Instytut „Pomnik - Centrum Zdrowia Dziecka”

Lekarz Izabela Knyziak-Mędrzycka

ANALIZA WYSTĘPOWANIA SWOISTYCH PRZECIWCIAŁ IgE OCENIANYCH TECHNIKĄ PRECYZYJNEJ DIAGNOSTYKI MOLEKULARNEJ U DZIECI Z PODEJRZENIEM ALERGII ORAZ U DZIECI Z ROZPOZNANĄ CELIAKIĄ

Rozprawa na stopień doktora nauk medycznych

Promotor: Prof. dr hab. n. med. Bożena Cukrowska
Promotor pomocniczy: dr hab. n. med. Emilia Majsiak

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„Co my wiemy, to tylko kropelka. Czego nie wiemy, to cały ocean.”

Isaac Newton

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Prace o charakterze artykułów poglądowych

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2. Majsiak E, Choina M, Knyziak-Mędrzycka I, Bierła JB, Janeczek K, Wykrota J, Cukrowska B. IgE-Dependent Allergy in Patients with Celiac Disease: A Systematic Review. *Nutrients.* 2023, 16;15(4): 995. doi: 10.3390/nu15040995.
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Prace o charakterze artykułów oryginalnych

3. Knyziak-Mędrzycka I, Majsiak E, Gromek W, Kozłowska D, Swadźba J, Bierła BJ, Kurzawa R, Cukrowska B. The Sensitization Profile for Selected Food Allergens in Polish Children Assessed with the Use of a Precision Allergy Molecular Diagnostic Technique. *Int. J. Mol. Sci.* 2024, 25(2): 825. doi. 10.3390/ijms25020825.
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Prace o charakterze przypadku klinicznego

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SPIS TREŚCI

WYKAZ ZASTOSOWANYCH SKRÓTÓW

A-IgE- alergia Ig-zależna

AIT- Allergen Immunotherapy – immunoterapia alergenowa

anty-DPG IgG- przeciwciała przeciw deaminowanym peptydom gliadyny

CD- celiakia

EMA-IgA- przeciwciała przeciwendomyzialne w klasie A

ESPGHAN- ang. European Society for Paediatric Gastroenterology Hepatology and Nutrition

IgA- immunoglobulina A

IgE- immunoglobulina E

IgG- immunoglobulina G

IgM- immunoglobulina M

IL- 4- interleukina 4

IL -5- interleukina 5

IL-13- interleukina 13

PAMD@- (od ang. słów precision allergy molecular diagnostic applications) - zastosowanie precyzyjnej diagnostyki molekularnej alergii

PTS- punktowe testy skórne

sIgE- swoiste IgE

Th1- limfocyty- T pomocnicze typu 1

Th2- limfocyty- T pomocnicze typu 2

tIgE- całkowite IgE

Treg- limfocyty T regulatorowe

tTG IgA- przeciwciała przeciw transglutaminazie tkankowej w klasie A

Skróty molekuł alergenowych

bloker anty-CCD- (ang. cross-reactive carbohydrate determinants) - determinanty węglowodanowe

Lol p 1 – (*Lolium pratense)* – molekuła życicy

nJug r 1, nJug r 2, nJug r 4, nJug r 6- (*Juglans regia)* – molekuły orzecha włoskiego

n- molekuła naturalna

nAra h 1, rAra h 2, nAra h 3, rAra h 6, nAra h 8, rAra h 9, rAra h 15- (*Arachis hypogaea)* – molekuły orzecha arachidowego

nBos d 4, nBos d 5, nBos d 8- (*Bos domesticus)*- molekuły mleka krowiego

nBos d 6- (*Bos domesticus)*- molekuły mięsa krowiego

nGal d 1, nGal d 2, nGal d 3, nGal d 4- (*Gallus domesticus)* – molekuły jaja kurzego

nGly m 6, rGly m 4- (*Glycine max)*- molekuły soi

nPen m 1, rPen m 2, rPen m 3, rPen m 4- (*Penaeus monodon)*- molekuły krewetki północnej

nTri a A_TI , rTri a 14 , rTri a 19- (*Triticum aestivum)*- molekuły pszenicy zwyczajnej

Ole e 1- (*Olea europaea)*- molekuły oliwki euopejskiej

Phl p 1- (*Phleum pratense)*- molekuły tymotki łąkowej

PR-10- (ang. pathogenesis releted proteins) - białka związane z patogenezą, np. rCor a 1.0401 (*Corylus avellana*- orzech laskowy) , rMal d 1 (*Malus domestica*- jabłko), rAra h 8 (*Arachis hypogaea*- orzech ziemny)

r- molekuła rekombinowana

rAna o 3- (*Anacardium occidetale)*- molekuła orzecha nerkowca

rAni s 1 – (*Anisakis simplex)*- molekuła nicienia

rApi g 1- (*Apium graveolens)*- molekuła selera

rChe a 1- (*Chenopodium album)*- molekuła komosy białej

rCor a 1.0401, nCor a 9, nCor a 14- (*Corylus avellana)*- molekuły orzecha laskowego

rCra c 6- (*Crangon crangon)* - molekuła garneli pospolitej

rDau c 1- (*Daucus carota)* - molekuła marchwi

rMal d 1, nMal d 2- (*Malus domestica)* - molekuły jabłka

rSco s 1 – (*Scomber scombrus)* - molekuła makreli atlantyckiej

STRESZCZENIE W JĘZYKU POLSKIM

ANALIZA WYSTĘPOWANIA SWOISTYCH PRZECIWCIAŁ IgE OCENIANYCH TECHNIKĄ PRECYZYJNEJ DIAGNOSTYKI MOLEKULARNEJ U DZIECI Z PODEJRZENIEM ALERGII ORAZ U DZIECI Z ROZPOZNANĄ CELIAKIĄ

Alergia zależna od immunoglobulin E (A-IgE) związana jest z produkcją przeciwciał w klasie IgE wobec uczulających substancji. W diagnostyce serologicznej A-IgE od wielu lat stosuje się badania swoistych IgE (sIgE) wobec ekstraktów alergenowych. Nowatorskim podejściem w diagnostyce A-IgE, które zostało wykorzystane w pracy doktorskiej, jest ocena występowania sIgE wobec molekuł alergenowych w oparciu o diagnostykę molekularną (test ALEX®). Do tej pory nie przeprowadzono kompleksowej analizy profilu uczulenia sIgE wobec molekuł alergenowych u dzieci w Polsce, dlatego ten obszar badań stał się głównym tematem pracy doktorskiej. Dodatkowo oceniono profil uczulenia sIgE w grupie dzieci z celiakią (CD) – chorobą o podłożu autoimmunizacyjnym, tj. chorobą o przeciwstawnym do A-IgE patomechanizmie.

Cel: Głównym celem pracy doktorskiej była ocena profilu uczulenia na podstawie występowania sIgE wobec alergenów pokarmowych u polskich dzieci z podejrzeniem alergii w zależności od wieku oraz w grupie dzieci z nowo rozpoznaną CD. Metody

Metody: Ocena występowania sIgE była przeprowadzona za pomocą multipleksowego testu III generacji ALEX®, który umożliwia ocenę tych przeciwciał wobec niemal 300 alergenów, w tym wobec ekstraktów i molekuł alergenowych pochodzących z różnych źródeł alergenowych (wziewnych i pokarmowych).

Wyniki: W pierwszej części rozprawy doktorskiej dokonano przeglądu literatury w zakresie zmian w profilu uczulenia związanych z wiekiem dziecka w procesie marszu alergicznego (*Knyziak-Mędrzycka i wsp., Nutrients 2023)* oraz w zakresie zagadnienia współwystępowania CD i uczulenia lub A-IgE *(Majsiak i wsp. Nutrients 2023)*. W drugiej części rozprawy przeprowadzono analizy opublikowane w 2 artykułach oryginalnych *(Knyziak-Mędrzycka i wsp. Int. J. Mol. Sci. 2024* oraz *Knyziak-Mędrzycka i wsp.*) oraz opisie przypadku *(Knyziak-Mędrzycka i wsp., J Asthma Allergy 2022)*.

W pracy *„The Sensitization Profile for Selected Food Allergens in Polish Children Assessed with the Use of a Precision Allergy Molecular Diagnostic Technique" (Knyziak-Mędrzycka i wsp., Int. J. Mol. Sci. 2024)* wykonano kompleksową ocenę profilu uczulenia w oparciu o sIgE wobec alergenów pokarmowych ze szczególnym uwzględnieniem ekstraktów i molekuł alergenów pokarmowych "wielkiej ósemki" (mleko krowie, jajo kurze, pszenica, soja, ryby i owoce morza, orzechy drzewne oraz orzech arachidowy). Do analizy wykorzystano

wyniki pobrane z wybranych laboratoriów z wybranych regionów w Polsce, które od 2019 do 2022 roku wykonywały test ALEX®. Uzyskano wyniki od 3715 dzieci. Średnia wieku badanych wynosiła 7.0 lat. Wyniki dzieci analizowano w następujących przedziałach wiekowych: <12m-cy (3.63%), 1-5 lat (39.54%), 6-13 lat (46.32%), 14-18 lat (10.0%). Najczęściej sIgE występowały wobec ekstraktów alergenów pokarmowych orzecha arachidowego (29.20%), orzecha laskowego (28.20%) oraz jabłka (23.60%) oraz wobec molekuł dla podrodziny PR10 (rCor a 1.0401 (23.77%), rMal d 1 (22.37%) i rAra h 8 (16.93%). Najrzadziej sIgE odnotowano dla ekstraktów truskawki (0.40%), oregano (0.30%) oraz płaszczki kolczystej (0.16%), a dla molekuł alergenowych wobec nMal d 2 (0.27%) (rodzina białek taumatynopodobnych, TLP), rAni s 1 (0.30%) (inhibitor proteazy serynowej typu Kunitz) oraz rChe a 1 (po 0.43%) (rodzina białek Ole e 1). Zaobserwowano istotnie statystyczny ($p<0.05$) spadek częstości uczulenia wraz z wiekiem wobec molekuł alergenów białek zapasowych: orzecha ziemnego (nAra h 1, rAra h 2, nAra h 3), soi (nGly m 6), orzechów drzew (nCor a 9, nJug r 4). Istotny statystycznie spadek częstości uczulenia wraz z wiekiem był obserwowany również dla molekuł mleka krowiego (nBos d 4, nBos d 5, nBos d 6, nBos d 8) i jaja kurzego (nGal d 1, nGal d 2). Istotnie statystyczny wzrost częstości uczulenia zaobserwowano wobec białek podrodziny PR10 (rAra h 8, rCor a 1.0401). Obserwowana zmiana profilu uczulenia w zależności od wieku została również ukazana w opisie przypadku 9. miesięcznego pacjenta, u którego początkowo stwierdzono alergię pokarmową, manifestującą się między innymi wstrząsem anafilaktycznym, a w ciągu 2 lat obserwacji stwierdzono rozwój astmy związanej z alergią wziewną co widoczne było również w jego profilu molekularnym (*Knyziak-Mędrzycka i wsp., J Asthma Allergy 2022*).

W pracy *„Sensitization profile in children with celiac disease assessed with the use of multiplex molecular diagnostic technique" (Knyziak-Mędrzycka i wsp.)* skoncentrowano się na ocenie występowania sIgE w grupie pacjentów z CD. Do badań włączono 108 dzieci (63% dziewcząt i 37% chłopców, średnia wieku 9.4 lat) z nowo rozpoznaną CD. Za pomocą testów multipleksowych stwierdzono występowanie sIgE wobec ekstraktów i/lub molekuł alergenów u prawie połowy dzieci (53/108; 49.1%). Najwięcej dzieci (22/53, 41.5%) było uczulonych jednocześnie na alergeny wziewne i pokarmowe. Wśród najczęstszych 10 alergenów uczulających dzieci z CD znalazło się 7 alergenów wziewnych i 2 alergeny pokarmowe. Trzy najczęściej występujące alergeny wziewne (pyłki tymotki łąkowej (26.9%), życica (24.1%), brzoza brodawkowata (18.5%)) to molekuły alergenowe Phl p 1, Lol p 1 (obydwie z rodziny B-ekspansyn) i Bet v 1 (podrodzina PR10). Najczęstsze alergeny pokarmowe (orzech laskowy, jabłko i orzech arachidowy) były molekułami (Cor a 1, Mal d 1 i Ara h 8) należącymi do podrodziny PR10. U badanych pacjentów nie wykazano uczulenia na alergeny zbóż

zawierające gluten. Analiza korelacji metodą rang Spearmana wśród pacjentów uczulonych wykazała istotną dodatnią zależność (r=0.31) między wiekiem badanych a liczbą pozytywnych sIgE (≥0.3 kU_A/L) dla molekuł alergenów wziewnych (p=0.045). Analiza kliniczna wybranych pacjentów z CD i uczuleniem wykazała, że w tej grupie pacjentów najczęściej występuje alergia wziewna, np. alergiczny nieżyt nosa, astma oskrzelowa.

Podsumowanie i wnioski: Przeprowadzone badania wykazały, że:

1. Profil uczulenia oparty na oznaczeniach sIgE wobec ekstraktów i molekuł alergenowych ze szczególnym uwzględnieniem alergenów pokarmowych wielkiej ósemki u dzieci w Polsce różnił się w porównaniu do innych krajów.
2. U polskich dzieci z podejrzeniem alergii na podstawie występowania sIgE najczęściej stwierdzano uczulenie na ekstrakt orzecha arachidowego, orzecha laskowego oraz jabłka, a wobec molekuł dla podrodziny PR10 (rCor a 1.0401, rMal d 1 i rAra h 8), na miejscu 4. znalazła się globulina 7/8S (nAra h 1).
3. Profil uczulenia wobec alergenów pokarmowych, analizowany na podstawie występowania sIgE zmieniał się wraz z wiekiem. Obserwowano wraz z wiekiem spadek uczulenia na alergeny pokarmowe oraz tendencje wzrostową uczulenia na alergeny wziewne. We wczesnym dzieciństwie dominowało uczulenie na białka zapasowe, a w późniejszych latach na białka z podrodziny PR10 z dominacją uczulenia na Bet v 1 z podrodziny PR10 pyłku brzozy.
4. Dzieci z CD były współuczulone na alergeny wziewne i pokarmowe.
5. Prawie połowa dzieci z nowo rozpoznaną CD była uczulona na minimum jeden alergen, przy czym nie stwierdzono u nich uczulenia na alergeny zbóż zawierających gluten.
6. Najczęstszymi alergenami pokarmowymi, które uczulały dzieci z CD były molekuły z podrodziny PR10 (Cor a 1, Mal d 1 i Ara h 8) co może wynikać z reakcji krzyżowych na brzozę, której marker pierwotnej alergii Bet v 1 (podrodzina PR10) był w trójce najczęstszych alergenów wziewnych, po tymotce łąkowej i życicy (Phl p 1, Lol p 1).

Na podstawie przeprowadzonych badań i analizy wyników wyciągnięto następujące wnioski:

1. Kompleksowa molekularna analiza uczuleń dzieci z podejrzeniem alergii zamieszkujących wszystkie regiony Polski pokazała, że profil uczulenia na alergeny pokarmowe w tej grupie różni się od profili obserwowanych w innych krajach; uzyskane wyniki badań mogą stanowić podstawę dla opracowania strategii profilaktyki oraz

leczenia alergii zarówno w aspekcie lokalnym w naszym kraju oraz globalnym w skali światowej.

2. Molekularna analiza uczulenia w wyselekcjonowanej grupie u dzieci z CD pozwala stwierdzić, że pomimo odmiennych mechanizmów immunologicznych A-IgE i CD współistnieją, oraz że istnieje potrzeba diagnostyki A-IgE u pacjentów z CD, nie tylko w zakresie alergii pokarmowej, ale również alergii wziewnej.

Słowa kluczowe: alergia; alergia IgE-zależna; sIgE; anafilaksja; marsz atopowy/alergiczny; PAMD@; precyzyjna diagnostyka molekularna alergii; multipleksowe testy molekularne; celiakia

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 295allergens, 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

1 WSTĘP

Alergia (z języka greckiego *allos* - inny + *ergos* - reakcja), polega na nadmiernej, nieprawidłowej reakcji układu immunologicznego na naturalnie występujące w środowisku, obce dla organizmu substancje, określane jako alergeny, które w normalnych warunkach powinny być przez organizm dobrze tolerowane [1, 2].

Najważniejsze terminy wiążące się z problemem alergii, które pojawiają się w rozprawie doktorskiej zdefiniowano zgodnie z obowiązującymi w literaturze polskiej określeniami [3-6] jako:

- **Nadwrażliwość** – powtarzalne, potwierdzone obiektywnie objawy ze strony określonych narządów i układów, wywołane przez ekspozycję na określony bodziec, który występuje w dawce tolerowanej przez osoby zdrowe. Podział: nadwrażliwość alergiczna (uwarunkowana mechanizmami immunologicznymi), nadwrażliwość niealergiczna (brak udziału mechanizmów immunologicznych).
- **Uczulenie** (sensytyzacja) – patologicznie zmieniona odpowiedź tkanek na oddziaływanie różnych obcych substancji, zwanych alergenami, polegająca na reakcji immunologicznej związanej z powstaniem swoistych przeciwciał, które po związaniu z antygenem mogą doprowadzić do uwolnienia różnych substancji – mediatorów stanu zapalnego.
- **Alergia** – swoista reakcja nadwrażliwości przebiegająca z udziałem mechanizmów immunologicznych w odpowiedzi na powtórny kontakt z alergenem. Reakcje alergiczne mogą przebiegać z udziałem immunoglobuliny E (IgE), np. alergia atopowa lub z udziałem mechanizmów niezależnych od IgE – alergia nieatopowa (przeciwciała innych klas, komórek zapalnych).
- **Antygen –** cząsteczka charakteryzująca się immunogennością, czyli zdolnością wywoływania przeciw sobie swoistej odpowiedzi immunologicznej, i antygenowością, czyli zdolnością do swoistego łączenia się z immunoglobulinami (zarówno wolnymi, jak i stanowiącymi receptory dla limfocytów B) i receptorami limfocytów T; wywołuje odpowiedź immunologiczną, humoralną, komórkową, tolerancję oraz pamięć immunologiczną.
- **Alergen** – antygen zdolny do wywołania ogólnej lub miejscowej reakcji alergicznej (zależnej od IgE i niezależnej od IgE) organizmu człowieka i innych ssaków.

- **Ekstrakt alergenowy** – wyciąg alergenowy poddawany obróbce chemicznej w celu uzyskania końcowego produktu do procedur diagnostycznych. Jest mieszanką różnych składowych alergenu, w tym zanieczyszczeń. Ekstrakt przez obróbkę, której jest poddawany może być pozbawiony istotnych komponent (molekuł) alergenowych.
- **Molekuła alergenowa** (komponent alergenowy) – zbiór łańcuchów polipeptydowych ułożony w sposób przestrzenny tworzący białka. Zbiór tych białek tworzy alergen źródłowy. Nazwę molekuły tworzy się od nazwy łacińskiej (np. dla alergenu brzozy *Betula verrucosa* – Bet v 1, dla alergenu pszenicy *Triticum aestivum* – Tri a 14, Tri a 19).
- **Epitop** (determinanty alergenowe) – fragment alergenu łączący się bezpośrednio z wolnym przeciwciałem, receptorem limfocytów B lub T wiążących antygen. Jeden alergen posiada zwykle wiele epitopów.
- **IgE –** immunoglobuliny klasy E, wiążą się z receptorami FcR na komórkach tucznych, co po przyłączeniu antygenu indukuje degranulację tych komórek i uwolnienie ich zawartości. IgE biorą udział w patomechanizmie alergii oraz obronie przeciw pasożytom.
- **Marsz alergiczny –** określona sekwencja występowania schorzeń atopowych, podkreślająca zmienność obrazu klinicznego na kolejnych etapach życia: od alergii na pokarmy przybierającej formę wyprysku atopowego w okresie wczesnego dzieciństwa do etapu uczuleń na alergeny wziewne manifestujących się astmą oskrzelową i alergicznym nieżytem nosa w wieku późniejszym.

1.1 Alergia – problem medyczny XXI wieku

Choroby alergiczne są coraz bardziej rozpowszechnione na świecie i stanowią główny problem medyczny nie tylko dla lekarzy alergologów, ale również lekarzy rodzinnych. Od kilku dekad obserwuje się wzrost odsetka osób chorujących na alergię oraz coraz cięższy przebieg alergii z koniecznością hospitalizacji [7,8]. Jednym z możliwych powikłań alergii jest astma alergiczna. Dane *International Study of Asthma and Allergies in Childhood* (ISAAC) z 1997 dotyczące występowania chorób alergicznych w 56 krajach pokazują, że wśród dzieci w wieku 13–14 lat, częstość występowania astmy w niektórych krajach zachodnich, w tym w Wielkiej Brytanii i Nowej Zelandii, przekraczała 20% [9]. Szacunki z *National Health and Nutrition Examination Survey* (NHANES) z lat 2007-2010 sugerowały, że 6.5% dzieci i 10% dorosłych miało alergię pokarmową [10]. Podawana częstość występowania alergii pokarmowej może

być jednak zależna od narzędzia wykorzystywanego do jej diagnozowania. Na podstawie danych *Food and Drug Administration* (FDA) *Food Safety Survey* z 2010 r. oszacowanych na podstawie ankiet przeprowadzonych wśród pacjentów na temat podejrzenia u siebie alergii, częstość występowania alergii pokarmowej dorosłych na 13%, a częstość występowania alergii pokarmowej zdiagnozowanej przez lekarza na 6.5% [11]. W Stanach Zjednoczonych populacyjne badanie przekrojowe Warrena i wsp. oszacowało, że alergia pokarmowa IgE-zależna może dotyczyć około 1 na 10 osób dorosłych i 1 na 12 dzieci [12]. W sumie dane te wskazują, że ponad 10% populacji Stanów Zjednoczonych może cierpieć na co najmniej jedną alergię pokarmową IgE-zależną [12].

W polskim badaniu ECAP (*Epidemiology of Allergic Diseases in Poland*) z 2006–2008 roku analizą objęto 22 700 osób w 9 regionach kraju, z czego niemal 25% osób przeszło szczegółowe badania lekarskie. W zależności od regionu i płci cechy alergii deklarowało do 40% osób, a dodatnie wyniki testów na powszechnie występujące alergeny uzyskano u takiego samego odsetka chorych. Choroby alergiczne stwierdzano o wiele częściej w miastach. W zależności od wieku alergiczny nieżyt nosa występował u około 22-25% osób, astma u 9-11%, pokrzywka u 5—8%, atopowe zapalenie skóry u 4-9%, a alergia pokarmowa u 5—13% [13]. W badaniu z Krakowa z lat 2014-2018 w grupie siedmio- i ośmiolatków z dodatnim wywiadem alergicznym u 52% stwierdzono alergiczny nieżyt nosa, a u 9.1% astmę oskrzelową. Natomiast w grupie wiekowej 16–17 lat alergiczny nieżyt nosa rozpoznano u 35.8%, a astmę u 4.9%. [14].

1.2 Mechanizmy immunologiczne alergii IgE-zależnej

Alergia to reakcja nabyta. Oznacza to, że pojawia się po kontakcie z alergenem, wówczas gdy komórki układu immunologicznego nauczyły się go już wcześniej rozpoznawać lub też rozpoznały wcześniej podobną do niego molekułę [15].

Według klasycznego podziału, pierwszym etapem reakcji alergicznej jest faza sensytyzacji (uczulenia), w której na skutek bezpośredniego kontaktu z alergenem układ immunologiczny programuje rozwój uczulenia. Dochodzi wówczas do ukierunkowanej odpowiedzi immunologicznej w stronę odpowiedzi z udziałem limfocytów pomocniczych (Th) typu 2 (odpowiedź Th2). Limfocyty Th2 wydzielają interleukiny (IL)-4, IL-5 i IL-13, co w konsekwencji prowadzi do aktywacji limfocytów i produkcji swoistych dla alergenu przeciwciał E. Po ponownej ekspozycji na alergen, czyli w fazie efektorowej, dochodzi do rozwoju zapalenia, które przebiega wielofazowo. Początkowa faza jest zależna od sIgE i

prowadzi do aktywacji mastocytów (komórek tucznych) z uwolnieniem chemoatraktantów dla leukocytów (histaminy, prostaglandyn, leukotrienów). Następnie zaczyna się migracja leukocytów z krwi obwodowej. Jest to wczesny etap reakcji alergicznej. W fazie późnej leukocyty przemieszczają się z krwi obwodowej do miejsca docelowego i tworzą naciek zapalny. Cechą charakterystyczną nacieku alergicznego jest duże skupisko eozynofilii w tym miejscu [16]. Zapalenie takie może wygasać albo jeśli dochodzi do powtarzającej się ekspozycji na dany alergen, może przechodzić w postać przewlekłą. W postaci przewlekłej może dochodzić do remodelingu, czyli trwałych zmian w strukturze tkanek, które prowadzą do dysfunkcji narządu lub układu. Permanentne uszkadzanie w kontekście immunologicznym i mechanicznym bariery nabłonkowej lub śluzówkowej przez różne czynniki zewnętrzne doprowadza do utrwalenia patologicznego błędnego koła. Dochodzi do rozwoju zapalenia podnabłonkowego. Jest to nowe określenie charakteryzujące minimalny, ciągły proces zapalny toczący się w warstwie podnabłonkowej w wyniku jej uszkodzenia. Proces ten podtrzymuje dezintegrację nabłonka oraz jego zapalną aktywację. Umożliwia w ten sposób niekontrolowane przenikanie i oddziaływanie szkodliwych czynników w głębszych warstwach błony śluzowej przewodu pokarmowego, dróg oddechowych czy skóry właściwej [17].

1.3 Objawy alergii i marsz alergiczny

Objawy alergii mogą dotyczyć wielu układów górnych dróg oddechowych (katar, kichanie, ból gardła), dolnych dróg oddechowych (kaszel, duszność), skóry (świąd, pokrzywka), oczu (świąd, przekrwienie spojówek), układu pokarmowego (wzdęcia, ból brzucha, nieprawidłowe stolce) lub mogą objawiać się jako reakcje uogólnione (anafilaksja) [18].

Alergia może wystąpić w każdym wieku. Jej objawy mogą również zmieniać się wraz z wiekiem pacjenta. Pierwsze objawy alergii pojawiają się już u niemowląt, najczęściej jako alergie pokarmowe i wyprysk atopowy [19]. Alergiczny nieżyt nosa, alergiczne zapalenie spojówek i astma atopowa zwykle występują u dzieci w wieku przedszkolnym i nastolatków, a pokrzywki i uczulenia na jady owadów błonkoskrzydłych u osób dorosłych [20, 21]. Zależność zmiany manifestacji klinicznej alergii od wieku określana jest terminem marszu alergicznego (atopowy) [22]. Marsz alergiczny stanowi naturalną ewolucją choroby atopowej, która zmienia się z upływem czasu. Proces ten obejmuje zmiany w lokalizacji alergicznego procesu zapalnego, pojawianie się nowych objawów oraz zmianę spektrum uczulających alergenów [22]. Zmiana profilu uczulenia na coraz większa liczbę molekuł alergenowych w danym źródle

alergenowym na przestrzeni czasu została nazwana rozprzestrzenianiem molekularnym [23]. Problematyka marszu alergicznego oraz rozważania nad najtrafniejszym postępowaniem terapeutycznym w kontekście tego procesu zostały dokładnie opisane w pracy poglądowej *Knyziak-Mędrzycka i wsp., Allergic March in Children: The Significance of Precision Allergy Molecular Diagnosis (PAMD@) in Predicting Atopy Development and Planning Allergen-Specific Immunotherapy (Nutrients. 2023)".* Natomiast w opisie przypadku klinicznego *Knyziak-Mędrzycka i wsp. „The Precision Allergy Molecular Diagnosis (PAMD@) in Monitoring the Atopic March in a Child with a Primary Food Allergy: Case Report (J Asthma Allergy 2022)* zobrazowano kolejne stadia marszu alergicznego u pacjenta z alergią pokarmową i początkowymi objawami anafilaktycznymi, a następnie rozwijającą się astmą oskrzelową.

Istnieją liczne doniesienia opisujące podejmowanie prób profilaktyki marszu alergicznego i obejmują one szerokie działania środowiskowe i dietetyczne, wpływające w jak najbardziej efektywny sposób na ograniczenie zapalenia alergicznego oraz na stworzeniu barier dla progresji marszu atopowego już na wczesnym etapie życia [24]. Jednak, aby zastosować odpowiednie działania w tym zakresie, ważne jest poznanie profilu uczulenia populacyjnego, które jest zależne od położenia geograficznego, stylu życia, czy ekspozycji na alergen [25,26,27].

Dokładne poznanie profilu uczulenia pozwala również na wczesną immunoterapię alergenową, która najskuteczniej zapobiega ciężkim następstwom zapalenia alergicznego [28,29]. Znaczenie poznania profilu uczulenia z zastosowaniem nowoczesnej diagnostyki molekularnej w aspekcie planowania swoistej immunoterapii alergenowej opisano w pracy poglądowej *Knyziak-Mędrzycka i wsp. (Allergic March in Children: The Significance of Precision Allergy Molecular Diagnosis (PAMD@) in Predicting Atopy Development and Planning Allergen-Specific Immunotherapy (Nutrients 2023).*

W Polsce do tej pory nie prowadzono kompleksowych badań dotyczących profilu uczulenia w zależności od wieku pacjenta z użyciem molekularnych technik diagnostycznych. Dlatego postanowiono podjąć tę tematykę w pracy doktorskiej.

1.4 Diagnostyka serologiczna A-IgE

W diagnostyce A-IgE oprócz badania podmiotowego i przedmiotowego stosuje się tradycyjnie punktowe testy skórne (PTS), a w przypadku alergii pokarmowej rozstrzygające często są próba eliminacji i prowokacji [19]. Poza wymienionymi PTS i próbami

prowokacyjnymi, diagnostyka A-IgE obejmuje badania serologiczne. Badania serologiczne zastosowane w diagnostyce uczulenia czy A-IgE opierają się na ocenie przeciwciał IgE. Około 80% IgE jest związanych przez receptor wysokiego powinowactwa do IgE (Fc epsilon RI) na powierzchni mastocytów, bazofilów i komórek prezentujących antygen [5]. Ta frakcja IgE nie jest dostępna do oznaczeń ilościowych w surowicy krwi [30**,** 31]. Ocenie podlega tylko wolna frakcja IgE. Oceniać możemy wszystkie dostępne w surowicy IgE czyli IgE całkowite (tIgE) oraz alergenowo-swoiste IgE. Stężenie tIgE zależy od wieku. sIgE jest skierowana specyficznie przeciwko określonym antygenom, a wzrost jej stężenia świadczy o uczuleniu na dany alergen i podejrzeniu A-IgE [31]. Diagnostykę sIgE w surowicy krwi pacjenta przeprowadza się za pomocą oznaczania tych przeciwciał najczęściej wobec ekstraktów alergenowych, które wykonuje się przy pomocy testów immunofluorescencyjnych, immunochemicznych lub enzymatycznych. Nowatorskim podejściem do diagnostyki A-IgE, które zostało wykorzystane w pracy doktorskiej, jest ocena molekuł alergenowych w oparciu o diagnostykę molekularną [32]. Precyzyjna diagnostyka molekularna (PAMD@, ang. the precision allergy molecular diagnosis) badająca sIgE dla poszczególnych molekuł alergenowych jest metodą nowoczesną i jednocześnie od niedawna dostępną w diagnostyce alergologicznej. Diagnostykę molekularną alergii można podzielić na monokomponentową i multikomponentową. Monokomponentowa PAMD@ (singleplex) służy do oznaczania stężenia sIgE dla konkretnej molekuły alergenowej w surowicy krwi. Taki rodzaj badań wymaga relatywnie dużej objętości surowicy do wykonania pomiarów (40-50 µl surowicy na jeden alergen plus tzw. dodatkowa objętość martwa materiału około 100 µl) i jest stosunkowo drogi w przeliczeniu na pojedyncze oznaczenie. Natomiast diagnostyka multikomponentowa polega na jednoczesnym oznaczeniu sIgE dla wielu różnych komponent alergenowych w jednym teście [33]. Pierwszym testem multipleksowym był test ImmunoCAP ISAC oceniający łącznie sIgE wobec 112 komponentów z 50 źródeł alergenowych. Następnie pojawiły się testy III-generacji oznaczające sIgE w nanotechnologii. Pierwszym z nich był FABER, oznaczający jednocześnie 122 molekuł i 122 ekstrakty alergenowe, a kolejnym test ALEX, który pozwala na ocenę 178 molekuł alergenowych i 117 ekstraktów alergenowych. Niewątpliwą przewagą testu ALEX, wykorzystanego w pracy doktorskiej, nad pozostałymi testami jest obecność blokera przeciwciał wobec reszt węglowodanowych (bloker anty-CCD, ang. cross-reactive carbohydrate determinants), dzięki któremu w znacznej mierze dochodzi do eliminacji wyników fałszywie dodatnich. Dodatkowo, w odróżnieniu od testów ImmunoCAP ISAC oraz FABER, które są testami półilościowymi, test ALEX jest metodą ilościową, pozwalającą na uzyskanie bardziej precyzyjnych wyników dla sIgE.

1.5 Współwystępowanie A-IgE i celiakii

Celiakia (CD) jest chorobą autoimmunizacyjną występującą u osób z predyspozycją genetyczną (obecność haplotypu HLA-DQ2 i/lub HLA-DQ8), wywołaną spożywaniem glutenu. Gluten to nazwa białek nierozpuszczalnych w wodzie należących do grupy prolamin, które obecne są w zbożach (pszenicy, życie, jęczmieniu). W CD dochodzi do aktywacji limfocytów T pomocniczych typu 1 (Th1) i sekrecji cytokin prozapalnych oraz do produkcji autoprzeciwciał przez limfocyty B, które są skierowane przeciwko transglutaminazie tkankowej (tTG) – enzymowi biorącemu udział w deaminacji peptydów glutenu [34]. Aktywacja procesów zapalnych i autoimmunizacyjnych doprowadza u chorych na CD do charakterystycznych zmian histopatologicznych w jelicie cienkim: wzrostu limfocytów śródnabłonkowych, przerostu krypt i zaniku kosmków jelitowych [35].

