

STRESZCZENIE W JĘZYKU ANGIELSKIM

ANALYSIS OF SPECIFIC IgE ANTIBODY OCCURRENCE ASSESSED BY PRECISION MOLECULAR DIAGNOSTIC TECHNIQUES IN CHILDREN SUSPECTED OF ALLERGY AND CHILDREN WITH DIAGNOSED CELIAC DISEASE

Immunoglobulin E dependent allergy (A-IgE) is associated with overproduction of IgE antibodies against allergenic substances. For serological diagnosis of sensitisation/A-IgE, specific IgE (sIgE) tests against allergen extracts have been used for many years. A novel approach in sensitisation/A-IgE diagnosis, utilized in this doctoral dissertation, is the evaluation of sIgE occurrence against allergenic molecules based on molecular diagnostics (ALEX® test). Until now, a comprehensive analysis of sIgE allergy profile against allergenic molecules in the pediatric population in Poland has not been conducted, hence this research area became the main topic of the doctoral thesis. Additionally, the sIgE allergy profile was evaluated in a group of children with celiac disease (CD) - an autoimmune disease, i.e., a disease with a pathomechanism opposite to A-IgE.

Objectives: The main objectives were to evaluate the allergy profile based on the occurrence of sIgE against food allergens in the Polish pediatric population suspected of allergy (depending on age), and in the group of children with newly diagnosed CD.

Methods: The assessment of sIgE occurrence was conducted using the third-generation multiplex test ALEX®, which allows for the evaluation of these sIgE antibodies against 295 allergens, including 117 extracts and 178 allergenic molecules from various allergen sources (inhaled and food).

Results: In the first part of the doctoral dissertation, a literature review was conducted regarding changes in allergy profile associated with the child's age in the allergic march process (Knyziak-Mędrzycka et al., *Nutrients* 2023), as well as regarding the coexistence of CD and sensitisation/A-IgE (Majsiak et al., *Nutrients* 2023). In the second part, analyses published in two original articles (Knyziak-Mędrzycka et al., *Int. J. Mol. Sci.* 2024 and Knyziak-Mędrzycka et al. *J. Clin. Med.* 2024) and a case report (Knyziak-Mędrzycka et al., *J. Asthma Allergy* 2022) were conducted.

In the work "*The Sensitization Profile for Selected Food Allergens in Polish Children Assessed with the Use of a Precision Allergy Molecular Diagnostic Technique*" (Knyziak-Mędrzycka et al., *Int. J. Mol. Sci.* 2024), a comprehensive assessment of the allergy profile based on sIgE against food allergens was performed, with particular emphasis on extracts and molecules of the "big eight" food allergens (cow's milk, hen's egg, wheat, soy, fish and seafood,

tree nuts, and peanut). The analysis utilized results obtained from selected laboratories in selected regions in Poland, which performed the ALEX® test from 2019 to 2022. Results were obtained from 3715 children. The mean age of the participants was 7.0 years. Children's results were analyzed in the following age groups: <12 months (3.63%), 1-5 years (39.54%), 6-13 years (46.32%), 14-18 years (10.0%). sIgE was most frequently observed against extracts of peanut (29.20%), hazelnut (28.20%), and apple (23.60%), and against molecules for the PR10 subfamily (rCor a 1.0401 (23.77%), rMal d 1 (22.37%), and rAra h 8 (16.93%)). sIgE was least frequently noted for strawberry extracts (0.40%), oregano (0.30%), and thistle (0.16%), and for allergenic molecules against nMal d 2 (0.27%) (thaumatin-like protein family, TLP), rAni s 1 (0.30%) (Kunitz-type serine protease inhibitor), and rChe a 1 (both 0.43%) (Ole e 1 protein family). A statistically significant decrease ($p < 0.05$) in allergy frequency with age was observed for allergenic molecules of reserve proteins: peanut (nAra h 1, rAra h 2, nAra h 3), soy (nGly m 6), tree nuts (nCor a 9, nJug r 4). A statistically significant decrease in allergy frequency with age was also observed for cow's milk (nBos d 4, nBos d 5, nBos d 6, nBos d 8) and hen's egg (nGal d 1, nGal d 2). A statistically significant increase in allergy frequency was observed for PR10 subfamily proteins (rAra h 8, rCor a 1.0401). The observed change in allergy profile depending on age was also demonstrated in the case description of a 9-month-old patient, initially diagnosed with food allergy, manifesting, among other things, anaphylactic shock, and within 2 years of observation, asthma development associated with inhalant allergy was noted, which was also visible in his molecular profile (Knyziak-Mędrzycka et al., *J Asthma Allergy* 2022).

