

Shellfish allergy: unmet needs in diagnosis and treatment

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Abstract

Allergy to seafood is an important cause of food allergy and anaphylaxis worldwide. Shellfish is included among the “big eight” food groups, which are responsible for more than 90% of all food allergy cases. Approximately 2.5% of the world population had experienced some adverse reaction to seafood. It is one of the most frequent and lethal allergies that exist.

Several allergenic proteins involved in the allergic reactions have been described in the last years: Tropomyosin, Arginine Kinase, Myosin Light Chain, Sarcoplasmic Calcium-binding Protein, among other. Despite all the information that has been obtained in the last few years, seafood allergy is still diagnosed and treated as 50 years ago. The only effective treatment to prevent allergic reactions to shellfish is avoidance.

This review aims to update everything that has been published in recent years and highlight all that remains to be resolved.

Key words: Shellfish, Shrimp, Allergy, Allergens, Diagnosis, Food allergy

Resumen

La alergia al marisco es una causa importante de alergia alimentaria y anafilaxia en todo el mundo. Los mariscos se incluyen entre los "ocho grandes" grupos de alimentos, responsables de más del 90% de todos los casos de alergia alimentaria. Aproximadamente el 2.5% de la población mundial ha experimentado alguna reacción adversa a los mariscos. La alergia al marisco es una de las alergias más frecuentes y letales que existen.

Se han descrito varias proteínas alergénicas involucradas en las reacciones alérgicas en los últimos años: Tropomiosina, Arginina quinasa, cadena ligera de la miosina, proteína de unión a calcio, entre otras. A pesar de la información obtenida en los últimos años, la alergia a los mariscos todavía se diagnostica y trata como hace 50 años. Actualmente, el único tratamiento efectivo para prevenir reacciones alérgicas a los mariscos es la evitación.

Esta revisión tiene como objetivo recoger todas las actualizaciones realizadas en las publicaciones de los últimos años y resaltar las cuestiones pendientes de resolver.

Palabras clave: Marisco, Gamba, Alergia, Alérgenos, Diagnóstico, Alergia alimentos

1. Introduction

Allergy to seafood is an important cause of food allergy and anaphylaxis worldwide. Although seafood and shellfish are often used interchangeably, their meaning is different. Seafood refers to several distinct groups of edible aquatic animals including fish, crustaceans and mollusks. Shellfish term includes both, crustaceans and mollusks.

Shellfish is included among the “big eight” food groups, which are responsible for more than 90% of all food allergy cases. Approximately 2.5% of the world population had experienced some adverse reaction to seafood[1]. The prevalence of shellfish allergy is estimated to vary from 0% to 10.3% depending on the area studied, and its geographical location. In general, it is higher in regions where seafood is frequently consumed[2,3].

In Spain, it is the third cause of food allergy in adults, behind fruit and nuts allergy [4]. In children the prevalence is lower than in adults.

Shellfish is defined as any eatable marine invertebrate. The crustaceans belong to the *Phylum Arthropoda*, and they are taxonomically classified alongside insects and arachnid [5]. In this Phylum are included: prawn, crab and lobster species. All of them may contain species specific as well as common allergenic proteins, known as pan-allergens. These molecules have a high sequence homology, which favors the appearance of cross-reactivity among crustaceans, and crustaceans with other arthropods like dust mites or cockroaches.

The group of Mollusks belong to the *Phylum Mollusca*[5]. It is divided into bivalves (clams, scallops, cockles, mussels, oysters), gastropods (snail, abalone, limped) and cephalopods (squid, octopus). The probability of cross-reactivity among them is not well established. There seems to be few common proteins between crustaceans and mollusks, and those that have been described, have a low amino acid sequence homology, and therefore a low probability of cross- reactivity.

For many years it has been suggested that tropomyosin is the most important allergen in shellfish. However, in the last 15 years several studies have shown the complexity and the variability of the allergenic composition of this food group. Today there is clear evidence that there are several proteins involved in the allergenicity and cross-reactivity of shellfish.

Within the shellfish family, the better studied group are the crustaceans. Most studies have been conducted with shrimp.

2. Shellfish allergens

Shellfish allergens comprise a large and increasingly growing list of allergens. Several allergens have been reported in various species. The description of the most important ones is shown in Table 1.