Częstość występowania CD w populacji ogólnej waha się od 0.5% do 2%, czyli średnio około 1% [36]. Według dokładnych danych z 2020r. w XXI wieku łączna częstość występowania CD u kobiet wynosiła 17.4, a u mężczyzn- 7.8 na 100 000 osobolat. Częstość występowania charakterystyczna dla dzieci wynosiła 21.3 na 100 000 osobolat w porównaniu z 12.9 u dorosłych [37]. Na podstawie średnich rocznych zmian procentowych stwierdzono, że częstość występowania CD wzrasta średnio o 7.5% rocznie w ciągu ostatnich dekad [37]. W >70% przypadków CD rozpoznaje się >20. roku życia, choć u części z tych chorych objawy mogą występować od dzieciństwa. Badania Majsiak i wsp. wykazały, że w Polsce diagnoza CD jest postawiona dopiero po ponad 9 latach od wystąpienia pierwszych objawów choroby, u dzieci czas ten jest znacznie krótszy i wynosi średnio ok. 3 lata [38]. Zachorowalność wśród kobiet jest dwukrotnie większa niż u mężczyzn [39].

Wyróżniamy następujące postaci CD: klasyczną, nieklasyczną, dawniej nazywaną nietypową lub atypową, subkliniczną oraz celiakię potencjalną [36]. CD klasyczna najczęściej występuje u małych dzieci do 2 roku życia i charakteryzuje się występowaniem przewlekłej biegunki tłuszczowej lub wodnistej, utratą masy ciała lub brakiem przyrostu masy ciała. CD nieklasyczna związana jest z występowaniem objawów zarówno ze strony przewodu pokarmowego (wymioty, zaparcia, bóle brzucha), jak i spoza przewodu pokarmowego takich jak: niedokrwistość, zmiany skórne i błon śluzowych (nawracające afty, zapalenia błony śluzowej jamy ustnej, opryszczkowe zapalenie skóry), zaburzenia związane z nieprawidłowym wchłanianiem wapnia (osteoporoza, złamania patologiczne, niedorozwój szkliwa zębowego, bóle kostno-stawowe), zapalenie i bóle stawów zaburzenia neurologiczne i psychiatryczne (padaczka, depresja, ataksja, nawracające bóle głowy, zaburzenia koncentracji uwagi, migrena)

zaburzenia układu rozrodczego (skłonność do poronień, idiopatyczna niepłodność męska i żeńska, obniżone libido, zaburzenia potencji, hipogonadyzm i hiperprolaktynemia u mężczyzn), podwyższenie aktywności transaminaz wątrobowych.

Zgodnie z obowiązującymi rekomendacjami ESPGHAN (*European Society for Paediatric Gastroenterology Hepatology and Nutrition*) [35] diagnoza CD jest stawiana na podstawie badania przeciwciał anty-tTG w surowicy krwi obwodowej. W pierwszej kolejności należy ocenić stężenie anty-tTG w klasie IgA oraz całkowite IgA. Jeśli zostanie stwierdzony niedobór IgA, diagnostykę poszerza się o przeciwciała anty-tTG w klasie IgG. U dzieci z wysokim stężeniami przeciwciał anty-tTG-IgA (≥10 × górnej granicy normy (GGN)) można rozpoznać CD bez wykonywania biopsji jelita cienkiego. Warunkiem tego jest stwierdzenie obecności przeciwciał endomyzialnych EMA-IgA ocenianych w próbce krwi z odrębnego pobrania. U dzieci i młodzieży z niedoborem IgA oraz u osób ze stężeniami anty-tTG-IgA <10 × GGN należy wykonać biopsję jelita cienkiego z oceną histopatologiczną wycinków jelita cienkiego [35].

Leczenie CD polega na stosowaniu przez całe życie diety bezglutenowej, która powoduje ustąpienie objawów oraz normalizację przeciwciał w surowicy i powrót do prawidłowej morfologii struktur jelita cienkiego. Jednak u niektórych pacjentów pomimo stosowania diety i normalizacji przeciwciał nadal utrzymują się objawy CD [40]. Nie można wykluczyć, że może to być spowodowane współistnieniem innych chorób, w tym alergii.

W przeglądzie systematycznym badań oceniających współwystępowanie uczulenia lub A-IgE i CD (wchodzącej w cykl publikacji rozprawy dokorskiej) *Majsiak i wsp. „IgE-Dependent Allergy in Patients with Celiac Disease: A Systematic Review" (Nutrients. 2023)*, zidentyfikowano 2 prace oryginalne wykluczające [41, 42] i zaledwie 4 oryginalne prace wskazujące na możliwość współwystępowania obu chorób [43, 44, 45, 46]. W zdecydowanej większości autorzy tych prac oceniali współwystępowanie CD i uczulenia na alergeny pokarmowe. Obraz kliniczny alergii u osób z CD był podobny do tego obserwowanego u osób bez CD: atopowe zapalenie skóry, wymioty, pokrzywka, obrzęk naczynioruchowy lub wstrząs anafilaktyczny. Pacjenci z CD najczęściej byli uczuleni na pszenicę, co może wynikać z faktu, że alergen ten był najczęściej badanym alergenem. W opisanych badaniach do oceny uczulenia wykorzystywano techniki oparte o ocenę sIgE wobec ekstraktów alergenowych. W pracy doktorskiej profil uczulenia oceniano przy użyciu najnowszej techniki badawczej – multipleksowego testu (ALEX) umożliwiającego jednoczesne oznaczenie sIgE wobec molekuł i ekstraktów alergenowych.

2 CELE ROZPRAWY DOKTORSKIEJ

Główne cele rozprawy doktorskiej to:

1. kompleksowa ocena profilu uczulenia ze szczególnym uwzględnieniem alergenów pokarmowych u polskich dzieci z podejrzeniem alergii w zależności od wieku na podstawie występowania sIgE;
2. kompleksowa ocena profilu uczulenia w grupie dzieci z nowo rozpoznaną CD.

3 MATERIAŁ I METODY

3.1 Pacjenci

Do oceny profilu uczulenia u polskich dzieci z podejrzeniem alergii wykorzystano wyniki pobrane z wybranych laboratoriów w Polsce, które od 2019 do 2022 roku włącznie wykonywały test ALEX® (Macro Array Diagnostics GmbH, Wiedeń, Austria). Dane uzyskano z Pracowni Immunologii, Instytutu „Pomnik – Centrum Zdrowia Dziecka" w Warszawie oraz z sieci laboratoriów Diagnostyka S.A. wykonujących badania na terenie całej Polski. Na podstawie danych przekazanych nie była możliwa identyfikacja pacjenta. Udostępniono jedynie dane dotyczące wieku i płci badanych oraz uzyskanych wyników badań testem ALEX®. Opracowano wyniki sIgE wobec ekstraktów i molekuł alergenowych od 3715 dzieci. W tym 58% badanych stanowili chłopcy, a 42% dziewczęta. Średnia wieku wynosiła 7.0 lat przy rozkładzie wiekowym 2 m-ce – 17.3 lat. Szczegółowa charakterystyka pacjentów została opisana w pracy *Knyziak-Mędrzycka i wsp. The Sensitization Profile for Selected Food Allergens in Polish Children Assessed with the Use of a Precision Allergy Molecular Diagnostic Technique. Int. J. Mol. Sci. 2024, 25, 825.*

Do badania oceny profilu uczulenia w grupie pacjentów z CD włączono dzieci z nowo rozpoznaną CD w Klinice Gastroenterologii, Hepatologii, Zaburzeń Odżywiania i Pediatrii Instytutu „Pomnik – Centrum Zdrowia Dziecka" w Warszawie oraz w Poradni Gastroenterologicznej (przychodnie przyszpitalne Uniwersyteckiego Szpitala Dziecięcego w Lublinie). Diagnozę CD przeprowadzono według obowiązujących wytycznych ESPGHAN [35]. Do badania włączono 108 dzieci (63% dziewcząt i 37% chłopców). Średnia wieku wynosiła 9.4 lat. Najmłodszy badany był w wieku 0.9 lat, najstarszy w wieku 17.4 lat. Dokładna charakterystyka pacjentów włączonych do badania została opisana w pracy *Knyziak-Mędrzycka i wsp. (Sensitization profile in children with celiac disease assessed with the use of multiplex molecular diagnostic technique.).*

3.2 Metody

W przypadku obu prac oryginalnych wchodzących w skład rozprawy doktorskiej (*Knyziak-Mędrzycka i wsp. The Sensitization Profile for Selected Food Allergens in Polish Children Assessed with the Use of a Precision Allergy Molecular Diagnostic Technique. Int. J. Mol. Sci. 2024, 25, 825.* oraz *Knyziak-Mędrzycka i wsp. Sensitization profile in children with*

celiac disease assessed with the use of multiplex molecular diagnostic technique …….) do oceny profilu uczulenia użyto test ALEX® (Macro Array Diagnostics GmbH, Wiedeń, Austria), który jest multipleksowym testem III generacji umożliwiającym jednoczesne oznaczenie sIgE wobec molekuł i ekstraktów alergenowych. Test ALEX®, dostępny od 2017 roku umożliwia oznaczanie sIgE wobec 282 komponent alergenowych pochodzących ze 167 źródeł: 156 ekstraktów oraz 126 molekuł alergenowych. W 2019 roku w składzie testu ALEX® dokonano zmiany: usunięto kilka alergenów (głównie ekstrakty) i dodano nowe alergeny (głównie molekuły alergenowe). Do nazwy testu dodano liczbę arabską (2) informującą o zmianie składu testu. ALEX2® od 2019 umożliwia oznaczanie sIgE wobec 295 alergenów, w tym dla 117 ekstraktów i 178 molekuł alergenowych pochodzącym z różnych źródeł alergenowych.

Zgodnie z zakresem norm dla oznaczeń sIgE podanych przez producenta, za dodatni wynik przyjęto wyniki ≥0,3 kUA/l. Wyniki zostały wyeksportowane z programu MADx Raptor Software do plików Excel. Wyniki uzyskane przy pomocy ww. testu są wynikami ilościowymi wyrażanymi w jednostkach arbitralnych (kUA/L).

4 ANALIZA STATYSTYCZNA

Analiza statystyczna została szczegółowo opisana w pracach oryginalnych (*Knyziak i wsp The Sensitization Profile for Selected Food Allergens in Polish Children Assessed with the Use of a Precision Allergy Molecular Diagnostic Technique. Int. J. Mol. Sci. 2024, 25, 825*. oraz *Knyziak-Mędrzycka i wsp. Sensitization profile in children with celiac disease assessed with the use of multiplex molecular diagnostic technique)*.

Analizę statystyczną przeprowadzono przy użyciu programu IBM® SPSS® Statistics. W obu badaniach zależności między zmiennymi ilościowymi sprawdzano za pomocą współczynnika korelacji rang Spearmana. W obu badaniach wartość $p < 0.05$ uznano za istotną statystycznie.

5 KOPIE OPUBLIKOWANYCH PRAC

1. Knyziak-Mędrzycka I, Majsiak E, Cukrowska B. Allergic March in Children: The Significance of Precision Allergy Molecular Diagnosis (PAMD@) in Predicting Atopy Development and Planning Allergen-Specific Immunotherapy. *Nutrients*. 2023, 15;15(4): 978. doi: 10.3390/nu15040978.

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Review

Allergic March in Children: The Significance of Precision Allergy Molecular Diagnosis (PAMD@) in Predicting Atopy Development and Planning Allergen-Specific Immunotherapy

Izabela Knyziak-Mędrzycka [1], Emilia Majsiak [2,*] and Bożena Cukrowska [3]

[1] Outpatient Allergology Clinic, The Children's Memorial Health Institute, Aleja Dzieci Polskich 20, 04-730 Warsaw, Poland
[2] Department of Health Promotion, Chair of Nursing Development, Faculty Health of Sciences, Medical University of Lublin, Staszica 4/6, 20-081 Lublin, Poland
[3] Department of Pathomorphology, The Children's Memorial Health Institute, Aleja Dzieci Polskich 20, 04-730 Warsaw, Poland
* Correspondence: emiliamajsiak@tu.rzhib.pl

Abstract: The allergic march is a progression of naturally occurring symptoms whose nature changes with age. The classic allergic march typically begins in infancy and manifests in the form of atopic dermatitis and food allergy. As immune tolerance develops over time, these conditions may resolve by the age of 3–5 years; however, they may evolve into allergic rhinitis and bronchial asthma. Traditional diagnostic assessments, such as skin prick testing or serum allergen-specific immunoglobulin E (sIgE) level testing, are conducted to introduce effective treatment. Recent years saw the emergence of precision allergy molecular diagnosis (PAMD@), which assesses sIgE against allergenic molecules. This new technology helps more accurately evaluate the patient's allergy profile, which helps create more precise dietary specifications and personalize allergen-specific immunotherapy. This review presents possible predictions regarding the allergic march and the means of controlling it based on PAMD@ results.

Keywords: precision allergy molecular diagnostic applications; PAMD@; allergic march; molecular spreading; allergen-specific immunotherapy

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1. Introduction

Allergy that occurs in the first years of a child's life is typically food allergy (FA) [1]. Due to its increasing incidence, FA is an important problem in clinical pediatrics. FA may manifest with mild symptoms, such as abdominal discomfort, nausea, vomiting, or diarrhea, but also with severe, life-threatening symptoms, which may be due to immunoglobulin E (IgE)-mediated hypersensitivity (e.g., anaphylactic shock) (Table 1) or due to severe dehydration and electrolyte imbalance resulting from intense vomiting or diarrhea caused by non-IgE-mediated reactions [2,3]. Hypersensitivity to foods is often also the first step in the so-called allergic march—a progression from FA to inhalant allergy, which leads to asthma [4]. Usually, one of the first FA manifestations is atopic dermatitis (AD), which is a common condition in early childhood, with an estimated prevalence of 15–25% in children [5]. Data from the literature show that approximately 45% of infants develop AD symptoms before the age of 6 months, with the proportion rising to 50% by the age of 1 year. Comorbid IgE-mediated FA and AD in infancy and early childhood are the earliest manifestations of the atopic march [6,7]. Symptomatic FA, especially severe or multiple ones, was shown to be closely associated with bronchial asthma in children aged ≥6 years [8]. Children with FA developed bronchial asthma earlier than children without this allergy [9]. Another study revealed that milk sensitivity in infancy predisposes the child to severe respiratory tract infections and airway hypersensitivity to histamine [10].

The authors of a pediatric cohort study [11] assessing the progression of symptoms in the allergic march observed a systematic relationship between FA in infancy and inhalant allergies at the age of 3–6 years.

There are a number of literature reports on wide-scope community-based and dietary interventions in an attempt to prevent the allergic march, to most effectively limit allergic inflammation, and to create barriers against allergic march progression at an early age [12–15]. Other allergy prevention strategies, ranging from dietary interventions to therapeutic procedures, are secondary in nature. They involve allergen-specific immunotherapy, which is instrumental in symptom management when the allergic condition has already developed [16].

The need for new management strategies drives researchers to look for biomarkers that could help identify the causative factors of food hypersensitivity. Identifying specific protein allergens responsible for hypersensitivity reactions helps estimate the risk of allergic symptom progression and the effectiveness of planned therapies. Precision allergy molecular diagnosis (PAMD®), which involves evaluating specific IgE (sIgE) against allergenic molecules, is a state-of-the-art technique available in everyday allergy practice.

Of course, it should be emphasized that the mere presence of sIgE in relation to allergen molecules, as in the case of allergen extracts, is not sufficient to diagnose allergies. The presence of sIgE antibody detected with the use of extracts or molecules in in vivo or in vitro assays is only an indicator of allergic sensitization and not allergic disease itself.

Undoubtedly, comparing the known benefits it brings, PAMD® will prove useful both in primary and secondary prevention of childhood allergies.

The purpose of this review was to discuss the significance of PAMD® in predicting atopy development and planning effective allergen-specific immunotherapy in the pediatric population.

Table 1. The most common food allergens involved in allergic manifestations in infants (based on [17]).

Species	Allergenic Molecule	Protein Family Name	Sensitization Rate in Specific Species (%)	Resistance to Heating and Chemical Denaturation	Allergic Symptoms [2]
Cow milk, *Bos domesticus*	Bos d 4	α-lactalbumin	51	Moderate	Oral allergy syndrome (OAS), abdominal pain, bloating, nausea, diarrhea/skin changes, atopic dermatitis, asthma, edema, allergic rhinitis/allergic conjunctivitis, anaphylactic shock
	Bos d 5	β-lactoglobulin	61	Low	
	Bos d 6	Serum albumin	43	Low	
	Bos d 8	Casein	63	High	
Eggs, *Gallus domesticus*	Gal d 1	Ovomucoid		High	
	Gal d 2	Ovoalbumin		Low	
	Gal d 3	Ovotransferrin		Low	
	Gal d 4	Lysozyme		Moderate	
Fish, e.g., *Gadus callarias*	Gad c 1	Parvalbumin	100	High	
Shellfish, e.g., *Penaeus monodon*	Pen m 1	Tropomyosin	62	High	
Nuts, e.g., *Corylus avellana*	Cor a 1	Bet v 1-like	90	Low	
	Cor a 8	11S globulin	36–83	High	
	Cor a 9	nsLTP [1]	5.8	High	
Peanuts, *Arachis hypogaea*	Ara h 1	Vicilin	63–80	High	
	Ara h 2	2S albumin	90	High	
	Ara h 3	Legumin		High	
	Ara h 6	2S albumin	76–96	High	
	Ara h 8	Bet v 1-like		Low	
	Ara h 9	nsLTP [1]		High	
Soy, *Glycine max*	Gly m 4	Bet v 1-like	10.3	Low	
	Gly m 5	Vicilin	33	High	
	Gly m 6	Legumin		High	
	Gly m 8	2S albumin		High	
Wheat, *Triticum aestivum*	Tri a 14	nsLTP		High	
	Tri a 19	Omega-5-gliadin	50–70	High	

[1] nsLTP—Non-specific lipid-transfer protein. [2] Not all of these symptoms may occur with all molecules listed, and other symptoms not listed here may also occur. The presence of sIgE for some molecules (e.g., Ara h 1, Ara h 2) is associated with a higher risk of severe reactions.

2. PAMD® Assays

The means of determining the cause of allergy in routine laboratory diagnostics involve measuring the levels of sIgE against the most common allergens (including allergens of foods, such as cow's milk, eggs, wheat, soy, nuts, and fish, and inhalant allergens, such as birch, timothy grass, house dust mites, molds, and animal allergens) [17]. Developed several years ago, PAMD® is a state-of-the-art form of allergy diagnostics, which helps establish the allergy type (primary/cross-reaction), course (depending on the type of protein allergens), and prognosis (transient/persistent allergy) [17,18]. The use of PAMD® makes it possible to identify individual allergen molecules and assess them comprehensively via multiplex testing. This approach seems more appropriate if we stop thinking about egg, milk, and other foods as allergens and begin to construe these foods as a source of many different allergens, as shown in Table 1.

Singleplex PAMD® is used for assessing the serum levels of sIgE against individual allergenic molecules. Depending on the technical characteristics (solid-phase and liquid-phase assays, various solid-phase substrates, native and recombinant components, different types of detection antibodies, and different types of enzyme interactions), the tests have varied sensitivity and specificity. Singleplex tests yield quantitative results but require the use of relatively large serum sample volumes (40–50 µL of serum per allergen, plus the so-called dead space volume of approximately 100 µL), and their cost per single assay is relatively high [19]. Conversely, multiplex PAMD® involves the simultaneous determination of sIgE for multiple allergen components in a single assay. The first multiplex assay was ImmunoCAP ISAC, capable of analyzing sIgE against a total of 112 allergen components from 50 allergen sources. This was followed by the emergence of third-generation nanotechnology applications. The first of such assays was FABER (which is now no longer produced), capable of simultaneously analyzing 122 molecules and 122 allergen extracts, and another was ALEX (after changes in 2019—ALEX2), capable of analyzing 178 allergen molecules and 117 allergen extracts. One unquestionable advantage of ALEX2 tests over other assays is the presence of a cross-reactive carbohydrate determinant (CCD) inhibitor, which greatly reduces false-positive results. Moreover, unlike the ImmunoCAP ISAC assay, which is semi-quantitative, ALEX2 is a quantitative assay [17].

3. Allergy Prognosis Based on PAMD®

Since the introduction of PAMD® several years ago, the usefulness of this technique in predicting the course of disease has been increasingly emphasized. Earlier assays to determine the sIgE against allergen extracts, which had been in use since the 1960s, offered only a rough overview of the patient's allergy status. Recent years brought the possibility of determining the patient's precise allergy profile, thanks to the introduction of PAMD®.

3.1. *PAMD®, Allergy Symptoms, and Provocation Testing*

PAMD® seems to be useful in predicting the type and severity of allergic symptoms. In the case of cow's milk allergy, determining the sIgE to individual allergen components helps identify patients allergic to casein (Bos d 8), who are at a high risk for anaphylactic reactions, and those allergic to alpha-lactalbumin (Bos d 4) or beta-lactoglobulin (Bos d 5), whose risk of severe anaphylaxis is lower and who can be expected to develop milder symptoms, mainly in the form of skin lesions or gastroenteritis [17]. Another example is egg allergy, where the detection of sIgE against ovomucoid (Gal d 1), which is an egg protein, is associated with high risk of anaphylaxis [17]. PAMD® can also be used in predicting the results of allergen provocation tests. Depending on the type of test used, the levels of casein-specific IgE that have been reported to be predictive of a positive oral cow's milk provocation test range from 0.95 kU/L to 10.0 kU/L [20–23]. Ando et al. reported Gal d 1-specific IgE levels of over 4.4 kU/L to be associated with positive egg provocation challenge results in children [24]. Conversely, other egg-derived molecules, such as ovalbumin (Gal d 2) and ovotransferrin (Gal d 3) very rarely produce a positive provocation test result [25].

Moreover, PAMD® helps predict if the allergy is temporary or persistent. In a prospective study, Dang et al. determined the levels of sIgE to egg molecules (Gal d 1, 2, 3, and 5) and to an egg protein extract in three subgroups of 12-month-old infants [25]. These subgroups were infants with egg white allergy confirmed via allergen provocation testing, infants with egg sensitivity, and those with egg tolerance. The study was followed up at the ages of 2 and 4 years and showed that Gal d 1 sensitization increased the risk of long-term egg allergy five-fold, and the presence of sIgE to all egg allergens (Gal d 1, 2, 3, and 5) increased the risk of persistent allergy to raw eggs four-fold. Moreover, the authors demonstrated that egg allergy in infants increased the risk of aeroallergen sensitization and was associated with an increased risk of developing a respiratory allergy (asthma, rhinitis) by the age of 4 years. An earlier study presented that an egg allergy in infancy, particularly if it coexisted with AD, increased the risk of developing respiratory symptoms and hypersensitivity to aeroallergens in early childhood [26].

Similarly, studies on peanut allergy have demonstrated a relationship between the severity and type of allergy and symptom progression. A study by Sicherer et al. conducted on 511 children showed that the cut-off sIgE levels associated with clinically significant peanut allergy were ≥5 kU/L in children aged ≤2 years and ≥14 kU/L in those older than 2 years. The authors concluded that high-risk groups include non-breastfed infants and infants with high Ara h 2-specific IgE levels [27]. A systematic review and meta-analysis involving component-resolved diagnostics of peanut allergy indicated that the use of PAMD® can lead to establishing the final diagnosis in a more rapid and safer way while reducing the number of unnecessary oral allergen provocation tests with peanut allergens [28].

3.2. *Assessment of Molecular Spreading*

PAMD® also helps us observe the phenomenon of molecular spreading, which involves progressive sensitization to other allergenic molecules from a given source in patients initially sensitized only to a single type of allergenic molecules. This phenomenon was described by Matricardi et al. who evaluated the course of sensitization to timothy grass (*Phleum pratense*) in a boy from the age of 3 to 10 years. Initially, at the age of 3 years, the boy was diagnosed only with sensitivity to Phl p 1 [29]. Subsequently, at the age of 6, he was also found to have Phl p 2 sensitivity. By the age of 10 years, the boy had become sensitized to Phl p 4, Phl p 5, Phl p 6, and Phl p 11 molecules. The authors of that study hypothesized that the introduction of allergen-specific immunotherapy would stop or inhibit this molecular spreading and the associated progression of allergy symptoms [29]. Posa et al. followed up with pediatric patients to evaluate the extent of their allergy by analyzing the sIgE against molecules of *Dermatophagoides pteronyssinus* over a period of 20 years [30]. The most common molecules (>40%) detected early in their lives were Der p 2, Der p 1, and Der p 23 (molecules of group A), followed by (15–30%) Der p 5, Der p 7, Der p 4, and Der p 21 (molecules of group B). The least common (<10%) sensitivities were to Der p 11, Der p 18, Der p 16, Der p 14, and Der p 15 (molecules of group C). Sensitization usually started with group A proteins. Over time, blood tests revealed the presence of sIgE to group B allergens and, eventually, to group C molecules. Early-onset sensitization, extensive exposure to house dust mites, and parental allergic rhinitis were associated with the development of overtly symptomatic allergy during the subsequent years. The patients sensitized to all house dust mite allergen groups listed above (groups A + B + C) were at a significantly higher risk of allergic rhinitis and bronchial asthma. The presence of serum sIgE against Der p 1 or Der p 23 at the age of 5 years or younger was a positive prognostic factor for the development of asthma by school age [30]. In the case of birch allergy, Westman et al. showed a correlation between the risk of emerging or persistent symptoms of allergic rhinitis in the evaluated children at the age of 16 and the presence of Bet v 1-specific IgE at the age of 4 years [31]. Moreover, they demonstrated that high Bet v 1-specific IgE levels at the age of 4 were associated with severe allergic rhinitis at the age of 16 years. Other evaluated PR10 proteins showed a hierarchic correlation: Bet v 1 > Mal d

1 > Cor a 1.04 > Ara h 8 > Pru p 1 > Aln g 1 > Api g 1 > Act d 8 > Gly m 4. The proteins from the PR10 group that were recognized by sIgE were also associated with the risk of developing allergic rhinitis and oral allergy syndrome [31].

4. PAMD® and Allergen-Specific Immunotherapy

The data verified based on the PAMD® results can be invaluable in preparing a patient for immunotherapy [32]. Such data help personalize the immunotherapy vaccine, which improves the effectiveness of the entire course of immunotherapy and increases the chances for successful management of atopy [33]. Diagnosing allergy based on allergenic molecules also helps assess if the patient's hypersensitivity is associated with the so-called true allergy or cross-reactivity, which may be very important in making decisions on causative treatment.

Until recently, the selection of vaccines for allergen-specific immunotherapy was very challenging in patients with both symptomatic allergic rhinitis in the season from March to June and the presence of sIgE against birch and timothy allergen extracts [34]. Currently, PAMD® helps determine whether the result obtained via conventional techniques is due to cross-reactivity between pollen allergens or to true allergy. Selecting the appropriate allergen, the one that causes the allergy, determines the achieved clinical response and the effectiveness of immunotherapy.

The content of whole-allergen extracts is highly variable. They may not even contain clinically significant allergen components, which may pose diagnostic difficulties and lead to suboptimal immunotherapy vaccine selection. Frick et al. evaluated Api m 1 and Api m 10 levels in commercially available whole-allergen extracts used for bee venom immunotherapy [35]. The absence of Api m 10 in the extract (found in 3 out of 5 analyzed samples) was associated with a ten-fold higher risk of immunotherapy failure. Determining the Api m 10 levels and, possibly, the use of an allergen extract containing Api m 10 are indicated in clinical practice in patients who have failed to respond to immunotherapy, which is supposed to protect the patient against an anaphylactic reaction to a bee sting [35].

It is also possible for a patient to present with obvious allergy symptoms but have undetectable serum sIgE levels or negative skin prick test results. Nonetheless, once individual allergen molecules are analyzed, the final diagnosis may be sensitivity to a molecule that is completely absent from standard whole-allergen extracts or whose levels in those extracts are very low. This may be due to the methods used to manufacture the test extract or to the physicochemical properties of the given allergen [36], which can be exemplified by Api m 10 components (which play an important role in bee venom immunotherapy) or Der p 10 and Der p 23 components (which play a role in house dust mite allergy) [37]. Casset et al. analyzed *D. pteronyssinus* allergen extracts from 10 different manufacturers. Only the Der p 1 and Der p 2 components were detected in all analyzed extracts. However, even then, their levels and relative proportions varied significantly. Moreover, at least one of the four expected molecules (Der p 5, 7, 10, and 21) was not detectable in 8 out of the 10 analyzed allergen extracts. Importantly, the lack of Der p 10 (tropomyosin) in diagnostic extracts makes it difficult to diagnose potentially clinically significant cross-reactivity allergy to shrimp and other seafood. The authors of that study concluded that the available house dust mite allergen extracts vary greatly in their allergen content and may not contain clinically significant allergen components, which may lead to diagnostic problems [38]. It is worth noting that the molecular makeup of both immunotherapy vaccines and whole-allergen extracts is similar [39]. Therefore, immunotherapy should not be initiated in patients who test negative for major house dust mite allergens (Der p 1, Der p 2, Der p 23, Der f 1, and Der f 2) [17].

5. The Lower Limit of Normal sIgE Levels in PAMD®

The discovery of IgE by Ishizaka and by Johansson and Bennich reported in 1960 was an important milestone in allergy diagnosis [40]. Another important milestone was a method of detecting the serum levels of IgE against individual allergens. Sensitivity and specificity analysis of the diagnostic tests available in the 1970s helped establish the lower

limit of normal sIgE levels to be 0.35 kU/L [41]. The issue of lowering this lower limit below 0.35 kU/L has been recently suggested and discussed [42]. Nilsson et al. demonstrated that food allergen-specific IgE levels of 0.1–0.34 kU/L (in the case of allergens such as eggs, milk, or nuts) detected in infancy increase the risk of developing inhalant allergies at the age of 5 years, and—in the case of low levels of egg-specific IgE—also the risk of developing AD in early childhood [43]. Therefore, sIgE levels in the first year of life, despite being below 0.35 kU/L, may be an additional predictive factor of allergy development. Recently, our research team described a case of a 9-month-old boy with an anaphylactic reaction following the intake of cow's milk and eggs. His blood tests showed high levels of sIgE against milk and egg components and Bet v 1-specific IgE levels below 0.35 kU/L [44]. The boy's Bet v 1-specific IgE levels measured at 26 months were low at 0.27 kU/L, whereas those measured at 37 months were 2.33 kU/L. Clinical manifestations of allergy, whose timing was consistent with the birch pollen season, occurred already at sIgE levels below 0.35 kU/L. There have also been studies conducted in adult patients that have shown the significance of low-allergen molecule-specific IgE levels (with a range of 0.1–0.34 kU/L) in diagnosing allergies. Balsells-Vives et al. demonstrated that although most patients with symptoms of peach allergy had Pru p 3-specific IgE levels of ≥0.35 kU/L, nearly 50% of the peach allergy patients had low Pru p 3-specific IgE levels of 0.1–0.34 kU/L [45].

Clinically evident allergic disease appears to be present when the IgE antibody level is greater than 0.35 kU/L, but not always, and it depends highly on the specific-to-total IgE ratio, the total IgE of the patient, the extent of allergen exposure, and the "sensitivity" of the patient's mast cells. Nevertheless, the interpretation of sIgE results in the range of 0.1 to 0.35 kU/L should be made with caution.

IgE is a marker of sensitization but not allergic disease. It is only one of many "risk" factors for the development of an allergic reaction. IgE antibody levels above 0.35 kUa/L have been associated with a higher probability or a greater risk of clinical allergic disease manifestations. However, allergic disease cannot be determined by the test itself but rather by the physician who takes into consideration the patient's clinical history of type 1 hypersensitivity-associated reactions following a relevant allergen exposure and other patient and environmental risk factors (e.g., IgE antibody levels, total serum IgE levels, the patient's family genetic history, the severity of the reported reactions, type of reactions, possible cross-reactive allergen exposure).

6. PAMD® Not for Everyone

Currently, PAMD® is not meant to be part of routine allergy diagnostics. Disease-management protocols, including those on patient eligibility to undergo immunotherapy, still require positive allergen extract-based sIgE or positive skin prick test results [46]. Nonetheless, this method serves the additional function of helping to make the results more precise. PAMD® is not intended for monosensitized patients with a predictable, seasonal pattern of allergy symptoms. These patients require only routine diagnostic tests involving sIgE levels or skin prick testing in order to receive immunotherapy.

With the development and greater availability of PAMD®, practical questions of clinicians are: "how to" and "when" to use molecular allergen diagnosis and whether such a diagnostic strategy is appropriate in terms of costs and predictive values. There is no single optimal answer to these questions. Each case should be considered individually based on the clinical condition of the patient. Nevertheless, we can identify certain groups of patients who benefit most from the use of PAMD® in the process of diagnosing their clinical symptoms. These are subjects classified for immunotherapy (patients allergic to single or several inhalant allergens, patients with multiple allergies to pollen or with allergy to *Hymenoptera insects*), patients with anaphylaxis (after food, with the participation of cofactors, with delayed anaphylaxis after red meat, idiopathic anaphylaxis), patients with latex allergy, with polysensitization (especially those with a co-existence of sensitization to inhalant and food allergens), and patients with food allergy (to assess the risk of the severity of allergic reactions and to identify unexpected sources of sensitization). At this

point, the importance of clinicians should also be emphasized. They are responsible for deciding when and which diagnostic strategy should be used, taking into account the patient's symptoms and the local law.

7. Limitations of PAMD®

One of the limitations is the number of molecules marked with PAMD®. Currently, over a thousand molecules are described, but we can routinely label fewer than 200 of them (Table 2). Due to cost constraints and the clinical value of the results, only very few component sIgE antibodies are routinely run on singleplex assays today. For example, peanut molecules (Ara h 1, Ara h 2, Ara h 3, Ara h 8, and Ara h 9) are routinely performed due to their established value in confirming risk for mild versus severe reactions and cross-reactivity with the PR10 and nsLTP allergen families. However, it should be emphasized that we can now determine sIgE against many clinically important molecules, but more studies are necessary to prove their usefulness in clinical practice. On the other hand, it should be noted that in some cases, e.g., cow's milk allergy, the determination of sIgE against allergen components does not bring greater benefits than the determination of sIge based on allergen extracts. The improper interpretation of sIgE results based on allergen extracts or individual molecules may lead to the overdiagnosis of the patient and unnecessary elimination of a given product from the patient's diet, which may result in a loss of tolerance to a given product. Therefore, sIgE testing should be ordered and interpreted very carefully by the clinicians based on the patient's clinical symptoms.

