ORIGINAL ARTICLE

Usefulness of the Nasal Allergen Provocation Test in the Diagnosis of Shellfish Allergy

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Abstract

Background: Shellfish allergy is a major cause of food allergy and anaphylaxis worldwide. Several allergenic proteins have been described in the last few years, but the only diagnostic tool that still enables discrimination between allergic and nonallergic sensitized persons is the oral food challenge (OFC).
Objective: The aim of this study was to evaluate the usefulness of the nasal allergen provocation test (NAPT) as a diagnostic tool in shellfish allergy.
Methods: Forty-five patients with confirmed sensitization to shrimp by a positive skin prick test (SPT) result with a commercial shrimp extract were recruited and classified as sensitized-allergic or sensitized-nonallergic based on current tolerance to shrimp intake, the result of an OFC with a freeze-dried cooked shrimp mixture extract, or a recent history of anaphylaxis induced by shrimp ingestion. These patients and 10 controls not sensitized to shrimp underwent NAPT with a freeze-dried cooked shrimp mixture extract. The response was evaluated using acoustic rhinometry and a visual analog scale.
Results: Significant differences (*P*=.001) were found between the sensitized-allergic group (18/20 positive NAPT, 90%) and both the sensitized-nonallergic group (2/18 positive NAPT, 11.1%) and controls (0/10 positive NAPT). NAPT enables differentiation between allergic and nonallergic persons with a sensitivity of 90%, specificity of 89%, positive predictive value of 90%, and negative predictive value of 89%.
Conclusions: Our results indicate that NAPT makes it possible to differentiate between sensitized symptomatic patients and sensitized tolerant patients and could be a valuable diagnostic tool when assessing shrimp allergy.

Key words: Nasal allergen provocation test. Nasal allergen challenge. Acoustic rhinometry. Oral food challenge. Shellfish allergy. Shrimp allergy.

Resumen

Antecedentes: El marisco es una de las causas más importantes de alergia alimentaria y anafilaxia en el mundo. Aunque se han descrito varias proteínas alergénicas implicadas en estas reacciones, la única prueba que permite discriminar entre sujetos alérgicos y no alérgicos sigue siendo la prueba de exposición oral controlada (PEOC).
Objetivo: Evaluar la utilidad de la prueba de exposición nasal con alérgeno como herramienta diagnóstica en el estudio de la alergia al marisco.
Metodología: Se reclutaron 45 sujetos con sensibilización a gamba confirmada mediante una prueba intraepidérmica positiva realizada con extracto comercial de gamba, y se clasificaron como alérgicos o no-alérgicos según el resultado de la PEOC realizada con extracto de mezcla de gambas, la tolerancia actual o la historia reciente de anafilaxia en relación con su ingesta. Estos sujetos y diez controles, sin sensibilización a gamba, se sometieron a una provocación nasal con un extracto de mezcla de gambas cocidas. La respuesta se evaluó mediante rinometría acústica y escala visual analógica.
Resultados: Se encontraron diferencias significativas (p=0,001) entre el grupo de sensibilizados alérgicos (18/20 NAPT positivos, 90%) frente a los sensibilizados no alérgicos (2/18 NAPT positivos, 11,1%) y los controles (0/10 NAPT positivos). La NAPT permite diferenciar entre sujetos alérgicos y no alérgicos con una S: 90%, E: 89%, PPV: 90% y VPN: 89%.
Conclusiones: Según los resultados del estudio, la NAPT es una prueba diagnóstica que permite diferenciar los sujetos sensibilizados alérgicos de los no alérgicos y podría ser una herramienta diagnóstica valiosa a la hora de realizar un estudio de alergia a gamba.

Palabras clave: Prueba de provocación nasal con alérgeno. Prueba de exposición nasal. Rinometría acústica. Prueba de exposición oral controlada. Alergia a crustáceos. Alergia a gamba.

Introduction

Food allergy has become increasingly common in recent years, to the extent that is now a major health problem [1,2]. Reported prevalence rates for shellfish allergy vary greatly depending on the area studied, with a global average value of 5.4% [3].

Shellfish is one of the main allergenic food groups, alongside milk, egg, fish, nuts, peanuts, wheat, and soy, which account for up to 90% of all reported cases of food allergy. The case of shellfish is noteworthy in that it is associated with life-threatening reactions.

The many shellfish allergens include tropomyosin, arginine kinase, myosin light chain, sarcoplasmic calcium-binding protein, troponin C, troponin I, triose phosphate isomerase, hemocyanin, α and β actinin, ubiquitin, myosin heavy chain, fatty acid binding protein, and enolase [4-7]. The clinical relevance of each of them is unclear, although tropomyosin, arginine kinase, sarcoplasmic calcium-binding protein, hemocyanin, ubiquitin, and α-actin are involved in cross-reactivity between shellfish and house dust mites [8-16].

The routine work-up for shrimp allergy is based on in vivo skin prick test (SPT) performed with either commercial extracts or the fresh food (prick-by-prick) and in vitro tests, mainly the measurement of serum specific IgE (sIgE) to the whole shrimp extract and to tropomyosin (Pen a 1 or Pen m 1) [17]. While these tests are useful for determining sensitization, they do not define clinical allergy, and although some publications suggest that a higher level of sIgE to shrimp and shrimp-tropomyosin could indicate allergy, there is no clear evidence to support this finding [18-20].

Component-resolved diagnosis is poorly developed in shellfish allergy, in contrast with other foods. Allergy and tolerance should be confirmed using an oral food challenge (OFC). This approach remains the gold standard, despite the consumption of resources and potential risk of inducing allergic events [21,22].

At present, there is no treatment or cure for shellfish allergy, with the only recommendation being strict avoidance [1]. For this reason and given the elevated risk of presenting life-threatening allergic reactions, reaching a definitive diagnosis is an absolute priority.

The nasal allergen provocation test (NAPT) with mites, epithelia, and pollens has been used for years to reproduce and study allergic rhinitis, to confirm the clinical relevance of environmental allergens [23,24], and as an outcome measure in clinical trials [24,25]. It is safe, simple, and inexpensive [26]. NAPT is not currently used in the food allergy work-up, and few studies have examined this approach. The main objective of the present study was to evaluate whether NAPT performs well in the diagnosis of shellfish allergy, making it possible to differentiate allergic patients from sensitized nonallergic individuals.

Methods

Patient Selection

Participants were recruited prospectively and consecutively from outpatient clinics of the allergy department of Hospital Clinic (Barcelona, Spain) over 1 year. The study was approved by the Ethics Committee of Hospital Clinic, and written informed consent was obtained from all the participants.

