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ALEX2 multiplex examination – results of specific IgE to fish and shrimps in patients suffering from atopic dermatitis

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ABSTRACT

The sensitivity of ALEX2 Allergy Explorer test in patients suffering from atopic dermatitis and clinical reactions to fish/shrimps was evaluated. We compared the results of specific IgE to allergen reagents (molecular components) and clinical reactions in the open exposure test (patient's history); the sensitivity was calculated with Fisher's Exact test (Rates and Confidence Intervals 95% C. I., value, lower, upper). Altogether 100 atopic dermatitis patients were examined. We confirmed significant relationship between the results of specific IgE to allergen reagents (molecular components) of fish and the results open exposure test/history; the sensitivity is in the range of 33.3% (7.49%–70.07%)–44.4% (13.7%–78.80%). We did not confirm the significant relationship between the results of specific IgE to allergen reagents (molecular components) of shrimps and the results open exposure test/history; the sensitivity is in the range of 0%–40% (5.27%–85.34%).

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Fish/shrimp allergy; ALEX2 allergy explorer; atopic dermatitis; specific IgE

Introduction

Various allergens play a role in the elicitation or exacerbation of eczematous skin lesions in atopic dermatitis patients, and much research effort has been focused on improving diagnostic tests to identify the causative allergens (Bergmann et al., 2013; Sweeney et al., 2021). Diagnostic sensitivity is related to a clinical history and physical examination which is based on the assessment of affected subjects. Requirements for proper calculation and interpretation of the diagnostic sensitivity of IgE antibody tests of sensitization require sound clinical data from the subject's case history and in some cases additional challenge tests to back up the clinical diagnosis. The clinical relevance of allergen-specific IgE detection in a patient's serum is strictly as a marker for allergic sensitization (risk for allergy). Although the gold standard for diagnosis of food allergy is an oral food challenge, clinically relevant biomarkers of IgE sensitization, including serum-specific IgE

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and skin prick testing, can aid in diagnosis (Bousquet et al., 2016; Jensen-Jarolim et al., 2017; Passalacqua et al., 2013; Patelis et al., 2016; Anto et al., 2017; Campana et al., 2017; Heffler et al., 2018; Matricardi et al., 2016; Ruethers et al., 2018; van Hage et al., 2017; WHO/IUIS Allergen Nomenclature Sub-Committee Allergen Nomenclature). Progress in laboratory diagnostics of IgE-mediated allergy is the use of component-resolved diagnosis (CRD) or molecular diagnosis (MD) of allergies (Bousquet et al., 2016; Jensen-Jarolim et al., 2017; Passalacqua et al., 2013; Patelis et al., 2016; Anto et al., 2017; Campana et al., 2017; Heffler et al., 2018; Matricardi et al., 2016; Ruethers et al., 2018; van Hage et al., 2017; WHO/IUIS Allergen Nomenclature Sub-Committee Allergen Nomenclature). Molecular allergy diagnosis using singleplex allergens or multiplex allergen microarrays are typical methods of precision medicine; MD uses pure, mainly recombinant and structurally defined allergen molecules and allergen-derived epitopes to study mechanisms of IgE-associated allergy, to diagnose, and even predict the development of allergic manifestations and to treat and prevent IgE-associated allergies (Campana et al., 2017). Research into the structural similarity between allergens and the amino acid sequence homology between food allergens also helps to explain cross-reactivity between allergens, which may be clinically relevant or irrelevant (Bousquet et al., 2016; Jensen-Jarolim et al., 2017; Passalacqua et al., 2013; Patelis et al., 2016; Anto et al., 2017; Campana et al., 2017; Heffler et al., 2018; Matricardi et al., 2016; Ruethers et al., 2018; van Hage et al., 2017; WHO/IUIS Allergen Nomenclature Sub-Committee Allergen Nomenclature). Some diagnostic modalities combine molecular allergens and extract testing in the forms of ImmunoCAP® Solid-phase Allergen Chip (ISAC) multiplex testing and ALEX® Allergy Explorer (most widely used in Europe) which utilizes microarray technology to perform multiple simultaneous analyses (Bousquet et al., 2016; Jensen-Jarolim et al., 2017; Passalacqua et al., 2013; Patelis et al., 2016; Anto et al., 2017; Campana et al., 2017; Heffler et al., 2018; Matricardi et al., 2016; Ruethers et al., 2018; van Hage et al., 2017; WHO/IUIS Allergen Nomenclature Sub-Committee Allergen Nomenclature). However, the diagnostic accuracy of identified components varies across studies, and thus, the diagnostic value and clinical utility of molecular diagnostics remains unclear (Bousquet et al., 2016; Jensen-Jarolim et al., 2017; Passalacqua et al., 2013; Patelis et al., 2016; Anto et al., 2017; Campana et al., 2017; Heffler et al., 2018; Matricardi et al., 2016; Ruethers et al., 2018; van Hage et al., 2017; WHO/IUIS Allergen Nomenclature Sub-Committee Allergen Nomenclature). ALEX 2 Allergy Explorer is an in vitro assay for the measurement of allergen specific IgE antibodies in human plasma (Heffler et al., 2018; van Hage et al., 2017). The major advantage of ALEX2 Allergy Explorer is the comprehensive IgE pattern obtained with a minute amount of serum (Heffler et al., 2018; van Hage et al., 2017). This new array contains 295 allergen reagents (117 allergenic extracts and 178 molecular components), with a large majority of aeroallergen families and cross-reactive food allergens being represented.

Shellfish (shrimps), along with fish are commonly termed as seafood, but these two groups are very distinct in evolutionary terms and contain different molecular repertoires of food allergens. All shellfish species are invertebrate animals, in comparison with fish, which are lower vertebrates. Comparing evolutionary distance, crustaceans are placed closer to insects and arachnids, and this seems to be the major factor for molecular cross-sensitization and clinical cross-reactivity between crustacean, house dust mites,

and insects (Campana et al., 2017; Chokshi & Sicherer, 2016; Khora, 2016; Lopata & Lehrer, 2009; Matricardi et al., 2016; Ruethers et al., 2018; Sharp & Lopata, 2014). The aim of our study is to analyse the sensitisation profile to fish/shrimp allergens in patients suffering from atopic dermatitis and to determine the sensitivity of ALEX2 Allergy Explorer test. We also evaluate the sensitisation to Der p 10 (tropomyosin) from house dust mites in patients with clinical reaction to shrimps.