2.1. Tropomyosin

In 1981, a 38 kDa thermostable protein was identified, which appeared to be responsible for shrimp allergy [6]. In the coming years several papers were published showing that patients with symptoms of immediate hypersensitivity after ingesting prawns had positive Skin Prick Test (SPT) and circulating specific IgE to crustaceans [7,8]. Tropomyosin (TM), the first allergen identified in seafood, was described in *Penaeus indicus* (Pen i 1), commonly known as Indian white prawn, in 1993 [9]. It is a pan-allergen involved in muscle contraction in invertebrates [10]. It is considered one of the most important pan-allergens within allergens of animal origin [11]. Tropomyosin has been described in numerous invertebrate species, in addition to crustaceans, it is also in mollusks, cockroach, nematodes such as *Anisakis simplex*, and dust mites [12,13,14,15,16,17]. TM is also described in vertebrates but without allergenic behavior [18,19].

TM has been considered the most important allergen of shrimp for many years. Several studies show that 72-98% of patients with shrimp sensitization have IgE binding to the purified allergen [20,21,22], although a recent Italian multicenter study has found less than 50% of sensitized patients recognize it [23].

Shrimp TM, prawn TM, lobster TM and crab TM, share sequence identity between 91% and 100%. The sequence identity between crustacean and mollusk tropomyosin is lower, near to 65% [11].

The tropomyosin of invertebrates is thermostable and resistant to digestion [24,25,26,27].

2.2. Arginine Kinase

Arginine Kinase (AK) was the second allergen identified, in 2008. It was first described in *Penaeus monodon* (Pen m 2) [28], commonly known as black tiger shrimp, and subsequently in many other crustaceans [29,30], such as crabs [31], octopuses [32], cockroaches [33] and dust mites [34,35]. Compared to tropomyosin is more unstable and less

resistant [24,36]. It is thermolabile and volatile, so it is considered one of the allergens responsible for steam inhalation respiratory symptoms [37,38].

The percentage of patients sensitized to prawn who recognize AK is not well defined and ranges between 10% to 51% according to different studies [22, 39].

2.3. Myosin Light Chain

The third allergen described was Myosin Light Chain (MLC), in 2008. Was identified in American white shrimp, *Litopenaeus vannamei* (Lit v 3) [40]. It was later described in other shrimp species, lobster [41], crabs [42] and cockroaches [20].

Like tropomyosin, it is highly resistant [24]. It is considered a minor allergen, with a frequency of sensitization oscillating from 19 to 55% [43,44], depending on the series. Although it usually accompanies Tropomyosin in the sensitization, there have been reports in subjects with allergy due to shrimp intake, including anaphylaxis, in whom MLC was the only responsible allergen [39,40].

2.4. Sarcoplasmic Calcium-binding Protein.

Described in 2008, immediately after than Lit v 3. Sarcoplasmic Calcium-binding Protein (SCP), was located first in *Penaeus monodon* (Pen m 4) [44]. Is highly resistant and stable [45]. It has high sequence homology with crustacean but low with mollusks [46,47].

As in the previous case, it is a minor allergen which could be clinically relevant regardless of sensitization to tropomyosin [39]. It seems to be a very important allergen in children. Submit in this group a high rate of sensitization, up to 85% [46,22].

2.5. Other allergens

During the last 15 years other allergens have been described, including: Troponin C [20,43,37,48,49,50], Triose phosphate isomerases (TIM) [20,22,51,52], hemocyanin [39,53,54,55,56], fructosebiphosphate aldolase [34], fatty acid binding protein (FABP), α -actinin and β -actinin [57,34], ubiquitin [34], paramyosin [58] and myosin heavy chain [54]. The clinical relevance to them still needs to be determined.

It is worth mentioning that hemocyanin, with an unclear relevance in shellfish allergy, seems to have a very important role in cross-reactivity with mites, cockroach and other invertebrates such as snails [59].

2.6. Epitopes.

The study of the peptides by means of microarray techniques has allowed to identify linear peptides involved in the sensitization to the different allergens. To date, 8 epitopes of Tropomyosin [60,22,61,62], 7 epitopes of Arginine Kinase, 5 epitopes of Myosin light chain and 3 epitopes of Sarcoplasmic Calcium Protein have been described [43].

Sensitization to different epitopes can determine the cross-reactivity between invertebrates. It can also condition the variety of symptoms that patients may suffer [62,22,43].

