Differentiation of IgE-dependent and IgE-independent reactions in children with bronchial asthma on the basis of TOP CAST Paediatric Allergen Mix test

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Abstract

Purpose: TOP CAST Paediatric Allergen Mix test is a new cellular *in vitro* test based on evaluation of leukotrienes synthesised by basophils under the influence of specific allergens. The aim of the study was evaluation of applicability of this test as screening examination in diagnosis of atopic asthma in children.

Material and methods: The study was carried out on a group of 30 children (56.7% boys and 43.3% girls) aged 6-15 yrs (mean age 8 years and 9 months, SD=2.1) with diagnosed bronchial asthma. In children qualified for the study clinical symptoms, subject examination as well as functional examination of the respiratory system (obturation with positive reversibility test) confirmed the disease. All the children had skin prick tests performed with the most popular aero- and troph-allergens, which results were expressed (+) according to the Skandinavian scale. In 15 cases asthma had atopic origin: in 11 children – mites were responsible for the contraction of bronchi, in 3 cases – tree-pollens allergens and in 1 case – grass pollens. In 15 next cases non-atopic asthma was diagnosed. The control group consisted of 10 children without clinical manifestations of asthma and negative results of the above tests. Test TOP CAST Paediatric Allergen Mix with mixture of 21 inhalatory and food allergens was performed according to the producer's procedure.

Results: Statistically significant differences of the values of released leukotrienes were noted at allergen concentration of both 100 ng/ml and 10 ng/ml in children with diagnosed atopic asthma compared to those with non-atopic asthma and control group. The sensitivity of TOP CAST Paediatric Allergen Mix test was 80% at both allergen concentrations while the specificity was higher (90%) at the lower concentration. There was also correlation between the number of released leukotriens and IgEc in the examined group of children, however, no statistically significant differences were observed between the concentration of the released leukotrienes and the size of the wheal and the number of positive skin prick tests.

Conclusions:

1. TOP CAST Paediatric Allergen Mix test is a good screening method in differentiation of atopic and non-atopic background of bronchial asthma in children.
2. At the present evaluation stage of this test, it may be applied as complementation of routine tests in allergological practice.

Key words: TOP CAST, leukotrienes, atopic asthma in children.

Introduction

Bronchial asthma is one of the most frequent chronic ailments of the respiratory system in children, which constitutes a serious problem of public health.

Diagnosis of bronchial asthma is based on history, recognition of typical clinical manifestations and additional tests. In most cases functional examination of respiratory system, skin prick tests and total IgE and allergen-specific IgE determination are sufficient for the diagnosis.

However, in some children a complex diagnosis is difficult or impossible (due to age, skin alterations, application of antihistamine drugs) or the results of widely used tests are equivocal. Regarding these, search for new methods applicable widely in diagnosis of allergic ailments, including atopic asthma present in most cases of asthma (80-90%) in population at developmental age, still remains an open issue.

CAST-ELISA (Cellular Allergen Stimulation Test) Method

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is based on measurement of cysteinyle leukotrienes generated by peripheral blood leucocytes under the influence of specific allergens or other factors (drugs, lipopolysaccharides, insect venom, food) [1,2]. In Poland this method was applied in adult patients suspected of allergy to mites, plant pollens, venoms of insects or drugs and in order to monitor specific immunotherapy [3-13]. Our own experience confirms usefulness of this test in diagnosis of asthma stimulated by allergens of home dust mites and pollens in children [14-16]. Therefore we carried on with the work to evaluate applicability of the new TOP CAST Paediatric Allergen Mix test, consisting of 21 inhalatory and food allergens, as screening examination in differentiation of the background of atopic bronchial asthma in children. The possibility of performing a screening examination of a small amount of blood (2 ml) detecting sensitivity to common inhalatory and food allergens would make a valuable diagnostic method in exogenous asthma in children.

The aim of the study was evaluation diagnostic applicability of TOP CAST Paediatric Allergen Mix test in differentiation of IgE-dependant and IgE-independent bronchospastic reactions in children.

Material and methods

The study was carried out on 30 children (17 boys – 56.7% and 13 girls – 43.3%) between 6-15 yrs (mean age 8 years and 9 months, SD=2.1) with diagnosed bronchial asthma. The duration of the disease was 2-8 years (mean 4 years). Diagnosis of bronchial asthma was based on data obtained from: history (characteristic features of the ailment), results of functional examination of the lungs during acute phase of the disease (obturation of the bronchial tree) confirmed by reversibility test (ΔFEV1>15%). In all patients skin prick tests were performed with inhalatory allergens (mites, grass, tree, weed pollens, cat and dog fur, mildew) and food allergens (milk, egg, hen meat, fish, orange, nuts, flour, soy). Skin tests with aero- and troph-allergens were performed with the prick method with Allergopharma kit. Skin reaction was compared to the diameter of the histamine wheal (the result was expressed in Scandinavian scale from 0 to 4+). For the study we qualified patients with skin reaction at least at 3+ or 4+.

