ORIGINAL ARTICLE

Shrimp Allergy: Analysis of Commercially Available Extracts for In Vivo Diagnosis

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Abstract

Background: Skin prick testing (SPT) with commercial extracts is the first step in the diagnosis of shrimp allergy, although its clinical efficiency is unknown.
Objective: To analyze the clinical usefulness of all commercial crustacean extracts available for SPT in Italy.
Methods: We performed a multicenter study of 157 shrimp-allergic patients who underwent SPT with 5 commercial crustacean extracts and with house dust mite (HDM) extract. Commercial extracts were analyzed using SDS-PAGE and compared with a freshly prepared in-house shrimp extract. IgE to Pen a 1/Pen m 1, Pen m 2, and Pen m 4 was determined, and immunoblot analysis was performed on a large number of sera.
Results: The skin reactions caused by commercial crustacean extracts were extremely heterogeneous, resulting in 32 clinical profiles, with marked differences in protein content and missing proteins at molecular weights corresponding to those of major shrimp allergens. Only strong Pen a 1/Pen m 1 reactors reacted to both HDM and all 5 commercial extracts in SPT. Most patients, including those who were tropomyosin-negative, reacted to HDM. Patients reacted to a large and variable array of proteins, and IgE reactivity was common at high molecular weights (>50 kDa).

Conclusions: The in vivo diagnosis of shrimp allergy must continue to be based on SPT with fresh material. Shrimp-allergic patients frequently react to a number of ill-defined high-molecular-weight allergens, thus leaving currently available materials for component-resolved diagnosis largely insufficient. Mites and crustaceans probably share several allergens other than tropomyosin.

Key words: Allergens. Allergy diagnosis. Food allergy. Shrimp allergy. Skin testing.

Resumen

Introducción: Las pruebas cutáneas con extractos comerciales representan el primer paso en el diagnóstico de alergia a gamba, si bien, su eficacia clínica no está bien definida.
Objetivos: El objetivo de este estudio fue analizar la utilidad clínica de todos los extractos comerciales disponibles en Italia frente a crustáceos en pruebas cutáneas.
Métodos: En un estudio multicéntrico, se incluyeron 157 pacientes alérgicos a gamba a los que se realizaron pruebas cutáneas con cinco extractos comerciales de crustáceos y con ácaros del polvo doméstico. Los extractos comerciales fueron analizados mediante SDS-PAGE y comparados con un extracto de gamba preparado en fresco. Se determinó IgE frente a Pen a 1/Pen m 1; Pen m 2, y Pen m 4; y el análisis mediante inmunoblotting se realizó en un amplio número de sueros.
Resultados: Los extractos de gamba comercializados dieron lugar a reacciones cutáneas muy poco homogéneas en 32 perfiles clínicos diferentes; así mismo, mostraron grandes diferencias en contenido proteico y, en algunos casos, a falta de proteína a pesos moleculares correspondientes a alérgenos mayoritarios de gamba. Únicamente los reactores más fuertes a Pen a1 /Pen m 1 reaccionaron tanto a ácaros del polvo de casa como a los cinco extractos comerciales en pruebas cutáneas. La mayoría de los pacientes, incluyendo los negativos a tropomiosina, reaccionaron a los ácaros del polvo. Los pacientes reaccionaron a un amplio y variable array de proteínas y se detectó con frecuencia reactividad de IgE en pesos moleculares altos (>50 kDa).
Conclusiones: El diagnóstico in vivo de alergia a gamba todavía debe estar basado en pruebas cutáneas prick con producto fresco. Los pacientes alérgicos a gamba a menudo reaccionan a un número de alérgenos de peso molecular alto poco definido, lo que hace que las moléculas disponibles hoy en día para el diagnóstico por componentes sean muy insuficiente. Ácaros y crustáceos probablemente comparten varios alérgenos además de la tropiomiosina.

Palabras clave: Alérgenos. Diagnóstico alergológico. Alergia alimentaria. Alergia a gamba. Pruebas cutáneas.