3. Cross-reactivity syndromes

3.1. Involvement of Tropomyosin

As previously mentioned, the cross-reactivity between crustaceans, between crustaceans and mollusks, and between crustaceans and mollusks with mites or cockroaches, is mainly due to the high sequence identity of the TM of the different species.

Cross-reactivity is attributed to the epitope that the subject recognizes. Seven TM epitopes have been described: epitope 1, 2, 3a, 3b, 4, 5a, 5b and 5c. Additionally, in depth analysis of these epitopes suggested that they can be classified into three groups; The first one, composed of the 5a epitope, is highly conserved among crustaceans, mollusks, insects and mites. The second group, composed of epitopes 2, 3 and 4, is found in arthropods but not in mollusks. And the third type, composed of epitopes 1, 5b and 5c, seems to be specific to crustaceans [61,62,63].

Sensitization to TM can occur in several ways. Namely through the digestive route by shellfish consumption, or through the respiratory tract by inhalation of mites or by inhalation of shellfish vapors. Some studies have shown the sensitization to shellfish can trigger dust mite sensitization and vice-versa. It seems that the prevalence of shrimp allergy is higher in regions with high prevalence of house dust mite (HDM) allergy. In fact, in these regions almost all patients sensitized to shrimp show positive SPT to HDM, with or without clinical relevance. Approximately 30% of HDM allergic patients are sensitized to Der p 10 [64].

Wong et al. reviewed the evidence to support the hypothesis that HDM inhaled tropomyosin is the main sensitizer for shellfish allergy in hot and humid tropical climates [65]. A study conducted in the United States by Wang et al. showed a positive significant correlation between high specific IgE levels to shrimps and high exposure to cockroach allergens in urban children [66]. Yang et al. obtained similar results in rural subjects in southern China

[67]. Furthermore, Fernandes et al. reported a series of Orthodox Jews individuals who presented sensitization to shrimp without ever being exposed to them [68]. Thus, it seems that the sensitization to shellfish may be justified and explained by the presence of mites or cockroaches in the environment and the consequent sensitization to these arthropods. Conversely, although less frequent, there seem to be patients allergic to shellfish who have presented positive SPT or specific IgE determinations against mites or cockroaches without having a previously demonstrated contact with these allergenic sources[34].

3.2. Involvement of other allergens

In addition to the TM, other allergens could justify cross-reactivity between dust mites and shrimps.

The allergens AK [28,34,69], SCBP[22,44,70] and hemocyanin[39,70] are proteins which may be also involved in this cross-reactivity syndrome.

Yang et al. reported that in some cases of shrimp sensitization explained by cross-reactivity with cockroaches, tropomyosin was not the dominant allergen responsible for such cross-reactivity[67].

Asero et al. conducted a multicenter study that included 116 Italian shrimp-allergic adult patients. Only 40% were positive to tropomyosin. In 52% of the cases, specific IgE binding to molecular weight component of >60kDa was detected[23].

Giuffrida et al. conducted a study to know the clinical relevance of hemocyanin in patients allergic to shrimp. Postulated that hemocyanin is a possible marker of cross-reactivity with mites [39].

Kamath et al. studied the importance of hemocyanin as an allergen in children as well as their cross-reactivity to house dust mite[70].

Although a sequence identity between the shellfish-hemocyanin and the HDM-hemocyanin has been demonstrated, there is a publication by Piboonpocanunet al. in which a selective allergy to the giant freshwater shrimp (*Macrobrachium rosenbergii*) by exclusive sensitization to hemocyanin in patients tolerating *Penaeus monodonis* reported [53].

More recently, Gámez et al. postulated that α -Actinin and Ubiquitin could be implicated in shrimp-mite cross-reactivity [34]. Finally, enolase could be an important allergen that justifies cross-reactivity in infants, according to Kamath et al[70].

3.3. Cross-reactivity between crustaceans and mollusks

Although the cross-reactivity between HDM and crustaceans is well documented, there are few studies that have analyzed cross-reactivity between crustaceans and mollusks.

Vidal et al. recruited subjects with crustacean anaphylaxis.

They noted that mollusk allergic patients had higher levels of specific IgE to TM (rPen a 1) and a more intense specific IgE binding in immunoblots to the shrimp extract. No differences were found between groups regarding AK, MLC, SCP, troponin-C and α/β actin[71].