Major and relevant minor fish allergenic molecules are recorded in Table 1 (WHO/IUIS Allergen Nomenclature Sub-Committee Allergen Nomenclature); allergenic molecules present in crustacean species are shown in Table 2; the molecular components that are included in the ALEX2 Allergy Explorer test are marked in bold (WHO/IUIS Allergen Nomenclature Sub-Committee Allergen Nomenclature). Allergen extracts from fish and shrimps for detection of specific IgE in ALEX2 Allergy Explorer are recorded in Table 3.

Methods

Patients and methods

In the period 2018–2020 100 patients suffering from atopic dermatitis were examined. All these patients were examined in the Department of Dermatology, Faculty Hospital Hradec Králové, Charles University, Czech republic. The diagnosis of atopic dermatitis was made with the Hanifin-Rajka criteria. Exclusion criteria were systemic therapy (cyclosporin, systemic corticoids, biological therapy), pregnancy, breastfeeding. Patients

Table 1. Major and relevant minor fish allergenic molecules (WHO/IUIS Allergen Nomenclature Sub-Committee Allergen Nomenclature).

	Allergen	Molecular component	Biochemical name Proten family
Clupeiformes	Herring (<i>Clupea harengus</i>)	Clu h 1*	Parvalbumin
	Pilchard (<i>Sardinops sagax</i>)	Sar sa 1	Parvalbumin
Cypriniformes	Carp (<i>Cyprinus carpio</i>)	Cyp c 1	Parvalbumin
Gadiformes	Atlantic cod (<i>Gadus callarias</i>)	Gad c 1	Parvalbumin
	Atlantic cod (<i>Gadus morhua</i>)	Gad m 1*	Parvalbumin
		Gad m 2*	Enolase
		Gad m 3*	Aldolase
Perciformes	Tuna (<i>Thunnus albacares</i>)	Thu a 1*	Parvalbumin
		Thu a 2	Enolase
		Thu a 3	Aldolase
		Xip g 1*	Parvalbumin
Pleuronectiformes	Swordfish	Lep w 1	Parvalbumin
	Megrim, whiff (<i>Lepidorhombus whiffiagonis</i>)		
Salmoniformes	Pacific salmon (<i>Oncorhynchus keta</i>)	Onc k 5	Vitellogenin
	Rainbow trout (<i>Oncorhynchus mykiss</i>)	Onc m 1	Parvalbumin
	Salmon (<i>Salmo salar</i>)	Sal s 1*	Parvalbumin
		Sal s 2	Enolase
		Sal s 3	Aldolase
Scorpaeniformes	Redfish (<i>Sebastes marinus</i>)	Seb m 1	Parvalbumin
Scombriformes	Atlantis macrel	Sco s 1*	Parvalbumin
Ascaridia	Herring worm	Ani s 1*	unknown function, similar to Kunitz serine protease inhibitors
Ascaridia	Herring worm	Ani s 3*	Tropomyosin

*Molecular components examined in ALEX 2 Allergy Explorer are shown extra bold.

Table 2. Allergenic molecules present in crustacean species. Registered allergen names are stated in accordance with WHO/IUIS Allergen Nomenclature ([WHO/IUIS Allergen Nomenclature Sub-Committee Allergen Nomenclature](#)).

Allergen	Molecular component	Biochemical name Proten family
Flowery crab	Cha f 1	Tropomyosin
North Sea shrimp	Cra c 1	Tropomyosin
	Cra c 2	Arginine kinase
	Cra c 4	Sarcoplasmic calcium-binding protein
	Cra c 5	Myosin, light chain 1
	Cra c 6	Troponin C
	Cra c 8	Triosephosphate isomerase
Chinese mitten crab	Eri s 2	Ovary development-related protein
American lobster	Hom a 1	Tropomyosin
	Hom a 3	Myosin light chain 2
	Hom a 6	Troponin C
White-leg shrimp	Lit v 1	Tropomyosin
	Lit v 2	Arginine kinase
	Lit v 3	Myosin, light chain 2
	Lit v 4	Sarcoplasmic calcium-binding protein
	Mac r 1	Tropomyosin
Giant freshwater prawn	Mel l 1	Tropomyosin
King prawn	Met e 1	Tropomyosin
Greasyback shrimp	Pan b 1	Tropomyosin
Northern shrimp	Pan s 1	Tropomyosin
Spiny lobster	Pen a 1	Tropomyosin
Brown shrimp	Pen i 1	Tropomyosin
Indian prawn	Pen m 1*	Tropomyosin
Black tiger prawn	Pen m 2*	Arginine kinase
	Pen m 3*	Myosin light chain 2
	Pen m 4*	Sarcoplasmic calcium binding protein
	Pen m 6	Troponin C
	Pon l 4	Sarcoplasmic calcium-binding protein
Narrowclawed crayfish	Pon l 17	Troponin I
Blue swimmer crab	Por p 1	Tropomyosin

*Molecular components examined in ALEX 2 Allergy Explorer testing are shown extra bold.

Table 3. Allergenic extracts from fish and shrimp in ALEX2 Allergy Explorer testing.

Allergenic extracts	
Clu h	Herring (<i>Clupea harengus</i>)
Gad m	Atlantic cod (<i>Gadus callarias</i>)
Hom g	Lobster
Chi spp	Crab
Lit s	Shrimp
Lol spp	Squid
Myt e	Common mussel
Ost e	Oyster
Pan b	Northern shrimp
Pecc spp.	Scallop
Raj c	Thornback ray
Raj c parvalbumin	Thornback ray
Rud spp	Clam
Sal s	Atlantic Salmon
Sco s	Atlantic mackerel
Thu a	Yellowfin tuna

with atopic dermatitis having other systemic diseases were excluded from the study as well. Complete dermatological and allergological examination was performed in patients included in the study. This study was approved by Ethics committee of Faculty Hospital Hradec Králové, Charles University of Prague, Czech Republic.