Introduction

Crustaceans are one of the most prevalent causes of food allergy worldwide, and in Italy they are the second cause of type 1 food allergy after plant foods [1] and the second cause of food-induced anaphylaxis after lipid transfer protein, particularly in adults [2]. The complexity of the allergenic profile of shrimp and other crustaceans has been increasingly recognized over the last 10 years. The muscle protein tropomyosin was the first shrimp allergen detected [3-5]. Its presence in a conserved form in invertebrates has led it to be considered a panallergen [5-7]. Tropomyosin (Pen m 1/Pen a 1; MW, 38-41 kDa) has been considered the major shrimp allergen ever since, although recent multicenter studies carried out in Italy found that fewer than 50% of shrimp-allergic patients from this geographic area react to the allergen [8]. Several shrimp allergens other than tropomyosin have been detected and characterized in recent years. Some of them, namely, arginine kinase (Pen m 2; 40 kDa) [9,10] and sarcoplasmic calcium-binding protein (Pen m 4; 20-22 kDa) [11,12], are currently available for in vitro diagnosis, whereas others, such as myosin light chain (Lit v 3; 20 kDa) [13], are not. The number of shrimp allergens that have been characterized was recently increased with the addition of hemocyanin (75 kDa) [14], troponin C (Cra c 6; 21 kDa), triose phosphate isomerase (Cra c 8; 28 kDa), paramyosin (100 kDa) [15], myosin heavy chain (225 kDa), alpha-actin (31-42 kDa), smooth endoplasmic reticulum calcium ATPase (113 kDa), glyceraldehyde phosphate dehydrogenase (37 kDa) (reviewed in [16]), pyruvate kinase, thioredoxin, and enolase. However, it is very likely that the spectrum of crustacean allergens is much wider, both in the low-molecular-weight range [17,18] and in the high-molecular-weight range [8,14].

Diagnosis of crustacean allergy in clinical practice is based on clinical history, skin prick tests (SPT) with fresh food or commercial extracts, serum specific IgE, and (where possible) oral food challenges. While the clinical diagnosis of shrimp allergy is virtually always possible using a combination of the tools listed above, only 3 shrimp allergens are currently available for component-resolved diagnosis of this condition (Pen m 1, Pen m 2, Pen m 4). All 3 are used in the ISAC microarray platform (Thermo Fisher Scientific), and only tropomyosin IgE can be measured using the singleplex ImmunoCAP (Pen a 1) (Thermo Fisher Scientific). Very little is known about the sensitivity of commercial extracts for SPT that are commonly used as a first step for in vivo diagnosis of shrimp allergy by almost all clinical allergologists. In the present multicenter study, the sensitivity of all commercial shrimp extracts for SPT available in Italy was analyzed in a large group of shrimp-allergic patients.

Materials and Methods

Patients

The study was performed in 18 allergy centers throughout Italy (no patients were recruited at UOC Clinical Allergy and Immunology, IRCCS Foundation Ca' Granda Ospedale

Maggiore Policlinico, Milan, Italy, which contributed to the drafting of the manuscript). Participating doctors enrolled all patients who were diagnosed with shrimp allergy in routine clinical practice during 2015 based on the following criteria: (*1*) unequivocal clinical history of allergy to shrimp (oral allergy syndrome, urticaria with or without angioedema, asthma, or anaphylaxis); and (*2*) unequivocally positive skin test result with fresh shrimp by the prick-prick technique, as defined in the criteria of the EAACI [19]. Since virtually only seawater shrimps are consumed in Italy, in view of the possible allergenic differences with freshwater shrimps [20], only seawater animals belonging to the *Penaeides* family (*Aristeus antennatus, Parapenaeus longirostris, Parapeneopsis cornuta*, and *Melicertus kerathurus*) were used for SPT with fresh shrimp and to prepare the extracts for in vitro analysis.

All patients provided their written informed consent to participate in the study. Blood samples were taken from all patients; after centrifugation, coded sera were kept at –20°C until use in the in vitro analyses.

Skin Tests

All patients underwent SPT with all 5 commercial crustacean extracts currently available in Italy, as follows: shrimp (ALK-Abelló); shrimp (Stallergenes); lobster (Lofarma); shrimp (Allergopharma); and crustaceans (Anallergo). These skin tests were carried out in parallel in each patient and read as previously described [19]. SPTs were also performed with commercial house dust mite (HDM) extracts (*Dermatophagoides pteronyssinus* and *Dermatophagoides farinae*). Clinically relevant mite-induced respiratory allergy was not assessed in this study.