There are no other trials that have demonstrated the usefulness of biomarkers (level of IgE to prawn or tropomyosin, or sensitization to specific allergens) to predict the likelihood of cross-reactivity between crustaceans and mollusks. The epitope mapping of the allergens seems to provide useful information, as mentioned above [43,62].

3.4. Shellfish sensitization by use of allergen immunotherapy

For many years there has been an ongoing discussion about the possibility of inducing allergy to shellfish in patients, previously tolerant, receiving specific-HDM immunotherapy. Several cases of subjects who developed a new allergy have been reported[72]. Likewise, tolerance to seafood, after HDM immunotherapy, has been described in allergic patients, who had previously presented severe allergy and even episodes of anaphylaxis[73,74]. Both reactions, the new induced shrimp allergy, and the apparent desensitization to shrimp, have been reported for SCIT (subcutaneous immunotherapy) and for SLIT (sublingual immunotherapy). It is still unknown why some patients improve their food allergy, and others initiate it.

Prospective studies suggest that it may depend on the level of tropomyosin in the immunotherapy extracts, but nobody knows what this level is[75,76]. The role of tropomyosin in HDM and shellfish allergies are an important field of research, as it can provide new immunotherapeutic insights and strategies to treat shellfish allergy[65].

4. Clinical manifestations

There is no pathognomonic symptom of shellfish allergy. The clinical manifestation associated with an allergic reaction after the ingestion of shellfish is the same as the one that may occur by the ingestion of other foods.

The clinical manifestations may appear as an oral allergy syndrome (OAS) or affecting the skin by way of rash, urticaria or angioedema.

If the affection is systemic may involve the gastrointestinal, respiratory or cardiovascular systems.

As in most food allergies, reactions begin immediately, in the first 15 or 20 minutes after seafood intake. It is considered that all allergic reactions IgE-mediated must occur within the first two hours. But there are always exceptions, also in shellfish⁷⁷. Late phase reactions have been reported from 2 to 8 hours after the ingestion of shrimps, limpets, snow crabs and abalone [77,78,79].

Some studies suggest that shellfish are one of the most frequent causes of allergic reactions to foods and cause more severe reactions.

The clinical form of presentation according to Alergológica 2015 report, an epidemiologic study based on the Spanish population, was: 72.9% of patients have skin involvement, 31.3% OAS, 10.4% digestive symptoms, 4.2% asthma, 2.1% rhinitis and 12.5% anaphylaxis [4]. Similar study conducted in Australia showed that patients suffered contact urticaria in 15% and anaphylaxis in 21% [80]. Another review conducted in Hong Kong showed a high percentage of skin involvement, 95.7%, followed by respiratory symptoms, 29.9%, gastrointestinal symptoms, 16.3%, cardiovascular symptoms, 3.3%, and anaphylaxis ,11.9% [81,82].

Besides the classical symptomatology originated by the ingestion of the food, other symptoms have been described by shellfish contact and steam inhalation. Exposure during processing in factories and inside the domestic environment, may cause other allergic symptoms such as contact urticaria[83,84], contact dermatitis or respiratory symptoms[85]. In the respiratory tract, the symptoms can be produced due to the inhalation of the vapor/smell of shellfish itself, or by the inhalation the steam during the cooking process.

There seems to be a strong correlation between a high concentration of allergens in the air and increased allergic sensitization[86]. Asthma induced by steam inhalation in fishermen or shellfish workers and seafood industry processing factories is considered occupational asthma[38,85,87,88].

4.1. The role of cofactors

Physical exercise, nonsteroidal anti-inflammatory drugs or alcohol consumption are enhancers of allergic reactions due to food intake[89,90,91]. The role in shellfish allergy is

not well established. Some cases of anaphylaxis after ingestion of shellfish followed by exercise have been described [92,93,94].

There are other factors such as stress, sleep deprivation, concomitant diseases, acute infections or menstruation that can boost too allergic reactions[89,95].

5. Diagnosis

The diagnosis of shellfish allergy is mainly based, as in all food allergies, on the medical history. After performing a complete interview, complementary examinations are used to confirm the suspected diagnosis.

As complementary examinations we have: Skin prick test, specific serum IgE determinations and oral food challenges (OFC).

The first step, in the medical consultation, is to perform SPT with one of the commercially available extracts. This procedure is safe and rapid, but according to different studies carried out, it could be unreliable. Asero et al. analyzed five different commercially crustacean extracts using SDS-PAGE and compared them with a fresh prawn extract[96]. The authors found that commercially available extracts contained less protein bands than the fresh prawns and a lack of bands of molecular weights corresponding to the major shrimp allergens.