Dermatological examination

Complete dermatological examination was performed in patients included in the study. Severity of atopic dermatitis was scored in agreement with SCORAD, with assessment of topography items (affected skin area), intensity criteria and subjective parameters (European Task Force on Atopic Dermatitis, 1993).

Examination of specific IgE to allergen reagents

The serum level of the sIgE was measured by the components resolved diagnostic microarray-based sIgE detection assay ImmunoCAP ALEX2 Allergy Explorer (Heffler et al., 2018; van Hage et al., 2017). It is based on a state-of-the-art proprietary nano-bead technology. The ALEX2 Allergy Explorer measuring range for specific IgE is 0.3–50 kU_A/L (quantitative) and for total IgE is 1–2500 kU/L (semiquantitative). The sample requirement is 100 µL serum or plasma. The results are expressed as Class 0 (< 0.3 kU_A/L) – negative, Class 1 (0.3–1 kU_A/L) – low positivity, Class 2 (1–5 kU_A/L) – moderate positivity, Class 3 (5–15 kU_A/L) – high positivity, and Class 4 (> 15 kU_A/L) – very high positivity. The ALEX2 Allergy Explorer is commercially available, having attained CE certification, which assures that the quality of the assay, regarding LoD, precision and repeatability as well as specificity and linearity, is in line with *in vitro* diagnostic features. There is no significant interference from high total IgE, haemoglobin, bilirubin or triglycerides.

Initially, allergens are coupled to activated nanoparticles, for coupling individual and combinatorial optimization. Each allergen is attached reflecting its biochemical properties and specific requirements for stability, thereby preserving the full epitope complexity. The nanoparticles multiply the surface of the solid-phase presenting the allergen during the immunoassay, enabling highly sensitive detection. In the next step, the allergen-bearing nanoparticles are deposited onto a solid-phase matrix, forming a macroscopic array of individual assay parameters. The different allergens and components, spotted onto a nitrocellulose membrane as immunosorbent in a cartridge chip, are incubated with 0.5 mL of a 1:5 dilution of serum under agitation, the serum diluent containing a Cross-reactive Carbohydrate Determinants (CCDs) inhibitor. After incubation for 2 h, the chips are extensively washed. A pretitrated dilution of anti-human IgE labeled with alkaline phosphatase is added and incubated for 30 min. Following another washing cycle, the enzyme substrate is added, and after a few minutes, the reaction is complete. After the membranes are dried, the quantification of this colorimetric enzyme assay is performed with an easy-to-use and affordable image explorer. The image acquisition and analysis of a single test takes only a few seconds. The assay time is 3.5 h, and tests per run are up to 50 per operator, with manual processing.

Confirmation of clinical reaction to fish

The fish/shrimp allergy was confirmed in these cases: (1) In patients with the clinical reaction in the open exposure test or in the history (early and/or late reactions). (2) Sensitisation to examined food allergens was confirmed in patients with positive results in ALEX2 Allergy Explorer and with the negative results in the open exposure test.

Open exposure test

In patients without previous history about food reactions to fish and/or shrimps and with the positive results in ALEX2 Allergy Explorer Multiplex testing we recommended the elimination diet with the elimination of suspected food allergens for the period of 5 weeks (Čelakovská, Bukač, Vaňková, Krcmova, Krejsek, et al., 2020; Čelakovská, Bukač, Vaňková, Krejsek, Andrýs, et al., 2020).

Open exposure test was performed after this diet in intervals without symptoms or during a consistent period with regard to atopic dermatitis (not in pollen season in patients with pollen allergy). In generally, the open exposure test was recommended to perform with three doses of examined foods given during two days. One dose of examined food was given in incremental dosages in 10 min intervals during one hour. The food challenge results was scored as positive if one or more of the following objective and subjective clinical reactions were noted: itching, rash, urticaria, angioedema, vomiting, wheezing, abdominal pain, diarrhoea, pruritus, or worsening of atopic dermatitis. Early reactions were defined as clinical symptoms within 2 h after the ingestion of food and late symptoms if occurring after more than 2 h. In the case the physician or the patient recorded worsening of the atopic dermatitis or some other reactions during the open exposure test, the patient continued in the elimination of suspected food and the severity of atopic dermatitis was evaluated every 3 month during 1 year. If the open exposure test was negative, the patient introduced this food in the diet regimen. The severity of atopic dermatitis was evaluated during the ingestion of food over a period of 3 months during 1 year. If there is a suspicion to food allergy to more than one food, the next open exposure test was performed in 2–3 weeks after the first exposure test (Čelakovská, Bukač, Vaňková, Krcmova, Krejsek, et al., 2020; Čelakovská, Bukač, Vaňková, Krejsek, Andrýs, et al., 2020).

Statistical analysis

We compared the results of specific IgE to allergen reagents (molecular components) in the examination of ALEX2 Allergy Explorer testing and clinical reactions to fish and shrimps in the open exposure test (patient's history) test; according to these results, the sensitivity was calculated with Fisher's Exact test (we show Rates and Confidence Intervals 95% C. I., value, lower, upper). Sensitivity was calculated as a proportion of positive IgE antibody tests in patients with allergic disease. We used statistical software: NCSS 2019 Statistical Software (2019). NCSS, LLC. Kaysville, Utah, USA, [css.com/software/ncss](https://www.ncss.com/software/ncss).

Results

Characteristic of patients

One hundred atopic dermatitis patients were included in the study (49 men and 51 women with the average age 40.6 years: min. age 14 years, max. age 67 years and with the average SCORAD 39 points, SD 13.5 points). The mild form of AD was recorded in 14 patients (14%), moderate form in 61 patients (61%), severe form in 25 patients (25%); 55 patients (55%) suffer from bronchial asthma and 74 patients (74%) suffer

from allergic rhinitis. Positive family history about atopy was recorded in 48 patients (48%). The onset of AD before 5 years of age in 61 patients (61%) and the persistent eczematous lesions in 57 patients (57%).