In Vitro Analyses

Commercial extracts for SPT were dialyzed overnight at 4°C against phosphate-buffered saline (PBS) to eliminate glycerol. Raw seawater shrimps were homogenized and extracted (5%) in 0.1 M PBS, pH 7.4, under shaking for 2 hours at 4°C. The protein content, measured after Bradford [21], was 1.2 mg/mL. The extract was divided into aliquots and freeze-dried at –20°C until use. SDS PAGE of the shrimp extract and of the commercial extracts for SPT was carried out.

The sera underwent immunoblot analysis at Lofarma Laboratories (Milan, Italy) under reducing and nonreducing conditions. The extract was mixed with LDS sample buffer (Nupage Bis-Tris; Novex, Prodotti Gianni) and 5% β-mercaptoethanol (β-mercaptoethanol was not used in experiments carried out under nonreducing conditions). The samples were then denaturized by heating at 100°C for 10 minutes. Electrophoresis of shrimp extracts (25 µg/lane) was carried out in a 10% polyacrylamide precast gel (Nupage Bis-Tris, Novex, Prodotti Gianni) at 180 mA for 1 hour. The SDS-PAGE gel was stained with Coomassie colloidal blue according to the manufacturer's instructions (Invitrogen). In the immunoblot analysis, the resolved proteins were transferred for 1 hour onto a nitrocellulose membrane according to Towbin et al [22]. The membrane was saturated with 0.1 mol/L tris-buffered saline containing 5% fat-free milk powder and incubated for 16 hours at 4°C with patients' sera (diluted 1:10 in saturation buffer). After 3 washes, bound specific IgE was detected by peroxidase-conjugated goat antihuman IgE antibody (1:5000 in saturation buffer [Biospacific]) and using an ECM western blotting kit (Amersham) as substrate.

Shrimp tropomyosin–specific IgE was measured in some patients using ImmunoCAP (Pen a 1; Thermo Fisher Scientific), whereas in other patients IgE to Pen m 1, Pen m 2, and Pen m 4 was detected using the ImmunoCAP-ISAC 112 microarray platform (Thermo Fisher) following the manufacturer's recommendations. The results were expressed in kU_A/L or ISU-E/L, respectively, and levels <0.1 and <0.3, respectively, were considered negative.

Statistics

The Pearson chi-square test or Fisher exact test (for 2-by-2 contingency tables with fewer than 50 cases) were used to assess whether allergen profiles and IgE specific for Pen m 1, Pen m 2, and Pen m 4 were independent of each other. The associations between independent covariates and dependent variables were further analyzed using binary logistic regression (Spearman test). Probability values less than 5% were considered statistically significant.

Ethics

The study was approved by the Ethics Committee of the leading center (Clinica San Carlo, Paderno Dugnano), with the code 306-052015.

Results

The participating centers enrolled 157 shrimp-allergic patients (male/female, 79/78; mean age, 36.3 years [range, 5-69 years]). Shrimp ingestion induced local symptoms (oral allergy syndrome/angioedema of the lips and/or pharynx [n=40], gastrointestinal symptoms [n=7], and isolated dyspnea [n=3]), as well as systemic symptoms (urticaria/angioedema [n=87], anaphylaxis [n=16], and food-dependent exercise-induced anaphylaxis [n=4]).

Skin Tests

A positive SPT result was recorded in 145 of the 157 patients (92%), with at least 1 of the 5 commercial crustacean extracts, and 132 of the 157 (84%) patients scored positive on SPT with the commercial HDM extract. However, the results of testing were extremely heterogeneous: when the results of SPT with the mite and crustacean extracts were combined, 32 different allergenic profiles were detected (Table 1). These were grouped as follows: A, HDM positive/all SPT negative; B, HDM positive/all SPT positive; C, HDM negative/all SPT positive; D, HDM negative/all SPT negative; E, HDM positive/SPT partially positive; F, HDM negative/SPT partially positive.