A similar study was conducted years earlier by Jirapongsananuruk et al.[82]. Skin prick test was performed in 68 children diagnosed of prawn allergy. SPT was made with a commercially available extract and prick-prick with fresh and raw prawns. This study demonstrated that crude extracts are useful tools for screening shrimp sensitization and are better than commercially extracts.

Carnés et al. evaluated how the cooking process may alter the *in vivo* and *in vitro* allergenicity of the shrimp and lobster extracts[97]. They showed that a greater number of patients could be identified using boiled extracts of shrimp and American and spiny lobsters than using raw extracts.

Additionally, the wheel sizes of the skin test reactions and specific IgE levels were also significantly higher using boiled extracts. Jirapongsananuruk et al. found similar results in the study mentioned before; therefore, the use of boiled extracts seems to be more effective in diagnosing seafood allergy[97]. However, there are studies that showed contradictory results concerning the effect of heating on these extracts[24,98]. Thus, a negative result in a

SPT could be a false negative. If there is a suspicion of real allergy it is necessary to perform a prick- prick test with the fresh food, raw and cooked, to confirm sensitization[99].

In vitro diagnostic methods could also be useful. The determination of specific IgE to complete extract provides similar information as SPT. The existence of specific IgE in serum implies sensitization but does not correlate with symptoms and/or severity[100].

Neither the size of the wheal, nor the level of IgE makes it possible to distinguish the sensitized patients from those who are allergic.

At this point the only test capable of differentiating an asymptomatic sensitized subject from allergic is the oral food challenge (OFC). The double-blind placebo-controlled food challenge (DBPCFC) is considered the gold standard for food allergy diagnosis[101]. Different kinds of protocols have been proposed. Jirapongsananuruk et al. described a three-step protocol with 15 min intervals between doses, starting with 500 mg of shrimp and reaching a cumulative dose of 15.5 grams[82]. Nordlee et al. conducted the DBPCF with shrimp incorporated into a seasoned ground cooked beef matrix[102]. Seven doses ranging from 7 µg to 4 g of shrimp were administered, and placebo was interspersed between doses. Currently, there is no standardized initial dose for the OFC, but the European Academy of Allergy and Clinical Immunology (EAACI) recommends an initial dose of 5 mg of shrimp, and increasing doses every 15-30 minutes, to reach the daily recommended dose according to the age of the patient[101]. It should be considered that these tests are not exempt from risk. Serious anaphylactic reactions can be induced in many of these challenges[82,101,102].

During the last 20 years molecular diagnostic techniques have been introduced[103]. These techniques have opened a new field in the study of allergy. In addition to diagnosing the complete allergenic source, it allows studying the allergens individually.

Applying single allergenic molecules from shellfish for allergen-specific IgE detection could potentially modify test sensitivity and analytical specificity: risk associated molecules, markers of primary species and indicators of cross-reactivity[104].

Component -resolved diagnosis and epitope mapping have been applied to a wide range of allergens to elucidate distinct sensitization profiles which more accurately reflect clinical reactivity, avoiding, in some cases, the need to perform an OFC[105].

For some foods, such as peanuts[106,107], milk[108,109] or egg [110], a positive connection has been found between the recognition of certain sequential IgE binding epitopes and the allergic reactivity of the patients. It has been suggested that subjects with persistent allergy or a history of more severe reactions recognize a larger number of

sequential IgE epitopes. In addition, certain pan-allergens have been correlated with a greater or less severity.

An example of the different manifestations of allergy symptoms regarding the sensitization to different allergens of the same source is allergy to apple. While sensitization to Mal d 4 (profilin) is associated with OAS, sensitization to Mal d 3 (Lipid Transfer Protein) correlates to the risk of suffering anaphylactic reactions after apple intake.

In shellfish allergy the significance of allergens is not that clear. It has also been described that subjects with persistent allergy or history of more severe reactions recognize a large number of sequential IgE epitopes [67]. In addition, it has been suggested a different recognition pattern of epitopes and allergens, in children and adults[22]. According to the study conducted by Ayuso et al., the recognition pattern in children was: 94% to TM, 70% to MLC, 67% to AK and 59% to SCP. In adult TM was detected in 61%, MLC in 31%, AK in 21% and SCP in 21%. TM was the most frequently recognized allergen in both groups of patients[22]. The other allergens were predominantly recognized by children. This might suggest that TM could be an allergen associated with the persistence of shrimp allergy in adulthood. However, as mentioned before, TM is pan-allergen which cross-reacts with other common allergens, such as mites or cockroaches. Therefore, the presence of crustacean TM could only be a cross-reactivity sensitization without any clinical relevance.