Patients with positive results of specific IgE to fish/shrimps allergen reagents (Classes 1–4).

The number of patients with positive results of specific IgE to fish/shrimps allergen reagents (Classes 1–4) in 100 atopic dermatitis patients is recorded in [Table 4](#).

Results of specific IgE to fish/shrimp molecular components and allergen extracts according to the level of specific IgE to fish/shrimps in Classes 0, 1, 2, 3, 4

The detailed review of sensitisation according to the level of specific IgE to fish/shrimps in Classes 0, 1, 2, 3, 4 to allergen reagents (molecular components) in 100 atopic dermatitis patients is recorded in [Table 5](#) and in [Complement to Table 5](#). Atopic dermatitis patients suffer mainly from sensitisation to parvalbumins (Clu h 1, Cyp c 1, Sal s 1, Sco s 1, Thu a 1, Xip g 1, Gad m 1), to arginin kinase (Pen m 2) and to tropomyosin (Pen m 1, Ani s 3) – we confirmed high and very high level of specific IgE to these molecular components. We also confirmed high and very high level of specific IgE to allergen extracts such as Hom g (Lobster), Chispp (Crab), Lit s (Shrimp) and Pan b (Northern shrimp), and Rudsp (Clam). The most common sensitization was recorded to Pen m 2 in 18% of patients, to Hom g in 15%, to Pan b in 12%.

Clinical reactions to fish/shrimps

The reactions to fish were confirmed in nine patients (9%), the reactions to shrimps in five patients (5%) in the open exposure test (history). In patients with clinical reaction to fish, gastrointestinal symptoms such as abdominal pain, diarrhoea, vomiting, cramps were recorded; the new lesions of atopic dermatitis were observed in 4 patients (4%). In patients with clinical reaction to shrimps, gastrointestinal symptoms such as abdominal pain and diarrhoea were recorded; in 2 patients (2%), the pruritus and new lesions of atopic dermatitis were observed.

Sensitivity of allergen reagents in ALEX 2 Allergy Explorer test in patients with clinical reaction to fish

The significant relation was confirmed between the results of open exposure test (history) and the results of specific IgE to these allergen reagents ([Table 6](#)): Sco s, Sco s 1 (Parvalbumin Atlantic mackerel), Clu h, Clu h 1 (Atlantic herring Beta parvalbumin), Cyp c 1 (Beta parvalbumin, carp), Gad m (Beta parvalbumin, Atlantic Cod), Thu a 1 (Beta parvalbumin, Yellowfin tuna), Xip g (Beta parvalbumin, Swordfish), Sal s, Sal s 1 (Beta parvalbumin 1 Atlantic salmon), Gad m 1 (Beta parvalbumin, Atlantic cod). We show the sensitivity (Rates and Confidence Intervals 95% C. I., value, lower, upper); for molecular components and allergen extracts from fish the sensitivity is in the range of 33.3%

Table 4. The Review of allergen reagents (molecular components) in ALEX2 Allergy Explorer. We show the number of patients with positive results of specific IgE Classes 1–4 to allergen reagents (molecular components, allergen extracts) in 100 atopic dermatitis patients.

Allergen reagents, molecular components	Protein family	Allergen	Number of patients with positive results of specific IgE (in Classes 1 – 4)
Ani s 1	Unknown function, similar to Kunitz serine protease inhibitors	Ascaridia, Herring worm	0%
Ani s 3	Tropomyosin	Ascaridia, Herring worm	4.0%
Clu h		Atlantic herring	3.0%
Clu h 1	Beta-parvalbumin	Atlantic herring	5.0%
Cra c 6	Troponin C	North Sea shrimp	7.0%
Cyp c 1	Beta-parvalbumin	Common carp	6.0%
Gad m		Atlantic cod	4.0%
Gad m 1	Beta-parvalbumin	Atlantic cod	3.0%
Gad m 2 + Gad m 3	Beta-enolase + Aldolase A	Atlantic cod	3.0%
Hom g		Lobster	15.0%
Chi spp		Crab	9.0%
Lit s		Shrimp	6.0%
Lol spp.		Squid	12.0%
Myt e		Common mussel	4.0%
Ost e		Oyster	4.0%
Pan b		Northern shrimp	12.0%
Pec spp.		Scallop	11.0%
Pen m 1	Tropomyosin	Black tiger shrimp	5.0%
Pen m 2	Arginine kinase	Black tiger shrimp	18.0%
Pen m 3	Myosin light chain 2	Black tiger shrimp	1.0%
Pen m 4	Sarcoplasmic calcium binding protein	Black tiger shrimp	2.0%
Raj c		Thornback ray	0%
Rud spp.		Clam	8.0%
Sal s		Atlantic Salmon	2.0%
Sal s 1	Beta-parvalbumin 1	Atlantic salmon	7.0%
Sco s		Atlantic mackerel	6.0%
Sco s 1	Beta-parvalbumin	Atlantic mackerel	6.0%
Thu a		Yellowfin tuna	5.0%
Thu a 1	Beta-parvalbumin	Yellowfin tuna	7.0%
Xip g 1	Beta-parvalbumin	Swordfish	6.0%

(7.49%–70.07%)–44.4% (13.7%–78.80%). We also recorded the significant relationship between the results of specific IgE to Cra c 6 (North sea shrimp, troponin C) and Pen m 2 (Black tiger shrimp, arginin kinase) from shrimps and clinical reactions to fish. Specific IgE to Hom g (Lobster) is also significantly higher in patients with reactions to fish.

Sensitivity of allergen reagents in ALEX 2 Allergy Explorer test in patients with clinical reaction to shrimp

The relationship between the results of open exposure test (history) to shrimps and results of specific IgE to allergen reagents (molecular components) of shrimps was not confirmed (Table 7). We show the sensitivity (Rates and Confidence Intervals 95% C. I., value, lower, upper); for molecular components and allergen extracts from

Table 5. The detailed overview of fish and shrimp allergen reagents (allergenic extracts and molecular components) according to the frequency of sensitisation in classes 0, 1, 2, 3, 4 in 100 atopic dermatitis patients (= 100%).

