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

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This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.18176/jiaci.0736
Abstract

Background: Shellfish allergy is an important cause of food allergy and anaphylaxis worldwide. Several allergenic proteins have been described in the last few years, but the only diagnostic tool that allows discrimination between allergic and non-allergic sensitized subjects is still the oral food challenge (OFC).

Objective: The aim of this study was to evaluate the usefulness of nasal allergen provocation test (NAPT) as a diagnostic tool in the diagnosis of shellfish allergy.

Methods: Forty-five subjects with confirmed sensitization to shrimp by a positive skin prick test (SPT) to a commercial shrimp extract were recruited and classified as Sensitized-Allergic or non-Allergic based on current tolerance to shrimp intake, the result of an OFC with a freeze-dried cooked shrimp mixture extract, or recent history of anaphylaxis from shrimp ingestion. These subjects and ten controls without shrimp sensitization were subjected to a NAPT with a freeze-dried cooked shrimp mixture extract. The response was evaluated by means of acoustic rhinometry (AcRh) and visual analogue scale scores (VAS).

Results: Significant differences (p=.001) were found between the Sensitized-Allergic group (18/20 positive NAPT, 90%) compared to both Sensitized-non-Allergic (2/18 positive NAPT, 11.1%) and Control (0/10 positive NAPT) groups. NAPT allows differentiation between allergic and non-allergic subjects with a S: 90%, E: 89%, PPV: 90% and NPV: 89%.

Conclusions: According to the study results NAPT may be a useful diagnostic tool that allows differentiating sensitized symptomatic subjects from sensitized tolerant. It could be a valuable test to consider when conducting a shrimp allergy study.

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=.001) entre el grupo de sensibilizados-alérgicos (18/20 NAPT positivos, 90%) frente a los sensibilizados-noalérgicos (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.

Podría ser una herramienta diagnóstica valiosa a la hora de realizar un estudio de alergia a gamba.

INTRODUCTION

Food allergy has increased considerably in recent years and has become an important health problem[1,2]. Reported prevalence rates of shellfish allergy vary greatly depending on the area studied, with a global average value of 5.4%[3].

Shellfish is part of the main allergenic food groups, beside milk, egg, fish, nuts, peanuts, wheat, and soy, account for up to 90% of all reported food allergy cases with the aggravating factor that shellfish is especially linked to life-threatening reactions.

There are several allergens described in shellfish: Tropomyosin (TM), Arginine kinase (AK), Myosin light chain, Sarcoplasmic Calcium-binding protein (SCPB), Troponin C, Troponin I, Triose phosphate isomerase, Hemocyanin (HC), α and β actinin, ubiquitin, myosin heavy chain , fatty acid binding protein, enolase, etc.[4-7]. The clinical relevance of each of them is unclear , although TM, AK , SCBP, HC, ubiquitin and α-actin have been shown to be the allergens involved in the cross-reactivity(CR) between shellfish and house dust mites (HDM)[8-16].

Currently, the routine allergologic work-up of shrimp allergy is based on in vivoSPT, performed with either commercial extracts or with 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 TM (Pen a 1 or Pen m 1)[17].All these tests are useful to determine sensitization, but do not define clinical allergy. Although there are publications suggesting that a higher level of sIgE to shrimp and shrimp-TM could indicate allergy, there is not clear evidence to support this finding [18-20].

Unlike what happens with other foods, the component resolved diagnosis is poorly developed In all cases, an OFC should be performed to confirm allergy or tolerance, remaining the gold standard, despite the consumption of resources and potential risk to induce allergic events[21,22].
At present, there is no treatment or cure for shellfish allergy, the only recommendation is strict avoidance[1]. For this reason, and due to the elevated risk of presenting life-threatening allergic reactions, a good diagnosis of certainty is an absolute priority.

NAPT with mites, epithelia or pollens have been used during years to reproduce and study allergic rhinitis, to confirm the clinical relevance of environmental allergens[23,24], and as an outcome parameter in therapeutic trials[24,25]. It is described as a safe, simple and inexpensive technique[26]. NAPT is not currently used in the food allergy study protocol and there are few studies on this matter. The main objective of the present study is to evaluate if the NAPT may be a good diagnostic tool in the study of shellfish allergy to differentiate allergic patients from sensitized non-allergic subjects.
METHODS
Patient selection
Subjects were recruited prospectively and consecutively from outpatient clinics of the allergy department of Hospital Clinic (Barcelona, Spain) for one year. The study was approved by the Hospital Clinic’s ethics committee, and written informed consent was obtained from all patients.