Patients sensitized to shrimp detected by SPT as defined in the European Academy of Allergy and Clinical Immunology (EAACI) criteria [27,28] and controls not sensitized to shrimp were enrolled and included in 1 of the following 4 groups:

- Sensitized-nonallergic group (S-nA). Participants with positive results in the shrimp SPT and
  - current tolerance to shrimp intake according to their clinical history, or
  - reporting clinical manifestations other than anaphylaxis in the previous 6 months (oral allergy syndrome, angioedema, urticaria, or digestive symptoms) and negative results in the OFC with shrimp. Participants with steam inhalation symptoms only were excluded from recruitment owing to the difficulty performing exposure tests.
- Sensitized-allergic group (S-A). Participants with positive results in the shrimp SPT and
  - a documented and unequivocal history of anaphylaxis due to shrimp consumption during the previous 6 months (patients were directly classified as S-A, without undergoing the OFC, according to the National Institute of Allergy and Infectious Diseases [NIAID]–sponsored expert panel criteria [29]), or
  - clinical manifestations other than anaphylaxis and a positive result in the OFC with shrimp.
- Atopic control group. Participants with a negative SPTs to shrimp but positive results for *Dermatophagoides pteronyssinus* and current tolerance to shrimp intake according to their clinical history.
- Nonatopic control group. Participants not sensitized to environmental or food allergens with, therefore, a negative SPT result for shrimp and *D pteronyssinus* and current tolerance to shrimp according to the clinical history.

Shrimp Extract Preparation for SPT, OFC, and NAPT

Shrimp extract was prepared with 25 g of peeled *Parapenaeopsis* species, *Parapenaeus* species, *Solenocera* species, and *Trachypenaeus* species. These species were chosen because they are the most consumed in our area. The sample was boiled for 10 minutes, crushed, homogenized, and incubated with continuous shaking with 0.01 molar phosphate-buffered saline (pH 7.2) at 4°C for 18 hours. It was then centrifuged at 10 000 rpm for 10 minutes, and the supernatant was clarified by vacuum filtration through cellulose acetate membrane filters with decreasing thicknesses: 1 µm, 0.7 µm, 0.45 µm, and 0.2 µm (Sartorius Stedim Biotech SA). The dilution obtained was dialyzed by tangential ultrafiltration with Omega polyether sulfone membranes (Cassette TFF series T, Pall Life Sciences) with a pore size of 5 kDa. Dialysis was performed with 7 volumes of distilled water (Inmunotek SL Laboratories).

The Bradford technique [30] was used to quantify the total protein concentration of the extract by extrapolating the absorbance values obtained at a wavelength of 595 manometers on a standard bovine serum albumin line (Sigma Aldrich).

The resulting material was the freeze-dried cooked shrimp mixture extract used for the NAPTs by reconstituting 10 mL of saline solution, leaving a final concentration of 1048 mg/mL.

To perform the OFCs, the vials contained the lyophilized preparation equivalent to 20 g of fresh product, corresponding to the 3 g of shrimp protein necessary to perform the test.

Skin Prick Tests

All the patients underwent SPTs with a panel of allergen extracts including a commercial shrimp extract (Leti) and *D pterynossinus* extract (Leti). According to EAACI recommendations, histamine hydrochloride (10 mg/mL) and saline solution were used as positive and negative controls, respectively. The SPT result was considered positive when the wheal diameter was greater than 3 mm compared to the negative control [27,28].

SPTs were also performed with the freeze-dried shrimp mixture extract prepared for the study at 1:100, 1:10, and 1:1. As in the previous case, the results were considered positive if they were >3 mm, although, in addition, the size of the wheal was categorized to perform comparisons between groups, as follows: "0", if the test result was negative; "1", if the wheal was less than half the size of that obtained with histamine; "2" if the wheal was half or more than half of that obtained with histamine; and "3", if the wheal was the same size or larger than that obtained with histamine.

Detection of Total and Specific IgE in Serum

sIgE to shrimp (f24 is a mixture of *Penaeus monodon*, *Metapenaeopsis barbata*, *Pandalus borealis*, and *Metapenaeus joyneri*), sIgE to *D pterynossinus* (d1), and total IgE, were measured using ImmunoCAP (Thermo Fisher Scientific). The cut-off for sIgE was ≥0.35 kU_A/L.

Double-Blind, Placebo-Controlled Food Challenges

OFCs were performed as double-blind, placebo-controlled food challenges (DBPCFCs) following the PRACTALL consensus report protocol proposed by EAACI and the American Academy of Allergy, Asthma & Immunology for standardization of this procedure [22]. The freeze-dried cooked shrimp mixture extract was reconstituted with 10 mL of saline solution and mixed with 100 mL of pineapple juice and 2 mL of vanilla extract. Identical blending, without the lyophilized mixture, was used for the placebo. A total of 7 increasing doses were administered until the cumulative dose of 3000 mg of shrimp protein was reached. All patients sensitized to shrimp and suspected of being allergic who had not experienced anaphylaxis in recent months had to undergo this test to be classified as allergic or nonallergic.

Nasal Allergen Provocation Test

NAPT was performed following the most recent EAACI position paper on the standardization of this procedure, published in 2018 [31]. A bilateral baseline measurement was made. Using a micropipette, 100 µL of SS saline solution (same diluent as that used to prepare the allergen solution) was instilled on the surface of the inferior turbinate of each nostril. Ten minutes later, the nasal response was assessed. If it was within pre-established reproducibility values, the test proceeded with the serial application of different concentrations of the freeze-dried shrimp mixture preparation, starting at 1:100, followed by 1:10, and finishing with 1:1 (Figure 1). The response to instillation of each of the concentrations was measured using acoustic rhinometry (AcRh) as an objective test and the VAS as a subjective test.

The NAPT was considered positive if there was a clear positive value on the objective scale, a clearly positive value