Allergen reagents	Results of specific IgE in classes 0–4 to allergen reagents (molecular components) of fish and shrimps in 100 atopic dermatitis patients (100 patients = 100%)				
	Class 0	Class 1	Class 2	Class 3	Class 4
Ani s 1	100.0%	0.0%	0.0%	0.0%	0.0%
Ani s 3	96.0%	0.0%	0.0%	1.0%	3.0%
Clu h	97.0%	0.0%	2.0%	1.0%	0.0%
Clu h 1	95.0%	2.0%	0.0%	0.0%	3.0%
Cra c 6	93.0%	0.0%	2.0%	2.0%	3.0%
Cyp c 1	94.0%	2.0%	1.0%	0.0%	3.0%
Gad m	96.0%	1.0%	1.0%	1.0%	1.0%
Gad m 1	97.0%	0.0%	1.0%	1.0%	1.0%
Gad m 2+3	97.0%	2.0%	1.0%	0.0%	0.0%
Hom g	85.0%	2.0%	7.0%	2.0%	4.0%
Chi spp_	91.0%	1.0%	2.0%	3.0%	3.0%
Lit s	94.0%	1.0%	2.0%	1.0%	2.0%
Lol spp	88.0%	4.0%	3.0%	3.0%	2.0%
Myt e	96.0%	3.0%	0.0%	1.0%	0.0%
Ost e	96.0%	1.0%	1.0%	1.0%	1.0%
Pan b	88.0%	0.0%	5.0%	5.0%	2.0%
Pan m	92.0%	5.0%	3.0%	0.0%	0.0%
Pec spp_	89.0%	7.0%	4.0%	0.0%	0.0%
Pen m 1	95.0%	0.0%	1.0%	1.0%	3.0%
Pen m 2	82.0%	0.0%	6.0%	5.0%	7.0%
Pen m 3	99.0%	0.0%	0.0%	1.0%	0.0%
Pen m 4	98.0%	0.0%	2.0%	0.0%	0.0%
Raj c	100.0%	0.0%	0.0%	0.0%	0.0%
RajcParvalbumin	99.0%	0.0%	0.0%	0.0%	1.0%
Rud spp_	92.0%	5.0%	1.0%	1.0%	1.0%
Sal s	98.0%	0.0%	1.0%	1.0%	0.0%
Sal s 1	93.0%	2.0%	1.0%	1.0%	3.0%
Sco s	94.0%	5.0%	1.0%	0.0%	0.0%
Sco s 1	94.0%	1.0%	2.0%	0.0%	3.0%
Thu a	95.0%	5.0%	0.0%	0.0%	0.0%
Thu a 1	93.0%	2.0%	2.0%	0.0%	3.0%
Xip g 1	94.0%	1.0%	0.0%	2.0%	3.0%

Explanation:

Class 0 – negative (< 0.3 kU_A/L)

Class 1 – low positivity (0.3–1 kU_A/L)

Class 2 – moderate positivity (1–5 kU_A/L)

Class 3 – high positivity (5–15 kU_A/L)

Class 4 – very high positivity (> 15 kU_A/L).

shrimp the sensitivity is in the range of 0%–40% (5.27%–85.34%). We evaluated also the sensitisation to Der p 10 (tropomyosin) in patients with reaction to shrimps; the significant correlation between the results of specific IgE to Der p 10 and clinical reaction to shrimps was not confirmed.

Discussion

The purpose of our study was to analyse the sensitisation to molecular components of fish/shrimps with the use of ALEX2 Allergy Explorer test and to evaluate the sensitivity of this test. The advantage of this work is that we show the sensitivity of the test in comparison with clinical symptoms. So far, no work has been done on comparing laboratory

Class – level of specific IgE	Allergen reagents (Molecular components, allergen extracts)	Number of patients with positive results of sIgE
The very high level of specific IgE (Class 4)	Pen m 2	7%
	Hom g	4%
	Ani s 3, Clu h 1, Cyp c 1, Chispp, Pen m 1, Sal s 1, Sco s 1, Thu a 1, Xip g 1	3%
	Lit s, Pan b	2%
	Gad m 1, Raj c, Rud spp	1%
The high level of specific IgE (Class 3)	Lol p 1	10%
	Pen m 2, Pan b	5%
	Chispp	3%
	Hom g, Xip g	2%
	Ani s 3, Clu h, Gad m, Gad m 1, to Lit s, Myt e, Ost e, Pen m 1, Pen m 3, Rud spp, Sal s, Sal s 1	1%
The moderate level of specific IgE (Class 2)	Hom g	7%
	Lol p 1, Pen m 2	6%
	Pan b	5%
	Clu h, Chispp, Pen m 4, Sco s 1, Thu a 1	2%
	Cyp c 1, Gad m 1, Gad m 2+3, Ost e, Pen m 1, Rud spp, Sal s, Sal s 1, Sco s	1%
The low level of specific IgE (Class 1)	Pec spp, Lol p 1	7%
	Pan m, Rud spp, Soc s, Thu a	5%
	Clu h 1, Cyp c 1, Gad m 2+3, Hom g, Sal s 1, Thu a 1	2%
	Gad m, Chi spp, Lit s, Ost e, Sco s 1, Xip g 1	1%

results in ALEX 2 Allergy explorer test with respect to clinical symptoms. Flores et al. performed the first systematic review analyzing the evidence on the diagnostic accuracy of molecular allergens for a range of food allergies. This systematic review included 11 studies that assessed the accuracy of molecular diagnostics in evaluating cow's milk, hen's egg, peanut, hazelnut, and shrimp allergies. According to Flores 's study, some molecular components have the potential to diagnose cow's milk, hen's egg, peanut, hazelnut, and shrimp allergies with high specificity, but low sensitivity. Nevertheless, at present, there is not enough methodologically robust evidence to draw definite conclusions (Flores Kim et al., 2018). In our study, we confirmed the good sensitivity of molecular components and allergen extracts in ALEX2 Allergy Explorer test in patients with reaction to fish. On the other hand, we did not confirm the significant relationship between the results of specific IgE to shrimps and the results of the open exposure test (history) to shrimps; these patients with clinical reactions to shrimps may suffer from food intolerance or can be sensitised to molecular allergens which are not represented in ALEX2 Allergy Explorer test. Also, the significant relationship between the results of specific IgE to Der p 10 and clinical reaction to shrimps was not confirmed.