Table 2. Characteristics of selected tests used for PAMD®.

Test	Manufacturer	The Technic of Determination	Type of Designation	Type of Test	Number of Molecules Possible to be Determined [3]	Lower Limit of Detection
ImmunoCap®	Thermo Fisher Scientific Inc., Waltham, MA, USA	Fluorescence enzyme immunoassay	Quantitative	Singleplex	100	0.1 kU/L
Polycheck®	Biocheck GmbH, Münster,	Solid-phase immunoassays	Quantitative	Multiparametric	34	0.15 kU/L
Euroline®	Euroimmun AG, Lübeck, Germany	Solid-phase immunoassays	Semiquantitative	Multiparametric	35	0.35 kU/L
ImmunoCap® ISAC	Thermo Fisher Scientific Inc., Waltham, MA, USA	Solid-phase immunoassay	Semiquantitative	Multiplex	112	0.35 ISU-E [1]
ALEX®2	MacroArray Diagnostics, Vienna, Austria	Solid-phase immunoassay	Quantitative	Multiplex	178 [2]	0.1 kU/L

[1] ISU-E—Standardized units for specific IgE. [2] In addition to allergen molecules, the ALEX®2 test also determines the sIgE to 117 extracts and the total IgE. [3] Information on the number of molecules comes from the official catalogs of the test manufacturers (or distributors) from their official websites.

Another limitation is that some important molecules cannot be determined otherwise than by means of multiplexes [17,47]. One example of such proteins is oleosins, which have been shown to be important in patients who have a history of anaphylaxis after consuming peanuts, sunflower seeds, or soy and who have skin or blood tests negative for allergen extracts. The determination of proteins from this group allows for the assessment of the risk of severe anaphylaxis and the clarification of ambiguous cases of allergy [47].

As in the case of allergen extract-based diagnostics, the results of PAMD® may not always be consistent with clinical manifestations. One of the possible explanations for this phenomenon involves the CCDs that are present in some proteins [17,48]. Anti-CCD antibodies may produce positive results in in vitro allergy tests, which may hinder the clinical interpretation of laboratory test results. This problem may affect up to 30% of patients. The source of the CCDs that in turn activate CCD-specific IgE synthesis are usually plant pollen allergens and insect venoms [19,48].

We have naturally glycosylated and non-glycosylated molecules and allergen extracts. Clinicians' knowledge of molecular structure seems to be of great importance in interpreting the effect of anti-CCD on sIgE results from allergen molecules and extracts. Currently,

we have several approaches to limit the impact of anti-CCD on sIgE results for allergens, such as: (1) the use of recombinant allergen molecules (if it does not reduce the diagnostic sensitivity for molecules); (2) the determination of sIgE against CCDs in the patients' sera; (3) the use of a blocker against anti-CCDs (as an additional reagent or routinely in a standard procedure). Of course, individual solutions have their advantages and disadvantages. For example, the use of CCD inhibitors or recombinant proteins in sIgE testing is not a good solution for all molecules. The use of deglycosylated forms of Tri aA_TI and Api g 5 molecules has been shown to reduce the sensitivity of wheat allergen-specific and celery allergen-specific IgE tests, respectively [49,50]. In addition, in case of doubt, in vivo skin tests can always be performed or a provocation test can be carried out. Obtaining the knowledge and experience necessary for interpreting PAMD® test results may require a certain amount of dedication [17]. Education on PAMD® seems to be necessary to help clinicians obtain the knowledge required for interpreting the results.

8. Costs of PAMD® in Allergy Diagnosis

It is also worth emphasizing the cost of PAMD® use in diagnosing allergies. Singleplex and multiplex PAMD® tests (Table 2) are relatively expensive, and this is why they are not used routinely. However, we can look at the cost of a multiplex test in terms of the number of results it provides. A multiplex test result provides more than a hundred individual test results, thanks to which we can obtain a personalized detailed allergic profile of the patient. This allows us to look at the cost of using PAMD® in allergy diagnostics from a different perspective.

Recent reports have underlined the potential of PAMD® in the field of health economics and have suggested that it can help to save costs for the diagnosis of allergies [51,52]. The detailed sensitization profile also improves the effectiveness of immunotherapy. Although immunotherapy is still clinically based, it is PAMD® that allows for a more precise selection of specific immunotherapy vaccines, which can reduce the cost [53].

We would like to especially highlight the role of multiplex tests to reduce costs. More than a hundred individual test results from one sample can present a comprehensive picture of allergen sensitization, which can be used for precise medical treatment. In fact, unrecognized and untreated allergy is a major cost factor for the management of allergic diseases [54–56]. Early detection of the disease and the implementation of an elimination diet can not only reduce the costs of diagnostic processes and subsequent therapy but can also significantly improve the quality of life of patients, as we have demonstrated in our research on autoimmune diseases [57,58]. With adherence to proper treatment and the precise diagnosis and proper management of allergic diseases, it has been estimated that even high costs can be saved [54–56].

9. Summary

Using PAMD® in allergy diagnostics and reducing the lower limit of normal sIgE levels are intended to diagnose allergies as early as possible and help assess the risk of molecular spreading and anaphylaxis. PAMD® is also a state-of-the-art tool that helps to make decisions on the introduction of causative treatment—allergen-specific immunotherapy and personalized selection of immunotherapy vaccines. The actions taken based on the information obtained via PAMD® may help stem allergy development. Considering the substantial usefulness of PAMD® in the clinical management of patients with inconclusive results of routine allergy tests, having this technology at our disposal makes allergic march control seem more achievable.

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Systematic Review

IgE-Dependent Allergy in Patients with Celiac Disease: A Systematic Review

Emilia Majsiak [1,*], Magdalena Choina [2], Izabela Knyziak-Mędrzycka [3], Joanna Beata Bierła [4], Kamil Janeczek [5], Julia Wykrota [6] and Bożena Cukrowska [4]

[1] Department of Health Promotion, Faculty Health of Sciences, Medical University of Lublin, Staszica 4/6, 20-081 Lublin, Poland
[2] Polish-Ukrainian Foundation of Medicine Development, Nałęczowska 14, 20-701 Lublin, Poland
[3] Outpatient Allergology Clinic, The Children's Memorial Health Institute, Aleja Dzieci Polskich 20, 04-730 Warsaw, Poland
[4] Department of Pathomorphology, The Children's Memorial Health Institute, Aleja Dzieci Polskich 20, 04-730 Warsaw, Poland
[5] Department of Pulmonary Diseases and Children Rheumatology, Medical University of Lublin, Profesora Antoniego Gębali Street, 20-093 Lublin, Poland
[6] Department of Biostatistics and Translational Medicine, Medical University of Lodz, Mazowiecka 15, 92-215 Lodz, Poland
* Correspondence: emiliamajsiak@umlub.pl

Abstract: In order to answer the question if an IgE-mediated allergy (A-IgE) may occur in subjects with celiac disease (CD), a systematic review was performed of available publications collected in the United States National Institute for Biotechnology Information/National Institutes of Health/National Library of Medicine/PubMed database up to 28 December 2022, with the use of the following keywords "allergy&celiac/coeliac", "sensitization&celiac/coeliac", and "anaphylaxis&celiac/coeliac" compared in the form of a conjunction. In total, the search returned 2013 publications from these keywords in any section of the article. As numerous review articles included the above-mentioned entries in the abstract, we decided to focus on the publications with the entries only in the title (n = 63). After rejecting studies unrelated to the topic, narrative reviews, book chapters, conference abstracts, symposium reports, letters to the editor, or non-English articles, 18 publications (6 observational original studies and 12 case reports describing a total of 15 cases of A-IgE developed after a diagnosis of CD) were included to this review. Our study is the first systematic review on allergy occurrence in CD patients. The analysis indicated that the possibility of a coexistence of A-IgE with any food and inhalant allergens in subjects diagnosed with CD should be considered. A sensitization to wheat was the most frequently described in subjects with CD. The clinical manifestation of A-IgE in CD was similar to that in subjects without CD; e.g., with possible atopic dermatitis, vomiting, urticaria, angioedema, or anaphylactic shock. Screening for allergies in subjects with CD should be considered, especially in those cases where symptoms persist after introducing a gluten-free diet. The elimination of wheat from the diet of patients with CD may lead to a loss of immune tolerance and to the development of sensitization, which may even manifest as anaphylaxis. In conclusion, although there are few studies assessing the occurrence of A-IgE in subjects with CD, they show the possibility of a coexistence of both diseases and the high clinical significance of this phenomenon, which indicates the need for further studies.

Keywords: celiac disease; IgE-mediated allergy; immunoglobulin E

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1. Introduction

Celiac disease (CD) is one of the most common autoimmune diseases and is characterized by non-specific symptoms both from and beyond the gastrointestinal tract [1]. Gastrological symptoms mainly include stomach aches, bloating, diarrhea, or constipation.

Symptoms beyond the gastrointestinal tract may include chronic fatigue, headaches, and anemia [2,3]. The basic and most effective method of CD treatment is a gluten-free diet (GFD) [4]. However, it has been shown that in more than 80% of subjects, the symptoms may persist despite a restrictive GFD [5]. The studies of our team have revealed that symptoms in Polish CD patients persisted for up to 6 years after introducing a GFD [5,6]. One of the possible causes of persisting symptoms despite the use of a GFD may be the co-existence of other diseases. In the above-mentioned study, the respondents most frequently mentioned anemia (34%) and allergies (13%) when asked about comorbidities.

Although anemia is quite common in CD, posing a problem if it is a symptom or a complication of the disease [7], the literature data on the coexistence of CD and allergies are rather scarce.

An allergy is described as an exaggerated response from the body's immune system to otherwise inert substances present in the environment. An allergy may be IgE-mediated, non-IgE-mediated, or mixed (IgE-dependent and non-IgE-dependent at the same time). Possible symptoms that may occur are itching; redness; a rash; hives; swelling of the lips, tongue, and airways (angioedema); nausea; vomiting; abdominal cramps; shortness of breath; wheezing; bronchospasms; stridor; fainting; or collapse [8]. Anaphylaxis is the most severe form of a hypersensitivity reaction, the very rapid progression of which can be life-threatening and fatal without an intervention [9].

Both CD and allergies are related to an abnormal function of the immune system, especially of the regulatory T cells in the gastrointestinal tract, which play a fundamental role in maintaining immune tolerance and preventing both autoimmunization and sensitization [10].

The proper development of the immune tolerance of a given organism determines that the food it consumes is not treated as a potentially pathogenic antigen [8]. However, in a few situations, the disturbance of this tolerance and the stimulation of the immune system with a food antigen may lead to an abnormal immune reaction and result in the occurrence of, for example, CD and/or a food allergy (FA) [8,11]. The clinical symptoms and biochemical abnormalities ameliorate after an elimination diet in both conditions [4]. A lifelong gluten-free diet and a diet with the temporary elimination of harmful food allergens are prescribed for CD and FA, respectively [12].

The coexistence of CD and allergies is not fully explained and the literature lacks data on the frequency of the coexistence of these diseases as well as the population distribution or clinical significance. That is why, in the present study, we aimed to review the available publications for the occurrence of an IgE-mediated allergy (A-IgE) in subjects with CD.

2. Materials and Methods

This systematic review was performed in accordance with Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) guidelines [13]. The selected papers were important to reach the research question: "Can A-IgE occur in CD patients?". The electronic search was performed by two pairs of independent reviewers. Any disagreement was resolved by a discussion until a consensus was reached or by consulting a third author. After the search, all articles were inserted into the EndNote program to exclude duplicate articles and then saved in an Excel data sheet, where the classification, selection, and screening took place depending on the type of study, year, and relevance. Subsequently, an evaluation of the titles and abstracts was performed that screened the papers according to the eligibility criteria for the full-text evaluation and then determined the included studies for the qualitative evaluation. The descriptive data of the clinical or methodological factors such as the location, type of study, sample, age, type of allergen, symptoms, type of tests used to diagnose allergies, and prevalence results were extracted. In the case of lost or confusing data, the authors were contacted via e-mail. In cases of discussions, the authors were contacted by e-mail. The review protocol was submitted for registration to the PROSPERO system with the number CRD42023384887.

Due to the limited number of original studies included in our analysis, the low precision of the effect sizes, and high variability of the data, calculations of the bias with the use of statistical methods based on asymmetry and regression such as funnel plots or Egger's test were not performed.

2.1. Search Strategy

In order to verify the possible occurrence of A-IgE with any food and inhalant allergens in patients with CD, available publications on this issue gathered in the United States National Institute for Biotechnology Information/National Institutes of Health/National Library of Medicine/PubMed database (https://www.ncbi.nlm.nih.gov/pubmed) accessed on 28 December 2022 were collected and reviewed. The search was based on the following keywords: "allergy&celiac/coeliac", "sensitization&celiac/coeliac", and "anaphylaxis&celiac/coeliac" compared in the form of a conjunction. Due to the multiplicity of the review articles on gluten-related diseases where the keywords often occurred in the abstract, it was decided to search for the above-mentioned entries only in the titles of the articles.

2.2. Inclusion and Exclusion Criteria

The inclusion criteria were as follows, based on peer-reviewed full-text papers: systematic reviews and meta-analyses; original articles; case studies; written in English; and a description of the presence of an allergy, sensitization, or anaphylaxis in children and/or adults with CD that was related to the research question (Figure 1). The exclusion criteria were papers not related to the topic, narrative reviews, papers not available in full-length, written in languages other than English, animal studies, conference abstracts, editorials, letters to the editor, book chapters, symposium reports, or articles describing only the psychological issues related to A-IgE and CD.

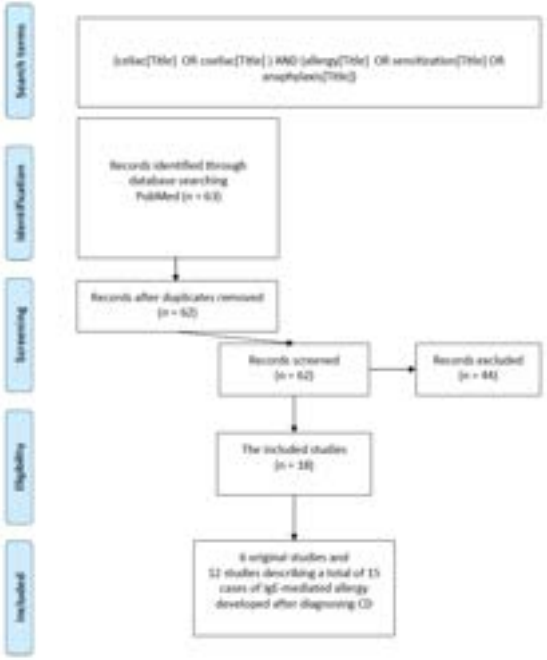

Figure 1. Flow chart showing the process for inclusion of studies [13].

3. Results

In total, a search using the search keywords ("allergy&celiac/coeliac", "sensitization&celiac/coeliac", and "anaphylaxis&celiac/coeliac") returned 2013 publications. After narrowing down the search to the content of these keywords in the titles, we obtained 63 publications (53 for "allergy&celiac/coeliac" keywords, 7 for "sensitization&celiac/coeliac", and 3 for "anaphylaxis&celiac/coeliac"). From a total of 53 articles containing the words "allergy&celiac/coeliac", 5 of them were deleted (4 articles, although containing the searched words, did not refer to the research topic after reading the content, and 1 was dated 2023). From the whole number of found articles, we also deleted one article with the use of "sensitization&celiac" and with "allergy&celiac" because its title contained all three words "allergy&sensitization&celiac". After rejecting the articles that were not related to the topic and did not meet the inclusion criteria, 18 publications remained: 6 original papers (observational studies) and 12 studies describing a total of 15 cases of A-IgE developed after a diagnosis of CD. These were included in the analysis. No systematic reviews or meta-analyses were found in the obtained materials. The number of obtained publications is presented in Table 1.

Table 1. The number of articles found as a result of searching article titles in the PubMed database using the keywords "allergy&celiac/coeliac", "sensitization&celiac/coeliac", and "anaphylaxis&celiac/coeliac" in the form of a conjunction as of 28 December 2022.

Keywords	All Articles in PubMed Included in the Analysis	Original Studies	Systematic Reviews	Case Studies	Review Articles	Inconsistent with the Search Topic	Other [3]
"allergy&celiac/coeliac"	48 [1]	4	0	9	5	17	13
"sensitization&celiac/coeliac"	6 (+1 [2])	2	0	0	0	1	3
"anaphylaxis&celiac/coeliac"	3	0	0	3	0	0	0
	57 (+1 [2])	6	0	12	5	18	16

[1] A total of 53 articles containing the words "allergy&celiac/coeliac" were found, but 4 of them, although containing the searched words, did not refer to the research topic after reading the content and one was dated 2023. [2] One article showed up both with the use of "sensitization&celiac" and with "allergy&celiac" because its title contained all three words "allergy&sensitization&celiac". [3] Others: chapter of a book, abstract of a conference, symposium report, letter to editor, unavailable articles (e.g., absent in the search engine or an article in a language other than English).

3.1. Incidence of A-IgE/Sensitization in Patients with CD

An analysis of six original studies (Table 2) revealed a possible coexistence of CD and A-IgE in four of them; however, two of them did not confirm this correlation. Among the papers confirming the possibility of an A-IgE occurrence in subjects with CD, two papers concerned allergies [14,15] and another two concerned sensitization [12,16]. Among the studies included in the analysis, there were four prospective [14,15] and two retrospective research studies [12,16]. Five of the six studies were conducted in Europe [12,14,15,17,18]. The study groups varied significantly and included 57 to 1044 people with CD [14,15]. In two studies, at the beginning, the study group consisted of subjects with a positive result of specific celiac antibodies;—i.e., antibodies to tissue transglutaminase (TG2) in immunoglobulin class A (IgA) and/or TG2 IgG—and CD was diagnosed in these patients during the study [17,18]. The groups of patients in the analyzed studies were also not homogeneous. In four out of the six analyzed original papers, the study groups included both adults and children [15–18]. However, in one of them, there were children over 14 years of age and adults [17]; the second included subjects from 1 to 20 years of age [16]. In the other two studies, only children [12] and adults [14] were analyzed.

Table 2. Summary of original studies assessing the coexistence of allergy/sensitization and CD.

No.	Study	Number of All Patients Tested/Number with CD	Age Cohort (Mean Age, y)	Region/Type of Study/Period	Tests Used for Diagnosis of Allergy/Sensitization	Tested Allergens	The Prevalence of Allergies/Sensitivities in Celiac Disease
1	Ciacci et al. [14]; 2004.	4114/1044 CD	Adults	Italy; prospective; 1992–2002	Reporting allergy symptoms; sIgE/ed; SPT/Stallergenes Srl, Saronno, Italy	*Gramineae*, *Parietaria officinalis*, *Dermatophagoides*, and *Alternaria*; skin: nickel, chrome, latex, cosmetics, soaps, and dye; milk, eggs, fish, shellfish, nuts/peanuts, tomato, citrus fruits, and soya; miscellaneous: all the reactions provoked by less common antigens (for example, pigeon's feathers, cloves, cinnamon, and olive tree pollen)	16.6% had sIgE for minimum of one tested allergen
2	Armentia et al. [15]; 2008.	88/57 CD	Children; adults	Spain; prospective; ND [1]	SPT/ALK-ABELLO Laboratories, Madrid, Spain sIgE/Pharmacia CAP System FEIA, (Uppsala, Sweden)	Pollen, mites, molds, and different foods: wheat, barley, and rye flours and a battery of food allergens: whole milk, α-lactalbumin, β-lactoglobulin, casein, eggs (white and yolk), legumes, nuts, and fish; Tri a 14, Tri a aA_TI (inhibitor a1 α-amylase (included CMD))	7.0% sIgE to wheat, but no patient in this group had IgE to other food allergens
3	Ensoth et al. [17]; 2013.	1068/24 people with TG2 IgA and/or TG2 IgG	Children (14+); adults	Sweden; prospective; 2006 and 2009	Self-reported allergy sIgE/ImmunoCAP®, F × 5, and Phadiatop Thermo Fisher Scientific/Phadia, Uppsala, Sweden	Grass/pollen, cow's milk, gluten, fur, fish, dust, cold air, mold, organic solvents, medicines, and others ND	No people with allergies were found in the study group with CD
4	Spoerl [18]; 2019.	2965/128 subjects with positive tTG IgA	Children; adults	Switzerland; retrospective; 2010–2016	sIgE/ImmunoCAP® Thermo Fisher Scientific/Phadia, Uppsala, Sweden)	Wheat extract, molecule Tri a 19, and molecular timothy grass Phl p 1, Phl p 5, Phl p 7, and Phl p 12	Wheat allergy did not seem to be associated with CD
5	Lanzarin et al. [16]; 2020.	74/74 CD	1–20 years of age	Brazil; prospective; NR	sIgE/ImmunoCAP®, Thermo Fisher Scientific/Phadia, Uppsala, Sweden))	Wheat, rye, barley, and malt	Frequency of sensitization to wheat, rye, barley, and malt among CD patients was 4, 10.8, 5.4, and 2.7%, respectively
6	Cudowska et al. [12]; 2021.	59/59 CD	Children (average age 8.1)	Poland; retrospective; 2016–2018	sIgE/Polycheck, Biocheck GmbH, Münster, Germany SPT/Allergopharma and Nevter	20 major food and airborne allergens Milk, eggs, soy, wheat, pork, cod, citrus fruits, peanuts, and airborne allergens	20.3% children were sensitized

[1] ND: no data.

The number of studies included in the current systematic review appeared to be too small to draw generalized conclusions about the prevalence of A-IgE in patients with CD. Additionally, none of the studies confirmed an allergy to a specific allergen with the use of a double-blind, placebo-controlled challenge. Therefore, based on the found and analyzed papers, we could only draw conclusions about the frequency of sensitization in people with CD.

In the subjects with CD included in this analysis, sensitization (based on the measurement of the concentration of specific immunoglobulin E (sIgE) in the blood serum) ranged from 16.6–20.0% when sIgE was tested to more than one allergen [12,14]. The most frequently tested allergens were those containing gluten (e.g., wheat, rye, and barley) [15,16,18]. The analysis showed that the frequency of a wheat allergy in subjects with CD ranged from 4.0–7.0% [15,16]. The incidence of sensitization to rye and barley in CD subjects was 10.8% and 5.4%, respectively [16]. The case studies presented in Table 3 also

reported that the most common allergen to provoke allergy symptoms in CD patients was wheat. Typically, an IgE-mediated reaction to this allergen appeared in early childhood and was overcome within 3–5 years of age, but in 3 out of 15 reported cases, a wheat allergy developed at a later age [19–21].

Other food allergens against which sensitization occurred in CD patients included cow's milk, hen's eggs, lupines, buck flour, barley, oats, lentils, fish, peanuts, rye, and corn [20,22–27]. Although a wheat allergy/sensitization was the most common in CD patients, especially in children, sIgE to other allergens was also found in sensitized CD subjects [14]. Ciacci et al. assessed the incidence of A-IgE in adult patients with CD (n = 1044), their relatives (n = 2752), and spouses (n = 318; as a control group) [14]. The study was based on questionnaires distributed among patients at the moment of a CD diagnosis. Subjects reporting any allergy underwent skin-prick tests and the determination of total IgE and sIgE in serum against 20 allergens, including inhaled (*Graminacee*, *Parietaria officinalis*, *Dermatophagoides*, and *Alternaria*) and food (milk, eggs, fish, shellfish, nuts/peanuts, tomatoes, citrus fruits, and soya) allergens. Among the study subjects, 173 (16.6%) with CD, 523 (19%) relatives, and 43 (13.5%) spouses had an allergy to at least 1 allergen, most often to pollen (53; 30.64%), mold (49; 28.32%), vegetables (12; 6.94%), cosmetics (11; 6.36%), and fish (6; 3.47%). Cudowska et al. [12] studied a group of 59 children aged from 10 months to 17 years (mean age 8.1 +/− 4.4 years) with diagnosed CD and demonstrated the presence of sIgE in more than 20% (n = 12) of the children. The children were most frequently sensitized to airborne allergens (66.7%), then to food allergens (58.3%) (a few subjects were sensitized both to airborne and food allergens). Of the airborne allergens, the most frequently sensitizing were house dust mites (6/12; 50%), grass (5/12; 41.7%), and birch pollen (4/12; 33.3%). Of the food allergens, the most frequently sensitizing were peanuts (5/12; 41.7%), cow's milk protein (3/12; 25%), and hen's egg protein (2/12; 16.7%).

It needs to be highlighted that in the analyzed studies, various tests were used to diagnose sensitization/allergies, from self-reporting questionnaires of allergy symptoms through skin-prick tests and the determination of sIgE in blood serum to the use of the most modern tool, which was molecular allergy diagnostics. Among the allergen molecules, serum sIgE was determined to Tri a 14, Tri a 19, and Tri a aA_TI (the inhibitor a1 α-amylase) from wheat, which may be associated with an anaphylaxis risk. Phl p 1, Phl p 5, Phl p 7, and Phl p 12 from timothy grass were associated with an inhalation allergy to this grass [15,18].

Among the analyzed original studies, two of them did not confirm the possibility of A-IgE in CD [17,18]. Enroth et al. did not find subjects with CD and A-IgE in their survey conducted as part of a health-state assessment in 1068 subjects above 14 years of age [17].

Similarly, Spoerl et al. [18], on the basis of a retrospective analysis of laboratory test results performed from 2010–2016, indicated that a wheat allergy did not seem to be related to CD. Nevertheless, of note is a comparison of the number of serological tests for CD with the number of sIgE determinations for wheat. Only 1% of patients tested for CD underwent an sIgE determination for wheat, despite the fact that approximately 20% of the patients tested for CD had intestinal symptoms (bloating or cramps) indicative of a wheat allergy. These findings were emphasized by the authors, who implied the underestimation of the allergy incidence in subjects with CD [18].

3.2. *Clinical Manifestation of A-IgE in CD Patients*

The analysis of the studies showed that A-IgE in patients with CD manifested similarly to non-CD subjects. The case studies (Table 3) repoeted that CD subjects presented with allergic symptoms such as diarrhea, abdominal pain, vomiting, urticaria, hypotension, a loss of consciousness, facial angioedema, dyspnea, coughs, or anaphylaxis. The cases also included seasonal rhinitis, asthma, and atopic dermatitis [20,24,27–30].

The symptoms of A-IgE in CD patients were also analyzed in selected original papers [12,14,16]. Lanzarin et al., in their group of 74 children and teenagers with CD, confirmed the occurrence of asthma, allergic rhinitis, and atopic dermatitis in more than 17%, 13.5%, and 5.4% of the study subjects, respectively [16]. Ciacci et al. presented that

atopic dermatitis occurred three times more often in subjects with CD (3.8%) and two times more often in their relatives (2.3%) than in their spouses (1.3%) [14]. Furthermore, no change in the incidence of allergies in the study subjects with CD was demonstrated after a year of adherence to a GFD [14]. Cudowska et al. [12] also presented that A-IgE in CD children often manifested as atopic dermatitis. It is noteworthy, however, that in this study, 1/3 of CD children had atopic dermatitis, but the symptoms were only caused by food allergens in 5 of them. On the other hand, the authors noticed that other skin diseases such as urticaria or herpetiform dermatitis could be claimed to be a skin form of CD, and gastrointestinal symptoms could be misinterpreted as those only related to CD.

Another group of allergic symptoms in patients with CD are gastrointestinal symptoms. Cudowska et al. [12] showed that almost 42% of CD children reported gastrointestinal symptoms despite using a GFD. The similarity of symptoms in A-IgE and CD can make it difficult to determine which of the diseases developed first, as shown in the descriptions from a few cases. Borghini et al. described a case of a 25-year-old woman who was diagnosed with both diseases at the same time [29]. Similarly, in the oldest reported case (1986) [31] of A-IgE in a 3-month-old infant, it was difficult to decide which disease developed first. Symptoms such as diarrhea and weight loss occurred after the introduction of a cow's milk and wheat diet [31]. Among the case studies presented in Table 3, there were 12 studies describing a total of 15 cases of A-IgE that developed after a diagnosis of CD. However, in 4 of these subjects, an allergy was also present before the CD diagnosis; after the diagnosis, an allergy to new allergens developed [20,27,30].

Regarding the fact that only 4 out of 6 original studies were designed in a way that enabled the reporting of effect sizes, with a low precision of the effect sizes and a high variability of the data, a calculation of the bias with the use of statistical methods was not possible. An overall risk of publication bias was plausible, but unclear.

Table 3. Comparison of case reports of IgE-mediated allergies in subjects with CD included in the literature review.

No.	Autor/Publication Year/n [1]	Age of CD Diagnosis/Gender	Symptoms [2]	Confirmed Allergens	Other Concomitant Allergic Diseases
1	Kuitunen et al. [31]; 1986; n = 1	10.9 years/boy	Vomiting, diarrhea	Cow's milk	
2	Rotinoti et al. [22]; 2007; n = 1	23 years/woman	Generalized urticaria, upper airway angioedema, wheezing, laryngeal edema, vomiting, profound hypotension, and loss of consciousness	Lupines	
3	Torres et al. [23]; 2008; n = 1	4 years/girl	Abdominal pain, gastric fullness, flatulence, and vomiting immediately	Wheat, gliadin, barley, and oat	
4	Sánchez-García et al. [24]; 2011; n = 1	2 years/girl	Abdominal pain, facial urticaria, and generalized urticaria	Cow's milk, eggs	AD; asthma
5	Wong et al. [19]; 2014; n = 1	18 months/girl	Urticaria, cough, shortness of breath with accidental exposures to wheat, tingly mouth, and wheezing	Wheat	
6	Heffler et al. [25]; 2014; n = 1	37 years/woman	Chronic urticaria	Buckwheat flour	

Table 3. *Cont.*

No.	Autor/Publication Year/n [1]	Age of CD Diagnosis/Gender	Symptoms [2]	Confirmed Allergens	Other Concomitant Allergic Diseases
7	Dondi et al. [25]; 2015; n = 1	9 years/boy	Oral allergy syndrome, respiratory impairment, hives, angioedema, abdominal pain or vomiting, and mild-to-moderate anaphylactic reactions	Cow's milk	AD, inhalant allergies, and asthma
8	Martín-Muñoz et al. [20]; 2016; n = 2	25 months/boy	Wheezing, urticarial, lip edema, vomiting, and bronchospasm	bdCD: hen's eggs, lentils, and fish adCD: wheat flour, hake, eggs, and lentils	AD, spring rhinitis, and asthma
		14 months/girl	Nasal pruritus, facial angioedema, dyspnea, and cough	Wheat flour	Spring rhinitis
9	Micozzi et al. [30]; 2018; n = 2	12 months/nd	Delayed growth, abdominal pain, vomiting, sneezing, and lacrimation	bdCD: hen's eggs, cow's milk adCD: wheat flour	Rhinoconjunctivitis; asthma
		6 months/nd	Delayed growth; eyelid angioedema	bdCD: hen's eggs, cow's milk adCD: wheat flour	Rhinoconjunctivitis; asthma
10	Borghini et al. [29]; 2018; n = 1	25 years/woman	Swelling, abdominal pain, diarrhea, and weight loss	Wheat	AD; erythematous skin lesions
11	Mennini et al. [27]; 2019; n = 1	3 years/boy	Anaphylaxis	bdCD: hen's eggs adCD: wheat	AD, rhinitis, and asthma
12	Lombardi et al. [26]; 2019; n = 2	5 years/girl	Generalized urticaria, lip swelling, abdominal pain, diarrhea, vomiting, and respiratory distress	Wheat	
		26 years/woman	Hypotension, generalized urticaria, lip swelling, abdominal pain, diarrhea, vomiting, and dyspnea	Wheat	

[1] n: Number of patients described. AD: atopic dermatitis; bdCD: before CD diagnosis; adCD: after CD diagnosis. [2] Symptoms associated with an allergic reaction to the allergen to which the allergy occurred after the diagnosis of CD.

4. Discussion

The analysis conducted on the basis of the literature indicated that a possible coexistence of A-IgE in CD subjects should be considered. Allergies in CD subjects manifested similarly to non-CD subjects; e.g., with atopic dermatitis, vomiting, urticaria, angioedema, or anaphylactic shock. The literature most often describes subjects with CD as allergic to wheat, which may have been because this allergen was the most frequently tested in the studies. Screening for allergies in subjects with CD should be considered, especially in those cases where symptoms persist after introducing a GFD. There are no available original studies on the effect of a GFD in subjects with CD on the development of a wheat allergy or an allergy to other gluten-containing cereals. A few reported cases indicated that the elimination of gluten-containing foods may induce the appearance of sIgE against these foods [26,27,30]. A hypothesis was even developed that the interrupted and accidental consumption of gluten by patients with CD may promote sensitization [30]. An example that could confirm this hypothesis was the case of a girl who was diagnosed with CD at the age of 6. After many years of a strict GFD, at the age of 15, the girl started to consume gluten occasionally, which could have contributed to the development of anaphylaxis when she ate pita bread. In the case report, the authors hypothesized that occasional gluten consumption by subjects on a GFD was likely to contribute to the development of an allergy to wheat [26]. Elimination diets, including a GFD, may lead to a reduced tolerance to the products excluded from the diet. This hypothesis was also supported by the described case

of a 13-year-old boy with CD and a GFD who developed anaphylaxis after accidentally ingesting gluten [27]. Another hypothesis that could be considered after analyzing the literature regarding the occurrence of A-IgE in subjects with CD was that it may be that patients with CD are already sensitized to wheat. Embarking on a GFD may lead to a loss of tolerance to wheat and, thereby, to the development of a wheat allergy [30]. Further investigations into this issue are necessary because avoiding eating wheat for subjects with CD and a wheat allergy may lead to fatal anaphylaxis [28]. Importantly, food products introduced as substitutes for gluten-containing cereals may also be a source of allergens responsible for the development of anaphylaxis in sensitized patients with CD. A case of a patient with CD who developed anaphylaxis after eating gluten-free pasta was described. It was established that sensitization to lupines caused the symptoms [22]. In the cases of unexplained anaphylaxis, it is essential to establish if the patients with CD were not allergic to food products introduced as substitutes for gluten-containing cereals and to monitor the risk of sensitization in these patients with CD.