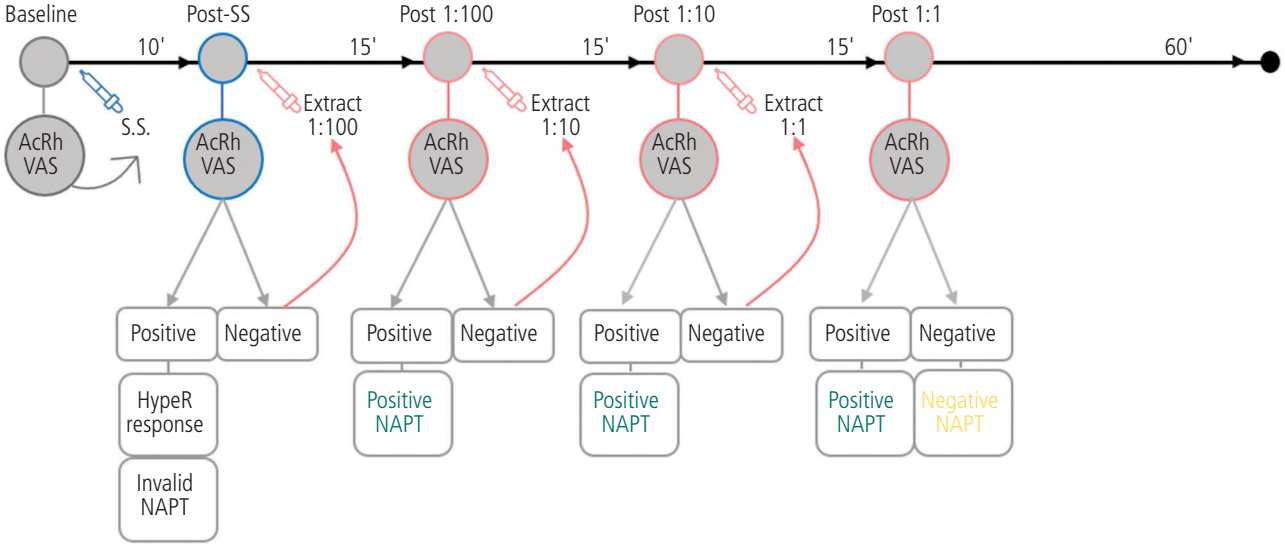

Figure 1. Protocol for performing the nasal allergen provocation test (NAPT) based on the position paper published by European Academy of Allergy and Clinical Immunology [31]. Acoustic rhinometry (AcRh) was performed and a visual analog scale (VAS) administered at baseline, 10 minutes after instillation of the saline solution (SS), and 15 minutes after instillation of the freeze-dried cooked shrimp mixture at concentrations of 1:100, 1:10, and 1:1. The test is considered invalid if the individual presents a hyperreactive response (HyperR) with the SS. The test ends when the individual presents a positive result at any concentration or a negative result after instillation of the undiluted 1:1 extract. In all cases, the individual remains under observation for 60 minutes.

on the subjective scale, or a moderate positive value in the 2 criteria. The test was terminated with a positive result or a negative result after administration of the undiluted 1:1 concentration.

This test was not blinded for the patient or for the investigator (in this case the person who performed the NAPT).

Acoustic Rhinometry

Nasal obstruction was assessed by means of AcRh (SRE 2000 rhinometer, Rhinometrics). The parameter evaluated was the volume of the nasal cavity between 2 cm and 5 cm, known as Volume 2 (Vol2), corresponding to the head of the inferior turbinate and the head of the middle turbinate [32,33,34,35]. The percentage decrease in volume in this portion of the nostril (PDVol2) was calculated, and the values obtained after instilling the lyophilized shrimp mixture preparation at the different concentrations were compared with the value obtained after instillation of saline solution (considered 100% in all participants).

AcRh was considered clearly positive if the volume of the nasal cavity decreased by ≥40% bilaterally and moderately positive if it decreased by ≥27% bilaterally.

Visual Analog Scale

The VAS provides participants with a 10-cm long (0-100 mm) line to rate the severity of symptoms caused by exposure to the allergen challenge (nasal obstruction, rhinorrhea, itching, and sneezing) by placing a vertical mark. The value 0 equals asymptomatic and 100 extremely bothersome. Following the EAACI criteria [31], the score was considered clearly positive if symptoms were ≥55 mm, and moderately positive if symptoms were ≥23 mm.

Statistical Analysis

Quantitative variables are expressed as median (IQR) or mean (SD). Qualitative variables are reported as absolute frequency or percentages. The primary outcome, ie, the usefulness of the NAPT for diagnosing the shellfish allergy in sensitized patients (S-A vs S-nA), was assessed using the sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), positive likelihood ratio (LR+), and negative likelihood ratio (LR–), calculated using 2×2 contingency tables and analyzed using the Fisher exact test.

The secondary outcomes were assessed as follows:

(*1*) The comparison between groups (S-A, S-nA, and controls) for the variables PDVol2 and VAS during the NAPT was performed using the Mann-Whitney test and the Wilcoxon signed rank test.

(*2*) Differences between groups (S-A and S-nA) in the SPT results with freeze-dried cooked shrimp mixture extract were analyzed using the receiver operating characteristic curve (cut-off points according to wheal size) and the levels of sIgE and the Fisher exact test (to compare the frequencies of sIgE).

Statistical significance was set at $P<.05$ and expressed with the 95%CI.

The analysis was performed with GraphPad Prism 8.1 Software (GraphPad Software Inc).

Results

Patient Population

A total of 55 participants were enrolled in the study (29 women and 26 men; median [IQR] age, 35 [28-42] years). Ten were controls (5 nonatopic [C1 to C5] and 5 sensitized to *D pteronyssinus* [C6 to C10]), and 45 were recruited as patients sensitized to shrimp, with and without clinical manifestations in relation to intake (Figure 2). The S-nA group included 21 patients: 15 recruited as tolerant according to their clinical history (S-nA1 to S-nA15) and 6 with negative results in the DBPCFC (S-nA16 to S-nA21). The S-A group included 22 patients: 7 with positive results in the DBPCFC (S-A1 to S-A7) and 15 diagnosed with anaphylaxis due to shrimp intake (S-A8 to S-A22). Two patients were ruled out of the study because of an inconclusive DBPCFC.

Table 1 shows the demographic and clinical characteristics of the 4 groups (individual values in Table S-I, Supplementary material).

Table 1. Baseline Demographic and Clinical Characteristics Stratified by Groups

Characteristic	S-nA	S-A	Control-DPT+	Control-nonatopic
Participants, No. (%)	21 (39.6)	22 (41.0)	5 (9.4)	5 (9.4)
Median (IQR) age, y	37 (30-46)	32 (25-41)	38 (34-44)	28 (22-40)
Female sex, No. (%)	10 (47.6)	10 (45.4)	3 (60.0)	3 (60.0)
Sensitization to shrimp, No. (%)	21 (100)	22 (100)	0 (0)	0 (0)
Sensitization to DPT, No. (%)	18 (86)	19 (86)	5 (100)	0 (0)
Anaphylaxis after shrimp intake, No. (%)	0 (0)	15 (68)	0 (0)	0 (0)
Clinical manifestations reported at recruitment, No. (%)	6 (28)	22 (100)	0 (0)	0 (0)
Tolerance reported at recruitment, No. (%)	15 (71)	0 (0)	5 (100)	5 (100)
OFC (DBPCFC) Positive/Performed	0 / 6	7 / 7	0 / 0	0 / 0

Abbreviations: DBPCFC, double-blind, placebo-controlled food challenge; DPT, Dermatophagoides pteronyssinus; OFC, oral food challenge; S-nA, sensitized-nonallergic; S-A, sensitized-allergic (S-A).