Only sensitisation to molecular components and allergen extracts from fish/shrimps was confirmed in patients with no clinical reaction after ingestion fish/shrimp, but with positive results of specific IgE to allergen reagents in ALEX2 Allergy Explorer Multiplex testing. It means, that although the positive result of specific IgE was recorded, these patients are without clinical symptoms. All these patients are informed about the positive results of specific IgE and they are always monitored. Atopic dermatitis patients suffer mainly from sensitisation to parvalbumins, to arginin kinase and to tropomyosin – we confirmed high and very high level of specific IgE to these molecular components. We also confirmed high and very high level of specific IgE to allergen extracts such as Hom g (Lobster), Chispp (Crab), Lit s (Shrimp) and Pan b (Northern shrimp), and

Table 6. Results of specific IgE to allergen reagents (molecular components) in atopic dermatitis patients with the clinical reaction to fish and in patients without clinical reaction to fish. Results of specific IgE to allergen reagents (molecular components) of fish/shrimps are evaluated as positive (classes of specific IgE 1–4) and negative (Class 0).

Specific IgE to allergen reagents (molecular components)	Number of patients – clinical reaction to fish			Sensitivity Rates and Confidence Intervals 95% C. I.		
	yes 9 (9%)	no 91 (91%)	p- value	value	lower	upper
Ani s 1 +	Tests cannot be computed for 0x2 tables.					
Ani s 1 –	9	91				
Ani s 3 +	1	3				
Ani s 3 –	8	88	0.31841	0.1111	0.0028	0.4825
Clu h +	3	0				
Clu h –	6	91	0.00052*	0.3333	0.0749	0.7007
Clu h 1 +	3	2				
Clu h 1 –	6	89	0.00472*	0.3333	0.0749	0.7007
Cra c 6 +	3	4				
Cra c 6 –	6	87	0.01501	0.3333	0.0749	0.7007
Cyp c 1 +	4	2				
Cyp c 1 –	5	89	0.00044*	0.4444	0.1370	0.7880
Gad m +	3	1				
Gad m –	6	90	0.00198*	0.3333	0.0749	0.7007
Gad m 1 +	3	0				
Gad m 1 –	6	91	0.00052*	0.3333	0.0749	0.7007
Gad m 2 +3 +	1	2				
Gad m 2 +3 –	8	89	0.24870	0.1111	0.0028	0.4825
Hom g +	4	11				
Hom g –	5	80	0.02701*	0.4444	0.1370	0.7880
Chispp +	2	7				
Chispp –	7	84	0.18588	0.2222	0.0281	0.6001
Lit s+	2	4				
Lit s –	7	87	0.08972	0.2222	0.0281	0.6001
Lolspp +	3	9				
Lolspp –	6	82	0.07390	0.3333	0.0749	0.7007
Myt e +	1	3				
Myt e –	8	88	0.31841	0.1111	0.0028	0.4825
Ost e +	1	3				
Ost e –	8	88	0.31841	0.1111	0.0028	0.4825
Pan b +	4	8				
Pan b –	5	83	0.01122*	0.4444	0.1370	0.7880
Pecs +	3	8				
Pecs –	6	83	0.05823	0.3333	0.0749	0.7007
Pen m 1+	1	4				
Pen m 1 –	8	87	0.38231	0.1111	0.0028	0.4825
Pen m 2 +	7	11				
Pen m 2 –	2	80	0.00006*	0.7778	0.3999	0.9719
Pen m 3 +	1	0				
Pen m 3 –	8	91	0.09000	0.1111	0.0028	0.4825
Pen m 4 +	0	2				
Pen m 4 –	9	89	1.00000	0.0000	0.0000	0.3363
Raj c +	Tests cannot be computed for 0x2 tables.					
Raj c –	9	91				
Rud ssp +	1	7				
Rud ssp –	8	84	0.54330	0.1111	0.0028	0.4825
Sal s +	2	0				
Sal s –	7	91	0.00727*	0.2222	0.0281	0.6001
Sal s 1 +	4	3				
Sal s 1 –	5	88	0.00099*	0.4444	0.1370	0.7880
Sco s +	3	3				
Sco s –	6	88	0.00900*	0.3333	0.0749	0.7007
Sco s 1 +	3	3				

(Continued)

Table 6. Continued.

Specific IgE to allergen reagents (molecular components)	Number of patients – clinical reaction to fish		<i>p</i> -value	Sensitivity Rates and Confidence Intervals 95% C. I.		
	yes 9 (9%)	no 91 (91%)		value	lower	upper
Sco s 1 –	6	88	0.00900*	0.3333	0.0749	0.7007
Thu a +	0	5				
Thu a –	9	86	1.00000	0.0000	0.0000	0.3363
Thu a 1+	4	3				
Thu a 1 –	5	88	0.00099*	0.4444	0.1370	0.7880
Xip g +	4	2				
Xip g –	5	89	0.00044*	0.4444	0.1370	0.7880
Der p 10 +	1	3				
Der p 10 –	8	88	0.31841	0.1111	0.0028	0.4825

Allergen reagents (molecular components) of fish are marked extra bold. Fisher's Exact is used for the calculation of the sensitivity of examination (Rates and Confidence Intervals 95% C. I., value, lower, upper); the significant relationship between results of ALEX2 Allergy explorer test and the results of the open exposure test/history are marked with* extra bold.

Explanation: Specific IgE to allergen reagents (molecular components) + positive, – negative

We confirmed significant relation between the results of specific IgE to allergen reagents (molecular components) of fish and the results open exposure test/history. The sensitivity allergen reagents (molecular components) of fish is 33.3%–44.4%.