The diagnosis of A-IgE in patients with CD may be difficult due to allergic symptoms being masked by symptoms interpreted as those related to CD. There are two main types of clinical A-IgE manifestations occurring in CD: skin and gastrointestinal symptoms. Unfortunately, both groups of symptoms are also typical in the course of CD without an allergy [3]. Therefore, it is important to consider allergies in patients with CD who fail to clinically improve with the introduction of a restrictive GFD.

The diagnostic tests used may also lead to a misinterpretation of the results and a failure to diagnose allergies. In the case of a 15-year-old girl with anaphylaxis after the consumption of pita bread, skin-prick tests gave a positive result for gliadin (8 mm), but a negative result for wheat extract [26]. It must be emphasized that the diagnostic tests are based on various allergen extracts that are soluble in water and that not all allergens prepared in this way contain allergic proteins. Several allergic proteins only dissolve in the presence of detergents or alcohol [32,33]. As wheat proteins are poorly water-soluble, the diagnostics based on their extracts may fail to establish the allergic source responsible for the symptoms. A new tool enabling a more detailed analysis of sIgE to particular allergen proteins and highly facilitating the diagnosis of wheat allergies in patients with CD is precision allergy molecular diagnosis (PAMD®) [34]. After the discovery of sIgE, the use of tests detecting antibodies to allergen molecules has become another milestone in the diagnosis of allergies. PAMD® provides more information than a diagnosis based on extracts and it enables both a differentiation between primary sensitizations and sensitizations resulting from cross-reactions and the determination of the risk of anaphylaxis [35]. Therefore, often only this tool can precisely establish what the cause of an allergic response is [32,34]. In the reported cases, apart from the determination of serum sIgE to the whole wheat extract, more and more often its particular proteins such as Tri a 14 (nsLTP), Tri a aA_TI (α-amylase), and Tri a 19 (ω-5-gliadin) had been determined [20,23,26–28,30]. In the case of an allergy to wheat in a girl with CD diagnosed when she was 5, which was reported by Lombardini et al., particular wheat molecules were also assessed. It was confirmed that she had serum sIgE to gliadin and ω-5-gliadin [26]. ω-5-gliadin is a marker of both a primary allergy to wheat and of severe reactions in children with an allergy to wheat, as well as a marker of wheat-dependent exercise-induced anaphylaxis [32]. PAMD®, as a new diagnostic method, may help diagnose patients with CD who, despite the use of a restrictive GFD, still experience bothersome symptoms resulting from their food intake.

5. Conclusions

Our study was the first systematic review on allergy occurrence in CD patients. Although there are few studies assessing the occurrence of A-IgE in subjects with CD, the current systemic review indicated the possible coexistence of A-IgE in CD. However, the occurrence of A-IgE in subjects with CD is a not fully understood problem; this requires

further studies in particular populations as well as studies that would explain a possible pathomechanism of a coexistence of these two diseases.

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The Sensitization Profile for Selected Food Allergens in Polish Children Assessed with the Use of a Precision Allergy Molecular Diagnostic Technique

Knyziak-Mędrzycka Izabela [1], Majsiak Emilia [2,*], Gromek Weronika [3], Kozłowska Danuta [4], Swadźba Jakub [4], Bierła Joanna Beata [5], Kurzawa Ryszard [6] and Cukrowska Bożena [5]

[1] Outpatient Allergology Clinic, the Children's Memorial Health Institute, Aleja Dzieci Polskich 20, 04-730 Warsaw, Poland
[2] Department of Health Promotion, Faculty Health of Sciences, Medical University of Lublin, Staszica 4/6, 20-081 Lublin, Poland
[3] Polish-Ukrainian Foundation of Medicine Development, Nałęczowska 14, 20-701 Lublin, Poland
[4] Diagnostyka S.A., Prof. M.Życzkowskiego 16, 31-864 Kraków, Poland; jakub.swadzba@diag.pl (S.J.)
[5] Department of Pathomorphology, the Children's Memorial Health Institute, Aleja Dzieci Polskich 20, 04-730 Warsaw, Poland; b.cukrowska@ipczd.pl (C.B.)
[6] Department of Allergology and Pneumonology of the National Research Institute for Tuberculosis and Lung Diseases, Regional Branch in Rabka-Zdrój, Profesora Rudnika 3B, 34-700 Rabka-Zdrój, Poland
* Correspondence: emiliamajsiak@uzzlub.pl

Abstract: Individual populations show a variety of sensitization patterns, which may be associated with the geographic region, climate, dietary habits, or ways of preparing food. The purpose of this study was to comprehensively assess the food allergy sensitization profile in Polish children, particularly to eight food allergens (so-called "the Big 8"): cow milk, eggs, wheat, soybeans, fish, crustacean shellfish, tree nuts, and peanuts. To assess the prevalence and serum levels of specific immunoglobulins E (sIgE), we analyzed the results obtained from selected laboratories located in all regions of Poland that used the multiplex ALEX® test in the period from 2019 to 2022. Results from 3715 children were obtained. The mean age of the study population was 7.0 years. The results were stratified by age: <12 months (3.63%), 1–5 years (39.54%), 6–13 years (46.32%), and 14–18 years (10.07%). The final analysis included the sIgE results obtained with 95 food extracts and 77 food allergen molecules. The highest rates of sIgE to food allergen extracts were found for peanut (29.20%), hazel (28.20%), and apple (23.60%), and those to allergenic molecules were found for the PR-10 family of molecules (Cor a 1.0401 (23.77%), Mal d 1 (22.37%), Ara h 8 (16.93%), and globulin 7/8S (Ara h 1; 15.59%)). The lowest rates of sIgE reactivity to extracts were found for strawberry (0.40%), oregano (0.30%), and thornback ray (0.16%), and those to allergenic molecules were found for Mal d 2 (0.27%) (thaumatin-like protein, TLP), Ani s 1 (0.30%) (Kunitz-type serine protease inhibitor), and Che a 1 (0.43%) (Che e 1 family). The rates of sensitization to storage proteins of the analyzed "the Big 8" molecules decreased significantly ($p < 0.05$) with age. Conversely, the rates of sensitization to PR-10 family proteins increased significantly with age. The three most common allergens in Poland, regardless of whether IgE was assayed against extracts or molecules of food allergens, were peanut, hazel, and apple (in different order depending on the ranking). A detailed analysis of sensitization to the extracts and molecules of main food allergens based on the results of a multiplex ALEX® test demonstrated the sensitization profile in Polish children (including molecular sensitization, particularly the "the Big 8" food allergen molecules), which shows considerable differences in comparison with those in other countries. Serum sIgE analysis of children from all regions of Poland revealed a food allergen molecular sensitization profile that changes with age.

Keywords: sensitization profile; food allergens; allergy molecular diagnostic; molecular sensitization map

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1. Introduction

Food allergies are an increasingly common problem. There is a growing body of evidence showing that food allergies are on the rise [1]. Geographic and temporal differences in allergy prevalence reported so far indicate the role of environmental factors in allergy development [2–4].

Molecular diagnostics allows us to identify specific proteins to which a patient is allergic. This enables us to manage each patient's allergy on an individual basis. By understanding the characteristics of the described proteins, we can predict the symptoms a patient may exhibit. An example is an allergy to profilins and polcalcins, which generally result in mild clinical symptoms. On the other hand, reserve proteins such as oleosins, nsLTP, tropomyosin, or parvalbumins can lead to anaphylaxis and even the death of the patient. Evaluating molecular sensitization helps better understand and track the allergic march that takes place in a given population and determine the most common allergenic molecules [5,6]. Molecular allergy diagnostics helps specify whether allergy symptoms are due to primary sensitization or cross-reactivity. Molecular sensitization assessment is a tool used to determine representative sensitization patterns in specific groups of patients. Such tests help develop diagnostic and therapeutic algorithms in early childhood.

Different populations have various sensitization patterns. This was demonstrated in the year 2023 in a multi-center (Sweden, Norway, the Netherlands, the United Kingdom, Germany, Italy, Spain) study by Kiewiet et al., who assessed the specific immunoglobulin E (sIgE)-reactivity profile based on allergenic molecules in children (with an ImmunoCAP ISAC test, Phadia Austria GmbH, part of Thermo Fisher Scientific ImmunoDiagnostics, Vienna, Austria) [7]. The authors observed regional, exposome- and climate-dependent differences in molecular IgE-reactivity profiles in Northern, Western, Central, and Southern Europe, which may be a molecular basis for precision medicine-based approaches to allergy management and prevention.

Earlier studies on molecular sIgE reactivity profiles conducted in cohorts from individual countries (*e.g.*, the United Kingdom, Germany, and Italy) also showed various patterns of molecular sensitization [8–10]. The differences in patterns may occur even within one country, as demonstrated in a study conducted in various regions of France [11]. That study showed significant differences in molecular sensitization profiles based on the differences in exposure to the given allergens, mainly as a result of the differences in the plants growing in different regions of the country. Likewise, in Kiewiet's study, the exposome (primarily the climate) seemed to be an important factor affecting the differences in sIgE profiles observed between the cities of Rome and Bologna, Italy, and Sabadell and Gipuzkoa, Spain [7].

The purpose of our study was to comprehensively assess pediatric sensitization profiles to food allergens, focusing particularly on eight food allergens (so-called "the Big 8"): cow milk, eggs, wheat, soybeans, fish, crustacean shellfish, tree nuts, and peanuts. Diagnostic tests were conducted with the use of precision molecular diagnostics, and the obtained data helped create an age-dependent sensitization profile of Polish children. Creating such a sensitization profile helps identify the most common allergens, determine their characteristics and the risk of anaphylaxis, and draw the conclusions necessary to manage allergy problems in entire populations. Our study provides a comprehensive review of IgE sensitization profiles based on both molecular and extract analyses of food allergens in children and adolescents living in Poland. To the best of our knowledge, this is the first analysis of the results of multiplex third-generation tests conducted on children from various regions of Poland.

2. Results

2.1. Subjects' Characteristics

We analyzed the test results of 3715 children who were diagnosed in search of allergic causes of their various symptoms in several Polish laboratories in the period from 2019 to 2022. More than half of the analyzed group (58.0%) were boys. The mean subject age was 7.0 years. The youngest subject was 2 months old, and the oldest was 17.3 years old. The

largest age groups were 6–13 year-olds (46.32%) and 1–5 year-olds (39.54%). A detailed analysis is in Table 1.

Table 1. Characteristics of the study group whose results were analyzed to assess sensitization.

		The Number of Results Analyzed	%
Sex	Female	1575	42%
	Male	2140	58%
Age	Mean	7.0	
	Standard deviation	4.16	
	Percentile 25	4.0	
	Median	6.0	
	Percentile 75	10.0	
	Minimum	0.2	
	Maximum	17.3	
	Up to 12 months old	135	4%
	1–5 years old	1469	40%
	>5–13 years old	1721	46%
	>13–18 years old	390	10%

Out of the collected test results, 572 results were obtained with the ALEX® test (assessing 156 extracts and 126 allergen molecules), and 3143 results were obtained with the ALEX^{2} test (assessing 117 extracts and 178 allergen molecules). A total of 1,088,489 individual sIgE tests were obtained and analyzed, including 631,526 sIgE tests (58.0%) for allergen molecules and 456,963 sIgE tests (42.0%) for allergen extracts. Three molecules were excluded from the overall allergen molecule analysis. Two of those were excluded because they were a mix of molecules from different groups, which made it impossible to assign them to a specific family of molecules—these were a mix of strawberry molecules (Fra a 1 + Fra a 3, mix) and a mix of cod molecules (Gad m 2 + Gad m 3)—and the third one was hazelnut molecule Cor a 11, which was due to a data transfer error resulting in only 197 test results being available for this molecule.

Ultimately, our analysis included sIgE test results for 95 food allergen extracts and 77 food allergen molecules. The list of all analyzed allergens has been made available as supplementary data (Tables S1 and S2).

2.2. Sensitization Profile to Selected Food Allergens Based on Allergen Extracts

Table 2 in Part A presents the ranking of selected allergen extracts, namely those against which the proportion of sIgE exceeded 10%. All data have been included in supplementary data (Table S1). Out of 3715 sIgE results obtained with the ALEX® test for 95 allergen extracts, the most common food allergens were peanuts (29.20%), hazelnuts (28.20%), and apples (23.60%). Conversely, the allergens that yielded the lowest proportion of positive tests were strawberry (0.40%), oregano (0.30%), and thornback ray (0.16%). The highest observed mean sIgE levels were for cow milk (9.86 kU_A/L), shrimp (8.55 kU_A/L), and peanut (8.54 kU_A/L), whereas the lowest mean sIgE levels were for lychee extract (0.49 kU_A/L), mushroom (0.45 kU_A/L), and strawberry (0.40 kU_A/L).

Apart from peanut and hazelnut, all other "the Big 8" food allergens were also analyzed; these included hen eggs, cow milk, fish, wheat, soybeans, shellfish, and other tree nuts. Out of these other "the Big 8" allergens, hen egg white ranked the highest, taking 4th place, whereas egg yolk ranked 35th, with mean levels of 5.46 kU_A/L and 3.26 kU_A/L, respectively. Out of the 3715 evaluated children, 383 (10.31%) were positive for cow milk sIgE (with mean levels of 9.86 kU_A/L) assessed with a cow milk extract, which placed this allergen at the top of the ranking for average sIgE concentration for extract-based sensitization.

Table 2. Analyses of sIgE, which occur at rates of more than 10% of all evaluated food allergen extracts and molecules.

Place in the Ranking of sIgE Frequency *	Tested Allergen	Protein Family	Allergen Source	Number of all sIgE Determinations **	Number and % of Positive sIgE		Place in the Ranking of sIgE Concentration ***	Mean Concentration of Positive sIgE [kU$_A$/L]
Allergen extracts								
1	Ara h	–	Peanut (*Arachis hypogaea*)	500	146	29.20%	3	8.54
2	Cor a_hazel	–	Hazel (*Corylus avellana*)	500	141	28.20%	24	4.25
3	Mal d	–	*Apple* (*Malus domestica*)	500	118	23.60%	14	5.69
4	Gal d_egg white	–	Egg white (*Gallus domesticus*)	3715	653	17.58%	18	5.46
5	Ana o	–	Cashew (*Anacardium occidentale*)	3715	570	15.34%	5	7.44
6	Pis v	–	Pistachio (*Pistacia vera*)	500	76	15.20%	10	6.37
7	Gly m	–	Soy (*Glycine max*)	500	73	14.60%	33	3.76
8	Api g	–	Celery (*Apium graveolens*)	500	72	14.40%	36	3.40
9	Jug r_nut	–	Walnut (*Juglans regia*)	500	70	14.00%	13	5.78
10	Cic a	–	Chickpea (*Cicer arietinum*)	3715	503	13.54%	23	4.37
11	Car i	–	Pecan (*Carya illinoensis*)	3715	478	12.87%	8	6.64
12	Dau c	–	Carrot (*Daucus carota*)	3715	476	12.81%	9	6.49
13	Ses i	–	Sesame (*Sesamum indicum*)	3715	439	11.82%	15	5.68
14	Pru du	–	Almond (*Prunus dulcis*)	3715	420	11.31%	29	4.05
15	Hel a	–	Sunflower seed (*Helianthus annuus*)	3715	404	10.87%	48	2.68
16	Pap s	–	Poppy seed (*Papaver somniferum*)	3715	392	10.55%	34	3.70
17	Bos d	–	Cattle (*Bos domesticus*)	3715	383	10.31%	1	9.86
18	Act d	–	Kiwi (*Actinidia deliciosa*)	621	63	10.14%	46	2.74
Allergen molecules								
1	Cor a 1.0401	PR-10	Hazel (*Corylus avellana*)	3715	883	23.77%	6	12.22
2	Mal d 1	PR-10	*Apple* (*Malus domestica*)	3715	831	22.37%	12	10.89
3	Ara h 8	PR-10	Peanut (*Arachis hypogaea*)	3715	629	16.93%	27	7.99
4	Ara h 1	7/8S Globulin	Peanut (*Arachis hypogaea*)	3715	579	15.59%	20	9.35
5	Gly m 4	PR-10	Soy (*Glycine max*)	3715	564	15.18%	15	9.91
6	Api g 1	PR-10	Celery (*Apium graveolens*)	3715	560	15.07%	21	9.08
7	Dau c 1	PR-10	Carrot (*Daucus carota*)	3715	511	13.76%	22	8.64
8	Jug r 4	11S Globulin	Walnut (*Juglans regia*)	3143	431	13.71%	52	4.05
9	Cor a 9	11S Globulin	Hazel (*Corylus avellana*)	3715	486	13.08%	50	4.49
10	Gal d 2	Ovalbumin	Egg white (*Gallus domesticus*)	3715	480	13.00%	47	4.80
11	Ses i 1	2S Albumin	Sesame (*Sesamum indicum*)	3715	453	12.19%	19	9.39
12	Ara h 2	2S Albumin	Peanut (*Arachis hypogaea*)	3715	449	12.09%	1	15.92
13	Gal d 4	Lysozyme C	Egg white (*Gallus domesticus*)	3715	425	11.44%	38	5.32
14	Gal d 1	Ovomucoid	Egg white (*Gallus domesticus*)	3715	422	11.36%	33	6.36
15	Ara h 3	11S Globulin	Peanut (*Arachis hypogaea*)	3715	410	11.04%	31	6.44
16	Jug r 2	7/8S Globulin	Walnut (*Juglans regia*)	3715	401	10.79%	36	5.85
17	Bos d 8	Casein	Cow's milk (*Bos domesticus*)	3715	389	10.47%	25	8.24

* Place in the ranking of frequency of positive sIgE (i.e., >0.3 kU$_A$/L) when all 95 extracts and 77 molecules were investigated (the whole ranking is presented in Table S1); ** The difference in the number of determinations results from the tests performed—ALEX and ALEX2 (explained in detail in the section on the method); *** Place in the ranking of mean concentrations of positive sIgE when all 95 extracts and 77 molecules were investigated (the whole ranking is presented in Table S1).

Analysis of sIgE against fish allergens ranked cod at the 41st place (4.23%), Atlantic herring at the 55th place (2.67%), salmon at the 62nd place (1.99%), tuna at the 75th place (0.94%), Atlantic mackerel at the 76th place (0.92%), and thornback ray at the 85th place, with the mean sIgE levels of 7.08 kU_A/L, 4.42 kU_A/L, 3.63 kU_A/L, 1.11 kU_A/L, 0.92 kU_A/L, and 0.78 kU_A/L, respectively.

Common wheat and spelt wheat ranked 39th and 40th, with similar rates of 4.60% and 4.50%, respectively. The mean sIgE levels were 4.24 kU_A/L against common wheat and 3.32 kU_A/L against spelt wheat. sIgE against soybean extract was detected in 73 children (14.60%) at a mean level of 3.76 kU_A/L, which made this allergen rank as the 7th most common positive sIgE test based on allergen extracts and in place of the 33rd in the analysis of IgE concentrations for food extracts.

Out of four crustacean shellfish extracts, the most common positive sIgE result was found for lobsters (2.21%), placing this allergen at the 60th rank with mean sIgE levels of 5.65 kU_A/L. Crab ranked 69th with the mean sIgE levels of 8.38 kU_A/L, and the extracts of various species of shrimp (Pan b and Lit s z 2) ranked 71st and 72nd, with the mean sIgE levels of 6.17 and 8.55 kU_A/L, respectively.

The test included six tree nut allergen extracts. Apart from hazel, which ranked 2nd, the remaining nuts, namely cashew, walnut, pecan, Brazil nut, and macadamia nut, ranked 5th, 9th, 11th, 19th, and 32nd, respectively, with mean sIgE levels of 7.44 kU_A/L, 5.78 kU_A/L, 6.64 kU_A/L, 4.06 kU_A/L, and 5.47 kU_A/L, respectively. Table 2 presents the ranking of sIgE in allergen extracts for food allergens, with rates of more than 10%.

2.3. Sensitization Profile for Selected Food Allergens Based on Allergen Molecules

Table 2 presents those 17 out of all 77 tested food allergen molecules that yielded positive sIgE results in over 10% of the analyzed population. The entire ranking of sIgE rates in response to all analyzed allergen molecules has been attached as supplementary data (Table S2). Out of the 3715 sIgE results obtained with the ALEX® test, the most common food allergen molecules yielding a positive sIgE response were those from the PR-10 family. Molecules from this family constituted six out of ten food allergen molecules with the highest sIgE rates; these six molecules were: Cor a 1.0401 (23.77%), Mal d 1 (22.37%), Ara h 8 (16.93%), Gly m 4 (15.18%), Api g 1 (15.07%), and Dau c 1 (13.76%). These six molecules ranked 1st, 2nd, 3rd, 5th, 6th, and 7th in the overall ranking of allergen molecules producing positive sIgE responses in the greatest proportion of the study population. One molecule from outside the PR-10 family that ranked in the top five positions was Ara h 1 (globulin 7/8S) (15.59%), a peanut molecule. The lowest proportion of the population showed sIgE to Mal d 2 (0.27%) (TLP), Ani s 1 (0.30%) (Kunitz-type serine protease inhibitor), and Che a 1 (0.43%) (Ole e 1 family).

We analyzed the mean levels of sIgE (kU_A/L) against the individual allergen molecules. The highest observed sIgE levels were against Ara h 2 (15.92 kU_A/L) (albumin 2S) and Ara h 6 (14.43 kU_A/L) (albumin 2S), Jug r 1 (14.19 kU_A/L) (globulin 7/8S), Cuc m 2 (12.92 kU_A/L) (profilin), Thu a 1 (12.26 kU_A/L) (β-parvalbumin), Cor a 1.0401 (12.22 kU_A/L) (PR-10), Clu h 1 (12.07 kU_A/L) (β-parvalbumin), Ana o 3 (11.93 kU_A/L) (globulin 11S), Pis v 1 (11.50 kU_A/L) (albumin 2S), and Sal s 1 (11.44 kU_A/L) (β-parvalbumin) molecules. The lowest observed mean sIgE levels were against Ara h 15 (1.00 kU_A/L) (oleosin), Mal d 2 (0.99 kU_A/L) (TLP), and Ani s 1 (0.78 kU_A/L) (Kunitz-type serine protease inhibitor) molecules.

2.4. sIgE against "the Big 8" Food Allergen Molecules Stratified by Age

Figure 1 shows the proportion of the evaluated individuals with sIgE against the "the Big 8" food allergens: cow milk, eggs, peanuts, soybeans, wheat, fish, crustaceans, and tree nuts.

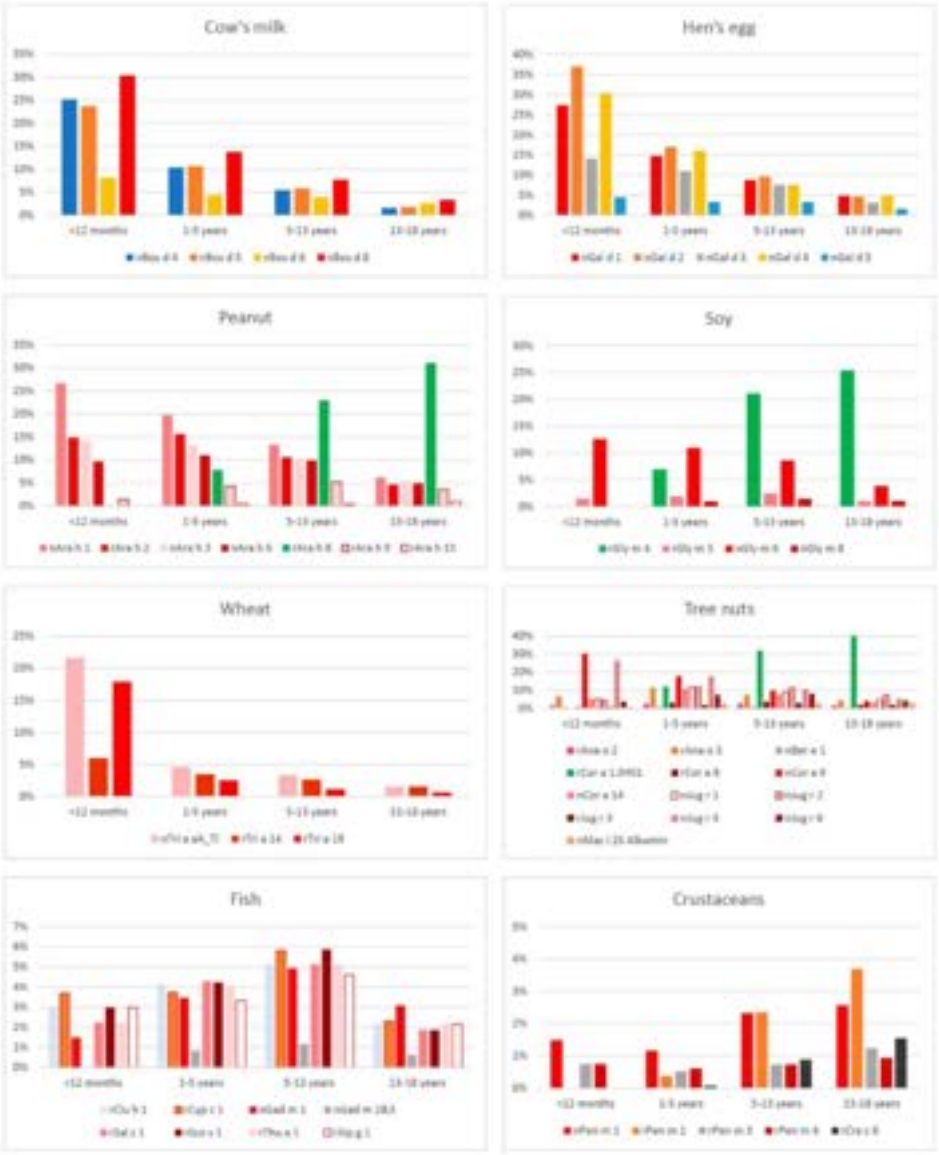

Figure 1. The sIgE rates against the "the Big 8" allergen molecules stratified by age. Blue columns represent serum albumins, green columns represent proteins of the PR-10 family, and red columns (of various shades and patterns) represent molecules associated with a high risk of anaphylactic shock (the remaining colors, even repeated ones, do not indicate any relationships between them).

Children from the youngest evaluated age range (<1 year of age) showed the highest rates of positive sIgE results against all cow milk molecules, with casein (Bos d 8) yielding the highest proportion of positive tests (30.37%). The age group with the lowest rates of positive sIgE against all analyzed cow milk molecules was the 13–18 year-olds. Molecules Bos d 4, Bos d 5, and Bos d 8 showed a significant decrease in positive sIgE rates with age in all analyzed age groups ($p < 0.05$). (All statistical differences with respect to the discussed number of children with sIgE against "the Big 8" food allergen molecules stratified by age can be found in "Table S3: sIgE against "the Big 8" food allergen molecules stratified by age"). Another molecule, Bos d 6, showed a similar decrease with age; however, only the difference between the <12 month-olds and 1–5 year-olds reached statistical significance.

A detailed analysis showed the egg molecule that yielded the highest rates of positive sIgE results was Gal d 2 ($n = 483$; 13.00%), with mean sIgE levels of 4.80 kU_A/L, followed by egg white molecules Gal d 4 (425; 11.44%), Gal d 1 ($n = 422$; 11.36%), and Gal d 3 (321; 8.64%). In the overall ranking of allergen molecules producing positive sIgE results, these egg molecules ranked 10th, 13th, 14th, and 22nd, respectively. The highest mean levels of sIgE against egg molecules were observed for Gal d 1 (6.36 kU_A/L). The lowest rates of sIgE were observed in response to the Gal d 5 ($n = 118$; 3.18%) egg yolk molecule; this allergen component also yielded the lowest mean sIgE levels of 3.07 kU_A/L. Among the analyzed food molecules, egg molecules Gal d 2, Gal d 4, Gal d 1, Gal d 3, and Gal d 5 based on average sIgE concentration ranked 47th, 38th, 33rd, 46th, and 61st place, respectively. The age group with the highest rates of sIgE against egg molecules was the group of infants, whereas the lowest sIgE rates were observed in 13–18 year-olds. Analysis of positive sIgE results for Gal d 1, Gal d 2, Gal d 3, and Gal d 4 of eggs showed a significant decrease in positive reactions with age ($p < 0.05$). This decrease was also observed, but did not reach statistical significance, for Gal d 3 and Gal d 5 between the groups of <12 month-olds and 1–5 year-olds and for Gal d 4 between the groups of 5–13 year-olds and 13–18 year-olds.

Peanut molecules ranked 3rd (Ara h 8), 4th (Ara h 1), 12th (Ara h 2), 15th (Ara h 3), 18th (Ara h 6), 39th (Ara h 9), and 72nd (Ara h 15) in the overall rates of sIgE to allergen molecules. Analysis of mean sIgE levels for all analyzed food molecules showed the highest levels of antibodies to Ara h 2 (15.92 kU_A/L), followed by Ara h 6 (14.43 kU_A/L). The lowest mean sIgE levels were observed for Ara h 15 (1.00 kU_A/L), which ranked third out of the mean sIgE levels for food allergen molecules. The number of positive sIgE results for Ara h 1 decreased with age, whereas those for Ara h 8 were absent in <12 month-olds, were first detected in 1–5 year-olds, and increased in consecutive older age groups. There was a significant ($p < 0.05$) decrease in the number of positive sIgE results for Ara h 1, Ara h 2, and Ara h 3 molecules between 1–5 year-olds and 5–13 year-olds and between 5–13 year-olds and 13–18 year-olds. Conversely, there was a significant ($p < 0.05$) increase in positive sIgE tests to Ara h 8 with age between each of the consecutive age groups.

The ranking of food allergen molecules with the highest positive sIgE test results revealed Gly m 4 to be in 5th place (15.18%), with mean levels of 9.91 kU_A/L. Although these antibodies were not detected in under 12 month-olds, their levels increased considerably in subsequent age groups (ages 1–5, 5–13, and 13–18 years). This increase was statistically significant ($p < 0.05$) between the groups of <12 month-olds and 1–5 year-olds and between the groups of 1–5 year-olds and 5–13 year-olds. The Gly m 6 molecule produced the 20th highest sIgE response rates of 9.18%, with a mean sIgE level of 4.63 kU_A/L; and the Gly m 5 molecule ranked 58th (2.07%), with a mean level of sIgE of 1.92 kU_A/L. Out of soybean molecules, the one that produced the lowest rates of sIgE (1.16%) was Gly m 8 (66th place), with a mean sIgE level of 2.39 kU_A/L. The number of positive sIgE results for the Gly m 6 protein decreased with age, with significant differences between the age groups of 1–5 and 5–13 years ($p = 0.025$) and between the age groups of 5–13 and 13–18 years ($p = 0.001$).

The wheat molecule that produced the highest sIgE response rates (4.45%) in the overall ranking of food molecules was Tri a A_TI (40th place), with a mean sIgE level of 5.78 kU_A/L. The next wheat molecule, Tri a 14, ranked 50th, producing 2.99% positive sIgE responses with a mean sIgE level of 6.52 kU_A/L. The final wheat molecule, Tri a 19, ranked

56th, with 2.35% positive sIgE responses and a mean sIgE level of 4.02 kU_A/L. Analysis of sIgE to wheat allergen molecules showed the highest positive sIgE levels to all evaluated allergen molecules in the <12 month age group, and then the levels of sIgE decreased with age for all analyzed wheat molecules, with the decrease reaching statistical significance for Tri a aA_TI between the groups of <12 month-olds and 1–5 year-olds ($p = 0.001$) and for Tri a 19 between <12 month-olds and 1–5 year-olds (<0.001) and between 1–5 year-olds and 5–13 year-olds ($p = 0.006$).

In terms of sIgE levels to fish molecules, the thornback ray molecule Raj c-parvalbumin ranked last, producing 0.64% positive responses, whereas the highest sIgE rates were due to the Sco s 1 molecule of Atlantic mackerel. The highest mean sIgE levels of fish allergen molecules were produced by Thu a 1 (12.26 kU_A/L). The sIgE rate-to-age analysis of fish allergen molecules showed the highest sIgE levels of all evaluated fish molecules in the 5–13 year age group.

An inverse tendency was observed with shrimp molecules, particularly Pen m 2 and Cra c 6. All evaluated shrimp molecules produced the highest rates of positive results in the age group of 13–18 year-olds. The ranking of sIgE to allergen molecules showed shrimp molecules taking the 60th (Pen m 1), 63rd (Pen m 2), 69th (Pen m 3 and Pen m 4, ex aequo), and 71st (Cra c 6) places. The highest observed mean sIgE levels were for Pen m 1 (9.98 kU_A/L) and Cra c 6 (9.65 kU_A/L), and the lowest were for Pen m 3 (3.16 kU_A/L). The differences in sIgE levels between Pen m 1 ($p = 0.013$) and Pen m 2 ($p < 0.001$) were statistically significant between the age groups of 1–5 year-olds and 5–13 year-olds.

A detailed analysis of tree nut allergens showed the allergen molecule most commonly producing a positive sIgE response was the Cor a 1.0401 molecule of hazelnut, with a mean sIgE level of 12.22 kU_A/L. Among the 30 food allergen molecules most commonly producing a positive sIgE response, there were as many as eight molecules of various tree nuts (Cor a 1.0401, Jug r 4, Cor a 9, Jug r 2, Jug r 1, Ana o 3, Cor a 14, and Jug r 6). The highest sIgE levels were due to the hazelnut molecule Jug r 1 (14.19 kU_A/L), which was characterized as producing the 3rd highest mean sIgE levels in the overall ranking of all allergen molecules. There was a statistically significant increase in the rates of positive sIgE responses to Cor a 0401 and a decrease in sIgE responses to Cor a 9 in all evaluated groups. The ranking of positive sIgE responses to "the Big 8" food allergen molecules has been presented in Table 3, while Table S3 presents sIgE against "the Big 8" food allergen molecules stratified by age.

Table 3. Detailed analysis of the "the Big 8" molecules of food allergens in Polish children.