Table 2. Results of the Nasal Allergen Provocation Test[a]

C	PDVol2		VAS		NAPT result
	Final V	Class	Final V	Class	
C 1	2.1%	N	0	N	-
C 2	1.4%	N	0	N	-
C 3	7.3%	N	0	N	-
C 4	+3.3%	N	0	N	-
C 5	6.2%	N	4	N	-
C 6	10%	N	0	N	-
C 7	7.5%	N	0	N	-
C 8	6.2%	N	0	N	-
C 9	1.1%	N	0	N	-
C 10	+8.3%	N	4	N	-

S-nA	PDVol2		VAS		NAPT result
	Final V	Class	Final V	Class	
S-nA 1	15.2%	N	15	N	-
S-nA 2	23.7%	N	0	N	-
S-nA 3	3.1%	N	0	N	-
S-nA 4	13.8%	N	14	N	-
S-nA 5	4.9%	N	0	N	-
S-nA 6	+2.8%	N	0	N	-
S-nA 7	11%	N	0	N	-
S-nA 8	+28 %	N	0	N	-
S-nA 9	1%	N	0	N	-
S-nA 10	27.6%	MP	33	MP	+
S-nA 11	12.5%	N	12	N	-
S-nA 12	11.6%	N	0	N	-
S-nA 16	34.3%	MP	9	N	-
S-nA 17	+11.5	N	17	N	-
S-nA 18	43.2%	CP	24	MP	+
S-nA 19	+0.1%	N	0	N	-
S-nA 20	16.6%	N	09	N	-
S-nA 21	23.2%	N	8	N	-

S-A	PDVol2		VAS		NAPT result
	Final V	Class	Final V	Class	
S-A 1	49.6%	CP	29	MP	+
S-A 2	51.7%	CP	43	MP	+
S-A 3	41.5%	CP	74	CP	+
S-A 4	37.7%	CP	73	CP	+
S-A 5	27.4%	MP	33	MP	+
S-A 6	36.2%	MP	57	CP	+
S-A 8	32.7%	MP	25	MP	+
S-A 9	34.3%	MP	31	MP	+
S-A 10	37.7%	MP	24	MP	+
S-A 11	27.8%	MP	32	MP	+
S -A 12	11.4%	N	0	N	-
S-A 13	32.1%	MP	25	MP	+
S-A 14	45.5%	CP	36	MP	+
S-A 15	18.1%	N	53	CP	+
S-A 16	65%	CP	69	CP	+
S-A 17	24.1%	N	33	MP	-
S-A 18	28.8%	MP	43	MP	+
S-A 19	27.5%	MP	53	CP	+
S-A 20	57%	CP	36	MP	+
S-A 21	45%	CP	29	MP	+

[a]Values for the objective measurements and the subjective measurements following the EAACI task group criteria [30] obtained in the control group, sensitized-nonallergic group (S-nA), and sensitized-allergic group (S-A). The participant's code is the result of the acronym of the group to which it belongs plus the order number. Participants S-nA14, S-nA15, S-A7, and S-A22, were removed because of a hyperreactive response to saline solution. Participant S-nA13 was removed because of a negative SPT result with the shrimp extract preparation.
Abbreviations: Class (classification); CP, clearly positive; Final V, final value; MP, moderately positive; N, negative; NAPT, nasal allergen provocation test; PDVol2, percentage decrease in nasal volume 2 measured using acoustic rhinometry; VAS, visual analog scale.

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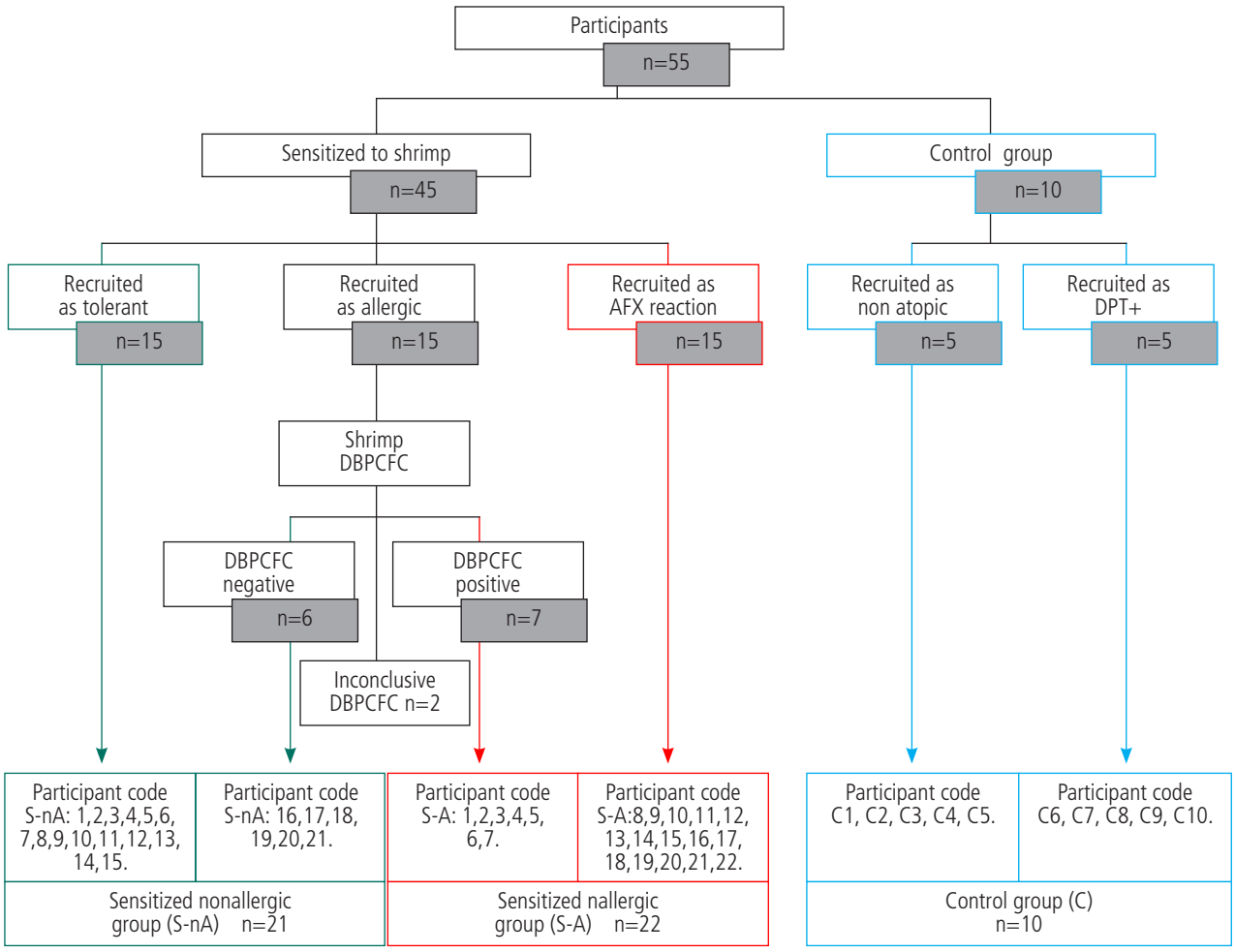

Figure 2. Classification of the study participants initially, at recruitment, and after the double-blind placebo-controlled food challenge (DBPCFC). Final groups: Sensitized-nonallergic (S-nA), sensitized-allergic (S-A), and controls. The participant code is the result of the acronym of the group to which it belongs plus the participant order number. AFX indicates anaphylaxis. DPT+, sensitization to *Dermatophagoides pteronyssinus*.

Nasal Allergen Provocation Test as a Diagnostic Tool

Of the 21 patients classified as S-nA, 3 were excluded (1 with a negative SPT for freeze-dried cooked shrimp mixture and 2 with nasal hyperreactivity to saline solution), 16 obtained a negative result (16/18 [88.9%]), and 2 obtained a positive result (2/18,11.1%), according to the criteria endorsed by the EAACI (PDVol2 ≥40% in AcRh or VAS ≥55 mm, or AcRh ≥27% plus VAS ≥23 mm) [31] (Table 2). Of the 22 patients classified as S-A, 2 were eliminated (nasal hyperresponsiveness), 18 obtained a positive result (18/20 [90%]), and 2 obtained a negative result (2/20 [10%]).