We did not confirm the significant relation between the results of sIgE to Gad m 2+3 and the open exposure test/history to fish. On the other hand, we confirmed the significant relationship between the results of specific IgE to molecular components and allergen extracts of shrimp (Hom g, Pan b, Pen m 2, Cra c 6) and the open exposure test/history to fish.

Rudsp (Clam). The most common sensitization was recorded to Pen m 2 in 18% of patients, to Hom g in 15%, to Pan b in 12%.

In general, the main clinical manifestations of allergic reactions to fish include vomiting and diarrhoea, whereas the extreme form of reaction is life-threatening anaphylactic shock (Chokshi et al., 2015; Magnusson et al., 2013). The gastrointestinal symptoms were confirmed in our study, but in addition, we confirmed late reactions to fish and shrimps, namely the feeling of itching and worsening of atopic dermatitis as a late reactions.

Epidemiological studies on fish allergy are missing to present consistent data of sensitization to fish and fish allergens. Prevalence rates to fish have been determined in studies of variable design and methodology (Chokshi et al., 2015). According to the literature, sensitization rates for parvalbumins were determined based on studies during allergen characterization (Kuehn, Fischer, et al., 2014; Kuehn, Metz-Favre, et al., 2014; Mazzucco et al., 2018; Rona et al., 2007).

Regarding the allergy to shrimps, previous sensitization rates were mainly based on skin or IgE testing to whole shellfish extracts (Nwaru et al., 2014) and some studies have identified the prevalence of shellfish allergy to be 2% for the general population and 0.1–0.9% for children (Pascal et al., 2015); 60% of individuals with confirmed allergy to shellfish elicit specific IgE binding to tropomyosin (Nwaru et al., 2014). More importantly, it has been demonstrated that serum specific IgE to tropomyosin is a better predictor of shrimp allergy than shrimp skin prick test or IgE to whole shrimp extract.

In our previous study we evaluated the sensitivity of ISAC Multiplex testing (Čelakovská, Bukač, Vaňková, Krcmova, Krejsek, et al., 2020; Čelakovská, Bukač, Vaňková, Krejsek, Andrýs, et al., 2020). Gad c 1 (parvalbumin) is only one component from fish represented in ISAC Multiplex testing. On the other hand, molecular components

Table 7. Results of specific IgE to allergen reagents (molecular components) in atopic dermatitis patients with the clinical reaction to shrimps and in patients without clinical reaction to shrimps. Results of specific IgE to allergen reagents (molecular components) are evaluated as positive (classes of specific IgE 1–4) and negative (Class 0).

Specific IgE to allergen reagents (molecular components)	Number of patients – clinical reaction to shrimps			Sensitivity Rates and Confidence Intervals 95% C. I.		
	Yes 5 (5%)	no 95 (95%)	<i>p- value</i>	value	lower	upper
Ani s 1 +	Tests cannot be computed for 0×2 tables.					
Ani s 1 –	5	95				
Ani s 3 +	0	4				
Ani s 3 –	5	91	1.0000	0.0000	0.0000	0.5218
Clu h +	0	3				
Clu h –	5	92	1.0000	0.0000	0.0000	0.5218
Clu h 1+	0	5				
Clu h 1–	5	90	1.0000	0.0000	0.0000	0.5218
Cra c 6+	0	7				
Cra c 6–	5	88	1.0000	0.0000	0.0000	0.5218
Cyp c 1+	0	6				
Cyp c 1–	5	89	1.0000	0.0000	0.0000	0.5218
Gad m+	0	5	1.0000	0.0000	0.0000	0.5218
Gad m–	5	90				
Gad m 1 +	0	3	1.0000	0.0000	0.0000	0.5218
Gad m 1 –	5	92				
Gad m 2 +3 +	0	3	1.0000	0.0000	0.0000	0.5218
Gad m 2 +3 –	5	92				
Hom g +	1	14	0.56432	0.2000	0.0051	0.7164
Hom g –	4	81				
Chispp +	2	7				
Chispp –	3	88	0.06281	0.40000	0.0527	0.8534
Lit s +	0	6				
Lit s –	5	89	1.0000	0.0000	0.0000	0.5218
Lolspp +	1	11	0.47965	0.2000	0.0051	0.7164
Lolspp –	4	84				
Myt e +	0	4	1.0000	0.0000	0.0000	0.5218
Myt e –	5	91				
Ost e +	0	4	1.0000	0.0000	0.0000	0.5218
Ost e –	5	91				
Pan b +	1	11	0.47965	0.2000	0.0051	0.7164
Pan b –	4	84				
Pecs +	1	10	0.44868	0.2000	0.0051	0.7164
Pecs –	4	85				
Pen m 1 +	0	5	1.0000	0.0000	0.0000	0.5218
Pen m 1 –	5	90				
Pen m 2 +	2	16	0.21941	0.40000	0.0527	0.8534
Pen m 2 –	3	79				
Pen m 3 +	0	1	1.0000	0.0000	0.0000	0.5218
Pen m 3 –	5	94				
Pen m 4 +	1	1	0.09798	0.2000	0.0051	0.7164
Pen m 4 –	4	94				
Raj c +	Tests cannot be computed for 0×2 tables.					
Raj c –	5	95				
Rud ssp +	1	7	0.34681	0.2000	0.0051	0.7164
Rud ssp –	4	88				
Sal s +	0	2	1.0000	0.0000	0.0000	0.5218
Sal s –	5	93				
Sal s 1+	0	7	1.0000	0.0000	0.0000	0.5218
Sal s 1–	5	88				
Sco s +	0	6				
Sco s –	5	89	1.0000	0.0000	0.0000	0.5218
Sco s 1+	0	6				

(Continued)

Table 7. Continued.