SDS-PAGE

The SDS-PAGE profiles of both the 5 commercially available extracts and the freshly prepared shrimp extract used to carry out the immunoblot analyses are shown in Figure 1. A large number of protein bands were observed for the fresh

Table 1. Allergenic Profiles Detected in 157 Shrimp-Allergic Patients Based on Hypersensitivity to HDM and Skin Reactivity to 5 Commercial Crustacean Extracts

Allergenic Profile	HDM	Extract #1	Extract #2	Extract #3	Extract #4	Extract #5	No.
A	*						10
B	*	*	*	*	*	*	67
C		*	*	*	*	*	11
D							2
E1	*	*					2
E2	*		*			*	5
E3	*					*	7
E4	*	*			*		2
E5	*	*	*				1
E6	*			*			1
E7	*	*	*		*		1
E8	*	*		*			1
E9	*	*				*	1
E10	*		*	*		*	2
E11	*		*	*			1
E12	*		*				6
E13	*	*	*	*			2
E14	*		*	*	*		1
E15	*				*		1
E16	*	*	*			*	1
E17	*	*	*	*		*	10
E18	*			*		*	1
E19	*		*	*	*	*	6
E20	*	*		*	*	*	2
E21	*	*		*	*		1
F1			*			*	3
F2			*	*			2
F3		*		*			1
F4			*				2
F5		*	*	*		*	2
F6			*	*		*	1
F7				*	*	*	1
Total	132	105	124	113	93	120	157
Sensitivity		66.8	78.9	71.9	59.2	76.4	

Abbreviation: HDM, house dust mite.
*, skin prick test positive

extract, whereas marked differences were observed between the commercial extracts, with a frequent absence of proteins at molecular weights corresponding to those of the major shrimp allergens. The largest number of proteins on SDS-PAGE was observed for commercial extract #2.

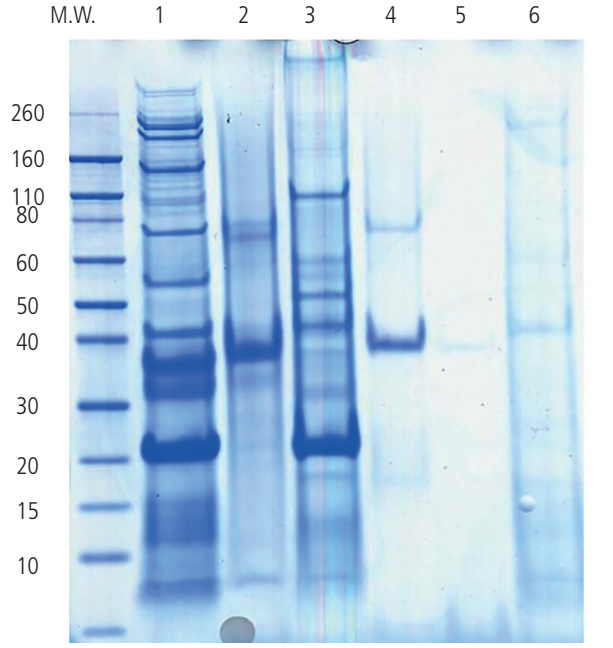

Figure 1. SDS-PAGE of the fresh shrimp extract (lane 1) and of commercial crustacean extracts for SPT. Lane 2, commercial extract #1 (Lofarma); lane 3, commercial extract #2 (ALK); lane 4, commercial extract #3 (Allergopharma); lane 5, commercial extract #4 (Anallergo); lane 6, commercial extract #5 (Stallergenes).

Specific IgE Measurements

Positive results were recorded in 41 (57%) of the 72 patients who underwent determination of specific IgE to Pen a 1/Pen m 1. When these results were plotted against the allergen profile of the corresponding patients, a clear association was observed between IgE reactivity to tropomyosin and allergenic profile B (skin reactivity to HDM and all 5 commercial crustacean extracts) (P<.001; Table 2). After analyzing the effect of specific IgE levels in the 23 Pen m 1–monosensitized patients (defined as Pen m 1–pos, Pen m 2–neg, Pen m 4–neg) on the allergen profile obtained by SPT with commercial crustacean extracts, it turned out that the 16 patients with low IgE levels (arbitrarily defined as <2.0 ISU/L) had a variable allergenic profile including profiles B (n=8), E20 (n=2), C, E6, E17, E19, and E21 (n=1 each), whereas all 7 strong responders with IgE levels >2.0 ISU/L had profile B. Interestingly, 24 of 31 (77%) patients who did not have Pen a 1/Pen m 1 hypersensitivity scored positive on SPT with HDM. SPT with extract #2, notably the commercial extract with the highest number of protein bands on SDS-PAGE (Figure 1) yielded positive results in patients with 7 of the 14 (50%) profiles that were negative for tropomyosin hypersensitivity (Table 2). The proportions for the other commercial extracts were 5/14 (commercial extracts # 1 and #5), 4/14 (extract #3), and 3/14 (extract #4).