The result was negative for all the participants in the control group, which was only included to demonstrate that the freeze-dried cooked shrimp mixture was not irritant and could not produce a positive local nasal response by cross-reactivity in individuals also sensitized to *D pteronyssinus*.

To evaluate the usefulness of NAPT as a diagnostic tool, we only considered the S-A and S-nA groups, which were the groups between which we were trying to differentiate. The sensitivity and specificity of the test were 90% and 89% respectively, the PPV was 90%, the NPV was 89%, the LR+ was 8.1, and the LH– was 0.1 (P<.0001 [see Table S-II, Supplementary material for 95%CI values]).

Acoustic Rhinometry

The 2 control groups were studied first (individual values in Table S-III, and group averages in Table S-IV, Supplementary material) Neither experienced changes in Vol2 after instillation of the lyophilized shrimp mixture. The S-nA group was then challenged, and no relevant changes were observed in most cases. No differences were found between this group and the controls (P>.05). A clear decrease in Vol2 was observed in the S-A group. Differences were found with respect to the previous groups, whether compared to controls (P<.001) or to S-nA (P<.001) (Figure 3).

Four patients, 2 from each group (S-nA14, S-nA15, S-A7, S-A22), experienced a hyperreactivity response to the diluent and were therefore excluded from the study.

Visual Analog Scale

The clinical symptoms produced by administration of saline solution and lyophilized shrimp mixture at different concentrations were evaluated using the VAS and compared between the 3 study groups. Hardly any symptoms were recorded for the control and S-nA groups, and no differences were detected between them. Patients in the S-A group did show symptoms or differences, although in this case they were only significant at concentrations 1:10 and 1:1 (Figure 4).

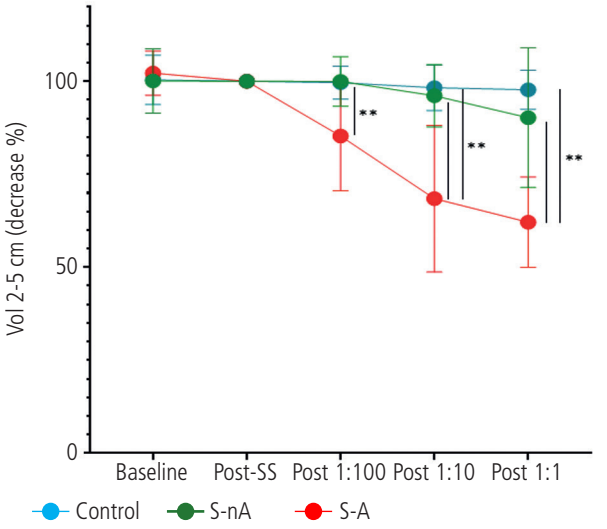

Figure 3. Nasal allergen provocation test with cooked shrimp mixture at 1:100, 1:10, and 1:1. Percentage of volume decrease measured by acoustic rhinometry. Comparative values between controls, sensitized-nonallergic patients(S-nA), and sensitized-allergic patients (S-A) at baseline, after instillation of saline solution, and after instillation of lyophilized shrimp mixture at concentrations of 1:100 (post 1:100), 1:10 (post 1:10), and 1:1 (post 1:1). No differences were found between the control and S-nA groups. Significant differences were found for control vs S-A and S-nA vs S-A. Vol 2-5 cm (decrease %) indicates the percentage decrease in nasal area 2-5.
***$P<.0001$

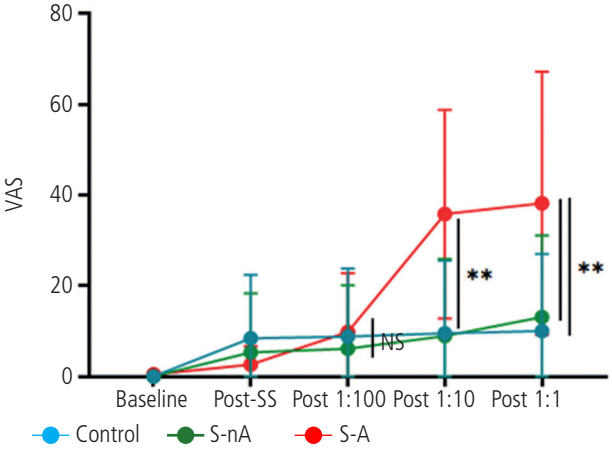

Figure 4. Nasal allergen provocation test with the cooked shrimp mixture at concentrations of 1:100, 1:10, and 1:1. Visual analog scale (VAS) values. Comparative values between control, sensitized-nonallergic patients (S-nA), and sensitized-allergic patients (S-A) at baseline, after instillation of saline solution, and after instillation of lyophilized shrimp mixture at concentrations of 1:100 (post 1:100), 1:10 (post 1:10), and 1:1 (post 1:1). No differences were found between the control group and S-nA or between the S-nA group and the S-A group at the 1:100 concentration. Significant differences were found for control vs S-A and S-nA vs S-A at the 1:10 and 1:1 concentrations. NS, indicates nonsignificant.
**: $P<.0001$