Specific IgE to allergen reagents (molecular components)	Number of patients – clinical reaction to shrimps		<i>p</i> -value	Sensitivity Rates and Confidence Intervals 95% C. I.		
	Yes 5 (5%)	no 95 (95%)		value	lower	upper
Sco s 1–	5	89	1.0000	0.0000	0.0000	0.5218
Thu a +	1	4	0.23041	0.2000	0.0051	0.7164
Thu a –	4	91				
Thu a 1+	0	7				
Thu a 1–	5	88	1.0000	0.0000	0.0000	0.5218
Xip g 1+	0	6				
Xip g 1–	5	89	1.0000	0.0000	0.0000	0.5218
Der p 10 +	0	4				
Der p 10 –	5	91	1.0000	0.0000	0.0000	0.5218

Allergen reagents (molecular components) of shrimps are marked extra bold. Fisher's Exact is used for the calculation of the sensitivity of examination (**Rates and Confidence Intervals 95% C. I., value, lower, upper**).

We did not confirm the significant relationship between the results of specific IgE to allergen reagents (molecular components) of shrimps and the results open exposure test/history.

Explanation: Specific IgE to allergen reagents (molecular components) + positive, – negative.

from parvalbumin (Xip g 1, Sal s 1, Sco s 1, Thu a 1, Clu h 1, Gad m 1), enolase (Gad m 2), aldolase (Gad m 3) are represented in ALEX2 Allergy Explorer test. Molecular components from shrimps Pen m 1 (tropomyosin), Pen m 2 (Arginin kinase) and Pen m 4 (Sarcoplasmic calcium-binding protein) are represented in ISAC Multiplex testing. These molecular components from shrimps are included in ALEX2 Allergy Explorer as well, and there is added Pen m 3 (Myosin light chain 2). Both ISAC and ALEX contain molecular components from Anisakis simplex such as Ani s 1 (unknown function, similar to Kunitz serine protease inhibitors) and Ani s 3 (Tropomyosin), (Čelakovská, Bukač, Vaňková, Krcmova, Krejsek, et al., 2020; Čelakovská, Bukač, Vaňková, Krejsek, Andrýs, et al., 2020).

In our previous study, we evaluated the occurrence of food hypersensitivity reactions to seafood in AD patients; we included 332 atopic dermatitis patients and the reactions to fish were recorded as mild oral allergic syndrome, vomiting, pruritus and worsening of AD altogether in 35 patients (11%), (Čelakovská, Bukač, Vaňková, Krejsek, Andrýs, et al., 2020).

Questionnaire-based studies revealed prevalence rates of reactions to fish 0–7% (USA 0.2%, Greece 1.5%, Finland 7%). Allergic sensitization (skin, serum) identified up to 2.9% of the individuals (China 0.2%, Norway 1.1%, Germany 2.9%). Food challenge-confirmed prevalence rates range up to 0.3% (Denmark 0.2%, Iceland 0.3%), (Dunlop & Keet, 2018; Moonesinghe et al., 2016). The prevalence of fish allergy is higher in regions with frequent fish exposure (diet, processing industries (Lyons et al., 2019; Renz et al., 2018). Food challenge-based studies on fish cross-reactivity are rare. Previous studies reported on high levels of cross-reactivity related to IgE testing and clinical history (Helbling et al., 1999; Hilger et al., 2017; Kalic et al., 2019; Kobayashi et al., 2016; Kuehn et al., 2011; Renz et al., 2018; Schulkes et al., 2014; Sørensen et al., 2017; Sten et al., 2004; Vázquez-Cortés, Eguiluz Gracia, et al., 2012; Vázquez-Cortés, Nuñez-Acevedo, et al., 2012). Scala et al. evaluated results of Allergy Explorer-ALEX2® macroarray and ImmunoCAP ISAC112. Despite the excellent concordance of the species-specific markers, the analysis of the

panallergens provided in both methods suggests a better performance of the ISAC® test on those components, while the ALEX2® test, which includes a larger number of allergens, allowing a broader molecular detection (Scala et al., 2021).

In our previous studies, we also demonstrated the detailed profile of sensitization to allergens reagents (allergen extracts and molecular components) in patients with atopic dermatitis with the use of ALEX 2 Allergy Explorer (Čelakovská et al., 2021) and we performed the cluster analysis of specific IgE results. Our results strongly pointed towards cross-reactivity for crustacean-allergic patients to desert locust, house cricket and stable flies (Čelakovská et al., 2021). Insects represent an alternative for meat and fish in satisfying the increasing demand for sustainable sources of nutrition. Various insect allergens have been identified including tropomyosin and arginine kinase, which are both pan-allergens known for their cross-reactivity with homologous proteins in crustaceans and house dust mite. Cross-reactivity and/or co-sensitization of insect tropomyosin and arginine kinase has been demonstrated in house dust mite and seafood (e.g. prawn, shrimp) allergic patients (de Gier & Verhoeckx, 2018; Verhoeckx et al., 2014).

Although food elimination is beneficial in one subset of atopic dermatitis patients, it has its own risk. Effects of elimination diet include nutritional deficiency, affect growth and development of the child, cause social isolation, and may cause anaphylaxis following reintroduction of restricted food and low-quality health. Recent findings from interventional studies have prompted a shift in the mind set from avoidance to early introduction of potentially allergenic foods (Elissa & Abrams Allan, 2015; Przybilla & Ring, 1990; Sampson, 1992).

Conclusion

Our study evaluated the sensitivity of ALEX 2 Allergy Explorer test in patients suffering from atopic dermatitis and clinical reaction to fish/shrimps. We confirmed the significant relationship between the results of specific IgE to allergen reagents (molecular components) of fish and the results open exposure test/history. The sensitivity of fish allergen reagents (molecular components) is 33.3%–44.4%. On the other hand, we did not confirm the significant relationship between the results of specific IgE to allergen reagents (molecular components) of shrimps and the results open exposure test/history.

Author contributions

Assoc. Prof. MUDr. Jarmila Čelakovská, Ph.D – the main investigator

- selection of the patients from the out- and in- patients dermatological departments
- dermatological examination and recommendations for the examination
- processing of all results
- publication of results

Mgr.Radka Vaňková

- laboratory examination

RNDr. Eva Čermáková

- statistical analysis

Doc. RNDr. Ctirad Andrýs, CSc

- control of laboratory examination

Prof. RNDr. Jan Krejsek, CSc

- professional cooperation.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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