IgE to Pen m 2 and Pen m 4 was measured using ISAC microarray in 46 patients. IgE reactivity to Pen m 2 and Pen m 4 was detected in 11 and 8 cases, respectively; 2 patients were cosensitized to Pen m 2 and Pen m 4. When the results were

Table 2. IgE Reactivity to Pen a 1 or Pen m 1 in 72 Shrimp-Allergic Patients With Different Allergen Profiles[a]

Allergenic Profile	HDM	Extract #1	Extract #2	Extract #3	Extract #4	Extract #5	No.	Pen a 1/ Pen m 1 IgE +
A	*						3	0
B	*	*	*	*	*	*	33	30
C		*	*	*	*	*	3	3
D							1	0
E1	*	*					1	0
E2	*		*			*	3	0
E3	*					*	2	0
E5	*	*	*				1	0
E6	*			*			1	1
E7	*	*	*		*		1	0
E8	*	*		*			1	0
E10	*		*	*		*	1	0
E12	*		*				3	1
E13	*	*	*	*			1	0
E15	*				*		1	0
E17	*	*	*	*		*	3	1
E19	*		*	*	*	*	6	1
E20	*	*		*	*	*	2	2
E21	*	*		*	*		1	1
F1			*			*	1	0
F4			*				1	0
F5		*	*	*		*	1	1
F7				*	*	*	1	0
Total							72	41

Abbreviation: HDM, house dust mite.
*, skin prick test positive.
[a]A strong association was observed between Profile B and Pen m 1 IgE ($P<.001$).

Table 3. IgE Reactivity to Pen m 1, Pen m 2, and Pen m 4 in 42 Shrimp-Allergic Patients With Different Allergenic Profiles

Profile	HDM	Extract #1	Extract #2	Extract #3	Extract #4	Extract #5	No.	Pen m 1 +	Pen m 2 +	Pen m 4 +
A	*						1	0	0	0
B	*	*	*	*	*	*	25	25	6	4
C		*	*	*	*	*	3	3	0	1
E2	*		*			*	1	0	0	0
E5	*	*	*				1	0	0	0
E6	*			*			1	1	0	0
E12	*		*				1	0	0	0
E17	*	*	*	*		*	2	1	1	1
E19	*		*	*	*	*	6	1	4	1
E20	*	*		*	*	*	2	1	0	0
E21	*	*		*	*		1	2	0	0
F5		*	*	*		*	1	1	0	0
F7				*	*	*	1	0	0	1
Total							46	35	11	8

Abbreviation: HDM, house dust mite.
*, skin prick test positive

Table 4. Results of Immunoblot Analysis With Sera From Patients With Different Allergenic Profiles

Profile	HDM	Extract #1	Extract #2	Extract #3	Extract #4	Extract #5	No. tested	<20 kDa	20 kDa	30 kDa	35 kDa	50 kDa	60 kDa	70 kDa	80 kDa	90 kDa	100 kDa	120 kDa	> 120 kDa	WB negative
A	*						7/10			3		2	2	2	3		3	3		1
B	*	*	*	*	*	*	31/67	7	7	1	14	2	4	3	7	6	5	7	2	3
C		*	*	*	*	*	5/11		1	3		1			2		1	1		2
E1	*	*					1/2													1
E2	*		*			*	2/5			1			1	1	1		2	1		0
E10	*		*	*		*	1/2													1
E12	*		*				2/6			1			1							1
E15	*				*		1/1													1
E17	*	*	*	*		*	2/10													2
E19	*		*	*	*	*	4/6	1									1			3
F5		*	*	*		*	2/2								2		1	2		0
F7				*	*	*	1/1													1
Total							59													

Abbreviations: HDM, house dust mite; WB, western blot.
*, skin prick test positive

plotted against the different allergen profiles, in many cases the 2 allergens were recognized by patients with allergen profile B (ie, the profile associated with tropomyosin hypersensitivity); however, a statistically significant association between profile E19 (ie, skin reactivity to HDM and all commercial crustacean extracts except #1) and hypersensitivity to Pen m 2 was detected ($P<.05$) (Table 3). In fact, all patients monosensitized to Pen m 2 (ie, Pen m 2+, Pen m 1–, and Pen m 4–) had the E19 profile. The 2 patients who were monosensitized to Pen m 4 (ie, Pen m 4+, Pen m 1–, Pen m 2–) had the E19 and F7 profiles, respectively. Interestingly, 1 Pen m 4 "monoreactor" (F7 profile) scored positive on SPT with extracts #3, 4, and 5, suggesting the presence of this molecule only in some commercial extracts.