In 15 cases (12 boys and 3 girls, mean age 7 years and 8 months, SD=2.5) asthma had atopic origin: in 11 cases the factors triggering asthma attack were mites, in 3 – tree pollens and in 1 – grass pollens. In 15 next cases (5 boys and 10 girls, mean age 7 years and 7 months, SD=1.7) non-atopic asthma was diagnosed (positive reversibility test, negative skin tests).

In all sick children any respiratory tract infections, inborn malformations of the bronchial tree and lung tissue, heart defects, gastroesophageal reflux or immunological insufficiencies were excluded.

The control group consisted of 10 healthy children (4 boys and 6 girls) aged between 6-17 yrs (mean age 10 yrs, SD=3.8) with negative history of allergic diseases or subject examination. Functional examination of the lungs didn't reveal any disorders of bronchi patency, and the results of skin tests were negative with inhalatory or food allergens.

Before CAST-ELISA Paediatric Allergen Mix procedure was applied, for a least 14 days the patients received no medical treatment nor any allergy symptoms were observed. After physical examination excluding any respiratory tract infections, venous blood was taken in the amount of 3 ml per 0.2 mmol of disodium versenate in volumetric proportion 20:1. Then it was mixed with dextran from a Buhlmann Laboratories A.G. kit in proportion 4:1 and incubated in room temperature for 90 minutes. After that the upper phase was collected and centrifuged for 15 minutes with 130 g. The supernatant was drained off and the sediment was mixed with stimulation buffer. The suspension was placed in test tubes, 200 µl in each and treated with 50 µl of specific inhalatory and food allergens in concentration of 100 and 10 ng/ml. To the test tubes labelled as spontaneous generation of leukotrienes we added 50 µl of stimulative buffer. The samples were incubated in 37°C for 40 minutes and centrifuged for 3 minutes at force of 1000 g. The supernatants collected from each test tube were frozen for 14 days. Then the wells coated with leukotriene antibodies were supplemented with 100 µl portions of: non-specific bond standard, zero standard, standard leukotriene solutions in 4 concentrations and supernatants after cells stimulation. To all the wells we also added 50 µl solution of leukotriene conjugated with alkaline phosphatase and 50 µl of leukotriene antibodies solution. The plate was incubated for 24 hours at 4°C, and then after emptying all wells and double-washing, 100 µl of substrate solution was added and incubated for 30 minutes at 20°C. Then 100 µl of inhibitor solution was applied, mixed and the absorbency was measured at 405 nm. Leukotriene concentration was recorded in relation to the standard curve.

Statistical analysis

The obtained results were analysed statistically – arithmetical mean and standard deviation were calculated for measurable traits and for qualitative traits – their quantitative-percentage distribution.

For the measurable traits in conformity with normal distribution, evaluated with Kolomogorov's conformity test, for comparison between the groups unifactor variation analysis was applied and for traits inconsistent with this distribution we applied respectively Kruskal-Wallis' test for many groups. In our calculation $p<0.05$ was assumed statistically significant.

The calculations were made with the statistical software SPSS 8.0 PL and Statistica 6.1 pl. Sensitivity and specificity were calculated and ROC curve was drawn.

Results

In the group of 40 examined children – 30 with diagnosed bronchial asthma and 10 without any symptoms (control) TOP CAST Paediatric Allergen Mix test was performed according to the manufacturer's instructions.

The concentration of leukotrienes (P) released under the influence of the mixture of 21 allergens (inhalatory and food) used in dilution 100 ng/ml and 10 ng/ml was considerably different in the group of children with diagnosed atopic asthma (1) than in the group of patients with non-atopic asthma (2)

Figure 1. **Leukotrienes values (P1) generated under the influence of allergens (concentration 100 ng/ml) in a group of children with atopic asthma (1), non-atopic asthma (2) and control group (3)**