Patients sensitized to shrimp detected by SPT as defined in the European Academy of Allergy and Clinical Immunology (EAACI) criteria \[27,28\], and control subjects without shrimp sensitization, were enrolled and included in one of these 4 groups:

- Sensitized-non allergic group (S-nA). Subjects resulting positive in shrimp SPT and:
  - current shrimp intake tolerance by clinical history,
  - or reporting clinical manifestations other than anaphylaxis in the last 6 months (oral allergy syndrome, angioedema, urticaria, or digestive symptoms) and result negative in the OFC to shrimp. Subjects with exclusive steam inhalation symptoms were excluded from recruitment due to the difficulty of performing exposure tests.

- Sensitized-allergic group (S-A). Subjects resulting positive in shrimp SPT and:
  - had a documented and unequivocal history of anaphylaxis due to shrimp consumption occurred in the last 6 months. They 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 reporting clinical manifestations other than anaphylaxis and result positive in the OFC to shrimp.

- Control atopic group. Subjects resulting negative in SPT to shrimp but positive to *Dermatophagoides ptenonysinus* (DPT), with current shrimp intake tolerance by clinical history.
Control non atopic group. Subjects without sensitization to environmental or food allergens, therefore negative in SPT to shrimp and DPT, and current shrimp intake tolerance by clinical history.

**Shrimp extract preparation for SPT, OFC, and NAPT.**
25 grams of peeled *Parapenaeopsis spp*, *Parapenaeus spp*, *Solenocera spp* and *Trachipenaeus spp*, were used. 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 PBS, Ph 7.2, at 4 °C for 18 hours. Subsequently, it was centrifuged at 10,000 rpm for 10 minutes and the supernatant was clarified by vacuum filtration through cellulose acetate membrane filters with decreasing filter: 1 micrometer (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 pore size 5 kDa. Dialysis was performed with 7 volumes of distilled water (Inmunotek SL Laboratories, Madrid, Spain).

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

The resulting material was the freeze-dried cooked shrimp mixture extract prepared to perform the nasal exposure tests by reconstituting 10 mL of saline serum (SS), leaving a final concentration of 1,048 mg/ml.

To perform the OFCs, the vials contained the lyophilized preparation equivalent to 20 grams of fresh product, corresponding to the 3 grams of shrimp protein necessary to perform the test.
Skin prick tests
All the patients underwent SPT with a panel of allergen extracts including a commercial shrimp extract (Leti, Madrid, Spain) and DPT extract (Leti, Madrid, Spain). According to the EAACI recommendations, histamine hydrochloride (10 mg/ml) and SS were used as positive and negative controls, respectively. The SPT were considered positive when the diameter of the papule was greater than 3 mm compared to the size of the negative control [27,28].

SPT were also performed with the freeze-dried shrimp mixture extract prepared for the study at 1:100, 1:10, and 1:1 concentration. As in the previous case the results were considered positive if they were >3 mm, but in addition the size of the papule was categorized to perform comparisons between groups. They were labeled as: “0”, if the test result was negative; “1”, if the papule size obtained was <½ the size of the papule size obtained with histamine; “2”, if the papule size was ≥½ of the papule size obtained with histamine, and “3”, if the size of the papule size was ≥ the papule size obtained with histamine.

Total and Specific IgE in serum detection
sIgE to shrimp (f24, is a mixture of Penaeus monodon, Metapenaeopsis barbata, Pandalus borealis, and Metapenaeus joyneri), sIgE to DPT(d1), and total IgE, were measured by ImmunoCAP system (Thermo Fisher Scientific, Uppsala, Sweden). Cut-off for sIgE was considered ≥0.35kU/A/L.