In the work *"Sensitization profile in children with celiac disease assessed with the use of multiplex molecular diagnostic technique"* (Knyziak-Mędrzycka et al. *J. Clin. Med.* 2024), the focus was on assessing the occurrence of sIgE in a group of CD patients. The study included 108 children (63% girls and 37% boys, mean age 9.4 years) with newly diagnosed CD. Using multiplex tests, sIgE occurrence against extracts and/or allergenic molecules was found in almost half of the children (53/108; 49.1%). Most children (22/53, 41.5%) were sensitized to both inhalant and food allergens. Among the most common 10 allergenic substances sensitizing children with CD, there were 7 inhalant allergens and 2 food allergens. The three most common inhalant allergens (timothy grass pollen (26.9%), rye grass (24.1%), birch (18.5%)) were allergenic molecules Phl p 1, Lol p 1 (both from the B-expansin family), and Bet v 1 (PR10 subfamily). The most common food allergens (hazelnut, apple, and peanut) were molecules (Cor a 1, Mal d 1, and Ara h 8) belonging to the PR10 subfamily. No sensitization to gluten-containing grain allergens was found in the tested patients. Correlation analysis using

Spearman's rank method among sensitized patients showed a significant positive relationship ($r=0.31$) between the age of the patients and the number of positive sIgE (≥ 0.3 kUA/L) for inhalant allergen molecules ($p=0.045$). Clinical analysis of selected CD patients with sensitization showed that allergic rhinitis and bronchial asthma were most common in this group of patients.

Summary and Conclusions: The conducted studies showed that:

1. The allergy profile based on sIgE against extracts and allergenic molecules, with particular emphasis on the big eight food allergens, differed in Polish children compared to other countries.
2. In Polish children suspected of allergy based on sIgE occurrence, the most common sensitization was to peanut extract, hazelnut, and apple, and against PR10 subfamily molecules (rCor a 1.0401, rMal d 1, and rAra h 8), globulin 7/8S (nAra h 1) was ranked 4th.
3. The allergy profile against food allergens, analyzed based on sIgE occurrence, changed with age. A decrease in food allergy and a rising trend in inhalant allergy sensitization with age were observed. In early childhood, sensitization to reserve proteins dominated, while in later years, sensitization to PR10 subfamily proteins with dominance of sensitization to Bet v 1 from the PR10 birch pollen subfamily prevailed.
4. Children with CD were co-sensitized to both inhalant and food allergens.
5. Almost half of the children with newly diagnosed CD were sensitized to at least one allergen, with no sensitization to gluten-containing grain allergens found among them.
6. The most common food allergens sensitizing children with CD were molecules from the PR10 subfamily (Cor a 1, Mal d 1, and Ara h 8), which may result from cross-reactions to birch, whose primary allergy marker Bet v 1 (PR10 subfamily) was among the three most common inhalant allergens, after timothy grass and rye grass (Phl p 1, Lol p 1).

Based on the conducted research and analysis of the results, the following conclusions were drawn:

1. Comprehensive molecular analysis of allergies in children suspected of allergy residing in all regions of Poland showed that the allergy profile for food allergens in

this group differs from profiles observed in other countries; the obtained research results may serve as a basis for developing allergy prevention and treatment strategies both locally in our country and on a global scale.

2. Molecular analysis of sensitization in a selected group of children with CD allows us to conclude that despite the different immunological mechanisms, A-IgE and CD coexist, and there is a need for A-IgE diagnostics in CD patients, not only in terms of food allergy but also inhalant allergy.

Key words: allergy; IgE-mediated allergy; sIgE; anaphylaxis; atopic/ allergic march; PAMD@; precision allergy molecular diagnostic; multiplex molecular tests; celiac disease