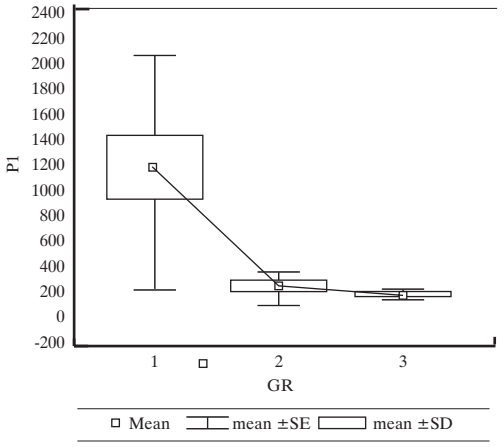


Figure 2. **Leukotrienes values (P1) generated under the influence of allergens (concentration 10 ng/ml) in a group of children with atopic asthma (1), non-atopic asthma (2) and control group (3)**

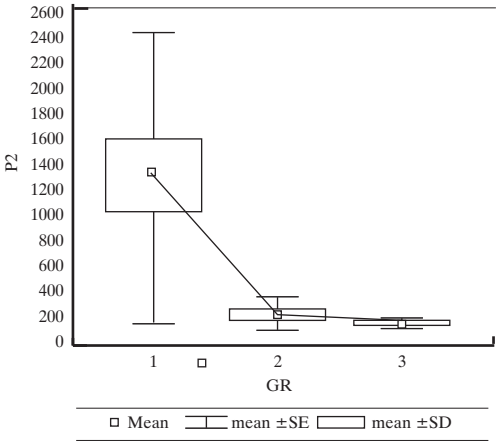


Figure 3. **Sensitivity and specificity of TOP CAST at allergen concentration 100 ng/ml**

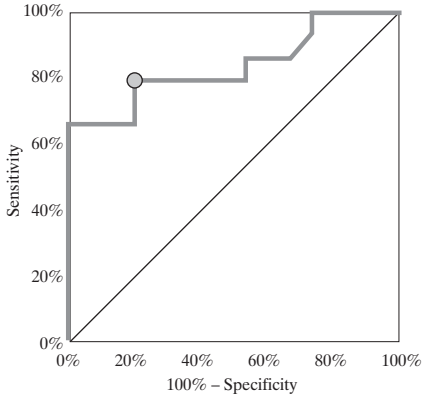


Figure 4. **Sensitivity and specificity of TOP CAST at allergen concentration 10 ng/ml**

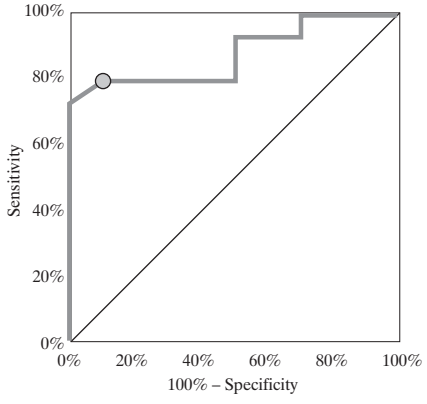
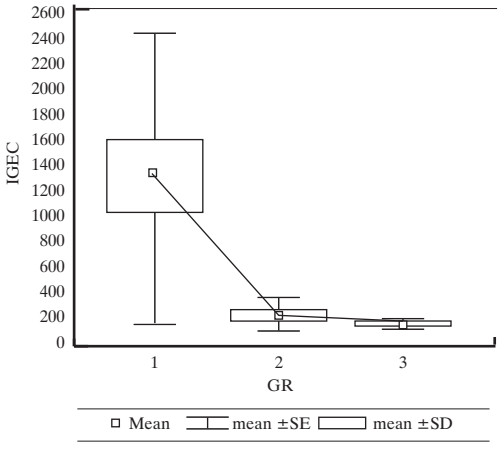


Figure 5. **Total IgE concetration in the group of children with atopic asthma (1), non-atopic asthma (2), and control group (3)**



or control group (3) (*Fig. 1, 2*). Mean concentration of leukotrienes P1 and P2 (at dilution of allergens 100 ng/ml and 10 ng/ml) were respectively (1)-1174 pg/ml (SD=973.8) and 1308 pg/ml (SD=1162), (2)-152 pg/ml (SD=81.5) and 179 pg/ml (SD=83.8), and in control group (3) – 117 pg/ml (SD=24.9) and 144.5 pg/ml (SD=44.8).

There was a statistically significant difference in the value of P1 (0.0014) and P2 (0.0021) between 1/2 and 1/3 group, but no statistically significant difference between 2 and 3 group (non-atopic asthmatics and controls).

Sensitivity and specificity of TOP CAST Paediatric Allergen Mix test for the value of P1 were 80% each, while for P2 – 80% and 90% respectively (*Fig 3*, *4*).