Analysis of SPT Extracts in the Light of Specific IgE Measurements

SPT with commercial extract #2 yielded a negative result in patients with weak Pen m 1 monoreactivity (profile E 20 in table 1). SPT with commercial extract #1 yielded a negative result in patients monosensitized to Pen m 4 or Pen m 2 (profile E19, Table 1) and only a weakly positive result in 1 patient who was reactive to both these allergens but not to Pen m 1 (profile E17). Of note, however, most patients had low specific IgE levels. In 2 strong Pen m 2 reactors (18.5 and 25.0 ISU/L, respectively, both belonging to profile B), the result of SPT with extract #1 was positive.

According to ISAC microarray, 4 patients did not react to Pen m 1, Pen m 2, or Pen m 4; on SPT, 3 had positive results with commercial extract #2 (in 1 case alone [profile E12, Table 1], and in 2 cases this was associated with weak reactivity to extracts #1 and #5, respectively [profiles E5 and E2, Table 1]), whereas in 1 case, the results were negative for all commercial extracts (profile A, Table 1).

Immunoblot Analysis

Sera from 59 patients underwent immunoblot analysis. As expected, IgE reactivity varied widely from one patient to another (Figure 2). No differences were observed between immunoblots carried out using the same sera under reducing and nonreducing conditions (data not shown). The results are summarized in Table 4, where they are plotted against the allergen profiles observed by SPT to HDM and commercial crustacean extracts. A highly significant association was found between profile B and IgE reactivity at 35 kDa (presumably tropomyosin) ($P<.001$). Notably, IgE reactivity at high molecular weights (>50 kDa) was observed in a large number of sera, whereas no serum appeared to react at 40 kDa, the molecular weight of shrimp arginine kinase (Pen m 2).

IgE reactivity at 20 kDa on immunoblot analysis was detected in 5 of 8 Pen m 4 reactors, whereas the remaining 3 sera were negative.

Discussion

SPTs with commercial allergen extracts are the mainstay of the diagnosis of clinical allergy and are used in virtually all clinical settings where allergic disorders are managed.

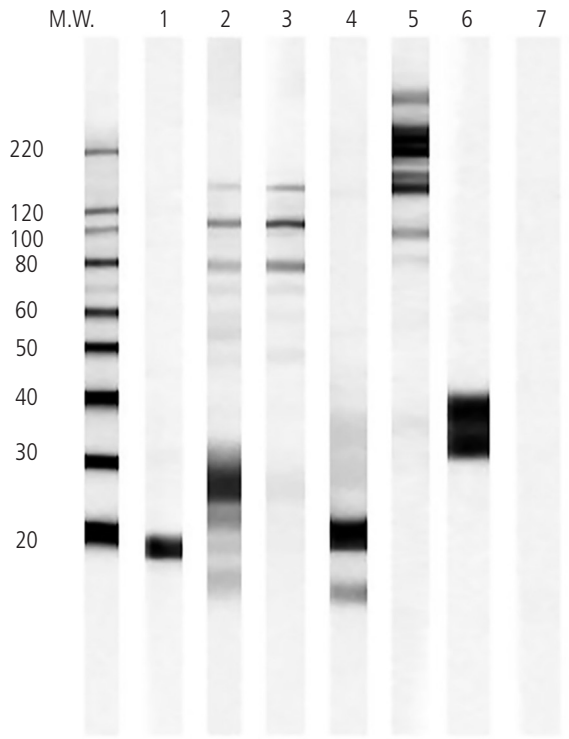

Figure 2. Selected immunoblots showing the variety of shrimp proteins recognized by different patients. Lane MW, molecular weight markers; Lanes 1-6, profiles for 6 different patients. Last lane, negative control serum.