Place in the Ranking of sIgE Frequency Food Allergen Molecules [a]	Molecule	Protein Family	Allergen Source	Number of All sIgE Determinations Performed	Number and % of "+" sIgE from All sIgE Determinations Performed		Place in the Ranking of Mean sIgE Concentration of Investigated [b]	Mean Concentration [kU_A/L]
			Bos domesticus					
17	Bos d 8	Casein	Cow's milk (*Bos domesticus*)	3715	389	10.47%	25	8.24
24	Bos d 5	β-Lactoglobulin	Cow's milk (*Bos domesticus*)	3715	296	7.97%	35	6.22
25	Bos d 4	α-Lactalbumin	Cow's milk (*Bos domesticus*)	3715	286	7.70%	32	6.57
44	Bos d 6	Serum Albumin	Beef (*Bos domesticus*)	3715	151	4.06%	43	5.28
			Gallus domesticus					
10	Gal d 2	Ovalbumin	Egg white (*Gallus domesticus*)	3715	483	13.00%	47	4.80
13	Gal d 4	Lysozyme C	Egg white (*Gallus domesticus*)	3715	423	11.44%	38	5.32
14	Gal d 1	Ovomucoid	Egg white (*Gallus domesticus*)	3715	422	11.36%	33	6.36
22	Gal d 3	Ovotransferrin	Egg white (*Gallus domesticus*)	3715	321	8.64%	46	4.81
48	Gal d 5	Serum Albumin	Egg yolk (*Gallus domesticus*)	3715	118	3.18%	61	3.07

Table 3. *Cont.*

Place in the Ranking of sIgE Frequency Food Allergen Molecules [a]	Molecule	Protein Family	Allergen Source	Number of All sIgE Determinations Performed	Number and % of "+" sIgE from All sIgE Determinations Performed		Place in the Ranking of Mean sIgE Concentration of Investigated [b]	Mean Concentration [kU$_A$/L]
			Arachis hypogaea					
3	Ara h 8	PR-10	Peanut (*Arachis hypogaea*)	3715	629	16.93%	27	7.99
4	Ara h 1	7/8S Globulin	Peanut (*Arachis hypogaea*)	3715	579	15.59%	20	9.35
12	Ara h 2	2S Albumin	Peanut (*Arachis hypogaea*)	3715	449	12.09%	1	15.92
15	Ara h 3	11S Globulin	Peanut (*Arachis hypogaea*)	3715	410	11.04%	31	6.44
18	Ara h 6	2S Albumin	Peanut (*Arachis hypogaea*)	3715	363	9.77%	2	14.43
39	Ara h 9	nsLTP	Peanut (*Arachis hypogaea*)	3715	167	4.50%	54	3.84
72	Ara h 15	Oleosin	Peanut (*Arachis hypogaea*)	3143	14	0.45%	72	1.00
			Glycine max					
5	Gly m 4	PR-10	Soy (*Glycine max*)	3715	564	15.18%	15	9.91
20	Gly m 6	11S Globulin	Soy (*Glycine max*)	3715	341	9.18%	49	4.63
58	Gly m 5	7/8S Globulin	Soy (*Glycine max*)	3715	77	2.07%	70	1.92
66	Gly m 8	2S Albumin	Soy (*Glycine max*)	3715	43	1.16%	66	2.39
			Triticum aestivum					
40	Tri a aA_TI	Alpha-Amylase Trypsin-Inhibitor	Wheat (*Triticum aestivum*)	3143	140	4.45%	37	5.78
50	Tri a 14	nsLTP	Wheat (*Triticum aestivum*)	3143	94	2.99%	50	6.52
55	Tri a Gliadin	Gliadin	Wheat (*Triticum aestivum*)	300	12	2.40%	39	5.31
56	Tri a 19	Omega-5-Gliadin	Wheat (*Triticum aestivum*)	3143	74	2.35%	53	4.02
			Fish					
37	Sco s 1	β-Parvalbumin	Atlantic mackerel (*Scomber scombrus*)	3143	146	4.65%	12	11.39
38	Cyp c 1	β-Parvalbumin	Carp (*Cyprinus carpio*)	3715	169	4.55%	14	10.10
41	Clu h 1	β-Parvalbumin	Atlantic herring (*Clupea harengus*)	3143	136	4.33%	7	12.07
41	Sal s 1	β-Parvalbumin	Salmon (*Salmo salar*)	3143	136	4.33%	10	11.44
42	Thu a 1	β-Parvalbumin	Tuna (*Thunnus albacares*)	3143	134	4.26%	5	12.26
45	Gad m 1	β-Parvalbumin	Atlantic cod (*Gadus morhua*)	3715	150	4.04%	24	8.31
46	Xip g 1	β-Parvalbumin	Swordfish (*Xiphias gladius*)	3143	118	3.75%	18	9.84
69	Raj c Parvalbumin	α-Parvalbumin	Thornback ray (*Thornback ray*)	3143	20	0.64%	46	4.84
			Crustaceans					
62	Pen m 1	Tropomyosin	Black tiger shrimp (*Penaeus monodon*)	3715	69	1.86%	15	9.98
64	Pen m 2	Arginine Kinase	Black tiger shrimp (*Penaeus monodon*)	3143	49	1.56%	25	8.27
71	Pen m 3	Myosin light chain	Black tiger shrimp (*Penaeus monodon*)	3143	22	0.70%	62	5.16
71	Pen m 4	Sarcoplasmic Calcium Binding Protein	Black tiger shrimp (*Penaeus monodon*)	3143	22	0.70%	30	7.78
73	Cra c 6	Troponin C	Brown shrimp (*Crangon crangon*)	3143	18	0.57%	19	9.63

Table 3. *Cont.*

Place in the Ranking of sIgE Frequency Food Allergen Molecules [a]	Molecule	Protein Family	Allergen Source	Number of All sIgE Determinations Performed	Number and % of "+" sIgE from All sIgE Determinations Performed		Place in the Ranking of Mean sIgE Concentration of Investigated [b]	Mean Concentration [kU$_A$/L]
			Nuts					
1	Cor a 1.0401	PR-10	Hazel (*Corylus avellana*)	3715	883	23.77%	6	12.22
3	Cor a 11	7/8S Globulin	Hazel (*Corylus avellana*)	3715	638	17.17%	52	4.33
10	Jug r 4	11S Globulin	Walnut (*Juglans regia*)	3143	431	13.71%	54	4.05
11	Cor a 9	11S Globulin	Hazel (*Corylus avellana*)	3715	486	13.08%	31	4.49
18	Jug r 2	7/8S Globulin	Walnut (*Juglans regia*)	3715	401	10.79%	37	5.65
21	Jug r 1	2S Albumin	Walnut (*Juglans regia*)	3715	354	9.53%	3	14.19
23	Ana o 3	2S Albumin	Cashew (*Anacardium occidentale*)	3715	324	8.72%	8	11.93
25	Cor a 14	2S Albumin	Hazel (*Corylus avellana*)	3715	316	8.51%	17	9.85
28	Jug r 6	7/8S Globulin	Walnut (*Juglans regia*)	3143	229	7.29%	43	5.08
49	Cor a 8	nsLTP	Hazel (*Corylus avellana*)	3715	119	3.20%	65	2.55
56	Ana o 2	11S Globulin	Cashew (*Anacardium occidentale*)	3143	77	2.45%	66	2.51
59	Jug r 3	nsLTP	Walnut (*Juglans regia*)	3143	70	2.23%	67	2.42
68	Ber e 1	2S Albumin	Brazil nut (*Bertholletia excelsa*)	3715	37	1.00%	42	5.27

[a] Place in the ranking of sIgE frequency of investigated 77 food allergen molecules; [b] Place in the ranking of average concentration sIgE of investigated 77 food allergen molecules.

3. Materials and Methods

3.1. Subjects and Study Design

The rates and levels of sIgE in Polish children were determined retrospectively based on blood test results obtained from selected laboratories located in various regions of Poland. The data were obtained from the Immunology Laboratory, Department of Pathomorphology of the Children's Memorial Health Institute in Warsaw, Poland, from the National Research Institute for Tuberculosis and Lung Diseases, Regional Branch in Rabka-Zdrój, Poland, and the network of Diagnostyka S.A. laboratories, conducting tests in all parts of Poland. The analysis of the prevalence of sIgE was based on the results of the ALEX® test (Macro Array Diagnostics GmbH, Vienna, Austria) of all children (0 to 18 years old) who were diagnosed at the above-mentioned Polish institutions in the period between 2019 and 2022. The obtained data were devoid of information that would allow the patient's identity to be revealed. Apart from the results of the ALEX® test, only the age and sex of each patient were included.

The analysis of the data was conducted as part of the Children's Memorial Health Institute internal project No. S168/2018 (principal investigator B.C.). The study was approved by the local ethics committees at the Children's Memorial Health Institute (approval No. 50/KBE/2018 of 21 November 2018) and Medical University in Lublin (approval No. KE-0254/86/03/2023 of 30 March 2023).

3.2. Multiplex ALEX® Test

The ALEX® test is a third-generation multiplex test for measuring the levels of sIgE against allergen extracts and molecular components simultaneously. In 2017, the ALEX® test allowed for measuring sIgE levels for 282 allergen components from 67 sources: 156 extracts and 126 molecular components. In 2019, the composition of the ALEX® test was altered: several allergens (mainly extracts) were removed, and new allergens (mainly allergen molecules) were added. The name of the test now also includes the numeral 2,

which indicates the altered composition. Since the year 2019, ALEX®2 has helped determine sIgE levels against 295 allergens, including 117 extracts and 178 allergen molecules from various sources, such as foods, animals, plants, molds, and others. Therefore, some of the patients had ALEX® and some had ALEX®2, which is important in terms of the number of tests for individual allergen extracts or molecular components (sIgE rates have been calculated with respect to the total number of tests conducted for the individual extract or molecule). For the purposes of this article, the name ALEX® is used throughout both tests. A detailed analysis of the incidence of sIgE included molecules of "the Big 8" allergens, namely cow's milk (nBos d 4, nBos d 5, nBos d 6, nBos d 8), hen egg (nGal d 1, nGal d 2, nGal d 3, nGal d 4, nGal d 5), wheat (nTri a aA_TI, r Tri a 14, nTri a Gliadin, rTri a 19), peanut (nAra h 1, rAra h 2, nAra h 3, nAra h 6, rAra h 8, rAra h9, rAra h 15), tree nut (rCor a 1.0401, nCor a 11, nJug r 4, nCor a 9, nJug r 2, nJug r 1, rAna o 3, nCor a 14, nJug r 6, rCor a 8, rAna o 2, rJug r 3, nBer e 1) fish (rSco s 1, rCyp c 1, rClu h 1, rSal s 1, rThu a 1, nGad m 1, rXip g 1, rRaj c Parvalbumin) and peanut–shellfish (nPen m 1, rPen m 2, rPen m 3, rPen m 4, rCra c 6). In accordance with the normal sIgE ranges provided by the manufacturer, a test was considered positive if its result was ≥0.3 kilounits of allergen sIgE per liter (kU_A/L). The results were exported from MADx Raptor Software to Excel spreadsheets. The results obtained with these tests are quantitative and are expressed in units of IgE response (kU_A/L).

3.3. Statistical Analysis

Statistical analysis was performed using IBM® SPSS® 24.0.0.0. Statistics. Chi-squared tests were used to define statistically significant correlations between qualitative variables. To measure the correlation between variables, Spearman's rank correlation coefficient was used. Continuous variables were summarized using mean values, while variability around mean values was reported in terms of standard deviations (SD). A p-value < 0.05 was considered statistically significant.

4. Discussion

The three most common allergens in Poland, regardless of whether IgE was assayed against extracts or molecules of food allergens, were peanut, hazel, and apple (in different order depending on the ranking).

Allergen extracts

There have been many sensitization assessments based on allergen extracts conducted over the years [3,4]. In our study, the most common food allergens detected with the use of allergen extracts were peanut, hazelnut, and apple. On the other hand, the allergen extracts producing the lowest rates of positive sIgE tests were strawberry, oregano, and thornback ray. The EuroPrevall study [3] showed geographic differences in the prevalence of food sensitization and food allergy in school-age children (7–10 years) across many European cities (Zurich, Madrid, Athens, Utrecht, Vilnius, Lodz, and Reykjavik). As part of that study, 24 foods considered to be the most sensitizing to children or most commonly consumed in the countries involved were selected for sIgE analysis. These were eggs, cow milk, fish, shrimp, peanuts, hazelnuts, walnuts, peach, apple, kiwi, melon, banana, tomato, celery, carrot, corn, lentils, soybeans, wheat, buckwheat, sesame, mustard, sunflower, and poppy seed. None of the cities involved in the EuroPrevall study reported peanuts to be among the top three sensitizing allergens, whereas in our study peanuts ranked first. The EuroPrevall study results, not unlike ours, showed hazelnuts to be among the allergens with the highest sIgE rates. In our study, it ranked second, and in the EuroPrevall study, it ranked first in Lodz, second in Utrecht, and third in Zurich and Vilnius. One of the most common food allergens was banana, which ranked first in Zurich and Madrid, second in Athens and Vilnius, third in Utrecht and Reykjavik, and fourth in Lodz. In our study, bananas ranked as the 73rd most common food allergen out of all evaluated allergen extracts [3].

Apple, which ranked third in our analysis, did not rank among the top three sensitizing allergens in any of the cities taking part in the EuroPrevall study. In our study, the rate of

sensitization to apples was 23.60%, whereas in the EuroPrevall study, it ranged from 2.05% to 11.95% (depending on the city). The rates of cow milk and egg sensitization were also higher in our study than those of shrimp (with the latter common in the Mediterranean and in Reykjavik). Despite the fact that peach allergens ranked second in Lodz (which is located in Poland—the country where our study was conducted) and Madrid, they ranked as the 26th most common cause of sensitization in our study [3].

In summary, our analysis, which was based on a very broad spectrum of allergen extracts, showed considerable differences in the sensitization profiles of Polish children in comparison with those reported by other authors.

Allergen molecules

Expanding the diagnostics to include allergen molecules allows for a more detailed assessment of the sensitization profile. Molecular diagnostics helps detect and quantify serum sIgE for a specific allergen molecule. An allergen extract is a collection of multiple proteins of a three-dimensional structure composed of polypeptide chains, namely allergen molecules, which are considered to be allergens proper in this type of diagnostic. Since various allergen molecules are of varied diagnostic and clinical significance, molecular diagnostics offers a much more accurate assessment of sensitization and its importance for the patient, along with the natural course of the given allergic condition [5]. Allergen component-based diagnostics increases assessment specificity, which helps assess primary sensitization, and increases test sensitivity, particularly if the allergens present in the extract are of insufficient quantity or are destroyed during the process of preparing the extract for analysis [6].

Our analysis of the rates of sIgE in food allergen molecules showed panallergen proteins of the PR-10 subfamily to produce the highest sIgE rates. The top 10 food allergen molecules producing the highest sIgE responses included as many as six proteins from this subfamily; these were Cor a 1.0401, Mal d 1, Ara h 8, Gly m 4, Api g 1, and Dau c 1. Allergy to these molecules is likely to be a result of cross-reactivity to a primary birch allergy (Bet v 1 molecule) [12]. A multicenter study by Kiewiet et al. showed that cross-reacting proteins of the PR-10 family, such as Cor a 1.0401, Mal d 1, and Pru p 1, are among the molecules most commonly bound by sIgE in cohorts with high rates of allergy to Bet v 1, due precisely to cross-reactivity [7]. Likewise, the EuroPrevall study showed that a hazelnut allergy, one of the most common allergies in various European cities, may be associated with cross-reactivity to birch pollens, which are common in Central/Northern Europe. One explanation for such high rates of proteins from the PR-10 family among the top ten allergen molecules may be provided by Westman et al. [13]. Those authors reported sIgE reactivity to PR-10 proteins to have a hierarchic intrarelationship, with Bet v 1 producing the highest sIgE reactivity and the following allergens producing gradually decreasing reactivity: Bet v 1 > Mal d 1 > Cor a 1.0401 > Ara h 8 > Pru p 1 > Aln g 1 > Api g 1 > Act d 8 > Gly m 4. In our study, the order of sIgE reactivity was only slightly different and was as follows: Bet v 1 > Cor a 1.0401 > Mal d 1 > Ara h 8 > Gly m 4 > Api g 1 > Dau c 1.

In summary, our analysis of sIgE reactivity profiles to allergen molecules showed the highest rates of sIgE to PR-10 proteins, which may be associated with environmental exposure to birch pollen in Poland (birch constitutes 7% of the 647,000 hectares of total forested area in Poland), as reported in the Forestry Almanac of 2021 issued by the Central Statistical Office [14].

sIgE reactivity to "the Big 8" molecules stratified by age

Cow's milk is one of the foods most commonly consumed by children. Therefore, sensitivity and allergy to cow milk proteins are the subject of a number of studies [7,15]. Our analysis showed the rates of sIgE reactivity to all cow milk proteins to be the highest in <12 month-olds and the lowest in 13–18 year-olds. Our analysis showed the highest rates of sIgE reactivity (10.47%) to cow milk casein (Bos d 8) and the lowest (4.06%) to Bos d 6. In contrast, Kiewiet et al., who assessed various European pediatric cohorts with the ImmunoCap ISAC method in a multicenter study, demonstrated the rates of allergy to cow

milk allergen molecules to be much lower, in most cases under 1% (with the exception of one Spanish group from Sabadell, where sIgE rates were 1.4–2.0%) [7]. In a study conducted in a Swedish cohort, the rates of sIgE reactivity to Bos d 4, Bos d 5, Bos d 6, and Bos d 8 increased with age [7], whereas analysis of our study cohort showed a significant decrease in the rates of positive sIgE responses to Bos d 4, Bos d 5, and Bos d 8 molecules with increasing age.

As with cow milk proteins, our analysis of sIgE reactivity to egg molecules showed a significant decrease in positive test results for Gal d 1, Gal d 2, Gal d 3, and Gal d 4 molecules with increasing age. In our study, the allergy rates to ovalbumin were 13.0% and to ovomucoid 11.4%. Interestingly, in the study by Kiewiet et al., the thermally stable egg allergen Gal d 1 was the most prominent in the Dutch study population over 1 year of age, whereas, in our study population, the highest rates of this allergen were observed in the under-12 month-olds [7]. Like in our study, Lin et al. reported sensitization rates to hen egg molecules to be decreasing with age in children with atopic dermatitis. The rates of serum sIgE to ovalbumin and ovomucoid in various age groups analyzed in that study were the highest in 0–2 year-olds and decreased with age [16].

The peanut molecules that showed the highest rates of sensitization in Polish children were Ara h 8 and Ara h 1, with an age-dependent sensitization profile. The rates of Ara h 8 sensitization increased significantly with age. Starting from absent sIgE (0.00%) to Ara h 8 in the group of <12 month-olds, subsequent age groups showed increasing rates of sIgE to reach the highest rates at 31.0% in the group of 13–18 year-olds. We believe that the increase in Ara h 8 sensitization rates with age observed in our cohort may be associated with sensitization to the birch Bet v 1 molecule. This is associated with the phenomenon of becoming sensitized to PR-10 proteins with increasing age, which may be related to environmental exposure to birch pollen. Studies conducted by other authors have shown considerable differences in the rates of sensitization to various peanut molecules, depending on the country. Patients with peanut allergy in the United States and Sweden were shown to have higher rates of allergen storage proteins Ara h 1–3 in comparison with those in patients from Spain, who showed higher rates of sensitization to lipid transfer protein Ara h 9 [17]. Kiewiet et al. demonstrated that Ara h 9 sensitization rates were higher in the cohort from southern Europe, like Spain (Gipuzkoa) and Italy (Rome), in comparison with those in the Swedish cohort [7]. In our study, the molecule with the highest sensitization rates in children up to 5 years old was Ara h 1 (26.7% in <12 month-olds and 19.7% in 1–5 year-olds). These results are partly consistent with those from studies conducted in English and Swedish populations, which showed Ara h 1 to be the molecule with the highest sensitization rates in 4 year-old children sensitized to peanuts. However, in contrast with Swedish studies, which showed a trend towards Ara h 1 sIgE rates increasing with age [7], our study showed the rates of sIgE not only to Ara h 1 but also to Ara h 2 and Ara h 3 to decrease with age. The differences in Ara h sensitization profiles may be due to the amounts consumed, the age of peanut introduction, dietary habits, and the different ways in which peanuts are prepared for consumption in various countries. The high peanut sensitization rates in Sweden, the United States, and other western countries are suspected to be a result of the fact that peanuts are most often consumed roasted (roasting increases the sensitizing potential of Ara h 1 and Ara h 2 molecules) or may expose proteins, such as Ara h 15 (oleosin), that would otherwise be enveloped in the fat that constitutes energy stores for the sprouting plants [18–20].

Our analysis of soybean-sIgE rates showed the Gly m 4 molecule to yield the highest positive sIgE rates of 15.2%. Similar results were obtained in a German cohort, where Gly m 4 was the most prevalent (10.5%) sensitizing soybean molecule [7]. In our study, Gly m 4 (as well as another PR-10 subfamily molecule, Ara h 8) produced no positive sIgE results in the youngest evaluated age group of up to 12 month-old infants; however, a statistically significant increase in the sIgE rates for this molecule was observed with increasing age. The observed increase in the rates of sensitization to Gly m 4 (as well as to Ara h 8) with age may be associated with the increase in the rates of birch allergen sensitization with

age. Studies show that a majority of patients with birch allergy are sensitized to the main allergen of birch pollen (Bet v 1), the main allergen of apple (Mal d 1), and soybeans (Gly m 4) [12]. Extended analysis of a correlation between Bet v 1 and Gly m 4 sIgE rates in our study group revealed only 0.88% of children (5/564) with positive sIgE reactivity to Gly m 4 who had no positive sIgE reactivity to Bet v 1 (data not shown). Like us in our study, Westman et al. also observed a close correlation between sIgE reactivity to Gly m 4 and Bet v 1 and the number of other IgE-reactive PR-10 proteins. Those authors showed that 100% of children's sIgE reactivity to Gly m 4 also had sIgE to Bet v 1, Mal d 1, and Cor a 1.0401 [13].

In our study, the wheat molecules with the highest sIgE rates were Tri a A_TI (4.45%), followed by Tri a 14 (2.99%), and Tri a 19 (2.35%). The highest rates of positive sIgE for all evaluated allergen molecules were observed in the <12 month-olds and the lowest ones in 13–18 year-olds. Kiewiet et al. reported sIgE rates ranging from 0.1% in the Swedish cohort to 0.4% in the Norwegian cohort, and no diminished sensitization to this molecule with age was observed. In the Swedish cohort, the groups of 4 year-olds, 7–12 year-olds, and 15–16-year-olds had a steady Tri a 14 sensitization rate of 0.1%. The Norwegian cohort showed sIgE levels to Tri a 19.0101 that were similar to those in our study, although they differed between age groups, with the highest sIgE rates of 3.3% in 15–16 year-olds and lower rates in 7–12 year-olds (3.0%) [7].

One of the most important fish proteins is parvalbumin. Our analysis of fish allergen molecule-sIgE rates with respect to age showed the highest rates of sIgE among all evaluated fish molecules found in the 5–13 year age group. The rates of sIgE in fish ß-parvalbumin ranged from 3.75% to 4.65% (with the lowest ß-parvalbumin sIgE rates for swordfish and the highest for Atlantic mackerel, carp, Atlantic herring, salmon, tuna, and cod). The ALEX® test allows for testing two parvalbumin variants: α and β. Despite the high homology between α- and β-parvalbumin, the allergenicity of fish α-parvalbumin is generally considered to be very low and well tolerated. Nonetheless, there are case reports demonstrating an allergic reaction to α-parvalbumin [21,22]. Out of all fish molecules evaluated in our study, the lowest sIgE rates were found in thornback ray α-parvalbumin. A study by Kiewiet et al. showed cod parvalbumin (Gad c 1) sensitization rates of 3.2% in British 4 year-olds [7]. However, other study cohorts (Spanish, Italian, Swedish, and Norwegian) in that study showed sensitization rates of 0.1–0.7%. The authors of that study suggested that this difference between the British cohort and cohorts from other countries may be a reflection of dietary habits. According to a European Market Observatory for Fisheries and Aquaculture Products (EUMOFA) report, the mean fish consumption in the European Union was 25.1 kg per person in 2018, with Spain, Italy, and Sweden showing higher consumption of approximately 45 kg, 28 kg, and 27 kg/person, respectively, whereas Poland has a considerably lower fish consumption of 15 kg/person [23]. Therefore, not only geographical regions but also the differences in dietary habits between countries should be evaluated to better understand and manage fish sensitization.

The observed high rates of shrimp sensitization may be attributed to high shrimp consumption [24]. However, despite geographical similarities, the protein sensitization profile in shrimp allergy shows variations. The major shrimp allergens among Hong Kong subjects were troponin C (Pen m 6) and glycogen phosphorylase (Pen m 14, 47.1%), tropomyosin (Pen m 1, 41.2%), and sarcoplasmic-calcium binding protein (Pen m 4, 35.3%), while those among Thai subjects were Pen m 1 (68.8%), Pen m 6 (50.0%), and fatty acid-binding protein (Pen m 13, 37.5%) [25]. In an Italian study conducted on individuals (aged 2–79 years) with a history of adverse reactions to shrimp, sensitization to Pen m 1 was predominant in the molecular sensitization profile; moreover, hypersensitivity to Pen m 1 was closely associated with an increased risk of a severe reaction to mollusks [26]. Shrimp tropomyosin (Pen m 1) is the leading IgE-binding protein in hypersensitivity to this allergen. Our study showed the highest Pen m 1–sIgE rates (1.86%) and levels (9.98 kU_A/L) out of all five evaluated shrimp molecules. Shellfish allergy often develops in late childhood or during adolescence [27]. In our study, the rates of sIgE in Pen m 1 and Pen m 2 showed a

significant increase between the age groups of 1–5 and 5–13 years, and all evaluated shrimp molecules showed the highest sIgE rates in the age group of 13–18-year-olds. Kiewiet et al. also reported increasing rates of sensitization to Pen m 1 with age in a Norwegian cohort (3.4% in 7–12 year-olds; 3.7% in 15–16 year-olds). Although Pen m 1 sIgE rates were not as high in the Swedish cohort, they still showed a tendency to increase with age [7]. Protein profiles in shrimp with hypersensitivity may differ depending on the population, which is likely due to differences in genetics, environmental exposure, and dietary preferences. Therefore, it is important to determine the molecular profile for individual populations to ensure an optimal protein profile in shrimp allergy diagnostic tests [7,24,26,27].

Our study showed the highest rates of tree nut allergen sIgE to be to hazelnut molecule Cor a 1.0401 (23.77%). Importantly, as many as eight various nut molecules (Cor a 1.0401, Jug r 4, Cor a 9, Jug r 2, Jug r 1, Ana o 3, Cor a 14, and Jug r 6) ranked among the 30 most common food allergen molecules. As in the case of peanuts, there was an observable age-related decrease in positive sIgE reactivity to molecules from the family of storage proteins, whereas the Cor a 1.0401 protein, which is a homolog of Bet v 1, showed an inverse tendency. We observed the highest mean sIgE levels for the hazelnut molecule Jug r 1, and this molecule ranked third in the general ranking of mean sIgE levels among all allergen molecules. One of the possible reasons for such a high Jug r 1-sIgE level may be the effect of antibodies to cross-reactive carbohydrate determinants (anti-CCDs) since this molecule is glycosylated and the ALEX® test contains its natural form. These antibodies may be, in part, blocked by the anti-CCD inhibitor that is routinely used in ALEX® [28]. However, the effect of high CCD-sIgE levels on Jug r 1 results cannot be excluded.

5. Conclusions

The three most common allergens in Poland, regardless of whether IgE was assayed against extracts or molecules of food allergens, were peanut, hazel, and apple (in different order depending on the ranking). A detailed analysis of sensitization to the extracts and molecules of main food allergens based on the results of a multiplex ALEX® test demonstrated the sensitization profile in Polish children (including molecular sensitization, particularly the "the Big 8" food allergen molecules), which shows considerable differences in comparison with those in other countries. Serum sIgE analysis of children from all regions of Poland revealed a food allergen molecular sensitization profile that changes with age.

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Informed Consent Statement: Informed consent was obtained from all subjects tested at the Immunology Laboratory at the Children's Memorial Health Institute. Patient consent from other subjects was waived because the results were obtained from a database and were devoid of information that would allow for the patient's identity to be revealed.

Data Availability Statement: Data supporting reports are available as additional appendices in Tables S1–S3 and from the corresponding.

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Sensitization profile in children with celiac disease assessed with the use of a multiplex molecular diagnostic technique

Izabela Knyziak-Mędrzycka[1], Bożena Cukrowska[2*], Wojciech Nazar[3], Joanna Beata Bierła[2,4], Kamil Janeczek[5], Paulina Krawiec[6], Weronika Gromek[7], Mariusz Wysokiński[8], Ewa Konopka[2,4], Ilona Trojanowska[2,4], Sylwia Smolińska[9], and Emilia Majsiak[10*]

[1] Outpatient Allergology Clinic, the Children's Memorial Health Institute, Aleja Dzieci Polskich 20, 04-730 Warsaw, Poland. (I.K.-M.) ORCID 0000-0003-2395-4096, i.knyziak-medrzycka@ipczd.pl
[2] Department of Pathomorphology, The Children's Memorial Health Institute, Aleja Dzieci Polskich 20, 04-730 Warsaw, Poland; (J.B.B.) ORCID 0000-0002-5772-5091; j.bierla@ipczd.pl; (E.K.) ORCID 0000-0002-5686-1112, e.konopka@ipczd.pl, (I.T.) ORCID 0000-0002-6573-7682, i.trojanowska@ipczd.pl; (B.C.) ORCID 0000-0001-6887-0094; b.cukrowska@ipczd.pl
[3] Faculty of Medicine, Medical University of Gdansk, Marii Sklodowskiej-Curie 3a, 80-210, Gdansk, Poland. (W.N.) ORCID 0000-0002-8448-0800, wojciech.nazar@gumed.edu.pl
[4] Department of Clinical Biochemistry, The Children's Memorial Health Institute, Aleja Dzieci Polskich 20, 04-730 Warsaw, Poland; (J.B.B.) ORCID 0000-0002-5772-5091; j.bierla@ipczd.pl; (E.K.) ORCID 0000-0002-5686-1112, e.konopka@ipczd.pl, (I.T.) ORCID 0000-0002-6573-7682, i.trojanowska@ipczd.pl
[5] Department of Pulmonary Diseases and Children Rheumatology, Medical University of Lublin, Profesora Antoniego Gębali Street, 20-093 Lublin, Poland, (K.J.) ORCID 0000-0002-8163-873X, kamil.janeczek@umlub.pl
[6] Department of Pediatrics and Gastroenterology, Medical University of Lublin, Profesora Antoniego Gębali Street, 20-093 Lublin, Poland, (P.K.) ORCID 0000-0002-2197-2197; paulina.krawiec@umlub.pl
[7] Polish-Ukrainian Foundation of Medicine Development, Nałęczowska 14, 20-701 Lublin, Poland; (W.G.) ORCID 0000-0001-5890-8159, weronikaa.gromek@gmail.com
[8] Department of Basic Nursing, Chair of Development in Nursing, Faculty of Health Sciences, Medical University, Staszica 4/6, 20-081 Lublin, Poland; (M.W.) ORCID 0000-0001-9939-2490, mariusz.wysokinski@umlub.pl
[9] Department of Clinical Immunology, Wroclaw Medical University, Parkowa 34, 51-616 Wroclaw, Poland, (S.S.) ORCID 0000-0001-6736-2696, sylwia.smolinska@umw.edu.pl
[10] Department of Health Promotion, Chair of Nursing Development, Faculty Health of Sciences, Medical University of Lublin, Staszica 4/6, 20-081 Lublin, Poland, (E.M.) ORCID 0000-0003-3638-9292; emiliamajsiak@umlub.pl
* **Correspondence:** emiliamajsiak@umlub.pl; b.cukrowska@ipczd.pl;

Abstract: Celiac disease (CD) often co-occurs with autoimmune conditions or genetic syndromes, but there are few studies on the co-existence of CD and immunoglobulin E (IgE)-mediated allergies. The purpose of this study was to assess sensitization profiles with a multiplex ALEX®2 test in pediatric CD patients. The study included 108 children with *newly* diagnosed CD. The presence of specific IgE (sIgE) towards allergen extracts and/or molecules was detected in 49.1% of children. Most children (41.3%) were allergic to both inhalant and food allergens. The three most common aeroallergens (timothy pollen, ryegrass, silver birch) were molecules Phl p 1, Lol p 1 and Bet v 1. The most common food allergens (hazelnut, apple and peanut) were Cor a 1, Mal d 1 and Aza h 8 molecules belonging to PR-10 subfamily. The tested patients were not sensitized to cereal allergens containing gluten. Spearman's rank correlation analysis among sensitized patients showed a significant positive relationship ($r = 0.31$) between the patients' age of and the number of positive sIgE (≥0.3 kUA/L) for inhalant allergen molecules ($p = 0.045$). In conclusion, the current study indicates the co-occurrence of IgE-related sensitization in children with celiac disease, both food and inhalational. These studies highlight the need to increase attention to the diagnosis of IgE allergy in patients with CD, which may contribute to better care for them and lead to a better understanding of the relationship between CD and IgE allergy.

Keywords: celiac disease, allergy, sensitization, specific immunoglobulin E, multiplex testing, molecular allergy diagnosis

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1. Introduction

Celiac disease (CD) is one of the most common gluten-induced food intolerances, affects people of all ages, and manifests with both gastrointestinal and extra-gastrointestinal symptoms [1]. The gastrointestinal symptoms of CD include primarily abdominal pain, bloating, diarrhea, or constipation. The extra-gastrointestinal symptoms may include chronic fatigue, headaches, anemia, and elevated liver enzyme levels. Although CD has long been known to co-occur with autoimmune conditions (such as type 1 diabetes mellitus, autoimmune thyroid or liver diseases) or genetic syndromes (such as Down syndrome, Turner syndrome, Williams syndrome) [2], there have been few studies on the co-existence of CD and immunoglobulin E (IgE)-mediated allergies [3]. In CD, the only factor that induces autoimmunity mechanisms are immunoreactive gluten peptides, whereas in allergies, the causative factors may be either inhalant or food allergens (the latter including gluten peptides). However, both types of conditions are associated with abnormal function of the immune system in general, and regulatory T-cells, which help maintain immune tolerance and prevent both autoimmune and allergic conditions [4-6]. The pathophysiology of CD involves activation of helper T-cells 1 (Th1) followed by secretion of pro-inflammatory cytokines and induction of the production of auto-antibodies directed against the enzyme tissue transglutaminase (tTG) [7]. The pathophysiology of IgE-mediated allergies involves helper T-cell 2 (Th2) activation with a different cytokine profile than in autoimmunity, which leads to overproduction of allergen specific IgE (sIgE), sensitization process activation with the release of allergic reaction mediators by basophils and mast cells [6]. For these reasons, CD and IgE-mediated allergy are believed to be mutually exclusive, and the nature of the condition (whether autoimmune or allergic) is determined by the imbalance in Th1 and Th2 activation.