Double-blind, placebo-controlled food challenges.
OFC were performed in form of double-blind, placebo-controlled food challenge (DBPCFC) following the PRACTALL consensus report protocol proposed by EAACI and American Academy of Allergy, Asthma & Immunology (AAAAI) to standardizing this procedure[22]. The freeze-dried cooked shrimp mixture extract was reconstituted with 10 ml of SS 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 seven increasing doses were administered until reaching the cumulative dose of 3000 mg of
shrimp protein. All the subjects sensitized to shrimp, with a suspicion of allergy, who had not presented anaphylaxis in recent months, had to undergo this test, to be classified as allergic or non-allergic.

**Nasal allergen provocation test.**

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

The NAPT was considered positive if there was a clearly positive (CP) value on the objective scale, a CP value on the subjective scale, or moderate positive (MP) value in the two criteria. The test was terminated on a positive result, or a negative result after administration of the undiluted 1:1 concentration.

This test was not blinded, neither for the patient nor for the investigator (in this case the same one that performs the NAPT).

**Acoustic rhinometry.**

Nasal obstruction was assessed by means of AcRh with the use of an SRE 2000 rhino meter (Rhinometrics, Lyng, Denmark). The parameter being evaluated is the volume of 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 volume in this portion of the nostril (PDVol2) has been calculated, comparing the values obtained after instilling the lyophilized shrimp mixture preparation at the different concentrations respect to the value obtained after instilling the SS, the value considered 100% in all subjects.
AcRh was considered CP if the volume of nasal cavity decrease ≥40% bilateral, and MP if decrease ≥ 27% bilateral.

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

**Statistics.**
Quantitative variables were described by median and interquartile range or mean and standard deviation. Qualitative variables were described with absolute frequency or percentages. The primary outcome, the usefulness of the NAPT for diagnosing the shellfish allergy in sensitized patients (S-A versus S-nA), was assessed using the sensitivity(S), specificity(E), positive predictive value (PPV), negative predictive value (NPV), and likelihood ratios positive (LR+) and negative (LR-), calculated using two-by-two contingency table and analyzed by Fisher’s exact test.

The secondary outcomes:
(1) the comparison between groups (S-A, S-nA and control) in the variables PDVol2 and VAS during the performance of the NAPT were analyzed using the Mann-Whitney U test and the Wilcoxon signed-rank test
(2) differences between groups (S-A and S-nA) in the SPT results performed by freeze-dried cooked shrimp mixture extract were analyzed by ROC (cut-off points in the papule size categorized item) and the levels of sIgE and Fisher exact test (to compare frequencies of sIgE).

Statistically significant difference was considered when p<.05, and the confidence interval (CI) has been 95% in all cases.
GraphPad Prism 8.1 Software (GraphPad Software Inc., San Diego, California, USA) was used.

RESULTS

Patient population
A total of 55 subjects were enrolled in the study, 29 females and 26 males, median [IQR] age: 35 [28-42] years-old. 10 of them recruited as controls: 5 non atopic (C1 to C5) and 5 sensitized to DPT (C6 to C10), and 45 recruited as subjects sensitized to shrimp, with a without clinical manifestations in relation to the intake.(Figure 2). TheS-nA group includes 21 subjects: 15 recruited as tolerant by clinical history (S-nA1 to S-nA15), and 6 with negative DBPCFC (S-nA16 to S-nA21). The S-A group includes 22 subjects: 7 with positive DBPCFC (S-A1 to S-A7), and 15 diagnosed to anaphylaxis due to prawn intake (S-A8 to S-A22). Two subjects were ruled out of the study because of an inconclusive DBPCFC.

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

Nasal Allergen Provocation Test as a diagnostic tool.
Of the 21 subjects classified as S-nA, 3 were eliminated (1 for negative SPT with freeze-dried cooked shrimp mixture, and 2 for presenting nasal hyperreactivity to SS), 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 (AcRh ≥ 40% in PDVol2, or VAS ≥55mm, or AcRh≥27% plus VAS ≥23mm) [31](Table II). Of the 22 subjects classified as S-A, 2 were eliminated (for presenting nasal hyperresponsiveness), 18 obtained a positive result (18/20, 90%), and 2 obtained a negative result (2/20, 10%).