Total IgE concentration was different in group 1 (mean 1123 IU/ml, SD=1462) and 2 (78.8 IU/ml, SD=88.5) and group 3 (15.1 IU/ml, SD=10.6) ($p<0.05$) (*Fig. 5*).

Comparison of skin prick test (wheal size and number of positive results in skin tests) and leukotriens concentration didn't reveal any statistically significant differences ($p=0.831$ and $p=0.774$).

Discussion

For over 30 years the diagnosis of atopic diseases is based on skin tests and evaluation of allergen-specific E class antibodies. Other methods, like tests evaluating basophils reactivity (histamine release test) are not widely used in allergological examinations. In the 1990s, Buhlmann laboratory elaborated a new method of cell tests (CAST ELISA), which is based on measurement of cysteinyle leukotriens generated by bazofiles stimulated by allergens at the presence of IL-3 [1,2].

So far this test has been used in diagnosis of allergic diseases (hypersensitivity to pollens, mites, insect venoms, drugs, polysaccharides) and to monitor immunotherapy in adult patients [3-13]. Our own experience proved applicability of this examination in diagnosis of mite asthma [14] and allergy to tree and grass pollens [15-16] in puerile population.

One application of this method is TOP CAST Paediatric Allergen Mix test, evaluating atopic status of patients. In this test peripheral blood leukocytes are stimulated with a mixture of 21 common inhalatory (grass mix, wheat, birch, hazel, mugwort, ribwort, *Alternaria sp.*, home dust and flour mites, dog, cat) and food allergens (egg, milk, fish, nuts, soy). In literature data regarding applicability of this test is incomplete and only few authors [17-23] have attempted to evaluate usefulness of this method in adult and juvenile patients.

The aim of our study was to evaluate the applicability of this test as screening examination in the direction of atopy in juvenile population with clinical manifestations of bronchial asthma.

For the study, children with diagnosed bronchial asthma were qualified on the basis of history (character of symptoms, recurrence and seasonal incidence), physical examination (typical asthmatic symptoms), functional examination of the lungs (bronchial obturation features, positive reversibility test). All the patients had skin prick tests performed with the most popular aero- and troph-allergens. In the group of children with positive skin tests, allergen-specific IgE was determined in the direction of the allergen triggering disease symptoms. TOP CAST Paediatric Allergen Mix test was performed with two allergen concentrations – 100 ng/ml and 10 ng/ml, according to recommendations of other authors [3,9,10]. In all patients with atopic asthma symptoms [1] we observed wide range of generated leukotrienes both at low and high allergen concentration, mean 1 174 and 1 308 pg/ml respectively and these values were significantly higher compared to the group of children with non-atopic asthma (2): 152 and 159 pg/ml and control group (3) where leukotrienes release under the influence of these allergens at both concentrations was below the test sensitivity (117 and 145.5 pg/ml).

Statistically significant difference of the value of P1 and P2 was found between these groups ($p<0.0014$ and $p<0.0021$ respectively).

Most authors have a positive opinion of CAST ELISA test in diagnosis of allergic ailments in case of single allergens or groups of allergens [1-3,7-8,11-13]. However, very scarce are studies regarding application of this test as a screening examination (mixture of 21 inhalatory and food allergens) in case of suspected atopic diseases, including exogenous asthma, especially in children [17-23].

In our own study sensitivity of TOP CAST Paediatric Allergen Mix test was 80% at both allergen concentrations (100 and 10 ng/ml), but specificity was better at lower concentration – 90%. In available literature the sensitivity of this test was determined at 86-100%, and the specificity at 80-90%, which makes the value of this test comparable to other multitests, such as, for example, Phadiatop [17-23].

We found correlation between total IgE concentration and quantity of released leukotrienes, however, there was no significant difference between the size of the wheal or number of positive results in skin tests and level of leukotrienes. This fact may be explained by qualitative differences of the allergens composition that was used in reagents for skin tests and in TOP CAST test and specificity and sensitivity of both methods.

To sum up, TOP CAST Paediatric Allergen Mix test could be used as a screening examination of the first choice in diagnosis of atopic ailments, especially in small children. This examination could also make a valuable diagnostic tool, e.g in cases when skin tests are impossible to perform (age restrictions, applied treatment, atopic dermatitis) which frequent in the population of developmental age, or where there are discrepancies between the history, skin test results and *in vitro* examinations.

Conclusions

1. TOP CAST Paediatric Allergen Mix test is a good screening method in differentiation of atopic and non-atopic background of bronchial asthma in children.
2. At the current stage of this test evaluation it is used as a complementary method of routine examinations in allergological practice.

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