It has been described that among all proteins, the determination of specific IgE against tropomyosin is the most specific and seems to have a higher positive predictive value when it comes to obtaining a positive oral provocation[111]. In addition to TM, SCP, could have clinical relevance in children[39,44].

Pascal et al.[43] conducted a study with the aim of identifying allergens and epitopes associated with clinical reactivity to shrimp. Subjects with positive DBPCFC recognized fundamentally tropomyosin alone or in combination with SCP and/or MLC. AK and hemocyanin were recognized for patients with SPT positive to HDM or cockroach and shrimp, but never had symptoms in relation to the ingestion of crustaceans.

They believed that AK and hemocyanin could be cross-reacting allergens between shrimp and arthropods without clinical significance. With the results obtained in their study based on 86 subjects, they proposed a protocol to diagnose allergic subjects based on a diagram that considers the outcome of SPC, specific IgE and the positivity of TM, SCP and MLC (Figure 1).

Currently the allergens marketed for *in vitro* diagnosis of crustaceans are: nPen m 1 and rPen a 1 (both TM), nPen m 2 (AK) and nPen m 4 (SCBP) in ImmunoCAP ISAC® multiple 112 p (ThermoFisherScientific), and nPen m 1 in the ALEX® multiplex allergy test (MADX).

Finally, as in other pathologies, knowing the genetic alterations that are associated with different types of allergies could facilitate diagnosis and treatment. Unfortunately, this type of study is still in a very immature phase in the allergy field. Several genome-wide association studies (GWAS) focused on European ancestry samples have identified food allergy-specific loci in the HLA class II region. Seik-Sonn et al. [112] conducted a study using the data from 11011 Japanese allergic women. They identified shrimp and peach allergy-specific loci in the HLA-DR/DQ. The research suggests that allergy to certain foods may be related to genetic differences that tag both HLA alleles, having particular epitope binding specificities as well as variants modulating the expression of specific HLA genes.

6. Treatment

The only effective treatment to prevent allergic reactions to shellfish is avoidance[113].

Allergen-specific Immunotherapy is currently being carried out on milk, egg, peanut and wheat allergic subjects with excellent results[114,115]. To the best of our knowledge, none of the active groups studying shellfish allergy have conducted studies with oral immunotherapy (OIT).

The cross-reactivity of tropomyosins among arthropods and the clinical contribution of the other shellfish allergens difficult the precise diagnosis and design ofAIT for shellfish allergy[116].

6. Discussion and unmet needs

Studying shellfish allergy is not an easy task.

It is one of the most frequent and lethal allergies that exist. Therefore, in patients who have presented symptoms compatible with allergy, either by intake, inhalation or contact, we tend to advise avoidance.

In those patients with suspected seafood allergy who present negative SPT and negative specific IgE serum determination, OFC (simple or DBPCFC) tends to be performed to

demonstrate tolerance, although in these circumstances there may be cases of positive OFC [117].

When sensitization is confirmed by positive SPT or by specific IgE, the same OFC should be performed as in the previous case, but in many occasions is not performed. Sometimes because of the patient refuses. In other occasions, it is the physician who decides not to subject the patient to a risky challenge. Finally, it may happen that the challenge cannot be carried out due to the lack of resources. It should be borne in mind that OFC requires adequate spaces within a hospital, skilled staff, and long-time investment. Consequently, food allergy, in general, is diagnosed excessively, without demonstrating it, and that entails having patients with unnecessary avoidance diets.

In the cases where the challenge can be performed, the avoidance diet is the treatment if the challenge is positive. Here come the first questions: If the challenge is realized with shrimp, the avoidance diet includes all the shellfish? Only crustacean? Only shrimps? Depending on the severity of the reaction? Depending on the results of the SPT realized with other prawns, or lobsters? Today we do not have accurate answers.