All subjects in the control group had a negative result. This group 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 CR in subjects with concomitant sensitization to DPT.
To evaluate the usefulness of NAPT as a diagnostic tool, only the S-A and S-nA groups were considered, which are, in fact, the groups that need to be discriminated. Thus, the S and E of the test are 90% and 89% respectively, the PPV: 90%, the NPV: 89%, the LR+: 8.1 and the LH-: 0.1 (p<.0001, CI: 95%) (Table S-II in supplementary material).

**Acoustic Rhinometry**
The 2 control groups were studied first (Individual values in TableS-III, and group averages in Table S-IV, in supplementary material.) None of them showed changes in the Vol2 of the nostrils after instilling the lyophilized shrimp mixture. S-nA group was then challenged and no relevant changes were observed either in most subjects. No differences were found between this group and the controls (p>.05). In the S-A group, a clear decrease in Vol2 was observed. Differences were found respect to the previous groups, whether compared to control (p<.001), or to S-nA (p<.001) (Figure 3).

Four subjects, two from each group (S-nA14, S-nA15, S-A7, S-A22) presented a hyperreactivity response due to diluent and therefore were excluded from the study.

**Visual Analogue Scale**
The clinical symptoms produced after the administration of SS and lyophilized shrimp mixture preparation at different concentrations were collected by VAS and compared between the three study groups. Control and S-nA groups showed hardly any symptoms and no differences were detected between them. Subjects in the S-A group did show symptoms and differences, although in this case they were only significant at 1:10 and 1:1 concentration (Figure 4). Some subjects in the latter group reported velopalatine and, to a lesser extent, otic pruritus. These symptoms are not clearly defined in the ARIA guide, so were computed as nasal itching [36,37]. (Individual and group values in TableS-III and S-IV, in supplementary material).
**Skin prick tests with freeze-dried cooked shrimp mixture**

Different results were obtained in the SPT performed on the study subjects (Individual results in Table S-V, in supplementary material). On the control group, as expected, all the results were negative.

In 1:100 concentration differences were found (p<.002) among S-A and S-nA groups (Figure 5-A). The cut-off point from which allergic subjects could be distinguished from non-allergic subjects was 2 (AUC (area under the curve): 0.81, YI (Youden index): 0.59, CI: 0.95). Thus, obtaining a papule size ≥ ½ than papule size obtained with histamine after performing the SPT with the reconstituted lyophilized extract of shrimp mixture at a concentration of 1:100, allows to differentiate the S-A from S-nA subjects with S: 65%, E: 94%, PPV: 93% and NPV: 71% (LR+: 11.6, LR−: 0.37).

The 1:10 concentration also allows to differentiate the S-A from the S-nA ones (p<.001) (Figure 5-B). The best cut-off point was 3 (AUC: 0.08, YI: 0.57, CI: 0.95). Having a papule size ≥ than histamine papule size enables the subject to be considered as allergic with a S: 68%, E: 89%, PPV: 87.5% and NPV: 72.5% (LR+: 3.4, LR−: 0.24).

The 1:1 concentration does not allow differentiating the symptomatic from the asymptomatic subjects (p>.05) (Figure 5-C).

The subject S-nA13 did not present a papule in front of the shrimp extract preparation, so it was eliminated from the study.

**Specific IgE in serum**

Shrimp sIgE values of S-nA subjects versus S-A were compared, finding no statistically significant differences between them (p>.5). Then mean value was 2.76 kU/L (± 2.59) in S-nA group, and 15.73 kU/L (± 28.12) in the S-A group. The median values and interquartile ranges were 1.71 kU/L (0.66-4.99), and 3.51 (0.99-9.73) respectively (Individual values in Table S-I, in supplementary material).
Safety
During or few minutes after conducting the NAPT only local allergic reaction was observed, which progressively decreased until disappearing in less than 90 minutes, spontaneously, or after administering oral antihistamine or topical nasal vasoconstrictor (8/38, 21% cases).

Only one subject, S-A7, who did not require drug treatment after positive NAPT, presented retro-nasal and palatal angioedema, which was initiated 5 hours after the NAPT and resolved with oral corticosteroid (30 mg prednisone/day) in 72h.