Despite the seemingly mutually exclusive pathogeneses of CD and IgE-mediated allergy, there have been studies indicating co-existence of both conditions. A systematic review of studies by our research group [3] revealed a handful of original articles [8-11] whose authors reported that patients with CD may develop an allergy that manifests in a similar way to that in non-CD subjects, i.e. with atopic dermatitis, vomiting, urticaria, angioedema, anaphylactic shock, etc. In those studies, CD patients were most often allergic to wheat, which may be due to the fact that this allergen was the one most frequently studied.

Co-occurrence of IgE-mediated allergy or sensitization in patients with CD has not been fully elucidated [6]. There is a need for studies on the comorbidity of CD and IgE-mediated allergy, not the least due to the fact that symptoms of CD often persist, despite the patients' restrictive gluten-free diet – the only effective treatment [12]. This phenomenon may be due to an undiagnosed allergy to food and inhalant allergens. Therefore, the purpose of this study was to comprehensively assess sensitization profiles to allergen extracts and molecules with a multiplex ALEX®2 test in pediatric patients with CD.

2. Materials and Methods

2.1. Subjects and Study Design

The study included children with newly diagnosed CD at the Department of Gastroenterology, Hepatology, Nutritional Disorders and Pediatrics, the Children's Memorial Health Institute, Warsaw, Poland and the gastroenterology outpatient clinic at the University Children's Hospital in Lublin, Poland. CD diagnosis was established according to European Society for Paediatric Gastroenterology Hepatology and Nutrition (ESPGHAN) guidelines [13]. In all children immunoglobulin A (IgA) anti-tTG and total IgA levels were tested (Thermo Scientific Phadia GmbH, Freiburg, Germany). In children with IgA deficit anti-tTG-IgG or anti-deaminated gluten peptides (DGP)-IgG were measured. Patients with high anti-tTG-IgA levels (>100 AU/ml, e.g. 10 times higher than the upper limit of normal) and positive anti-endomysial antibody tests (Euroimmun, Lübeck, Germany) (from a different blood sample) were diagnosed with CD without intestinal biopsy and a histopathological examination of small-intestinal specimens. In other patients biopsies were taken during endoscopy (at least 1 specimen from the duodenal bulb and 4 specimens from the distal part of the duodenum) and histological analyses were performed by two independent pathologists using a modified Marsh-Oberhuber classification [14]. CD was recognized in children with positive antibodies (≥10 AU/ml) and histological changes described at least as Marsh 2.

Finally, 108 out of 123 children were included into the study. Patients were excluded because of the lack of a written informed consent (n = 2) or of a proper CD diagnostic procedure (n = 18). Serum sensitization profiles were performed with the use of a multiplex molecular diagnostic technique (ALEX®2 test, MacroArray Diagnostics GmbH, Vienna, Austria) in the same sera that were used for CD diagnosis (anti-tTG test).

The study was approved by a local Ethics Committee (KE-0254/222/10/2023) and a written informed consent was obtained from each patient's parents or guardians, and from patients aged ≥ 16 years old, with respect to the use of their blood for scientific purposes.

2.2. *ALEX®2 test*

The ALEX®2 test (Macro Array Diagnostics GmbH, Vienna, Austria) is a third-generation multiplex test for measuring the levels of sIgE against allergen extracts and molecular components simultaneously. ALEX®2 helps determine sIgE levels against 295 allergens, including 117 extracts and 178 allergen molecules from various sources, such as foods, animals, plants, and molds. The tested extracts and molecules were classified into 3 allergen groups: inhalant, food, and other (with the last group comprising allergens whose route of exposure is other than via inhalation or ingestion, for instance via insect stings or via direct dermal contact, such as *Malassezia sympodialis*). The results were exported from MADx Raptor Software to Excel spreadsheets. The results obtained with these tests are quantitative and are expressed in kilounits of sIgE per liter (kU_A/L). In accordance with the normal sIgE ranges provided by the manufacturer, a test was considered positive if its result was ≥0.3 kU_A/L.

2.3. *Statistical analysis*

Statistical analysis was performed using IBM® SPSS® 24.0.0.0. Statistics. Relationships between qualitative variables were tested with Spearman's rank coefficient. The Mann–Whitney U test was used to test for differences in the number of positive results between females and males. The level of statistical significance was set at $p = 0.05$.

3. Results

3.1. *Patient characteristics*

Test results from 108 children with CD, including 63% girls and 37% boys, were analyzed. The mean age was 9.4 years. The age of subjects ranged from 0.9 to 17.4 years. Out

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of all evaluated patients with CD, the diagnostic protocol without duodenal biopsy was followed in nearly 78% of cases. IgA deficit was detected in 2.8% of study subjects (Table 1). Patients with CD exhibited predominantly gastrointestinal manifestations: abdominal pain (n=39; 36.1%), abdominal distension (n=37; 34.3%), diarrhea (n=32; 29.6%), and constipation (n=6; 5.6%). Other manifestations included weight loss (n=21; 19.4%), growth retardation (n=10; 9.3%), and other, listed in Table 1.

Table 1. Characteristics of the study group.

		Number	%
Sex	Female	68	63%
	Male	40	37%
Age (in years)	Mean	9.4	
	Standard deviation	4.43	
	Median	9.3	
	Minimum	0.9	
	Maximum	17.4	
CD diagnosis according to ESPGHAN guidelines	Without biopsy	84	78%
	With biopsy	24	22%
IgA deficiency	Yes	3	3%
	No	105	97%
Symptoms[1]	Abdominal pain	39	36.1%
	Diarrhea	32	29.6%
	Abdominal distension	37	34.3%
	Constipation	6	5.6%
	Growth retardation	15	9.3%
	Weight loss	21	19.4%
	Anemia	15	13.9%
	Skin changes	13	12.0%
	Other[2]	19	17.6%

ESPGHAN = European Society for Paediatric Gastroenterology Hepatology and Nutrition
[1] Multiple symptoms have been observed in individual patients;
[2] Other symptoms include headaches, chronic fatigue, hypersensitivity, and hypertransaminasemia.

3.2. Sensitization profile

Multiplex tests revealed sIgE to allergen extracts and/or molecules in 53 out of 108 (53/108) children with CD (49.1%). These children showed sensitization to 145 (44 extracts and 101 molecules) out of all 295 extracts and molecules assessed with the multiplex test.

A large subgroup of children with CD (22/53; 41.5%) showed simultaneous sensitivity to food and inhalant allergens (Figure 1). A majority of children (37/53; 69.8%) showed sensitivity to three or more allergens, whereas sIgE to a single allergen (extract and/or molecule) was observed in twelve (22.6%) and to two or more allergens in four children (7.5%).

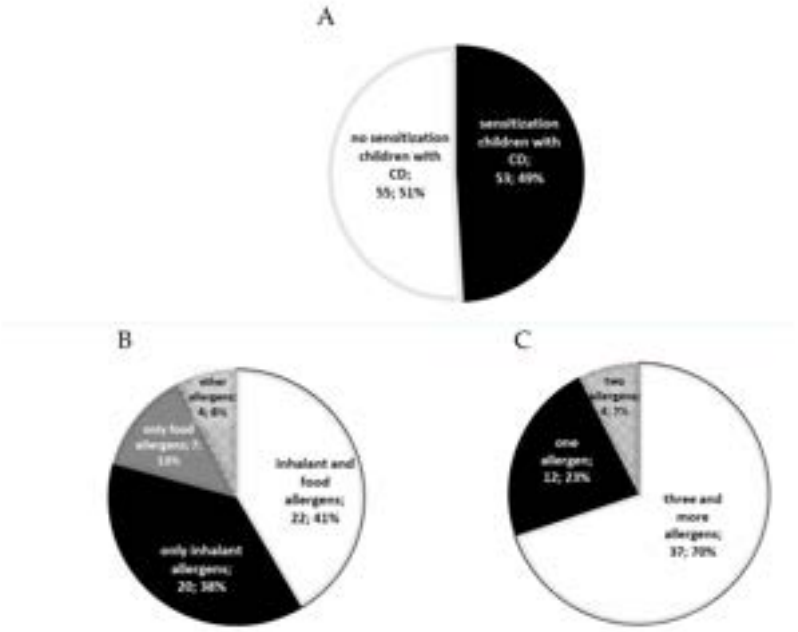

Figure 1. A. Prevalence of sensitization in children with CD. B. Prevalence of sensitization to food and inhalant allergens in children with CD. C. The number of sensitizing allergens in children with CD. Sensitization was assessed using the ALEX®2 test with the cut-off point of ≥0.3 kUA/L for a positive result.

Inhalant allergens produced the highest mean sIgE levels, with the highest levels produced by black alder (46.71 kU$_A$/L) and timothy grass (44.35 kU$_A$/L) (Poaceae family) molecules and by *Dermatophagoides farinae* (42.99 kU$_A$/L) dust mite (NPC2 family) molecules. The highest mean sIgE levels to food allergens were produced by salmon (β-parvalbumin), muskmelon (profilin), and apple (PR-10) molecules (12.38 kU$_A$/L, 11.26 kU$_A$/L, and 9.09 kU$_A$/L, respectively). The 10 most sensitizing allergens in children with CD included 7 inhalant and 2 food allergens. Table 2 shows a list of the common sensitizing allergens in children with CD.

The presence of sIgE to inhalant allergens (extracts and molecules) was detected in 38.8% of children with CD (48/108). Most of those children (37/42; 88.1%) showed sensitization to more than one inhalant allergen. Analysis of all extracts and molecules demonstrated the most common sIgE in children with CD to be against timothy (26.9%), perennial ryegrass (24.1%), and silver birch (18.5%) pollens (Table 2.). These three most common inhalant allergens are allergen molecules Phl p 1, Lol p 1 (both from the beta-expansin subfamily), and Bet v 1 (PR-10 subfamily). Over one half of the children with CD who showed sensitization to inhalant allergens (22/42; 52.4%) had a coexisting sensitization to

food allergens. Sensitization to food allergens (extracts and molecules) was detected in over one quarter of the study group (29/108; 26.8%), and three quarters of children (22/29; 75.9%) showed sIgE to more than one food allergen. The most common food allergen was hazelnut (15/108; 13.95%), which placed as low as eighth in terms of prevalence among all the analyzed allergens listed in Table 2. Subsequent in the list of sensitizing agents were apple and peanut allergens (with these allergens placing twelfth and sixteenth, respectively; Table 2). These three highest-placing food allergens were molecules (Cor a 1, Mal d 1, Ara h 8) from the PR-10 subfamily. Over three quarters of the children with CD and sensitization to food allergens (22/29; 75.9%) showed co-sensitization to inhalant allergens.

Evaluated children with CD had no sIgE against 150 out of all 295 allergens (73 extracts and 77 molecules) tested, among which were allergens of all gluten-containing cereals, including wheat (*Triticum aestivum*) molecules (Tri a aA_TI (alpha-amylase trypsin inhibitor), Tri a 14 (nsLTP), and Tri a 19 (Omega-5-gliadin)), as well as spelt (*Triticum spelta*), cultivated rye (*Secale cereale*), barley (*Hordeum vulgare*), and oat (*Avena sativa*) extracts.

Table 2. The most common sensitizing allergens in children with CD assessed with an ALEX®2 test (cut-off point for a positive result was ≥0.3 kUA/L).

	Allergen	Molecule or extract	Route of exposure	Family of molecules	n (%)	Mean sIgE levels (in kUA/L)
1	Timothy grass	Phl p 1	Inhalation	Beta-expansin	29 (26.9%)	18.08
2	Perennial ryegrass	Lol p 1	Inhalation	Beta-expansin	26 (24.1%)	16.03
3	Silver birch	Bet v 1	Inhalation	PR-10	20 (18.5%)	18.20
4	Bermuda grass	Extract	Inhalation		18 (16.7%)	3.96
5	Bermuda grass	Cyn d 1	Inhalation	Beta-expansin	18 (16.7%)	7.02
6	Beech	Fag s 1	Inhalation	PR-10	16 (14.8%)	12.26
7	Timothy grass	Phl p 2	Inhalation	Expansin	16 (14.8%)	16.39
8	Hazelnut	Cor a 1.0401	Ingestion	PR-10	15 (13.9%)	7.57
9	Hazel	Cor a 1.0103	Inhalation	PR-10	15 (13.9%)	13.36
10	*Dermatophagoides pteronyssinus*	Der p 23	Inhalation	Chitinase class III	14 (13.0%)	15.18
11	Timothy grass	Phl p 5.0101	Inhalation	Group 5/6 grass	14 (13.0%)	24,82
12	Apple	Mal d 1	Ingestion	PR-10	13 (12.0%)	9,09
13	Black alder	Aln g 1	Inhalation	PR-10	13 (12.0%)	6,71
14	Hazel	Extract	Inhalation		12 (11.1%)	6,77
15	Rye pollen	Extract	Inhalation		12 (11.1%)	7,33
16	Peanut	Ara h 8	Ingestion	PR-10	12 (11.1%)	3.32
17	Strawberry	Fra a 1+3	Ingestion	PR-10+LTP	12 (11.1%)	7.01
18	Timothy grass	Phl p 6	Inhalation	Group 5/7 grass	11 (10.2%)	23.06
19	*Dermatophagoides farinae*	Der f 2	Inhalation	NPC2 family	10 (9.3%)	42.99
20	*Dermatophagoides pteronyssinus*	Der p 2	Inhalation	NPC2 family	10 (9.3%)	42.14
21	Dog urine (including Can f 5)	Extract	Inhalation		9 (8.3%)	8.46

22	*Paspalum notatum*	Extract	Inhalation		9 (8.3%)	2.23
23	Cat	Fel d 1	Inhalation	Secretoglobin	9 (8.3%)	21.52
24	*Glycyphagus domesticus*	Gly d 2	Inhalation	NPC2 family	9 (8.3%)	7.47
25	Celery	Api g 1	Ingestion	PR-10	8 (7.4%)	6.93
26	Carrot	Dau c 1	Ingestion	PR-10	8 (7.4%)	6.75
27	Soy	Gly m 4	Ingestion	PR-10	8 (7.4%)	2.81
28	*Dermatophagoides farinae*	Der f 1	Inhalation	Cysteine protease	8 (7.4%)	21.92
29	Carrot	Extract	Ingestion		7 (6.5%)	3.35
30	European ash	Extract	Inhalation		7 (6.5%)	9.69
31	*Dermatophagoides pteronyssinus*	Der p 1	Inhalation	Cysteine protease	7 (6.5%)	22.38
32	Olive	Ole e 1	Inhalation	Ole e 1 family	7 (6.5%)	11.10
33	Walnut pollen	Extract	Inhalation		6 (5.6%)	2.74
34	Melon	Cuc m 2	Ingestion	Profilin	6 (5.6%)	11.26
35	European ash	Fra e 1	Inhalation	Ole e 1 family	6 (5.6%)	8.47
36	Annual mercury	Mer a 1	Inhalation	Profilin	6 (5.6%)	3.13
37	Date palm	Pho d 2	Inhalation	Profilin	6 (5.6%)	12.26
38	Common wasp venom	Ves v 5	Other	Antigen 5	6 (5.6%)	1.91

3.3. Relationship between sensitization and patients' age and sex

Statistical analysis showed no significant differences in sensitization rates between girls and boys in children with CD and sensitivity to any extracts or molecules, whether food, inhalant, or other allergens ($p = 0.462$). Moreover, there were no significant differences between boys and girls in the rates of sensitization to food (extracts and molecules) ($p = 0.674$) or inhalant (extracts and molecules) allergens ($p = 0.665$).

Spearman's rank correlation analysis (Table 3) in sensitized patients showed a significant correlation only between the patient's age and the number of positive sIgE (≥0.3 kU_A/L) test results for inhalant allergen molecules ($p = 0.045$). The correlation was positive and weak ($r = 0.31$), which means that the rate of sensitization to inhalant allergen molecules increased with age. There was, however, no significant correlation between the patient's age and sensitization to food allergens either extracts or molecules (Table 3).

Table 3. Relationship between age and sensitization rates in children with CD (Spearman rank correlation).

Type of allergen	**Spearman rank correlation**		
	Number of sensitized children	***r***	***p-value***
all tested allergens	53	0.21	0.124

food extracts and molecules	29	0.13	0.491
inhalant extracts and molecules	42	0.30	0.055
food extracts	20	0.20	0.403
food molecules	23	0.18	0.411
inhalant extracts	28	0.27	0.161
inhalant molecules	42	0.31	0.045[1]

[1]the statistically significant result ($p < 0.05$).

4. Discussion

A systematic review of studies assessing co-existence of IgE-mediated allergy/sensitization and CD [6] revealed two original articles that ruled out [15, 16] and only four original articles that suggested a possibility of allergy and CD co-occurrence [8-11], with a vast majority of authors assessing the co-occurrence of CD and sensitization to food allergens. Our study was conducted in a group of pediatric patients with CD with the use of multiplex tests to assess the levels of serum sIgE against 295 allergens, including 117 extracts and 178 allergen molecules from various sources, such as foods, animals, plants, and molds. Assessments conducted in 108 children with CD showed IgE-mediated sensitization in nearly half of the study group (53/108; 49.1%). Other authors reported considerably lower sensitization rates in patients with CD [8, 11]. Cudowska et al. demonstrated IgE-mediated allergy/sensitization in just over 20% (12/59; 20.3%) of children with CD [11]. Ciacci et al., who evaluated patients older than 17 years newly diagnosed with CD based on questionnaire-reported allergy symptoms (n=1,044), their relatives (n =2,752) and spouses (n =318), showed that 16.6% of patients with CD were allergic to at least one of the evaluated allergens [8]. The higher sensitization rates shown in our study group with CD may be due to the number and type of assessed allergens. The analysis by Cudowska et al. was based solely on sIgE against 20 food and inhalant allergens [11]. Similarly, Ciacci et al. assessed sensitization to 20 inhalant and food allergens and conducted skin tests in selected patients [8]. Additionally, unlike previous studies, our study assessed sIgE against both allergen extracts and molecules, which may increase screening test sensitivity [17, 18].

Our study showed children with CD to have the highest rates of sensitization to inhalant allergens. The three allergens with the highest sensitization rates in children with CD were timothy grass (Phl p 1), perennial ryegrass (Lol p 1), and silver birch (Bet v 1) pollens. Cudowska et al. [11], who evaluated the relationship between sensitization to inhalant allergen extracts, showed a similar tendency: the allergens with the second and third highest sensitization rates were grass and birch pollens. However, the highest rates of sensitization in that study were shown to be due to dust mites, whereas in our study, the same dust mite allergen (*Dermatophagoides pteronyssinus* molecule Der p 23) placed 7 in the ranking of the most common sources of inhalation allergies.

The food allergen with the highest sensitization rates in children with CD was hazelnut (Cor a 1), followed by apple (Mal d 1), and peanut (Ara h 8). These allergens placed eighth, twelfth, and sixteenth, respectively, among all the food and inhalant allergen extracts and molecules presented in Table 2. Peanut allergens were also among the most common food allergens in a study by Cudowska et al. [11], who demonstrated that nearly half of the evaluated children showing sensitization to any allergen (5/12; 41.7%) were sensitized to peanuts. However, that analysis was based solely on allergen extracts, and it is difficult to draw conclusions as to this having being a primary peanut sensitization or a result of secondary reaction (cross-reactivity with inhalant allergens).

In our study the three food allergens that placed highest in the ranking presenting in Table 2 (Cor a 1, Mal d 1, and Ara h 8) belong to the PR-10 subfamily, which has been demonstrated to produce cross-reactivity, with primary sensitivity to birch allergens (specifically Bet v 1 of the PR-10 subfamily) [19]. Our previous research shows that cases of sensitization to food allergens from plant sources may result from a primary allergy to inhalant allergens [20, 21]. Therefore, it is worth noting that in over three quarters of the current study group of children with CD and food allergen sensitization showed a co-existing sensitization to inhalant allergens. A reverse situation, namely food allergen sensitization in children with CD and sensitivity to inhalant allergens was less common, though still a large proportion (over half) of the children with sensitivity to food allergens was also sensitive to inhalant allergens. Nonetheless, the greatest proportion of children with CD (41.5%) was simultaneously sensitive to inhalant and food allergens. Most children with CD demonstrated sensitivity to two or more allergens, whereas less than one quarter of the study group was sensitive to a single allergen. Importantly, the same allergen molecules (Cor a 1.0401, Mal d 1, and Ara h 8) were the most common allergens in another of our study, where we assessed the levels of sIgE against food allergen extracts and molecules in 3,715 Polish children being diagnosed for a suspected allergy (the mean age of the study population was 7.0 years) [22], which may suggest that the food allergen sensitization profile in patients with CD is similar to that observed in the general Polish population.

In the current study in children with CD we observed no sIgE against 150 (73 extracts or 77 molecules) out of all 295 allergens assessed with the multiplex test. Interestingly, the tested allergens included all gluten-containing cereal allergens: wheat (*Triticum aestivum*) molecules (Tri a aA_TI, alpha-amylase trypsin inhibitor; Tri a 14, nsLTP; and Tri a 19, omega-5-gliadin) as well as spelt (*Triticum spelta*), cultivated rye (*Secale cereale*), barley (*Hordeum vulgare*), and oat (*Avena sativa*) extracts. Studies investigating the co-existence of CD and sensitivity to wheat or other cereal have been inconclusive. Using skin prick and sIgE tests, Armentia et al. [9] demonstrated that 7.0% out of 57 patients (adults and children) with CD were sensitive to wheat [8]. A study conducted by Lanzarin et al. [10] in 74 patients with CD (aged 1–20 years) showed the rates of sensitization to wheat, rye, barley, and barley malt to be 4%, 10.8%, 5.4%, and 2.7%, respectively. Our results are consistent with those reported by other authors, who indicated no sensitization to gluten-containing cereals in patients with CD [11, 16]. Interestingly, just like our research team, Spoerl et al. [16], who assessed allergy to wheat extract and wheat molecule Tri a 19, showed no sensitization to the evaluated allergens in patients with CD.

The incidence of CD is higher in women than in men, a fact which has been corroborated in an epidemiological study by Makharia et al. [23], where the pooled incidence rates in women and men were 17.4 (95% CI: 13.7–21.1) and 7.8 (95% CI: 6.3–9.2) per 100,000 person-years, respectively. This trend was also observed in our study group, whose majority (63%) were females. Studies regarding the incidence of sensitization with respect to patient's sex suggest a relationship with the patient's age and hormone levels [24]. Boys may be susceptible to allergies at an earlier age than girls, a phenomenon that may be absent later in life. In our study, a statistical analysis of those children with CD who had any sensitivity (to extracts and/or molecules of food, inhalant, or other allergens) showed no significant differences between girls and boys in the incidence of sensitization. The sensitization profile in children changes with age, and the rates of sensitization to inhalant allergens increased with patient age [25]. The observations from the current study also presents a significant relationship between the age of children with CD and the number of positive sIgE test results against inhalant allergen molecules.

Study limitation

We are aware of the fact that our study has certain limitations. One such limitation is a lack of clinical examination records. All patients were invited to the allergy outpatient clinic to undergo a clinical examination. However, so far only seven patients have

returned for their visit. This situation is due to the way the allergy outpatient clinic operates and the feasibility of accommodating all children with CD included into this study. Nonetheless, preliminary data indicate that all children who already had their visit at the clinic reported gastrointestinal symptoms (including abdominal pain, bloating, diarrhea/constipation) or exhibited inadequate weight gain. These children most commonly reported inhalation allergy and were diagnosed with allergic rhinitis, and/or conjunctivitis, or allergic bronchial asthma. Other reported allergy symptoms were skin changes in the form of hives or atopic dermatitis. We are planning to conduct a detailed analysis of the clinical forms of allergy in children with CD.

5. Conclusions

Our analysis presented that nearly a half of the children with CD was sensitized to at least one allergen, with no cases of sensitivity to gluten-containing cereals. Moreover, children with CD were usually sensitized simultaneously to both inhalant and food allergens. The food allergens with the highest sensitization rates were molecules of the PR-10 subfamily (Cor a 1, Mal d 1, and Ara h 8), which may be due to cross-reactivity with birch, whose primary-allergy marker, Bet v 1 (PR-10 subfamily), was among the three most common inhalant allergens after timothy grass and perennial ryegrass (Phl p 1, Lol p1). In conclusion, the current study indicates a need for IgE-mediated allergy diagnostics in patients with CD, in terms of not only food allergy but also inhalant allergy.

These studies highlight the need to increase attention to the diagnosis of IgE allergy in patients with CD, which may contribute to better care for them and lead to a better understanding of the relationship between CD and IgE allergy.

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Informed Consent Statement: A written informed consent was obtained from each patient's parents or guardians, and from patients aged ≥ 16 years old, with respect to the use of their blood for scientific purposes.

Conflicts of Interest: E.M. is an employee of the EMMA MDT Sp. z o.o. company. For the remaining authors, none are declared.

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CASE REPORT

The Precision Allergy Molecular Diagnosis (PAMD@) in Monitoring the Atopic March in a Child with a Primary Food Allergy: Case Report

Izabela Knyziak-Mędrzycka[1,2], Monika Szychta[3], Emilia Majsiak[4], Andrzej M Fal[2,5,6], Zbigniew Doniec[7], Bożena Cukrowska[8]

[1]Allergy Clinic, Children's Memorial Health Institute, Warsaw, Poland; [2]The Department of Allergy, Pulmonary Diseases and Internal Medicine, Central Clinical Hospital of the Ministry of Interior and Administration in Warsaw, Warsaw, Poland; [3]Department of Gastroenterology, Hepatology, Nutritional Disturbances and Pediatrics, Children's Memorial Health Institute, Warsaw, Poland; [4]Department of Health Promotion, Chair of Nursing Development, Faculty Health of Sciences, Medical University of Lublin, Lublin, Poland; [5]Collegium Medicum, Faculty of Medicine, Cardinal Stefan Wyszyński University, Warsaw, Poland; [6]Department of Public Health, Wroclaw Medical University, Wroclaw, Poland; [7]The Institute of Tuberculosis and Lung Diseases, Regional Branch in Rabka-Zdrój, Rabka-Zdrój, Poland; [8]Department of Pathomorphology, Children's Memorial Health Institute, Warsaw, Poland

Correspondence: Emilia Majsiak, Department of Health Promotion, Chair of Nursing Development, Faculty Health of Sciences, Medical University of Lublin, Staszica 4 m.6 (Collegium Maximum), Lublin, 20-081, Poland. Tel +48 81 448 6700, Fax +48 48 814 4867, Email emiliamajsiak@umlub.pl; wnoz@umlub.pl

Abstract: The case of a 9-month-old boy with an initial diagnosis of atopic dermatitis and confirmed allergy to hen's egg, cow's milk allergens with episodes of anaphylaxis who developed birch allergy whilst under observation with asthma symptoms was presented. The precision allergy molecular diagnosis (PAMD @) allowed for individualisation of dietary recommendations and observing the early progression of food sensitisation to the main birch molecule. The presented identification of major allergic molecules with PAMD@ in the preclinical phase of asthma contributes to the discussion related to early specific immunotherapy to suppress molecular spread and allergic march. However, more research is needed to verify this hypothesis.

Keywords: atopic march, allergy, anaphylaxis, sIgE, PAMD@, multiplex molecular tests

Introduction

Food allergy (FA) is often the first step of other atopic manifestations later in life, and this phenomenon is commonly named the atopic march.[1] It has been proposed that the precision allergy molecular diagnosis (PAMD@) could help identify some risks for the atopic child and predict the development of the atopic march.[2]

The article presents a case of a patient with an initially diagnosed FA manifested as atopic dermatitis (AD) and anaphylaxis, which evolved into childhood asthma. The patient's diagnostic process was based on PAMD@ and allergen challenge.

Clinical Case

In September 2019, a 9-month-old boy with AD and anaphylaxis who ate scrambled eggs on clarified butter after eating for the first time in his life was admitted to the Central Clinical Hospital of the Ministry of Inferior and Administration in Warsaw.

Paediatric history: the child was born by caesarean section at 38 Hbd, 10 Apgar score, the father has a pollen allergy and bronchial asthma, the mother is allergic to nickel, and one older brother is without allergy. The boy was breastfed for 9 months, and at the age of 6 months, the diet was extended with fruits, vegetables and meat. A month later, emesis after eating the cooked yolk, ear oedema and hives after homogenised cheese were observed. Symptoms regressed after

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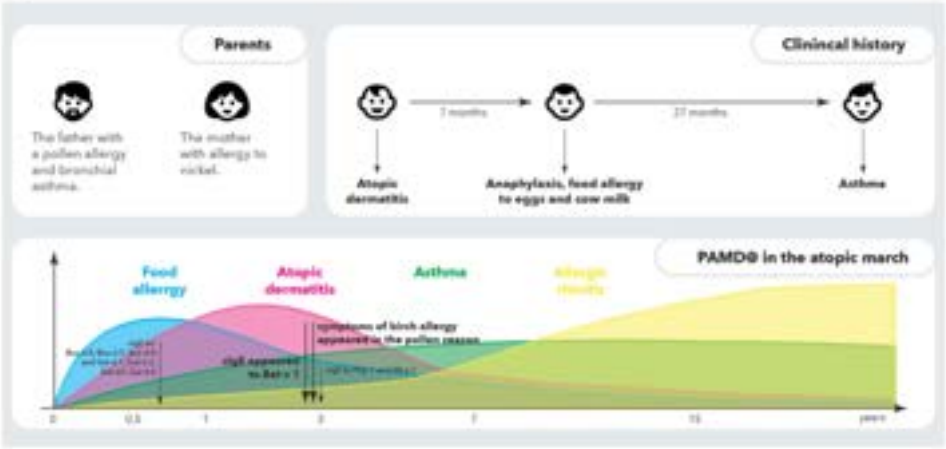
Graphical Abstract



administering cetirizine (5 mg/day). Laboratory tests showed eosinophilia in peripheral blood (8.6%), total IgE <20 kU/L and specific IgE (sIgE) to cow's milk, hen's egg, soybean, peanut and potato (Table 1).

Recommendations included an elimination diet without milk and eggs for the mother and child, further breastfeeding, feeding with amino acid formula, taking cetirizine as needed and avoiding soybean and nuts. The child was equipped with an anti-shock kit.

During 30 months of observation, dietary recommendations were followed, and the severity of skin lesions reduced (↓SCORAD from 33 to 9 points) without episodes of anaphylaxis. An attempt to discontinue cetirizine, despite the use of a restrictive elimination diet, resulted in the appearance of allergic symptoms. In 2019–2020, the patient had three bronchitis episodes with wheezing, treated with antibiotics. In 2021, in the period of birch pollination, the patient had bronchitis without fever, with preceding symptoms of acute conjunctivitis and rhinitis. He was treated with inhaled fluticasone preparation via low volume inhalation chamber, fenoterol with ipratropium bromide as nebulisation, nasal preparation of mometasone furoate and intraconjunctival hydrocortisone with a good clinical effect. In April 2022, the patient developed symptoms of full-blown asthma (cough with shortness of breath). Inhalation treatment with fluticasone propionate with salmeterol was intensified, treatment with oral antihistamine and nasal steroid was continued, and the patient's condition improved.

The first single-blind, placebo-controlled challenge with baked milk after 12 months of elimination diet was positive (after eating 1/2 of a muffin), as was the second one after 18 months (after eating 1/12 of a muffin). A challenge with a chicken egg after 19 months of the elimination diet was also positive.

The PAMD® was performed prior to the milk challenge (FABER multiplex; 11/2019) and then repeated twice (ALEX multiplex; 02/2021, 01/2022) (Table 2). ALEX multiplex (02/2021) done after 14 months of FABER test showed persisted positivity against hen's egg extract and components (ovomucoid, ovalbumin, ovotransferrin), cow's milk extract and components (beta-lactoglobulin, casein) and the milk of other ungulates. sIgE to birch (Bet v 1) was 0.27 kU/L, but it was still below the cut-off (0.35kU/L). Re-performed ALEX test (01/2022) presented new sensitisation to grass and *Alternaria alternata*, and progression of sensitisation to Bet v 1 (2.33 kU/L) (Table 2). Due to increased sensitisation to milk components, including casein, another milk challenge was not attempted, and the elimination diet without milk, eggs and nuts was maintained.

We have obtained the signed consent from the patient's parents and the hospital to publish the case details.