Another assumption would be to perform the challenge and it results negative. Here come more questions: for subjects that suffered the reaction in context of cofactor, the OFC is reliable? In these subjects the recommendation would be eat free? Or eat without any cofactor? Could we be ensuring that the tolerance to the crustacean that we used for the challenge implies tolerance for all the crustaceans? Returning to the vegetable model, demonstrating tolerance to apples does not imply being tolerant to peaches, even if they share the same allergen bread.

More diagnostic tools to reduce the number of subjects who avoid shellfish unnecessarily and to avoid fatal reactions in subjects who are misdiagnosed are absolutely necessary. (Table 2).

Commercially extracts must be perfected.

It is mandatory to continue working on the molecular diagnosis. It is essential to know the meaning of allergens.

Alternative diagnostic challenge to those we have should be sought. Could Nasal Provocation Tests be performed using Acoustic Rhinometry?

Finally, it is worth mentioning the need to find an option that allows our patients to be cured. Maybe knowing the exact significance of each of the shellfish allergens would permit us to propose an OIT as in the case of milk, egg or peanut.

Conflicts of interest

The authors declare that they have no conflicts of interest with publication of this paper.

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Table 1. Description of Shellfish Allergens

COMPONENT	Allergen described	Route Exposure	Molecular	Resistance	Disponibile for
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			weight		diagnosis
Tropomyosin	Pen a 1 Lit v 1 Pen m 1 Cra c 1 Mel l 1 Pan b 1 Pen i 1 Met e 1 Por p 1 Hom a 1 Scy o 1 Scy p 1 Scy s 1 Cha f 1	Ingestion Inhalation	34-38 kDa	Highly heat stable and IgE reactive	rPen a 1 ¹ nPen m 1 ²
Arginine kinase	Pen a 2 Pen m 2 Cra c 2 Lit v 2 Scy o 2 Scy p 2 Scy s 2 Cha f 2 Met e 2 Por p 2	Ingestion Inhalation	40-45 kDa	Labile. Can elicit IgE binding.	nPen m 2 ²
Myosin light chain	Pen m 3 Lit v 3 Cra c 3 Hom a 3	Ingestion	17-20 kDa	Stable	
Sarcoplasmic calcium calcium binding protein	Pen m 4 Lit v 4 Cra c 4 Mel l 4 Pon l 4 Scy p 4 Cha f 4 Met e 4 Por p 4	Ingestion	20-25kDa	Stable	nPen m 4 ²
Troponin C	Lit v 6 Cra c 6 Hom a 6 Pen m 6 Scy o 6 Pan b 6	Ingestion	20-21 kDa	Unknown	
Triose phosphate isomerase	Pen m 8 Cra c 8 Arc s 8 Pro c 8 Scy p 8	Ingestion Inhalation	26-29 kDa	Labile	
Hemocyanin	Lit v Hemocyanin Pan b Hemocyanin Mac r Hemocyanin	Ingestion	72-75 kDa	Stable	
Paramyosin	Myt g PM Oct v PM		100 kDa		
Fructose 1,6 Biphosphate aldolase		Ingestion Inhalation	39-43	Labile	