Allergic reactions derived from DBPCFC were OAS (3/15, 20%), lip AE (3/15,20%), urticaria (2/15,13%), abdominal pain (2/15,13%), erythema (1/15,6.5%), and pharyngeal occupation(1/15,6.5%). All symptoms resolved in less than 90 minutes with the administration of oral antihistamines or corticosteroids(9/15,60%).

None of the subjects presented a systemic reaction, neither in NAPT nor in OFC, but it should be noted that patients with a history of anaphylaxis did not undergo this last test.

DISCUSION
This is a study describing the diagnostic ability of NAP compared to OFC in 45 shrimp SPT-sensitized subjects and 10 controls. The data obtained in this study show that NAPT can differentiate allergic patients from non-allergic, in a group of subjects sensitized to shrimp.

There are few publications about nasal provocations with food. The first one was published in 1993 by Seppey et al.[38], and subsequently there were 2 more publications by Clarck et al. in 2007[39]and 2012[40]. All of them used facial thermography to measure the increase in the temperature of the nasal mucosa, 0.8º to 0.9ºC, after instilling egg extract or peanut protein, in the nose of allergic subjects to these substances. Although they 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. used Pru p 3 to perform NAPTs in subjects who had
presented anaphylaxis after ingesting peach[41]. Although the objective of the study was not the food allergy study, they were the first to use RhAc to measure the decrease of nasal volume by means of a food allergen.

Therefore, using these data and the position paper published on the standardization of NAPT [31], this study was conducted to test the hypothesis that NAPT could allow us to differentiate among shrimp sensitized subjects which would, or not, experience symptoms after being challenged with shrimp allergens.

The decision to use a cooked extract and not raw, was based on previous works published by Asero [42], Jirapongsananuruk [43], or Carnes[44] in which the authors demonstrated that cooked extracts were more powerful and were recognized by a greater number of subjects.

The reason for diluting the freeze-dried shrimp mixture extract to 1:100, 1:10 and 1:1 was mainly, because it could not be foreseen how subjects would respond to allergen application, what the dilution might be to trigger a local response, and whether, the extract might be absorbed and cause systemic allergic reactions.

A recent publication has demonstrated the great variability in the SPT response that the shrimp-sensitized subjects may have in front of the different commercial extracts[42].For this reason, the freeze-dried cooked shrimp mixture extract was tested in all subjects. It had to be checked that all subjects recognized the extract with which SPT, NAPT, or OFC, was to be performed (or that they did not really recognize it in the case of controls).

The results obtained from RhAc and VAS have been analyzed separately, since these two scales, objective and subjective, are designed to evaluate the local allergic reaction in the form of rhinitis produced after instilling an environmental allergen[45]. In the present study, we did not know if the nasal mucosa would behave in the same way after instilling a food allergen. The results obtained show the capacity of both scales to establish differences between groups, although RhAc show differences in the three concentrations while VAS
shows these differences in 1:10 and 1:1, but not in 1:100. The concentration at which the subject presented a positive NAPT was not considered. Was followed the same model as in the OFC, where the positive or non-positive test is considered without evaluating 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 which recognizes 90% of sensitized subjects that are truly allergic, and 89% of sensitized subjects that are effectively tolerant. It is also safe, because of the high PPV and NPV (90% and 89%). We acknowledge, that 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 publications on inhalant allergens [46].

In addition, the LR were calculated (LR +: 8.1 and LR --: 0.1). These variables report the diagnostic capacity of a test regardless of the prevalence of the disease being studied. Both values indicate that the usefulness of this test as a diagnostic tool is good.

Mention must be made of the SPTs performed with the shrimp mixture extract. As occurs in real life, SPTs allow to diagnose sensitization but not to differentiate between S-A and S-nA at 1:1 concentration. A 1:100 and 1:10 concentrations, differences between groups have been found, but the S, E, PPV, NPV and LH values obtained are insufficient to be considered good diagnostic tools.