Table 1 Study of Specific IgE in 29-Month Lasting Follow-Up

Allergen Component		Euroline Pediatric Profile*	Polycheck Egg Components **	FABER***	ALEX****	ALEX****
Unit of measure		kU/L	kU/L	FIU/mL	kU/L	kU/L
Date of execution		09/2019	10/2019	11/2019	02/2021	01/2022
Time since introducing an elimination diet		Without an elimination diet		After 2 months	After 17 months	After 28 months
Age of the patient in months		9	10	11	26	37
Food allergens	Alpha-lactalbumin (Bos d 4)	**1.70**		**0.54 (n)**	≤0.1 (n)	≤0.10 (n)
	Beta-lactoglobulin (Bos d 5)	**36.00**		**0.81 (n)**	0.32 (n)	**1.10 (n)**
	Casein (Bos d 8)	**16.80**		**1.62 (n)**	**1.15 (n)**	**4.52 (n)**
	Ovomucoid (Gal d 1)		**3.10 (n)**	**4.29 (n)**	**2.74 (n)**	**2.39 (n)**
	Ovalbumin (Gal d 2)		**3.50 (n)**	<0.01 (n)	**3.66 (n)**	**0.79 (n)**
	Ovotransferrin (Gal d 3)		**5.80 (n)**	**0.81 (n)**	**0.66 (n)**	**0.43 (n)**
	Lysozyme (Gal d 4)		**23.00 (n)**	<0.01 (n)	≤0.10 (n)	≤0.10 (n)
Inhalation allergens	Birch (Bet v 1)			<0.01 (r)	0.27 (r)	**2.33 (r)**
	Timothy (Phl p 1)			<0.01 (r)	≤0.10 (r)	**1.51 (r)**
	Alternaria alternata (Alt a 1)			<0.01 (r)	≤0.10 (r)	**5.35 (r)**

Notes: (r) – recombinant (n) – natural; filling colour grey – no data, due to non-performance of the measurement at a given time or no such measurement on a given test. *The results were obtained with the use of a multi-parameter Euroline immunoassay (Euroimmun, PerkinElmer Germany Diagnostics GmbH containing 27 allergens). Positive result ≥ 0.35 kU/L. **The results were obtained with the use of multi-parameter Polycheck assay (Biocheck GmBH, Germany). Positive result ≥0.35 kU/L. ***Multi-parameter FABER immunoassay (CAAM, Roma, Italy) – negative result <0.01 FIU/mL, borderline >0.01÷<0.30 FIU/mL, positive ≥0.30 FIU/mL. ****Multi-parameter ALEX immunoassay (MacroArray Diagnostics, Vienna, Austria) – negative or borderline result <0.3 kU/L, positive result ≥0.3 kU/L. Bold numbers – positive result (result ≥0.35 kU/L). Underlined numbers – borderline result (result between 0.1 kU/L and <0.35 kU/L).

Table 2 Assessment of Asthma Predictive Index in the Presented Patient

Assessed Criteria*	Patient's Signs and Symptoms
Major Criteria	
Asthma in parents	Yes
Atopic eczema	Yes
Minor Criteria	
Allergic rhinitis	Yes
Wheezing without infection	Yes
Peripheral eosinophilia >4%	Yes

Notes: *The criteria for the diagnosis of asthma according to Rodriguez JA, Hallberg CJ, Wright AL and Martinez FD: showed in article A clinical index to define risk of asthma in young children with recurrent wheezing. *Am J Respir Crit Care Med.* 2000; 162: 1403–1406.[3]

Discussion

The described case shows a typical route of an allergy march from FA manifested as AD to inhalation allergy and asthma. The reported case presented several risk factors for the classic atopic march: AD in infancy, atopy in family, male gender and primary sensitisation to many food allergens.[1] The Asthma Predictive Index assessment[3] confirmed a high

probability of asthma development: the patients met two major criteria (AD, asthma in the father) and three minor criteria (allergic rhinitis, peripheral eosinophilia, wheezing without infection) (Table 2). After considering the patient's age, the diagnosis of asthma was carried out in accordance with the applicable guidelines of COMPAS GP (ReCOMmendations for management of Preschool AStbma for General Practitioners).[4]

In the presented case, dietary treatment was applied after identifying food allergens and the food challenges with baked cow's milk and hen's eggs that were positively correlated with the presence of sIgE in the milk and egg molecules responsible for the persistent allergy. In addition, we observed that the concentration of sIgE to Bet v 1 increased from 0.27 kU/L to 2.33 kU/L. Since the boy showed symptoms at the time of birch pollination, we were able to recognise allergy to birch when the concentration of sIgE to Bet v1 was at the level of 0.27 kU/L. It is believed that the cut-off for positive results is the value of 0.35 kU/L, but it is necessary to underline that the limit of 0.35 kU/L for positive serological tests established by Johansson in the 1970s resulted from the sensitivity of diagnostic methods used at that time. A change in standard values for specific IgE has been proposed recently, and the cut-off value for sIgE at the level of 0.1 kU/L has been suggested.[5] In our patient, clinical observations of evident allergy to birch were confirmed by the result of a re-performed ALEX test, which showed sIgE to Bet v 1 at the level of 2.33 kU/L. Thus, in the presented case, low sIgE values (<0.35 kU/L) to allergen components may be significant with regard to future allergies. Thanks to the possibility of determining sIgE to allergen molecules, we observed the process of conversion from asymptomatic allergy to full clinical manifestation of birch allergy.

There are not many observations of molecular spread in FA and inhalation allergy. One of such reports was presented by Matricardi et al, who assessed the dynamics of specific component sensitisation of Phleum pratense timothy allergen[6] in 7 years in a boy who was initially allergic only to Phl p 1 at 3 years of age. Then, at the age of 6, the patient was already allergic to Phl p 1 and Phl p 2, and at the age of 10, to molecules Phl p 4, Phl p 5, Phl p 6 and Phl p 11. The authors wondered whether the introduction of allergen-specific immunotherapy could inhibit or limit the process of allergen spreading. The present case is another one that allows us to think about the need to verify the effectiveness of such early allergen immunotherapy in the context of inhibiting the allergic march.[6]

Therefore, the presented case is important as one of the few described in the literature that contributes to considering the optimal timing of the introduction of immunotherapy. It also presents the benefits of PAMD@ in dietary treatment, predicting the risk of anaphylaxis, and assisting in making decisions about provoking selected allergens.

Limitations

During the patient's diagnosis, various diagnostic methods were used, which were dictated by their current availability (eg, since 2021, the possibility to measure sIgE with FABER has been abolished in Poland and worldwide). Different tests make it impossible to compare the obtained sIgE values. In contrast to the semi-quantitative FABER and Euroline tests, Polycheck and ALEX are quantitative tests. In addition, sIgE measurement was performed at different stages of treatment: the FABER test was done on a dairy-free and egg-free diet, and the ALEX test when the elimination of nuts and legumes was added, which could have influenced the obtained sIgE results for these allergens. Thus, the comparison of sIgE values against allergen components (Table 1) is only indicative.

Conclusion

In conclusion, the use of PAMD@ in clinically manifested FA allowed for individualisation of dietary recommendations and revealed an early progression of sensitisation to the main molecule of birch.

The identification of major allergic molecules with PAMD@ in the early preclinical phase of the disease contributes to the discussion related to early specific immunotherapy to suppress molecular spread and allergic march. However, more research is needed to verify this hypothesis.

Disclosure

The authors report no conflicts of interest in this work.

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6 OMÓWIENIE WYNIKÓW

Szczegółowa analiza wyników została przedstawiona w 2 artykułach oryginalnych *(Knyziak-Mędrzycka i wsp The Sensitization Profile for Selected Food Allergens in Polish Children Assessed with the Use of a Precision Allergy Molecular Diagnostic Technique. Int. J. Mol. Sci. 2024, 25, 825* oraz *Knyziak-Mędrzycka i wsp Sensitization profile in children with celiac disease assessed with the use of multiplex molecular diagnostic technique*) zamieszczonych w rozdziale VIII. W tej części pracy skupiono się na omówieniu najważniejszych wyników i przeprowadzeniu analizy w porównaniu z dostępną literaturą przedmiotu.

6.1 Profil uczulenia na alergeny pokarmowe „wielkiej ósemki"

W pracy *Knyziak-Mędrzycka i wsp The Sensitization Profile for Selected Food Allergens in Polish Children Assessed with the Use of a Precision Allergy Molecular Diagnostic Technique. Int. J. Mol. Sci. 2024, 25, 825* przeanalizowano wyniki sIgE dla 96 ekstraktów pokarmowych i 77 molekuł alergenów pokarmowych. Wyniki testu ALEX® wobec alergenów pokarmowych „wielkiej ósemki" uzyskano od 3715 pacjentów. Najczęstszymi alergenami pokarmowymi, które uczulały badaną populację polskich dzieci były: orzech arachidowy (29.20%), orzech laskowy (28.20%) oraz jabłko (23.60%). Natomiast najrzadziej wyniki dodatnie odnotowano dla truskawki (0.40%), oregano (0.30%) oraz płaszczki kolczystej (0.16%). Najwyższe średnie stężenia sIgE odnotowano dla mleka krowiego (9.86 kUA/L), krewetki (8.55 kUA/L) oraz dla orzecha arachidowego (8.54 kUA/L), podczas gdy najniższe średnie stężenia sIgE obserwowano dla ekstraktu liczi (0.49 kUA/L), pieczarki (0.45 kUA/L) oraz truskawki (0.40 kUA/L). Spośród "wielkiej ósemki" alergenów pokarmowych, poza orzechem arachidowym i orzechem laskowym analizie poddano pozostałe alergeny, jak jajo kurze, mleko krowie, ryby, pszenica, soja, skorupiaki i inne orzechy drzew. Najwyżej w rankingu występowania uczulenia spośród tych pozostałych alergenów "wielkiej ósemki" znalazło się białko jaja kurzego, które uplasowało się na 4. miejscu, podczas gdy żółtko jaja znalazło się na 36. miejscu.

Opisany powyżej profil uczulenia dzieci z różnych rejonów Polski wykazuje znaczne różnice w porównaniu z badaniami w innych populacjach. W badaniu EuroPrevall [26], do którego włączono dzieci w wieku szkolnym (7-10 lat) z europejskich miast (Zurych, Madryt, Ateny, Utrecht, Wilno, Łódź i Reykjavik) analizowano wyniki dotyczące uczulenia na dwadzieścia cztery pokarmy uznane za najczęściej uczulające dzieci lub często spożywane w

badanych krajach. Były to: jaja kurze, mleko krowie, ryby, krewetki, orzeszki ziemne, orzechy laskowe, orzechy włoskie, brzoskwinie, jabłka, kiwi, melony, banany, pomidor, seler, marchew, kukurydza, soczewica, soja, pszenica, gryka, sezam, gorczyca, słonecznik oraz mak. W żadnym z analizowanych miast w badaniu EuroPrevall w pierwszej trójce najczęściej uczulających alergenów nie znalazł się alergen orzecha ziemnego, podczas gdy w naszej analizie znalazł się on na miejscu pierwszym. Drugim najczęstszym alergenem w naszym badaniu był orzech laskowy, który równie wysoko występował w badaniu EuroPrevall (miejsce w rankingu zależało od badanego miasta. W Łodzi znalazł się on na pierwszym miejscu, w Utrecht na drugim, a w Zurychu i Wilnie na trzecim miejscu. Jabłko, które było na miejscu trzecim w naszej analizie, w żadnym z analizowanych miast w badaniu EuroPrevall nie znalazło się w gronie 3 najczęściej uczulających alergenów. Różnice w profilach uczuleniowych na ekstrakty alergenów pokarmowych są jeszcze bardziej widoczne, gdy porównamy wyniki uzyskane poza Europą. W badaniu Segura i wsp., którzy oceniali występowanie uczulenia na ekstrakty alergenów pokarmowych (analiza sIgE przy pomocy ImmunoCAP, Thermo Fisher) w grupie pacjentów z różnymi alergiami w Meksyku analiza wykazała, że najczęściej uczulającymi pokarmami były: orzechy laskowe, jabłka, krewetki, orzeszki ziemne, białka jaj, żółtka jaj, brzoskwinie, migdały, pomidory, fasola, mleko, truskawki, kiwi, kukurydza i pszenica [27].

Analiza uczulenia wobec molekuł wykazała, że najczęstszymi molekułami alergenów pokarmowych, wobec których stwierdzono występowanie sIgE były molekuły należące do podrodziny białek PR10. Wśród 10 molekuł alergenów pokarmowych wobec których najczęściej stwierdzono występowanie sIgE znalazło się aż 6 białek z tej podrodziny: rCor a 1.0401 (23.77%), rMal d 1 (22.37%), rAra h 8 (16.93%), rGly m 4 (15.18%), rApi g 1 (15.07%) oraz rDau c 1 (13.76%). Jedyną molekułą nienależącą do podrodziny PR10 w pierwszej piątce najczęstszych molekuł uczulających była molekuła nAra h 1 (globulina 7/8S) orzecha ziemnego. Najrzadziej stwierdzono występowanie sIgE wobec nMal d 2 (0.27%) (rodzina białek taumatynopodobnych, TLP), rAni s 1 (0.30%) (inhibitor proteazy serynowej typu Kunitz) oraz rChe a 1 (po 0.43%) (rodzina białek Ole e 1).

Można założyć, z dużym prawdopodobieństwem, że powszechne uczulenie na białka z podrodziny PR10 może wynikać z reakcji krzyżowej na pierwotne uczulenie na alergen brzozy (molekułę Bet v 1). W wieloośrodkowym badaniu Kiewiet'a, wykazano, że reagujące krzyżowo białka PR10, takie jak Cor a 1.0401, Mal d 1 i Pru p 1, należą do najczęściej rozpoznawanych przez sIgE cząsteczek w kohortach o wysokim wskaźniku uczulenia na Bet v 1, właśnie ze względu na reaktywność krzyżową [47]. Podobnie w badaniu EuroPrevall stwierdzono, że jedno z najczęstszych uczuleń w różnych miastach europejskich na orzeszki laskowe może być

związane z reaktywnością krzyżową z pyłkiem brzozy, które było powszechne w Europie Środkowo-Północnej.

W naszym badaniu przeprowadzono również analizę występowania sIgE wobec molekuł alergenowych "wielkiej ósemki" alergenów pokarmowych w zależności od wieku. Zaobserwowano istotnie statystyczny (p <0.05) spadek częstości uczulenia wraz z wiekiem dla białek zapasowych wobec molekuł alergenów białek zapasowych: orzecha ziemnego (nAra h 1, rAra h 2, nAra h 3), soi (nGly m 6), orzechów drzew (nCor a 9, nJug r 4). Istotny statystycznie spadek częstości uczulenia wraz z wiekiem był obserwowany również dla molekuł mleka krowiego (nBos d 4, nBos d 5, nBos d 6, nBos d 8) i jaja kurzego (nGal d 1, nGal d 2). Podobny trend zmiany uczulenia, zwłaszcza dotyczący alergenów mleka krowiego i jaja obserwowany był przez innych badaczy [47, 48], chociaż w wieloośrodkowym badaniu Kiwiet'a i wsp. częstość występowania uczulenia w różnych europejskich pediatrycznych kohortach na molekuły alergenu mleka krowiego oceniane metodą ImmunoCap ISAC była znacząco niższa, bo przeważnie poniżej 1% (z wyjątkiem jednej grupy hiszpańskiej z Sabadell, gdzie częstość ta wynosiła 1.4%-2.0%) [47].

W naszym badaniu istotny statystycznie wzrost częstości uczulenia wraz z wiekiem zaobserwowano wobec białek podrodziny PR10 (rAra h 8, rCor a 1.0401). Wyniki dodatnie dla białka rAra h 8 nie występowały w grupie dzieci w wieku do 12 miesięcy, pojawiły się dopiero u dzieci w wieku 1-5 lat i sukcesywnie rosły w kolejnych grupach wiekowych.

Badania przeprowadzone przez innych badaczy pokazują znaczne różnice w częstości występowania uczulenia na różne molekuły orzecha arachidowego w zależności od badanego kraju. Wykazano, że u pacjentów z alergią na orzeszki ziemne w USA i Szwecji częściej rozpoznawano jako alergen białka zapasowe rAra h 1-3 w porównaniu z pacjentami z Hiszpanii, którzy byli częściej uczuleni na białko przenoszące lipidy rAra h 9 [49]. W badaniu Kiewiet i wsp. stwierdzono, że uczulenie na Ara h 9 było częstsze w południowej kohorcie hiszpańskiej Gipuzkoa i włoskiej Rome w porównaniu z kohortą szwedzką [47]. Różnice w profilu uczulenia na Ara h mogą wynikać z ilości spożycia, wieku wprowadzenia orzeszków ziemnych do diety, nawyków żywieniowych i różnic w przygotowaniu do spożycia orzeszków ziemnych w poszczególnych krajach. Wykazano, że w Szwecji, Stanach Zjednoczonych i innych krajach zachodnich, duża liczba nadwrażliwości na orzeszki ziemne może wynikać z tego, że spożywane są one najczęściej w formie prażonej. Prażenie orzechów wzmacnia potencjał alergizujący molekuł Ara h 1 i Ara h 2 lub może odkryć białka jak np. Ara h 15 (oleozyna), które w surowej postaci orzeszków zanurzone są w tłuszczu, stanowiącym materiał energetyczny dla kiełkujących roślin [50, 51, 52].

W naszym badaniu spośród molekuł pszenicy najwyżej (40. miejsce) w rankingu molekuł alergenów pokarmowych uplasowała się nTri a A_TI (4.45%). Analiza występowania sIgE wobec molekuł alergenowych pszenicy wykazała, że najczęściej wyniki dodatnie dla wszystkich analizowanych molekuł alergenowych odnotowano w grupie wiekowej do 12. miesięcy i wraz z wiekiem ilość sIgE spadała dla wszystkich analizowanych molekuł pszenicy. W badaniu Kiewieta i wsp. częstość występowania sIgE wynosiła od 0.1% w kohorcie szwedzkiej do 0.4% w kohorcie norweskiej i nie obserwowano spadku uczulenia dla tej molekuły wraz z wiekiem [47].

6.2 Znaczenie precyzyjnej diagnostyki molekularnej w praktyce klinicznej

W kontekście analizy klinicznej alergii u dzieci, w cyklu prac włączonych do pracy doktorskiej znalazł się również opis przypadku (*Knyziak-Mędrzycka I, Szychta M, Majsiak E, Fal AM, Doniec Z, Cukrowska B. The Precision Allergy Molecular Diagnosis (PAMD@) in Monitoring the Atopic March in a Child with a Primary Food Allergy: Case Report. J Asthma Allergy. 2022, 7;15:1263-1267*) jako prezentacja praktycznego podejścia do zmieniającego się z wiekiem profilu uczulenia ocenianego techniką diagnostyki molekularnej. Przedstawiony obraz kliniczny chłopca z początkowymi objawami alergii pokarmowej na mleko i jajo, a następnie rozwijającymi się objawami alergii wziewnej na pyłki w postaci alergicznego nieżytu nosa i astmy oskrzelowej jest zgodny z obserwacjami dotyczącymi sensytyzacji u dzieci w Polsce opisanymi w pracy oryginalnej (*Knyziak-Mędrzycka i wsp. The Sensitization Profile for Selected Food Allergens in Polish Children Assessed with the Use of a Precision Allergy Molecular Diagnostic Technique. Int. J. Mol. Sci. 2024, 25, 825*).

Analiza badań serologicznych przeprowadzonych u chłopca od 9 miesiąca życia przez 2 lata obserwacji pokazała znaczenie wdrożenia wczesnej diagnostyki u dzieci z objawami alergii. Istotnym wnioskiem z tej obserwacji jest to, że już na wczesnym etapie życia można było rozpoznać u pacjenta uczulenie na ekstrakt brzozy oraz na molekułę Bet v 1, które w kolejnych latach obserwacji istotnie się zwiększało, co potwierdzało się w objawach klinicznych u pacjenta, które z roku na rok przybierały na sile. Opis przepadku pokazał, że wczesna kompleksowa diagnostyka pozwala na jak najszybsze rozpoczęcie odpowiedniej terapii zapobiegającej rozwojowi alergicznego zapalenia, a co za tym idzie progresji objawów alergicznych. Wczesne podjęcie immunoterapii mogłoby przysłużyć się ograniczeniu

występowania ciężkich objawów alergii, takich jak astma, anafilaksja u młodzieży i osób dorosłych.

6.3 Profil uczulenia u dzieci z CD

Oceniając profil uczulenia w grupie dzieci z nowo rozpoznaną CD *(Knyziak-Mędrzycka i wsp Sensitization profile in children with celiac disease assessed with the use of multiplex molecular diagnostic technique ...)* stwierdzono, że u prawie połowy dzieci z CD (53/108; 49.1%) występują sIgE wobec ekstraktów i/lub molekuł alergenów. Tak wysoki odsetek uczulenia nie był do tej pory opisywany. Przegląd systematyczny badań w zakresie współwystępowania CD i uczulenia/A-IgE, będący częścią rozprawy doktorskiej *(Majsiak i wsp. IgE-Dependent Allergy in Patients with Celiac Disease: A Systematic Review. Nutrients. 2023)* zidentyfikował zaledwie 4 prace oryginalne [43, 44, 45, 46], które pokazały możliwość współistnienia CD i uczulenia lub A-IgE. Jednak odsetek uczulonych pacjentów w tych pracach był znacznie niższy. Cudowska i wsp. wykazali uczulenie u nieco ponad 20% (12/59; 20.3%) dzieci z CD [46]. Ciacci i wsp., którzy oceniali pacjentów w wieku powyżej 17 lat z nowo zdiagnozowaną CD na podstawie objawów alergii zgłaszanych w kwestionariuszu (n =1044), wykazali, że 16.6% pacjentów z CD było uczulonych na co najmniej jeden z ocenianych alergenów [43]. Ponad dwukrotnie wyższy odsetek pacjentów z pozytywnymi sIgE wykazanych w naszej grupie badawczej może wynikać z liczby i rodzaju ocenianych alergenów. Analiza Cudowskiej i in. opierała się wyłącznie na sIgE wobec 20 alergenów pokarmowych i wziewnych [46]. Podobnie Ciacci i in. oceniali uczulenie na 20 alergenów wziewnych i pokarmowych oraz przeprowadzali testy skórne u wybranych pacjentów [43]. W naszym badaniu ocenialiśmy sIgE wobec 295 alergenów, w tym 117 ekstraktów i 178 molekuł alergenowych z różnych źródeł. Taka kompleksowa analiza, obejmująca molekuły alergenowe znacznie zwiększa czułość testów przesiewowych [53, 54].

Najwięcej dzieci z CD (41.5%) było uczulonych jednocześnie na alergeny wziewne i pokarmowe, a najwyższe średnie stężenia sIgE stwierdzono wobec alergenów wziewnych. Analiza wszystkich ekstraktów i molekuł alergenowych wykazała, że najczęściej u dzieci z CD obecne były sIgE wobec pyłkom tymotki łąkowej (26.9%), życicy (24.1%) oraz brzozy brodawkowatej (18.5%). Wszystkie te 3 najczęstsze alergeny wziewne to molekuły alergenowe Phl p 1, Lol p 1 (obydwie z rodziny B-ekspansyn) i Bet v 1 (podrodzina białek PR10). Uczulenie wobec alergenów pokarmowych (ekstraktów i molekuł) występowało u ponad jednej czwartej badanej grupy. Najczęstszym alergenem pokarmowym był orzech laskowy (13.95%), który był

dopiero na 8 pozycji, jeżeli chodzi o częstość występowania uczulenia na wszystkie analizowane alergeny. Wszystkie trzy pierwsze alergeny pokarmowe były molekułami (Cor a 1, Mal d 1 i Ara h 8) należącymi do podrodziny PR10.

Podobnie jednym z najczęstszych alergenów pokarmowych w badaniu Cudowskiej i in. był orzech arachidowy. Wykazali oni, że prawie połowa z badanych dzieci z CD i z jakimkolwiek uczuleniem (5/12; 41.7%) była uczulona na orzechy arachidowe. Jednak analiza ta oparta była jedynie o ekstrakty alergenowe i trudno jest wyciągnąć wnioski czy było to uczulenie pierwotne na orzech arachidowy, czy wynik reakcji krzyżowej uczulenia na alergeny wziewne.

Pomimo wysokiego odsetka uczulonych dzieci z CD, nie stwierdzono obecności sIgE wobec alergenów zbóż zawierających gluten: molekuły pszenicy zwyczajnej (*Triticum aestivum*) – Tri a aA_TI (inhibitor α-amylazy I trypsyny), Tri a 14 (nsLTP) i Tri a 19 (omega-5-gliadyna) oraz ekstraktów pszenicy (*Triticum spelta*), żyta (*Secale cereale*), jęczmienia *Hordeum vulgare*), jak również owsa (*Avena sativa*). Badania dotyczące współistnienia CD i uczulenia na pszenicę lub inne zboża nie są jednoznaczne. W pracy Lanzarin i in. wykazano za pomocą oceny sIgE w grupie 74 osób (1-20 lat) z CD uczulenie na pszenicę, żyto, jęczmień i słód jęczmienny na odpowiednio poziomie 4%, 10.8%, 5.4 i 2.7% [45]. Nasze wyniki są zgodne jednak z wynikami badaczy wykazujących na brak występowania uczulenia u osób z CD na alergeny zbóż zawierających gluten [46, 42].

Analiza korelacji wykazała istotną statystycznie, zależność między wiekiem badanych a liczbą pozytywnych sIgE (≥0.3 kUA/L) dla molekuł alergenów wziewnych, wskazując na wzrost uczuleń na molekuły alergenów wziewnych wraz z wiekiem. Nie stwierdzono istotnej statystycznie korelacji pomiędzy wiekiem pacjentów a uczuleniem na alergeny pokarmowe (zarówno ekstrakty, jak i molekuły).

W pracy *Knyziak-Mędrzycka i wsp Sensitization profile in children with celiac disease assessed with the use of multiplex molecular diagnostic technique* podjęto również wstępną alergologiczną ocenę kliniczną pacjentów z CD (niestety zaledwie 7 z 53 uczulonych). Przebadane klinicznie dzieci zgłaszały objawy alergii wziewnej i rozpoznano u nich alergiczny nieżyt nosa, spojówek czy alergiczną astmę oskrzelową. Inne deklarowane objawy alergii to zmiany skórne pod postacią pokrzywki lub wyprysku atopowego. Dokładna analiza postaci klinicznych alergii u dzieci z CD została zaplanowana na większej grupie dzieci i będzie przeprowadzona w przyszłości.

7 PODSUMOWANIE WYNIKÓW I WNIOSKI

Przeprowadzone w toku przewodu doktorskiego badania dostarczają pierwszego kompleksowego przeglądu profilu uczulenia IgE-zależnego zarówno na poziomie molekularnym, jak i ekstraktów alergenów pokarmowych u polskich dzieci i młodzieży z podejrzeniem alergii oraz u dzieci z nowo rozpoznaną CD. Zgodnie z aktualną wiedzą, jest to pierwsza kompleksowa analiza oparta na wynikach multipleksowego testu III generacji u dzieci zamieszkujących wszystkie regiony Polski oraz wybranej grupy dzieci z CD.

Przeprowadzone badania pokazały, że:

1. Profil uczulenia oparty na oznaczeniach sIgE wobec ekstraktów i molekuł alergenowych ze szczególnym uwzględnieniem alergenów pokarmowych wielkiej ósemki u dzieci w Polsce różnił się w porównaniu do innych krajów.
2. U polskich dzieci z podejrzeniem alergii na podstawie występowania sIgE najczęściej stwierdzano uczulenie na ekstrakt orzecha arachidowego, orzecha laskowego oraz jabłka, a wobec molekuł dla podrodziny PR10 (rCor a 1.0401, rMal d 1 i rAra h 8), na miejscu 4. znalazła się globulina 7/8S (nAra h 1).
3. Profil uczulenia wobec alergenów pokarmowych, analizowany na podstawie występowania sIgE zmieniał się wraz z wiekiem. Obserwowano wraz z wiekiem spadek uczulenia na alergeny pokarmowe oraz tendencje wzrostową uczulenia na alergeny wziewne. We wczesnym dzieciństwie dominowało uczulenie na białka zapasowe, a w późniejszych latach na białka z podrodziny PR10 z dominacją uczulenia na Bet v 1 z podrodziny PR10 pyłku brzozy.
4. Dzieci z CD były współuczulone na alergeny wziewne i pokarmowe.
5. Prawie połowa dzieci z nowo rozpoznaną CD była uczulona na minimum jeden alergen, przy czym nie stwierdzono u nich uczulenia na alergeny zbóż zawierających gluten.
6. Najczęstszymi alergenami pokarmowymi, które uczulały dzieci z CD były molekuły z podrodziny PR10 (Cor a 1, Mal d 1 i Ara h 8) co może wynikać z reakcji krzyżowych na brzozę, której marker pierwotnej alergii Bet v 1 (podrodzina PR10) był w trójce najczęstszych alergenów wziewnych, po tymotce łąkowej i życicy (Phl p 1, Lol p 1).

Na podstawie przeprowadzonych badań i analizy wyników wyciągnięto następujące wnioski:

1. Kompleksowa molekularna analiza uczuleń dzieci z podejrzeniem alergii zamieszkujących wszystkie regiony Polski pokazała, że profil uczulenia na alergeny pokarmowe w tej grupie różni się od profili obserwowanych w innych krajach; uzyskane wyniki badań mogą stanowić podstawę dla opracowania strategii profilaktyki oraz leczenia alergii zarówno w aspekcie lokalnym w naszym kraju oraz globalnym w skali światowej.
2. Molekularna analiza uczulenia w wyselekcjonowanej grupie u dzieci z CD pozwala twierdzić, że pomimo odmiennych mechanizmów immunologicznych uczulenie lub A-IgE i CD współistnieją, oraz że istnieje potrzeba diagnostyki A-IgE u pacjentów z CD, nie tylko w zakresie alergii pokarmowej, ale również alergii wziewnej.

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9 ZGODA KOMISJI BIOETYCZNEJ

KOMISJA BIOETYCZNA
przy Instytucie „Pomnik-Centrum Zdrowia Dziecka"
Al. Dzieci Polskich 20
04-730 Warszawa-Międzylesie
T. (22) 815-16-03

UCHWAŁA nr 50 /KBE/2018

OPINIA KOMISJI BIOETYCZNEJ
przy INSTYTUCIE „POMNIK-CENTRUM ZDROWIA DZIECKA"

Komisja Bioetyczna przy Instytucie Pomnik-Centrum Zdrowia Dziecka" **na posiedzeniu w dniu 21.11.2018** rozpatrzyła badanie pt: **Diagnostyka molekularna w alergii pokarmowej – ocena profilu uczulenia u dzieci przy użyciu nowoczesnej technologii nanomacierzy ALEX**

Gł. Badacz: prof. dr hab. Bożena Cukrowska

Opracowanie i wdrożenie metody CRD (ang. component-resolved diagnostics) z użyciem nanomacierzy ALEX w Pracowni Immunologii Zakładu Patologii IPCZD oraz kompleksowa ocena molekularnego profilu uczulenia u dzieci z alergią pokarmową.
Czas trwania projektu: 36 miesięcy
Grupa badana: łącznie 80 dzieci w wieku od 3 do 7 lat z alergią pokarmową IgE-zależną, u których potwierdzono obecność w surowicy swoistych przeciwciał w klasie IgE przeciwko ekstraktom alergenowym przy użyciu standardowych metod diagnostycznych.
Pobranie krwi: jednorazowa próbka krwi o objętości 2 ml pobrana na skrzep.
Metody badawcze:
1. Ocena swoistych IgE skierowanych przeciwko ekstraktom alergenowym techniką immunoblot
2. Ocena swoistych IgE skierowanych przeciwko komponentom (molekułom) alergenowym metodą CRD
- Diagnostyka molekularna CRD na nanomacierzach ALEX firmy MacroarrayDx,
- Analiza obrazu przy pomocy urządzenia ImageExplorer,
- Analiza wyników testu z zastosowaniem programu MADx Raptor Software (jednostki kUA/L).

Wyposażenie do wykonania i analizy mikromacierzy ALEX zostanie dostarczone nieodpłatnie przez dystrybutora testów w Polsce firmę EMMA MDT sp. z o.o.
Analiza korelacji pomiędzy wynikami uzyskanymi techniką immunoblot a CRD zostanie wykonana przy pomocy analizy regresji oraz współczynnika Pearsona r w programie Statistica.
Wprowadzenie nowoczesnej metody diagnostyki alergii, tj. oznaczania CRD przy pomocy mikromacierzy, znacznie zwiększa możliwości precyzyjnego oznaczenia cząsteczek biorących udział w reakcjach alergicznych u konkretnej osoby, jak również predykcji stopnia nasilenia ich ciężkości.
Ponadto, poznanie molekularnego profilu uczuleń w Polsce i porównanie ich z wynikami badań w innych krajach ułatwi wybór komponent molekularnych do opracowania szczepionek p/alergicznych najbardziej korzystnych dla danej populacji.
Realizacja projektu przyniesie wymierne efekty naukowe oraz wyjście Pracowni Immunologii Zakładu Patologii IPCZD z szeroką ofertą nowoczesnej diagnostyki molekularnej alergii IgE-zależnej na zewnątrz (działalność komercyjna).
Badacze informują o poufnym traktowaniu i przetwarzaniu danych osobowych – zgodnie z ustawą o ochronie danych osobowych.

Do zespołu badaczy należą:

Dr n med. Barbara Surowska
Dr n wet Joanna Bierła
Prof. dr hab. n med. Elżbieta Maciorkowska
Dr n med Emilia Majsiak

Dr n med. Ewa Konopka
Dr n med. Ilona Trojanowska

Badanie będzie prowadzone w kilku jednostkach:

- Pracowni Immunologii Zakładu Patologii IPCZD,
- Poradni Alergologicznej IPCZD,
- Zakładzie Medycyny Wieku Rozwojowego i Pielęgniarstwa Pediatrycznego Uniwersytetu Medycznego w Białymstoku,
- Polsko-Ukraińskiej Fundacji Rozwoju Medycyny

Przedłożone dokumenty:

1. Wniosek do Komisji Bioetycznej o zaopiniowanie zgłaszanego badania z dnia 23.10.2018 Załącznik nr 5 do Regulaminu Komisji Bioetycznej przy IPCZD)
2. Streszczenie protokołu badania (Załącznik nr 1 do Wniosku do Komisji Bioetycznej przy PCZD)
3. Formularz informacji pisemnej o badaniu (Załącznik nr 2 do Wniosku do Komisji Bioetycznej rzy IPCZD) dla Rodziców / Przedstawicieli ustawowych dziecka
4. Formularz deklaracji wyrażenia zgody na udział dziecka w projekcie badawczym (Załącznik 8a do Regulaminu Komisji Bioetycznej przy IPCZD)
5. Cztery Oświadczenia zgody na udział w projekcie wewnętrznym IPCZD podpisane przez:
   - Prof. nadzw. dr hab. n. med. Macieja Pronickiego, Kierownika Zakładu Patologii IPCZD,
   - Mgr Agnieszkę Pieniak, Kierownika Zespołu Poradni Specjalistycznych IPCZD,
   - Prof. dr hab. n. med. Elżbietę Maciorkowską, Kierownika Zakładu Medycyny Wieku Rozwojowego i Pielęgniarstwa Pediatrycznego UM w Białymstoku,
   - Pawła Majsaka, Prezesa Polsko-Ukraińskiej Fundacji Rozwoju Medycyny
6. Opinia Eksperta

Na podstawie przedłożonej dokumentacji, dodatkowych wyjaśnień przedstawionych przez prof. dr hab. n med. Bożenę Cukrowską oraz w dyskusji i wyniku tajnego głosowania Komisja Bioetyczna przy IPCZD wyraziła zgodę na przeprowadzenie badania. Skład i działanie Komisji zgodne z GCP oraz wymogami lokalnymi. Tekst uchwały został sporządzony w 2 jednobrzmiących egz. po jednym dla wnioskodawcy i Komisji Bioetycznej. Lista członków Komisji biorących udział w posiedzeniu stanowi załącznik do niniejszego dokumentu.