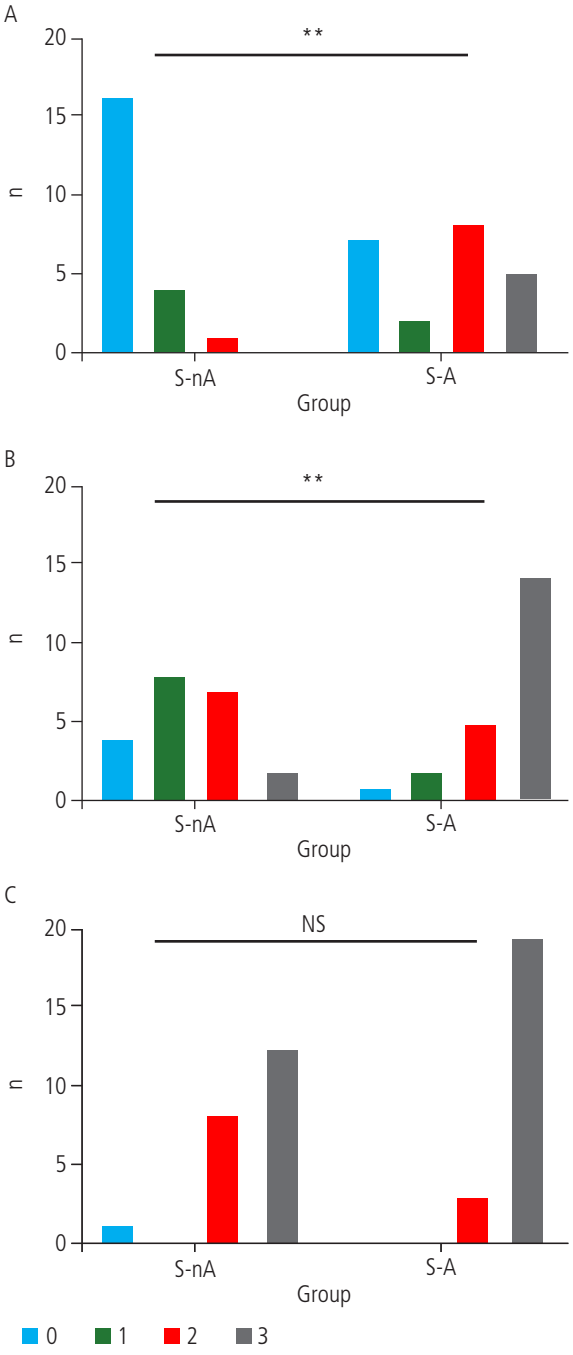

Figure 5. Comparison of skin prick test results between sensitized-nonallergic individuals (S-nA) and sensitized-allergic individuals (S-A) at the 3 concentrations of cooked shrimp mixture extract: 1:100 (A), 1:10 (B), and 1:1(C). Wheals were classified as not present (0), less than or equal to half the histamine control wheal size (1), more than half the histamine wheal size (2), and equal to or greater than the histamine wheal size (3). Significant differences were found for the 1:100 and 1:10 concentrations. **$P<.00$. NS indicates nonsignificant.

Some patients in the latter group reported palatal pruritus and, to a lesser extent, itchy ear. As these symptoms are not clearly defined in the ARIA guide, they were computed as nasal itching [36,37] (see Table S-III and S-IV, Supplementary material for individual and group values).

Skin Prick Tests With Freeze-Dried Cooked Shrimp Mixture

The results obtained in the SPTs performed on the study participants differed to some extent (individual results in Table S-V, Supplementary material). In the control group, as expected, all the results were negative.

In the 1:100 concentration, differences were found (P<.002) in the S-A and S-nA groups (Figure 5A). The cut-off point from which allergic individuals could be distinguished from nonallergic individuals was 2 (area under the curve [AUC], 0.81; Youden index [YI], 0.59). Thus, obtaining a wheal size equal to or greater than half the histamine wheal size after SPT with the reconstituted lyophilized extract of shrimp mixture at a concentration of 1:100 enabled us to differentiate the S-A group from the S-nA group with a sensitivity of 65%, specificity of 94%, PPV of 93%, and NPV of 71% (LR+, 11.6; LR–, 0.37).

The 1:10 concentration also makes it possible to differentiate the S-A group from the S-nA group (P<.001) (Figure 5B). The best cut-off point was 3 (AUC, 0.08; YI, 0.57). A wheal size greater than the histamine wheal size enabled the individual to be considered allergic with a sensitivity of 68%, specificity of 89%, PPV of 87.5%, and NPV of 72.5% (LR+, 3.4; LR–, 0.24).

The 1:1 concentration does not make it possible to differentiate symptomatic from asymptomatic individuals (P>.05) (Figure 5C).

Participant S-nA13 did not develop a wheal with the shrimp extract preparation and was therefore excluded from the study.

Specific IgE in Serum

Shrimp sIgE values were compared between the S-nA group and the S-A group, with no statistically significant differences being identified (P>.5). The mean value was 2.76 (2.59) kU_A/L in the S-nA group and 15.73 (28.12) kU_A/L in the S-A group. The median (IQR) was 1.71 (0.66-4.99) kU_A/L and 3.51 (0.99-9.73) kU_A/L, respectively (individual values in Table S-I, Supplementary material).

Safety

The only adverse effect observed during or shortly after the NAPT was a local allergic reaction. This resolved progressively, disappearing spontaneously in less than 90 minutes or after administration of oral antihistamine or topical nasal vasoconstrictor (8/38 [21%]).

Only 1 participant, S-A7, who did not require drug treatment after a positive NAPT result, presented retronasal and palatal angioedema, which first appeared 5 hours after the NAPT and resolved with an oral corticosteroid (prednisone 30 mg/d) in 72 hours.

The allergic reactions resulting from the DBPCFC included oral allergy syndrome (3/15 [20%]), lip angioedema (3/15 [20%]), urticaria (2/15 [13%]), abdominal pain (2/15 [13%]), erythema (1/15 [6.5%]), and globus sensation (1/15 [6.5%]). All symptoms resolved in less than 90 minutes with the administration of oral antihistamines or corticosteroids (9/15 [60%]).

No systemic reactions were recorded in the NAPT or the OFC, although it should be noted that patients with a history of anaphylaxis did not undergo the latter test.

Discussion

We compare the diagnostic ability of NAPT with that of OFC in 45 shrimp-sensitized patients (SPT) and 10 controls. Our data show that NAPT can differentiate allergic patients from nonallergic patients in a group of shrimp-sensitized individuals.

Few publications address nasal provocations with food. The first was published in 1993 by Seppey et al [38], followed by 2 publications by Clark et al in 2007 [39] and 2012 [40]. The authors used facial thermography to measure the increase in the temperature of the nasal mucosa (0.8ºC to 0.9ºC) after instilling egg extract or peanut protein in the nose of persons who were allergic to these substances. Although the authors showed that this technique was fast, safe, and objective, no other studies on food allergies have been carried out. In 2013, Sánchez-López et al [41] used Pru p 3 to perform NAPTs in persons who experienced anaphylaxis after ingesting peach. Although the objective of the study was not to perform a food allergy work-up, the authors were the first to use AcRh to measure the decrease in nasal volume by means of a food allergen.

Therefore, using these data and the position paper published on the standardization of NAPT [31], we tested the hypothesis that NAPT would enable us to differentiate between shrimp-sensitized individuals who would or would not experience symptoms after being challenged with shrimp allergens.

The decision to use a cooked extract as opposed to a raw one was based on previous studies by Asero et al [42], Jirapongsananuruk et al [43], and Carnes et al [44], in which the authors demonstrated that cooked extracts were more potent and were recognized by more patients.

The main reason for diluting the freeze-dried shrimp mixture extract to 1:100, 1:10, and 1:1 was that we could not predict how participants would respond to instillation of the allergen, which dilution would trigger a local response, and whether the extract would be absorbed and cause systemic allergic reactions.

A recent publication demonstrated marked variability in the SPT response of shrimp-sensitized patients to commercial extracts [42]. Therefore, the freeze-dried cooked shrimp mixture extract was tested in all participants. We had to ensure that all participants recognized the extract with which SPT, NAPT, or OFC was to be performed (or that they genuinely did not recognize it in the case of controls).

The results obtained from AcRh and VAS were analyzed separately, since each—objective and subjective—is designed to evaluate the local allergic reaction in the form of rhinitis produced after instillation of an environmental allergen [45].

We did not know whether the nasal mucosa would behave in the same way after instillation of a food allergen. The results obtained show the capacity of both approaches to establish differences between groups, although AcRh show differences in the 3 concentrations, while VAS shows these differences for 1:10 and 1:1, but not for 1:100. The concentration at which the participant presented a positive NAPT result was not considered relevant. We followed the same model as in the OFC, where the positive or nonpositive test result does not take account of the amount of protein ingested.