Crustaceans are one of the most frequent causes of food allergy, and our study is the first to investigate the sensitivity of a series of commercial shrimp/lobster extracts. To this end, all 5 commercial extracts currently available in Italy for the diagnosis of allergy to crustaceans were analyzed for their protein content using SDS-PAGE and applied in SPTs in more than 150 shrimp-allergic patients. Furthermore, sera from a large number of study patients were analyzed to determine IgE to all currently available shrimp allergens (Pen a 1/Pen m 1, Pen m 2, and Pen m 4) and immunoblot analysis in order to assess their IgE reactivity profile. Finally, hypersensitivity to HDM (both *D pteronyssinus* and *D farinae*) was detected in the whole study population. In vivo tests produced an extremely complex picture, including 32 different profiles that were combined with the results of in vitro studies.

Several aspects of this study are worthy of discussion. One impressive finding was the high proportion of HDM-hypersensitive patients (84% of the study population), which changed very little (77%) in patients who did not react to Pen a 1/Pen m 1, suggesting that mites and crustaceans might share allergens other than tropomyosin. In fact, in a previous study [8], we demonstrated allergenic cross-reactivity between high-molecular-weight allergens in shrimp and mites. Furthermore, as long as 15 years ago, Binder et al [23] reported the cross-reactive nature of arginine kinase in various invertebrates. Also interesting is the observation that hypersensitivity to tropomyosin did not correlate fully with hypersensitivity to HDM; in fact, the results of SPT with HDM were negative in more than 10% of Pen m 1 reactors (Table 3). Whether this depends on a low concentration of Der p 10, the HDM tropomyosin, in commercial mite extracts for SPT or to allergenic differences between tropomyosins from different invertebrates remains to be established. In any case, this study shows that a negative SPT with HDM in a shrimp-allergic patient does not rule out hypersensitivity to tropomyosin.

Surprisingly, in comparison with fresh shrimp extract, commercial extracts for SPT showed a dramatic loss of protein bands, which was so pronounced in some cases that it suggested that the diagnosis of hypersensitivity to shrimp using certain extracts would have been severely hampered. In fact, the commercial extract with the poorest protein profile on SDS-PAGE (extract #4) was the one that scored negative in the largest proportion (>40%) of allergic patients.

In view of the notable differences in protein content of commercial extracts, it is not surprising that SPTs eventually yielded so many different allergenic profiles, with every possible combination, ranging from “all negative” to “all positive”. Interestingly, although some extracts appeared to lack proteins at about 35 kDa, the molecular weight of tropomyosin, all of them had positive results in patients with strong hypersensitivity to Pen m 1. Some commercial extracts seemed to lack specific allergens; for instance, extract #1 showed low sensitivity in the detection of hypersensitivity to Pen m 2. However, this was also the case for the immunoblot analysis that was carried out using an in-house fresh shrimp extract. Since commercial extract #1 and fresh shrimp extract for immunoblot were prepared by the same laboratories, one might hypothesize that specific features of the extraction procedures may lead to the loss of arginine kinase in the final product. Further studies are needed to clarify this point.

The immunoblot analysis confirmed that shrimp-allergic patients frequently react to high-molecular-weight allergens. We recently detected hemocyanin as a high-molecular-weight shrimp allergen [14], although other allergens, including paramyosin (100 kDa), myosin heavy chain (225 kDa), and calcium ATPase (113 kDa), have been described [15,16]. Hypersensitivity to these high-molecular-weight allergens was commonly associated with IgE reactivity to tropomyosin or other low-molecular-weight allergens; however, they seem clinically relevant (eg, allergenic profiles A, E2, or E5). Future studies by our group aim to better characterize these allergenic proteins. The immunoblot analysis yielded negative results in a significant proportion of shrimp-allergic patients, as observed in several previous studies, probably because immunoblot is less sensitive than SPT or specific IgE measurement.

In conclusion, the in vivo diagnosis of shrimp allergy must still be based on SPT with fresh material, as the sensitivity of current commercial crustacean extracts may be rather low and their allergen content highly variable. Nonetheless, performing SPT with all available commercial extracts alongside SPT with fresh material and with detection of IgE to the few currently available recombinant shrimp molecules may provide useful information about hypersensitivity to minor shrimp allergens. In clinical practice, the use of extract #2 in combination with extract #1 led to slightly better sensitivity of commercial SPTs, since most of the allergen profiles were detected.

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Conflicts of Interest

The authors declare that they have no conflicts of interest.

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