PRZEWODNICZĄCA KOMISJI BIOETYCZNEJ

przy Instytucie „Pomnik-Centrum Zdrowia Dziecka"

Prof. nadzw dr hab. n. med. Wiesława Grajkowska

W. Grajk

KOMISJA BIOETYCZNA
PRZY UNIWERSYTECIE MEDYCZNYM W LUBLINIE
Al. Racławickie 1, 20-059 Lublin, tel. + 48 81 448-51-15
e-mail: komisja.bioetyczna@umlub.pl

UCHWAŁA nr KE-0254/222/10/2023
z dnia 26 października 2023 roku

Na podstawie art. 29 ustawy z dnia 5 grudnia 1996 r. o zawodzie lekarza i lekarza dentysty (Dz.U. z 2022 r., poz. 171, z póź. zm.), ustawy z dnia 6 września 2001 r. Prawo farmaceutyczne, rozporządzenia Ministra Zdrowia z dnia 26 stycznia 2023 r. w sprawie komisji bioetycznej oraz Odwoławczej Komisji Bioetycznej (Dz.U. z 2023 r., poz. 218).

Komisja Bioetyczna przy Uniwersytecie Medycznym w Lublinie, w składzie według listy dołączonej do niniejszej uchwały, na posiedzeniu w dniu 26.10.2023 r. zapoznała się z projektem badania:

„Zastosowanie precyzyjnej diagnostyki molekularnej (PMD@) w ocenie alergii u dzieci z celiakią"

Kierownik tematu badawczego:
Dr n. med. Emilia Majsiak
Zakład Promocji Zdrowia
Katedry Rozwoju Pielęgniarstwa
Uniwersytetu Medycznego w Lublinie
ul. Staszica 4/6
20-081 Lublin

Do Komisji wpłynęły następujące dokumenty:
1. Wniosek do Komisji Bioetycznej.
2. Protokół badania.
3. Zgody kierowników jednostek na przeprowadzenie badania.
4. Wniosek o zwolnienie z opłaty za wydanie opinii.

Po zapoznaniu się z całością dokumentacji Komisja Bioetyczna przy Uniwersytecie Medycznym w Lublinie wyraża pozytywną opinię o przedstawionym projekcie badania.

Komisja działa zgodnie z zasadami Prawidłowego Prowadzenia Badań Klinicznych GCP (Guidelines for Good Clinical Practice). Od niniejszej opinii przysługuje odwołanie do Odwoławczej Komisji Bioetycznej przy Ministrze Zdrowia za pośrednictwem Komisji Bioetycznej przy Uniwersytecie Medycznym w Lublinie.
Niniejsza opinia traci moc z chwilą ukończenia badania.

PRZEWODNICZĄCY
Komisji Bioetycznej
dr hab. Andrzej Głowniak, prof. uczelni

.....
Przewodniczący Komisji Bioetycznej

26.10.2023 r.
.....
data

KOMISJA BIOETYCZNA
przy Uniwersytecie Medycznym w Lublinie
20-059 Lublin, Al. Racławickie 1
tel. 81 448 55 15

LISTA OBECNOŚCI CZŁONKÓW KOMISJI BIOETYCZNEJ PRZY UNIWERSYTECIE MEDYCZNYM W LUBLINIE Z POSIEDZENIA W DNIU 26 PAŹDZIERNIKA 2023 r.

Lp.	Imię i nazwisko	Miejsce pracy	Podpis
1.	Dr hab. Anna Belcarz, prof. uczelni	Katedra i Zakład Biochemii i Biotechnologii UM w Lublinie	
2.	Dr n. med. Monika Bojarska-Łoś	Oddział Chorób Wewnętrznych, Endokrynologii i Diabetologii, Wojewódzki Szpital Specjalistyczny w Lublinie Przedstawiciel Okręgowej Rady Lekarskiej	
3.	Dr hab. Barbara Budzyńska, prof. uczelni	Samodzielna Pracownia Badań Behawioralnych UM w Lublinie	
4.	Prof. dr hab. Grzegorz Dzida	Katedra i Klinika Chorób Wewnętrznych UM w Lublinie	
5.	Prof. dr hab. Agata Filip	Katedra Genetyki Nowotworów z Pracownią Cytogenetyczną UM w Lublinie	
6.	Dr hab. Andrzej Głowniak, prof. uczelni	Katedra i Klinika Kardiologii UM w Lublinie	
7.	Prof. dr hab. Ewelina Grywalska	Zakład Immunologii Doświadczalnej UM w Lublinie	
8.	Prof. dr hab. Hanna Karakuła-Juchnowicz	I Katedra Psychiatrii, Psychoterapii i Wczesnej Interwencji UM w Lublinie	
9.	Prof. dr hab. Jerzy Kowalczyk	Klinika Hematologii, Onkologii i Transplantologii Dziecięcej UM w Lublinie	
10.	Prof. dr hab. Paweł Krawczyk	Pracownia Immunologii i Genetyki UM w Lublinie	
11.	Prof. dr hab. Tomasz Plech	Katedra Farmakologii i Biologii UM w Lublinie	
12.	Dr hab. Paweł Rybojad	Katedra i Klinika Chirurgii Klatki Piersiowej UM w Lublinie	
13.	Dr hab. Marzena Samardakiewicz, prof. uczelni	Katedra Psychologii UM w Lublinie	
14.	Ks. dr Krzysztof Smykowski	Katedra Bioetyki Teologicznej Katolicki Uniwersytet Lubelski	
15.	Prof. dr hab. Anna Torres	Klinika Ginekologii Dziecięcej UM w Lublinie	
16.	Dr hab. Paulina Własiuk	Zakład Hematoonkologii Doświadczalnej UM w Lublinie	
17.	Dr hab. Witold Zgodziński, prof. uczelni	I Katedra i Klinika Chirurgii Ogólnej, Transplantacyjnej i Leczenia Żywieniowego UM w Lublinie	

PRZEWODNICZĄCY
Komisji Bioetycznej
dr hab. Andrzej Głowniak, prof. uczelni

Potwierdzam zgodność z załączoną listą

UCHWAŁA KOMISJI BIOETYCZNEJ

numer KE-0254/ 86 /03/2023

Na posiedzeniu w dniu **30 marca 2023 r.** Komisja Bioetyczna przy Uniwersytecie Medycznym w Lublinie, Al. Racławickie 1, zapoznała się z projektem badania:

„Retrospektywna analiza występowania przeciwciał E wobec ekstraktów i molekuł alergenowych w surowicy krwi dzieci i dorosłych populacji polskiej"

Kierownik tematu badawczego:
Dr n. med. Emilia Majsiak
Zakład Promocji Zdrowia
Katedry Rozwoju Pielęgniarstwa
Uniwersytetu Medycznego w Lublinie
ul. Staszica 4/6
20-081 Lublin

Do Komisji wpłynęły następujące dokumenty:

1. Wniosek do Komisji Bioetycznej.
2. Protokół badania.
3. Zgoda kierownika jednostki na przeprowadzenie badania.
4. Wniosek o zwolnienie z opłaty za wydanie opinii.

Po zapoznaniu się z całością dokumentacji Komisja Bioetyczna wyraziła **pozytywną** opinię o przedstawionym projekcie badania.

Niniejsza opinia traci moc z chwilą ukończenia badania.

PRZEWODNICZĄCA
Komisji Bioetycznej

.....

Przewodnicząca Komisji

30.03.2023 r.

data

Członkowie Komisji:

LISTA CZŁONKÓW KOMISJI BIOETYCZNEJ PRZY UNIWERSYTECIE MEDYCZNYM W LUBLINIE opiniujących Uchwałę KE-0254/ 86 /03/2023 z dnia 30.03.2023 r.

Lp.	Imię i nazwisko	zawód	podpis
1.	**prof. dr hab. Jolanta Szymańska**	Stomatolog	
2.	**dr n. med. Hanna Czekajska-Łuckiewicz**	Farmaceuta	
3.	**prof. dr hab. Iwona Beń-Skowronek**	Pediatra	
4.	**prof. dr hab. Hanna Karakuła-Juchnowicz**	Psychiatra	
5	**prof. dr hab. Elżbieta Czekajska-Chehab**	Radiolog	
6.	**dr hab. n. med. Witold Zgodziński, prof. ucz.**	Chirurg	
7.	**prof. dr hab. Paweł Krawczyk**	Internista	
8.	**prof. dr hab. Wojciech Polkowski**	Chirurg onkologiczny	
9.	**prof. dr hab. Janusz Kocki**	Genetyk	
10.	**prof. dr hab. Grzegorz Dzida**	Internista diabetolog	
11.	**ks. dr Krzysztof Smykowski**	Duchowny	
12.	**dr n. med. Lidia Sierpińska**	Pielęgniarka	
13.	**mec. Stanisław Pęzioł**	Radca prawny	

KOMISJA BIOETYCZNA
PRZY UNIWERSYTECIE MEDYCZNYM W LUBLINIE
Al. Racławickie 1, 20-059 Lublin, tel. + 48 81 448-51-15
e-mail: komisja.bioetyczna@umlub.pl

UCHWAŁA nr KE-0254/222/10/2023
z dnia 26 października 2023 roku

Na podstawie art. 29 ustawy z dnia 5 grudnia 1996 r. o zawodzie lekarza i lekarza dentysty (Dz.U. z 2022 r., poz. 171, z póź. zm.), ustawy z dnia 6 września 2001 r. Prawo farmaceutyczne, rozporządzenia Ministra Zdrowia z dnia 26 stycznia 2023 r. w sprawie komisji bioetycznej oraz Odwoławczej Komisji Bioetycznej (Dz.U. z 2023 r., poz. 218).

Komisja Bioetyczna przy Uniwersytecie Medycznym w Lublinie, w składzie według listy dołączonej do niniejszej uchwały, na posiedzeniu w dniu 26.10.2023 r. zapoznała się z projektem badania:

„Zastosowanie precyzyjnej diagnostyki molekularnej (PMD@) w ocenie alergii u dzieci z celiakią"

Kierownik tematu badawczego:
Dr n. med. Emilia Majsiak
Zakład Promocji Zdrowia
Katedry Rozwoju Pielęgniarstwa
Uniwersytetu Medycznego w Lublinie
ul. Staszica 4/6
20-081 Lublin

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1. Wniosek do Komisji Bioetycznej.
2. Protokół badania.
3. Zgody kierowników jednostek na przeprowadzenie badania.
4. Wniosek o zwolnienie z opłaty za wydanie opinii.

Po zapoznaniu się z całością dokumentacji Komisja Bioetyczna przy Uniwersytecie Medycznym w Lublinie wyraża pozytywną opinię o przedstawionym projekcie badania.

Komisja działa zgodnie z zasadami Prawidłowego Prowadzenia Badań Klinicznych GCP (Guidelines for Good Clinical Practice). Od niniejszej opinii przysługuje odwołanie do Odwoławczej Komisji Bioetycznej przy Ministrze Zdrowia za pośrednictwem Komisji Bioetycznej przy Uniwersytecie Medycznym w Lublinie.
Niniejsza opinia traci moc z chwilą ukończenia badania.

PRZEWODNICZĄCY
Komisji Bioetycznej
dr hab. Andrzej Głowniak, prof. uczelni

Przewodniczący Komisji Bioetycznej

26.10.2023 r.
data

KOMISJA BIOETYCZNA
przy Uniwersytecie Medycznym w Lublinie
20-059 Lublin, Al. Racławickie 1
tel. 81 448 51 13

LISTA OBECNOŚCI CZŁONKÓW KOMISJI BIOETYCZNEJ PRZY UNIWERSYTECIE MEDYCZNYM W LUBLINIE Z POSIEDZENIA W DNIU 26 PAŹDZIERNIKA 2023 r.

Lp.	Imię i nazwisko	Miejsce pracy	Podpis
1.	Dr hab. Anna Belcarz, prof. uczelni	Katedra i Zakład Biochemii i Biotechnologii UM w Lublinie	
2.	Dr n. med. Monika Bojarska-Łoś	Oddział Chorób Wewnętrznych, Endokrynologii i Diabetologii, Wojewódzki Szpital Specjalistyczny w Lublinie Przedstawiciel Okręgowej Rady Lekarskiej	
3.	Dr hab. Barbara Budzyńska, prof. uczelni	Samodzielna Pracownia Badań Behawioralnych UM w Lublinie	
4.	Prof. dr hab. Grzegorz Dzida	Katedra i Klinika Chorób Wewnętrznych UM w Lublinie	
5.	Prof. dr hab. Agata Filip	Katedra Genetyki Nowotworów z Pracownią Cytogenetyczną UM w Lublinie	
6.	Dr hab. Andrzej Głowniak, prof. uczelni	Katedra i Klinika Kardiologii UM w Lublinie	
7.	Prof. dr hab. Ewelina Grywalska	Zakład Immunologii Doświadczalnej UM w Lublinie	
8.	Prof. dr hab. Hanna Karakuła-Juchnowicz	I Katedra Psychiatrii, Psychoterapii i Wczesnej Interwencji UM w Lublinie	
9.	Prof. dr hab. Jerzy Kowalczyk	Klinika Hematologii, Onkologii i Transplantologii Dziecięcej UM w Lublinie	
10.	Prof. dr hab. Paweł Krawczyk	Pracownia Immunologii i Genetyki UM w Lublinie	
11.	Prof. dr hab. Tomasz Plech	Katedra Farmakologii i Biologii UM w Lublinie	
12.	Dr hab. Paweł Rybojad	Katedra i Klinika Chirurgii Klatki Piersiowej UM w Lublinie	
13.	Dr hab. Marzena Samardakiewicz, prof. uczelni	Katedra Psychologii UM w Lublinie	
14.	Ks. dr Krzysztof Smykowski	Katedra Bioetyki Teolgicznej Katolicki Uniwersytet Lubelski	
15.	Prof. dr hab. Anna Torres	Klinika Ginekologii Dziecięcej UM w Lublinie	
16.	Dr hab. Paulina Własiuk	Zakład Hematoonkologii Doświadczalnej UM w Lublinie	
17.	Dr hab. Witold Zgodziński, prof. uczelni	I Katedra i Klinika Chirurgii Ogólnej, Transplantacyjnej i Leczenia Żywieniowego UM w Lublinie	

PRZEWODNICZĄCY
Komisji Bioetycznej
dr hab. Andrzej Głowniak, prof. uczelni

Potwierdzam zgodność z załączoną listą

KOMISJA BIOETYCZNA

posiedzenie w dniu 21 XI 2018

Przy Instytucie „Pomnik- Centrum Zdrowia Dziecka"
Al. Dzieci Polskich 20
04-730 Warszawa- Międzylesie
e-mail : komisja.bioetyczna@ipczd.pl
Tel:(22)815-16-03

LISTA OBECNOŚCI

Lp.	Imię, nazwisko, zawód /specjalność, funkcja	podpis
1.	**Prof. dr hab Jacek Hołówka** Filozof	
2.	**Mgr praw. Marcjanna Dębska** Adwokat	
3.	**Ks. Paweł Śmierzchalski** Teolog, Duszpasterstwo Służby Zdrowia	
4.	**Dr hab., prof. APS Irena Jelonkiewicz-Sterianos** psycholog	
5.	**Prof. dr hab. n. med. Rafał Paluszkiewicz** Lekarz (chirurg, transplantolog Kliniczny)- WUM, Okręgowa Rada Lekarska w Warszawie	
6.	**Mgr. Piel Ewa Szkiela** Pielęgniarka	
7.	**Mgr. farm. Bożena Tondys**	
8.	**Dr n. med. Ludmiła Bacewicz** Lekarz chirurg- IPCZD	
9.	**Prof. dr hab. n med. Krystyna Chrzanowska** Lekarz (pediatra, genetyk Kliniczny) - IPCZD	
10.	**Prof. Dr hab. n. med. Katarzyna Kotulska-Jóźwiak** Lekarz (neurolog, neurolog dziecięcy)- IPCZD	
11.	**Prof. nadzw dr hab. n med. Katarzyna Dzierzanowska-Fangrat** IPCZD	
12.	**Dr n. med. Małgorzata Łyszkowska** Lekarz (pediatra, transplantolog Kliniczny) - IPCZD	
13.	**Prof. nadzw dr hab. n med. Wiesława Grajkowska** IPCZD	
14.	**Dr hab. n med. Sylwester Prokurat, prof. nadzw** Lekarz (pediatra, nefrolog, transplantolog Kliniczny)- IPCZD	
15.	**Prof. zw dr hab. n med Andrzej Piotrowski** IPCZD	

10 OŚWIADCZENIA WSPÓŁAUTORÓW

OŚWIADCZENIE WSPÓŁAUTORA

O INDYWIDUALNYM WKŁADZIE PRACY W POWSTANIE ARTYKUŁU

TUTUŁ: . IgE-Dependent Allergy in Patients with Celiac Disease: A Systematic Review

AUTORZY: Majsiak E, Choina M, Knyziak-Mędrzycka I, Bierła JB, Janeczek K, Wykrota J, Cukrowska B.

czasopismo lub wydawca, rok wydania, tom, strony: Nutrients. 2023 Feb 16;15(4):995.

AUTOR: Cukrowska Bożena

Oświadczam, że mój wkład w powstanie artykułu polegał na opracowaniu metodologii, analizie formalnej, prowadzeniu badania, pisaniu – przygotowaniu wstępnej wersji artykułu oraz odpowiedzi recenzentom, nadzorze merytorycznym i administrowaniu projektem, w ramach którego powstała publikacja.

Wkład Izabeli Knyziak-Mędrzyckiej polegał na pisaniu i przygotowaniu wstępnej wersji artykułu, wysłania artykułu do redakcji.

Wyrażam zgodę, żeby publikacja ta była częścią rozprawy doktorskiej lek. Izabeli Knyziak-Mędrzyckiej.

Data: 13.03.2024

Podpis: B. Cukrowska

O INDYWIDUALNYM WKŁADZIE PRACY W POWSTANIE ARTYKUŁU

TUTUŁ: The Sensitization Profile for Selected Food Allergens in Polish Children Assessed with the Use of a Precision Allergy Molecular Diagnostic Technique

AUTORZY: Knyziak-Mędrzycka I., Majsiak E., Gromek W., Kozłowska D., Swadźba J., Bierła J.B., Kurzawa R., Cukrowska B.

czasopismo lub wydawca, rok wydania, tom, strony: Int. J. Mol. Sci. 2024, 25, 825.

AUTOR: Cukrowska Bożena

Oświadczam, że mój wkład w powstanie artykułu polegał na koncepcji publikacji, opracowaniu metodologii, analizie formalnej, prowadzeniu badania, gromadzeniu zasobów, analizie danych, redakcji artykułu, przygotowaniu wersji ostatecznej artykułu, wizualizacji, nadzorze merytorycznym, administrowaniu projektem, w ramach którego powstała publikacja i pozyskaniu finansowania.

Wkład Izabeli Knyziak-Mędrzyckiej polegał na koncepcji publikacji, opracowaniu metodologii, analizie formalnej, prowadzeniu badania, gromadzeniu zasobów, pisaniu – przygotowaniu wersji wstępnej artykułu oraz odpowiedzi recenzentom i redakcji tekstu.

Wyrażam zgodę, żeby publikacja ta była częścią rozprawy doktorskiej lek. Izabeli Knyziak-Mędrzyckiej.

Data: 13.03.2024

Podpis: B. Cukrowska

O INDYWIDUALNYM WKŁADZIE PRACY W POWSTANIE ARTYKUŁU

TUTUŁ: The Precision Allergy Molecular Diagnosis (PAMD@) in Monitoring the Atopic March in a Child with a Primary Food Allergy: Case Report

AUTORZY: Knyziak-Mędrzycka I, Szychta M, Majsiak E, Fal AM, Doniec Z, Cukrowska B.

czasopismo lub wydawca, rok wydania, tom, strony: J Asthma Allergy. 2022 Sep 7;15:1263-1267.

AUTOR: Cukrowska Bożena

Oświadczam, że mój wkład w powstanie pracy polegał na koncepcji publikacji, korekcie wersji wysłanej do redakcji i odpowiedzi recenzentom.

Wkład Izabeli Knyziak-Mędrzyckiej polegał na prowadzeniu i diagnostyce pacjenta, napisaniu wersji wstępnej artykułu, edycji wersji wysłanej do redakcji oraz odpowiedzi recenzentom.

Wyrażam zgodę, żeby publikacja ta była częścią rozprawy doktorskiej lek. Izabeli Knyziak-Mędrzyckiej.

Data: 13.03.2024

Podpis: B. Cukrowska

O INDYWIDUALNYM WKŁADZIE PRACY W POWSTANIE ARTYKUŁU

TUTUŁ: . **Allergic March in Children: The Significance of Precision Allergy Molecular Diagnosis (PAMD@) in Predicting Atopy Development and Planning Allergen-Specific Immunotherapy**

AUTORZY: Knyziak-Mędrzycka Izabela, Majsiak Emilia, Cukrowska Bożena

czasopismo lub wydawca, rok wydania, tom, strony: Nutrients. 2023 Feb 15;15(4):978.

AUTOR: **Cukrowska Bożena**

Oświadczam, że mój wkład w powstanie artykułu polegał na koncepcji publikacji, edycji wstępnej wersji artykułu i redakcji tekstu, odpowiedzi recenzentom oraz nadzorze merytorycznym.

Wkład Izabeli Knyziak-Mędrzyckiej polegał na pisaniu i przygotowaniu wstępnej wersji artykułu, wysłania artykułu do redakcji oraz odpowiedzi recenzentom.

Wyrażam zgodę, żeby publikacja ta była częścią rozprawy doktorskiej lek. Izabeli Knyziak-Mędrzyckiej.

Data: 13.03.2024

Podpis: B. Cukrowska

OŚWIADCZENIE WSPÓŁAUTORA

O INDYWIDUALNYM WKŁADZIE PRACY W POWSTANIE ARTYKUŁU

TUTUŁ: The Precision Allergy Molecular Diagnosis (PAMD@) in Monitoring the Atopic March in a Child with a Primary Food Allergy: Case Report.

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czasopismo lub wydawca, rok wydania, tom, strony: J Asthma Allergy. 2022 Sep 7;15:1263-1267.

AUTOR: Majsiak Emilia

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Wyrażam zgodę, żeby publikacja ta była częścią rozprawy doktorskiej lek. Izabeli Knyziak-Mędrzyckiej.

Data: 10.03.20024

Podpis: Emilia Majsiak

OŚWIADCZENIE WSPÓŁAUTORA

O INDYWIDUALNYM WKŁADZIE PRACY W POWSTANIE ARTYKUŁU

TUTUŁ: The Sensitization Profile for Selected Food Allergens in Polish Children Assessed with the Use of a Precision Allergy Molecular Diagnostic Technique.

AUTORZY: Knyziak-Mędrzycka I., Majsiak E., Gromek W., Kozłowska D., Swadźba J., Bierta J.B., Kurzawa R., Cukrowska B.

czasopismo lub wydawca, rok wydania, tom, strony: Int. J. Mol. Sci. 2024, 25, 825.

AUTOR: Majsiak Emilia

Oświadczam, że mój wkład w powstanie artykułu polegał na koncepcji publikacji, opracowaniu metodologii, analizie formalnej, prowadzeniu badania, gromadzeniu zasobów, analizie danych, użyciu oprogramowania, redakcji artykułu, przygotowaniu wersji ostatecznej artykułu, wizualizacji, nadzorze merytorycznym, administrowaniu projektem, w ramach którego powstała publikacja i pozyskaniu finansowania.

Wkład Izabeli Knyziak-Mędrzyckiej polegał na koncepcji publikacji, opracowaniu metodologii, analizie formalnej, prowadzeniu badania, gromadzeniu zasobów, pisaniu – przygotowaniu wersji wstępnej artykułu oraz odpowiedzi recenzentom i redakcji tekstu.

Wyrażam zgodę, żeby publikacja ta była częścią rozprawy doktorskiej lek. Izabeli Knyziak-Mędrzyckiej.

Data: 10.03.20024

Podpis: Emilia Majsiak

OŚWIADCZENIE WSPÓŁAUTORA

O INDYWIDUALNYM WKŁADZIE PRACY W POWSTANIE ARTYKUŁU

TUTUŁ: . IgE-Dependent Allergy in Patients with Celiac Disease: A Systematic Review.

AUTORZY: Majsiak E, Choina M, Knyziak-Mędrzycka I, Bierła JB, Janeczek K, Wykrota J, Cukrowska B.

czasopismo lub wydawca, rok wydania, tom, strony: Nutrients. 2023 Feb 16;15(4):995.

AUTOR: Majsiak Emilia

Oświadczam, że mój wkład w powstanie artykułu polegał na opracowaniu metodologii, analizie formalnej, prowadzeniu badania, gromadzeniu zasobów, analizie danych, pisaniu – przygotowaniu wstępnej wersji artykułu oraz odpowiedzi recenzentom, wizualizacji, nadzorze merytorycznym i administrowaniu projektem, w ramach którego powstała publikacja.

Wkład Izabeli Knyziak-Mędrzyckiej polegał na pisaniu i przygotowaniu wstępnej wersji artykułu, wysłania artykułu do redakcji.

Wyrażam zgodę, żeby publikacja ta była częścią rozprawy doktorskiej lek. Izabeli Knyziak-Mędrzyckiej.

Data: 10.03.20024

Podpis: Emilia Majsiak

OŚWIADCZENIE WSPÓŁAUTORA

O INDYWIDUALNYM WKŁADZIE PRACY W POWSTANIE ARTYKUŁU

TUTUŁ: . Allergic March in Children: The Significance of Precision Allergy Molecular Diagnosis (PAMD@) in Predicting Atopy Development and Planning Allergen-Specific Immunotherapy

AUTORZY: Knyziak-Mędrzycka Izabela, Majsiak Emilia, Cukrowska Bożena

czasopismo lub wydawca, rok wydania, tom, strony: Nutrients. 2023 Feb 15;15(4):978.

AUTOR: Majsiak Emilia

Oświadczam, że mój wkład w powstanie artykułu polegał na koncepcji publikacji, edycji wstępnej wersji artykułu i redakcji tekstu, odpowiedzi recenzentom oraz nadzorze merytorycznym.

Wkład Izabeli Knyziak-Mędrzyckiej polegał na pisaniu i przygotowaniu wstępnej wersji artykułu, wysłania artykułu do redakcji oraz odpowiedzi recenzentom.

Wyrażam zgodę, żeby publikacja ta była częścią rozprawy doktorskiej lek. Izabeli Knyziak-Mędrzyckiej.

Data: 10.03.20024

Podpis: Emilia Majsiak

OŚWIADCZENIE WSPÓŁAUTORA

O INDYWIDUALNYM WKŁADZIE PRACY W POWSTANIE ARTYKUŁU

TUTUŁ: The Precision Allergy Molecular Diagnosis (PAMD@) in Monitoring the Atopic March in a Child with a Primary Food Allergy: Case Report.

AUTORZY: Knyziak-Mędrzycka I, Szychta M, Majsiak E, Fal AM, Doniec Z, Cukrowska B.

czasopismo lub wydawca, rok wydania, tom, strony: J Asthma Allergy. 2022 Sep 7;15:1263-1267.

AUTOR: Doniec Zbigniew

Oświadczam, że mój wkład w powstanie pracy polegał na napisaniu wersji wstępnej artykułu, edycji wersji wysłanej do redakcji i korekcie wersji wysłanej do redakcji.

Wkład Izabeli Knyziak-Mędrzyckiej polegał na prowadzeniu i diagnostyce pacjenta, napisaniu wersji wstępnej artykułu, edycji wersji wysłanej do redakcji oraz odpowiedzi recenzentom.

Wyrażam zgodę, żeby publikacja ta była częścią rozprawy doktorskiej lek. Izabeli Knyziak-Mędrzyckiej.

Data: 10.03.2024

Podpis: .....

OŚWIADCZENIE WSPÓŁAUTORA

O INDYWIDUALNYM WKŁADZIE PRACY W POWSTANIE ARTYKUŁU

TUTUŁ: The Sensitization Profile for Selected Food Allergens in Polish Children Assessed with the Use of a Precision Allergy Molecular Diagnostic Technique.

AUTORZY: Knyziak-Mędrzycka I., Majsiak E., Gromek W., Kozłowska D., Swadźba J., Bierła J.B., Kurzawa R., Cukrowska B.

czasopismo lub wydawca, rok wydania, tom, strony: Int. J. Mol. Sci. 2024, 25, 825.

AUTOR: Kozłowska Danuta

Oświadczam, że mój wkład w powstanie pracy polegał na gromadzeniu zasobów i analizie danych.

Wkład Izabeli Knyziak-Mędrzyckiej polegał na koncepcji publikacji, opracowaniu metodologii, analizie formalnej, prowadzeniu badania, gromadzeniu zasobów, pisaniu – przygotowaniu wersji wstępnej artykułu oraz odpowiedzi recenzentom i redakcji tekstu.

Wyrażam zgodę, żeby publikacja ta była częścią rozprawy doktorskiej lek. Izabeli Knyziak-Mędrzyckiej.

Data: 11.03.2024

Podpis: .....

OŚWIADCZENIE WSPÓŁAUTORA
O INDYWIDUALNYM WKŁADZIE W PRACY W POWSTANIE ARTYKUŁU

TUTUŁ: . IgE-Dependent Allergy in Patients with Celiac Disease: A Systematic Review.
AUTORZY: Majsiak E, Choina M, Knyziak-Mędrzycka I, Bierła JB, Janeczek K, Wykrota J, Cukrowska B.

czasopismo lub wydawca, rok wydania, tom, strony:
Nutrients. 2023 Feb 16;15(4):995.

AUTOR: Wykrota Julia

Oświadczam, że mój wkład w powstanie pracy polegał na analizie formalnej danych.
Wkład Izabeli Knyziak-Mędrzyckiej polegał na pisaniu i przygotowaniu oryginalnego projektu do wysłania do redakcji.
Wyrażam zgodę, żeby publikacja ta była częścią rozprawy doktorskiej lek. Izabeli Knyziak-Mędrzyckiej.

Data: 06.03.2024
Podpis: Julia Wykrota

OŚWIADCZENIE WSPÓŁAUTORA

O INDYWIDUALNYM WKŁADZIE W PRACY W POWSTANIE ARTYKUŁU

TUTUŁ: The Sensitization Profile for Selected Food Allergens in Polish Children Assessed with the Use of a Precision Allergy Molecular Diagnostic Technique.

AUTORZY: Knyziak-Mędrzycka I., Majsiak E., Gromek W., Kozłowska D., Swadźba J., Bierła J.B., Kurzawa R., Cukrowska B.

czasopismo lub wydawca, rok wydania, tom, strony: Int. J. Mol. Sci. 2024, 25, 825.

AUTOR: Gromek Weronika

Oświadczam, że mój wkład w powstanie pracy polegał na użyciu oprogramowania, wizualizacji i pisaniu – przygotowaniu oryginalnego projektu.

Wkład Izabeli Knyziak-Mędrzyckiej polegał na koncepcji publikacji, metodologii, analizie formalnej, prowadzeniu badania, gromadzeniu zasobów, pisaniu – przygotowaniu oryginalnego projektu oraz recenzji i redakcji tekstu.

Wyrażam zgodę, żeby publikacja ta była częścią rozprawy doktorskiej lek. Izabeli Knyziak-Mędrzyckiej.

Data: 05.03.2024 r.

Podpis: Weronika Gromek

OŚWIADCZENIE WSPÓŁAUTORA

O INDYWIDUALNYM WKŁADZIE W PRACY W POWSTANIE ARTYKUŁU

TUTUŁ: . IgE-Dependent Allergy in Patients with Celiac Disease: A Systematic Review.

AUTORZY: Majsiak E, Choina M, Knyziak-Mędrzycka I, Bierła JB, Janeczek K, Wykrota J, Cukrowska B.

czasopismo lub wydawca, rok wydania, tom, strony: Nutrients. 2023 Feb 16;15(4):995.

AUTOR: Choina Magdalena

Oświadczam, że mój wkład w powstanie pracy polegał na pisaniu – przygotowaniu oryginalnego projektu.

Wkład Izabeli Knyziak-Mędrzyckiej polegał na pisaniu i przygotowaniu oryginalnego projektu do wysłania do redakcji.

Wyrażam zgodę, żeby publikacja ta była częścią rozprawy doktorskiej lek. Izabeli Knyziak-Mędrzyckiej.

Data: 13.03.2024

Podpis: Choina

OŚWIADCZENIE WSPÓŁAUTORA

O INDYWIDUALNYM WKŁADZIE W PRACY W POWSTANIE ARTYKUŁU

TUTUŁ: The Precision Allergy Molecular Diagnosis (PAMD@) in Monitoring the Atopic March in a Child with a Primary Food Allergy: Case Report.

AUTORZY: Knyziak-Mędrzycka I, Szychta M, Majsiak E, Fal AM, Doniec Z, Cukrowska B.

czasopismo lub wydawca, rok wydania, tom, strony: J Asthma Allergy. 2022 Sep 7;15:1263-1267.

AUTOR: Szychta Monika

Oświadczam, że mój wkład w powstanie pracy polegał na diagnostyce alergii pokarmowej i wykonaniu próby prowokacji u pacjenta.

Wkład Izabeli Knyziak-Mędrzyckiej polegał prowadzeniu i diagnostyce pacjenta, napisaniu wersji wstępnej artykułu, edycji wersji wysłanej do redakcji oraz odpowiedzi recenzentom.

Wyrażam zgodę, żeby publikacja ta była częścią rozprawy doktorskiej lek. Izabeli Knyziak-Mędrzyckiej.

Data: 5 03 2024

Podpis: Monika Szychta