A test is considered a good diagnostic tool if it is valid, safe, and reproducible. In this case, the NAPT is a valid test that recognizes 90% of sensitized individuals who are truly allergic and 89% of sensitized individuals who are effectively tolerant. It is also safe, because of the high PPV and NPV (90% and 89%). We do not know if our observation is reproducible, since we do not have another series with which to compare, although NAPT itself is reproducible according to existing data on inhalant allergens [46].

In addition, we calculated the LRs (LR+, 8.1; and LR–, 0.1), which are indicative of the diagnostic capacity of a test regardless of the prevalence of the disease being studied. Both values indicate that NAPT is useful as a diagnostic tool.

Mention must be made of the SPTs performed with the shrimp mixture extract. As occurs in real life, SPTs make it possible to diagnose sensitization but not to differentiate between S-A and S-nA at the 1:1 concentration. Differences were found between the groups at the 1:100 and 1:10 concentrations, although the sensitivity, specificity, PPV, NPV, and LH values obtained are insufficient for the SPTs to be considered good diagnostic tools.

Our study is subject to a series of limitations. First, recruitment could have been affected by selection bias. Participants were selected based on the positivity or negativity of the SPT with a specific commercial extract. Our results may have differed if the tests had been carried out with another shrimp extract [42]; therefore, the sample selected would also have been different. In any case, this does not affect the validity of the study, since the performance of the SPT with the lyophilized sample confirms the classification as sensitized or not sensitized (the only participant whose results were different was excluded from the study). Moreover, we used a shrimp mixture suitable for our population and with a high degree of recognition (44/45, 97.8%); we do not know if recognition would be as high in another sample. Second, the study could be subject to performance bias, since the NAPTs were performed after the OFC to classify participants as S-A or S-nA; in addition, the challenge was not blinded. While this did not affect the objective scale, it may have polarized the results of the subjective scale. Finally, there may be bias as a result of our directly classifying patients with a history of anaphylaxis as allergic, and although the NIAID allows this classification [29], such a claim has not been demonstrated.

In conclusion, NAPT with freeze-dried shrimp mixture was a good diagnostic tool for differentiating between S-A and S-nA in this population. Despite the small study sample and the fact that the results do not allow for broader generalization, our finding could pave the way for a new diagnostic strategy in individuals with food allergies.

There is a great need for a low-risk, non–OFC-based tool for diagnosis of food allergy. More studies with much larger samples should be performed to assess whether NAPT could be this tool.

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Conflicts of Interest

The authors declare that they have no conflicts of interest.

References

1. Sicherer SH, Sampson HA. Food allergy: A review and update on epidemiology, pathogenesis, diagnosis, prevention, and management. J Allergy Clin Immunol. 2018;141:41-58.
2. De Blok BMJ, Vlieg-Boerstra BJ, Oude Elberink JNG, Duiverman EJ, DunnGalvin A, Hourihane JOB, et al. A framework for measuring the social impact of food allergy across Europe: A EuroPrevall state of the art paper. V Allergy. 2007;62:733-7.
3. Moonesinghe H, Mackenzie H, Venter C, Kilburn S, Turner P, Weir K, et al. Prevalence of fish and shellfish allergy: A systematic review. Ann Allergy Asthma Immunol. 2016;117:264-72.e4.
4. Bauermeister K, Wangorsch A, Garoffo LP, Reuter A, Conti A, Taylor SL, et al. Generation of a comprehensive panel of crustacean allergens from the North Sea Shrimp Crangon crangon. Mol Immunol. 2011;48:1983-92.
5. Faber MA, Pascal M, El Kharbouchi O, Sabato V, Hagendorens MM, Decuyper II, et al. Shellfish allergens: tropomyosin and beyond. Allergy. 2017;72:842-8.
6. Khora SS. Seafood-Associated Shellfish Allergy: A Comprehensive Review. Immunol Invest. 2016;45:504-30.
7. Gelis S, Rueda M, Valero A, Fernández EA, Moran M, Fernández-Caldas E. Shellfish allergy: unmet needs in diagnosis and treatment. J Investig Allergol Clin Immunol. 2020;30:409-20.
8. Reese G, Ayuso R, Lehrer SB. Tropomyosin: An invertebrate pan-allergen. Int Arch Allergy Immunol.1999;119:247-58.
9. Yi FC, Cheong N, Shek PCL, Wang DY, Chua KY, Lee BW. Identification of shared and unique immunoglobulin E epitopes of the highly conserved tropomyosins in Blomia tropicalis and Dermatophagoides pteronyssinus. Clin Exp Allergy. 2002;32:1203-10.
10. Wang J, Calatroni A, Visness CM, Sampson HA. Correlation of specific IgE to shrimp with cockroach and dust mite exposure and sensitization in an inner-city population. J Allergy Clin Immunol. 2011;128:834-7.
11. Asero R, Mistrello G, Amato S, Ariano R, Colombo G, Conte ME, et al. Shrimp allergy in Italian adults: A multicenter study showing a high prevalence of sensitivity to novel high molecular weight allergens. Int Arch Allergy Immunol. 2011;157:3-10.
12. Ayuso R, Sánchez-Garcia S, Pascal M, Lin J, Grishina G, Fu Z, et al. Is epitope recognition of shrimp allergens useful to predict clinical reactivity? Clin Exp Allergy. 2012;42:293-304.
13. Gámez C, Zafra MP, Boquete M, Sanz V, Mazzeo C, Ibáñez MD, et al. New shrimp IgE-binding proteins involved in mite-