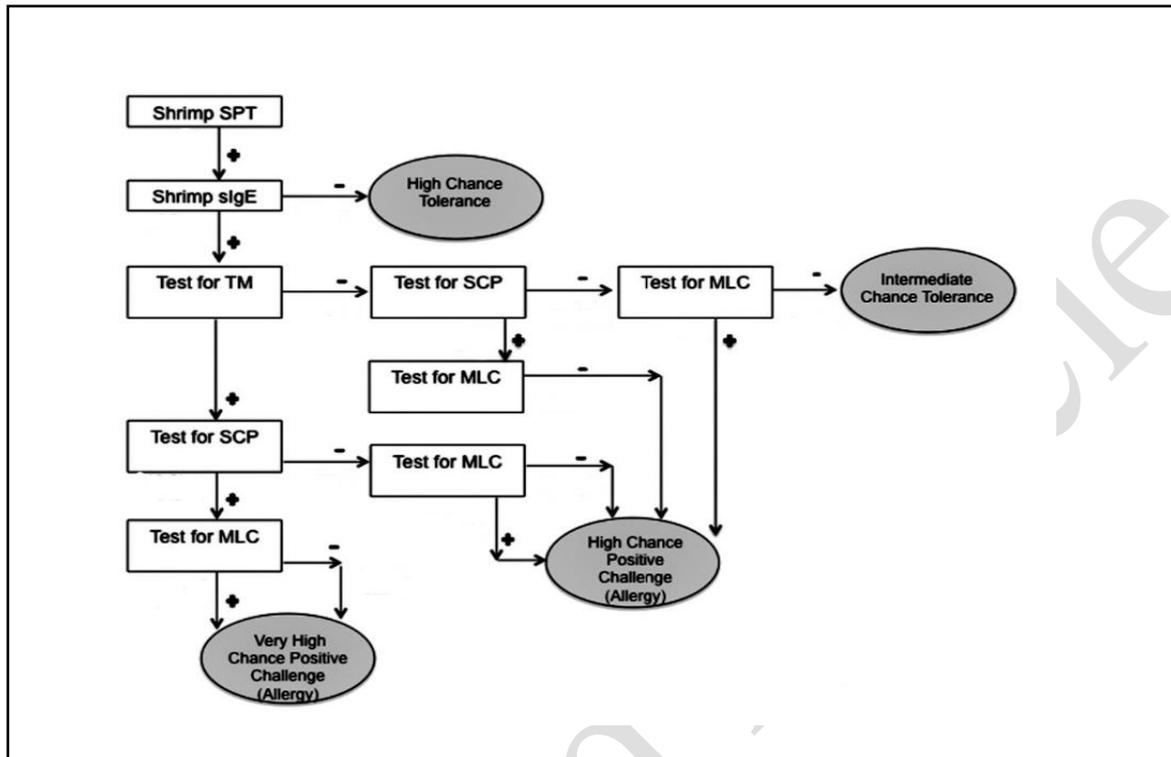
Pen a *: *Penaeusztecus*, Brown shrimp. **Lit v** *: *Litopenaeusvannamei*, pacific white shrimp. **Pen m** *: *Penaeus monodon*, Black tiger shrimp. **Cra c** *: *Crangoncrangon*, Commom shrimp. **Mel l** *: *Melicertuslatisulcatus*, king prawn. **Cha f** *: *Charybdis feriata*, Crucifix crab. **Pan b** *: *Panadalus borealis*, Red shrimp. **Pro c** *: *Procambarus clarkia*, red swamp crawfish. **Scy p** *: *Scylla paramamosain*, Green Mud Crab. **Scy o** *: *Scylla okivacea*, Mud Crab. **Scy s** *: *Scylla serrata*, Mangrove crab. **Pen i** *: *Penaeusindicus*, Indian white prawn. **Met e** *: *Metapenaeusensis*, Sand shrimp. **Por p** *: *Portunuspelagicus*, Blue swimmer crab. **Mac r** *: *Macrobrachiumroserbergii*, Giant Freshwater Prawn. **Pon l** *: *Pontastacusleptodactulus*, Narrow claxed crayfish. **Mit g** *: *Mytilusgalloprovinialis*, Blak mussel. **Oct v** *: *Octopus vulgaris*, Common octopus.

¹Recombinant allergens:originally identified innative allergenic extracts and obtained by molecular biology techniques.

²Native allergens:obtained from the allergenic source.

Accepted Article

Figure 1. Flow diagram from shrimp allergy diagnosis. Suggested protocol by Pascal et al. published in 2015[29].



SPT: Skin Prick Test. **TM**: Tropomyosin. **SCP**:Sarcoplasmic Calcium-binding Protein. **MLC**: Myosin Light Chain.

Table 2. Research needs in shellfish allergy

1. Determine the type of commercial extract with more sensitivity to detect allergic subjects.
 - Raw extract.
 - Cooked extract.
 - Single species.
 - A mixture of species.
2. Look for marks that determine the real probability of cross-reactivity between crustaceans, cephalopods and bivalves.
3. Assess the possibility of using nasal provocation tests as a diagnostic tool:
 - As a previous step in the OFC.
 - To replace the OFC.
 - In subjects who have presented Anaphylaxis, and in whom OFCs are contraindicated, to ratify diagnosis.
 - Determine the type of extract to be used in a nasal provocation.
 - Cooked or raw extract.
 - Single species or a mixture.
 - The amount of protein to be applied.
4. Carry out oral provocation studies with lyophilized extracts of different crustacean species containing all known allergens.
5. Determine how subjects who have had an allergic reaction in the presence of a cofactor should be studied.
6. Find a mark of mite-shrimp cross-reactivity in house dust mites allergic patients non allergic to shrimps.