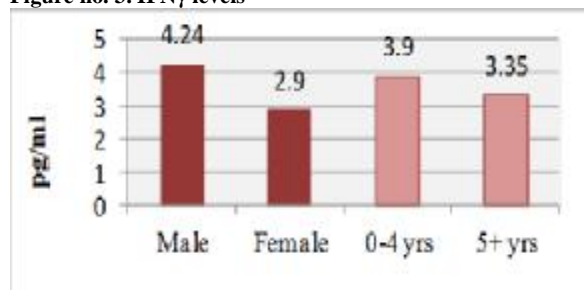
For the individual values of IFNγ, there were observed slightly lower average values in girls (2.90 *vs.* 4.24 pg/ml) and in the group below 5 years old (3.35 *vs.* 3,90 pg/ml).

Analyzing the relation between IFNγ and total IgE, Pearson's correlation shows a very weak indirect correlation (r= -0.036; R^2=0.0013; p=0.673), which is statistically insignificant.

Table no. 1. IL-4 and IFNγ correlations

Cytokines	Occasional wheezing		Recurrent wheezing		Asthma	
	mean	SD	mean	SD	mean	SD
IL-4	6,77	10,09	9,97	9,13	13,54	10,53
IFNγ	2,81	1,55	3,19	2,75	5,15	2,33

In the personal study, serum levels of IL-4 and IFNγ were above the detection limit in all diagnostic subgroups. The mean values of IL-4 were significantly increased in asthma group, while in subgroups with wheezing diagnosis, they were lower. In contrast, the levels of IFNγ were low in patients diagnosed with asthma. The two parameters were indirectly correlated, the results being consistent with the observations from other studies.(14,15)

CONCLUSIONS

The serum level of IL-4 may be elevated in contrast with decreased level of IFNγ in asthma. There is a correlation between increased levels of total IgE, and number of eosinophils on asthmatics, which may be used in clinical practice.

Determination of serum levels of IL-4 and IFNγ and correlations between inflammation markers, may be helpful for understanding the immunological mechanism in asthma.

Understanding early-life immune mechanisms responsible for allergic diseases, the cytokines role in balance the Th1 and Th2 immune response, continues to be a generous area of research.

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