seafood cross-reactivity. Mol Nutr Food Res. 2014;58:1915-25.
14. Pascal M, Grishina G, Yang AC, Sánchez-García S, Lin J, Towle D, et al. Molecular Diagnosis of Shrimp Allergy: Efficiency of Several Allergens to Predict Clinical Reactivity. J Allergy Clin Immunol Pract. 2015;3:521-9.
15. Wong L, Huang CH, Lee BW. Shellfish and House Dust Mite Allergies: Is the Link Tropomyosin? Allergy Asthma Immunol Res. 2016;8:101-6.
16. Martins TF, Mendonça TN, Melo JML, Moreno AS, Januário YC, DaSilva LLP, et al. Reactions to shrimp including severe anaphylaxis in mite- and cockroach-allergic patients who have never eaten shrimp: Clinical significance of ige cross-reactivity to tropomyosins from different sources. J Investig Allergol Clin Immunol. 2019;29:302-5.
17. Muraro A, Werfel T, Hoffmann-Sommergruber K, Roberts G, Beyer K, Bindslev-Jensen C, et al. EAACI Food Allergy and Anaphylaxis Guidelines: Diagnosis and management of food allergy. Allergy Eur J Allergy Clin Immunol. 2014;69:1008-25.
18. Ballmer-Weber BK, Fernandez-Rivas M, Beyer K, Defernez M, Sperrin M, Mackie AR, et al. How much is too much? Threshold dose distributions for 5 food allergens. J Allergy Clin Immunol. 2015;135:964-71.
19. Chokshi NY, Sicherer. Interpreting IgE sensitization tests in food allergy. Exp Rav Clin Immunol. 2015;12:389-403.
20. Yang AC, Arruda LK, Santos ABR, Barbosa MCR, Chapman MD, Galvão CES, et al. Measurement of IgE antibodies to shrimp tropomyosin is superior to skin prick testing with commercial extract and measurement of IgE to shrimp for predicting clinically relevant allergic reactions after shrimp ingestion. J Allergy Clin Immunol. 2010;125:872-8.
21. Bindslev-Jensen C, Ballmer-Welser BK, Bengtsson U, Blanco C, Ebner C, Hourihane J, et al. Standardization of food challenges in patients with immediate reactions to foods - Position paper from the European Academy of Allergology and Clinical Immunology. Allergy. 2004;59:690-7.
22. Sampson HA, Gerth Van Wijk R, Bindslev-Jensen C, Sicherer S, Teuber SS, Burks AW, et al. Standardizing double-blind, placebo-controlled oral food challenges: American Academy of Allergy, Asthma & Immunology-European Academy of Allergy and Clinical Immunology PRACTALL consensus report. J Allergy Clin Immunol. 2012;130:1260-74.
23. Agache I, Bilò M, Braunstahl GJ, Delgado L, Demoly P, Eigenmann P, et al. In vivo diagnosis of allergic diseases - Allergen provocation tests. Allergy Eur J Allergy Clin Immunol. 2015;70:355-65.
24. Tenn MW, Rawls M, Ellis AK. Nasal challenges in allergen immunotherapy Curr Opin Allergy and Clinical Immun. 2018;18:489-94.
25. Gosepath J, Amedee RG, Mann WJ. Nasal provocation testing as an international standard for evaluation of allergic and nonallergic rhinitis. Laryngoscope. 2005;115:512-6.
26. Scadding G, Hellings P, Alobid I, Bachert C, Fokkens W, Van Wijk RG, et al. Diagnostic tools in Rhinology EAACI position paper. Clin Transl Allergy. 2011;1:1-39.
27. Dreborg S, Backman A, Basomba A, Bousquet J, Dieges P MH. Skin test used in type I allergy testing Position paper. Sub-Committee on Skin Tests of the European Academy of Allergology and Clinical Immunology. Allergy. 1989;44:1-59.
28. Heinzerling L, Mari A, Bergmann KC, Bresciani M, Burbach G, Darsow U, et al. The skin prick test - European standards. Clin Transl Allergy. 2013;3:1-10.
29. Boyce JA, Assa'ad A, Burks AW, Jones SM, Sampson HA, Wood RA, et al. Guidelines for the diagnosis and management of food allergy in the United States: Summary of the NIAID-sponsored expert panel report. J Allergy Clin Immunol. 2010;126:1105-18.
30. Bradford M. A Rapid and Sensitive Method for the Quantitation of Microgram Quantities of Protein Utilizing the Principle of Protein-Dye Binding. Anal Biochem. 1976;72:248-54.
31. Augé J, Vent J, Agache I, Airaksinen L, Campo Mozo P, Chaker A, et al. EAACI Position paper on the standardization of nasal allergen challenges. Allergy. 2018;73:1597-608.
32. Dordal MT, Lluch-Bernal M, Sánchez MC, Rondón C, Navarro A, Montoro J, et al. Allergen-specific nasal provocation testing: Review by the rhinoconjunctivitis committee of the Spanish society of allergy and clinical immunology. J Investig Allergol Clin Immunol. 2011;21:1-12.
33. Nathan RA, Eccles R, Howarth PH, Steinsvåg SK, Togias A. Objective monitoring of nasal patency and nasal physiology in rhinitis. J Allergy Clin Immunol. 2005;115:442-545.
34. Malm L, Gerth van Wijk R Bachert C. Guidelines for nasal provocations with aspects on nasal patency, airflow, and airflow resistance. International Committee on Objective Assessment of the Nasal Airways, International Rhinologic Society. Rhinology. 2000;38:1-6.
35. Gotlib T, Samoliński B, Grzanka A. Bilateral nasal allergen provocation monitored with acoustic rhinometry. Assessment of both nasal passages and the side reacting with greater congestion: Relation to the nasal cycle. Clin Exp Allergy. 2005;35:313-8.
36. Bousquet PJ, Combescure C, Neukirch F, Klossek JM, Méchin H, Daures JP, et al. Visual analog scales can assess the severity of rhinitis graded according to ARIA guidelines. Allergy Eur J Allergy Clin Immunol. 2007;62:367-72.
37. Bousquet J, Hellings PW, Agache I, Bedbrook A, Bachert C, Bergmann KC, et al. ARIA 2016: Care pathways implementing emerging technologies for predictive medicine in rhinitis and asthma across the life cycle. Clin Transl Allergy. 2016;6:45.
38. Seppey M, Hessler C, Bruchez M, Savary M, Pécoud A. Facial thermography during nasal provocation tests with histamine and allergen. Allergy. 1993;48:314-8.
39. Clark AT, Mangat JS, Tay SS, King Y, Monk CJ, White PA, et al. Facial thermography is a sensitive and specific method for assessing food challenge outcome. Allergy Eur J Allergy Clin Immunol. 2007;62(7):744-9.
40. Clark A, Mangat J, King Y, Islam S, Anagnostou K, Foley L, et al. Thermographic imaging during nasal peanut challenge may be useful in the diagnosis of peanut allergy. Allergy. 2012;67:574-6.
41. Sánchez-López J, Tordesillas L, Pascal M, Muñoz-Cano R, Garrido M, Rueda M, et al. Role of Art v 3 in pollinosis of patients allergic to Pru p 3. J Allergy Clin Immunol. 2014;133:1018-25.
42. Asero R, Scala E, Villalta D, Pravettoni V, Arena A, Billeri L, et al. Shrimp allergy: Analysis of commercially available extracts for in vivo diagnosis. J Investig Allergol Clin Immunol. 2017;27:175-82.

43. Jirapongsananuruk O, Sripramong C, Pacharn P, Udompunturak S, Chinratanapisit S, Piboonpocanun S, et al. Specific allergy to Penaeus monodon (seawater shrimp) or Macrobrachium rosenbergii (freshwater shrimp) in shrimp-allergic children. Clin Exp Allergy. 2008;38:1038-47.
44. Carnés J, Ferrer Á, Huertas ÁJ, Andreu C, Larramendi CH, Fernández-Caldas E. The use of raw or boiled crustacean extracts for the diagnosis of seafood allergic individuals. Ann Allergy Asthma Immunol. 2007;98:349-54.
45. Yepes-Nuñez JJ, Bartra J, Muñoz-Cano R, Sánchez-López J, Serrano C, Mullol J, et al. Assessment of nasal obstruction: Correlation between subjective and objective techniques. Allergol Immunopathol. 2013;41:397-401.
46. Eguiluz-Gracia I, Testera-Montes A, González M, Pérez-Sánchez N, Ariza A, Salas M, et al. Safety and reproducibility of nasal allergen challenge. Allergy Eur J Allergy Clin Immunol. 2019;74:1125-34.

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