This study has some limitations. There could be a selection bias in patient recruitment. They have been selected based on the positivity or negativity in SPT to specific commercial extract. These results could have been different if they had been carried out with another shrimp extract[42], and therefore the selected sample would also have been different. Either way, this does not affect the validity of the study since the performance of the SPT with the lyophilized sample confirms the classification as sensitized and not sensitized (the only subject who showed different results was eliminated from the study). Moreover, it has been worked with a shrimp mixture suitable for our population and with a high degree of recognition (44/45, 97.8%), we do not know if in another sample of subjects, the
recognition would be as high. There could also exist a performance bias, since the NAPT s were performed after subjecting the subjects to the OFC to classify them into S-A or S-nA, and, in addition, the challenge was not blinded. Although this did not affect the objective scale, it may have polarized the results of the subjective scale. Finally, we must mention the possibility of having committed another bias by directly classifying subjects with a history of AFX as allergic although the NIAID allows this classification [29], this claim has not been demonstrated.

In conclusion, NAPT with freeze-dried shrimp mixture has proven to be a good diagnostic tool to differentiate S-A subjects from S-nA in this population. Even though it is a small study and the results do not allow for a broader generalization, the results obtained could open a new diagnostic strategy in subjects with food allergies.

There is a great need for a lower risk, non-OFC food allergy diagnostic method. More studies with much larger samples are needed to assess whether NAPT could be this tool.

**Financial sources**
This work was supported by a grand from the Spanish Society of Allergy.

**Conflicts of Interests**
The author declare no conflicts of interests.
Bibliography


35. Gotlib T, Samoliński B, Grzanka A. Bilateral nasal allergen provocation monitored


44. Carnés J, Ferrer Á, Huertas ÁJ, Andreu C, Larramendi CH, Fernández-Caldas E. The


The acoustic rhinometry (AcRh) and visual analogue scale (VAS) determinations were performed at basal state, 10 minutes after the instillation of the saline solution (SS), and 15 minutes after instillation of the freeze-dried cooked shrimp mixture at 1:100, 1:10 and 1:1 concentration.

The test is considered invalid if the subject presents a hyper-reactive response (hyperR) with the SS.

The test ends when the subject presents a positive result, at any concentration, or a negative result after the undiluted 1:1 extract application.

In all cases, the subject is kept under observation for 60 minutes.

NAPT: nasal allergen challenge test.
Initially at the time of recruitment, and after performed the oral food challenge: double-blind placebo-controlled food challenge (DBPCFC). Final groups: Sensitized-non-Allergic, Sensitized-Allergic, and Control.

The subject code (ID) is the result of the acronym of the group to which it belongs plus the subject order number.

AFX: Anaphylaxis. DPT+: Sensitized to *Dermatophagoides pteronyssinus*. 

**Figure 2. Classification of the subjects participating in the study.**
Figure 3. Nasal provocation test with shrimp cooked mixture at 1:100, 1:10 and 1:1 concentration. Percentage of volume decrease measured by acoustic rhinometry

Comparative values between control, sensitized-non-allergic (S-nA), and sensitized-allergic (S-A) groups, in baseline state, after saline solution (SS) instillation, and after lyophilized shrimp mixture at 1:100 (post 1:100), 1:10 (post 1:10), and 1:1 (post 1:1) concentration instillation.

No differences found between Control and S-nA group. Significant differences found between Control versus S-A, and S-nA versus S-A.

Vol 2-5 cm (decrease %): percentage decrease in area nasal 2-5.

***: p<.0001
Figure 4. Nasal provocation test with shrimp cooked mixture at 1:100, 1:10 and 1:1 concentration. Visual analogue scale (VAS) values.

Comparative values between control, sensitized-non-allergic (S-nA), and sensitized-allergic (S-A) groups, in baseline state, after saline solution (SS) instillation, and after lyophilized shrimp mixture at 1:100 (post 1:100), 1:10 (post 1:10), and 1:1 (post 1:1) concentration instillation.

No differences were found between the Control group and S-nA, nor between the S-nA group vs. S-A at the 1:100 concentration. Significant differences were found between Control vs. S-A, and S-nA vs. S-A at the 1:10 and 1:1 concentration.

***: p<.0001  ns: no significant
Figure 5. Comparison of skin prick test results between sensitized-non-allergic (S-nA) and sensitized-allergic (S-A) subjects at the three concentrations of cooked shrimp mixture extract: 1:100 (A), 1:10 (B) and 1:1(C).

Papule size categorization: “0” equals no papule, “1” equals less than half the histamine control papule size, “2” equals more than half the histamine size, and “3” equals similar or greater histamine size.

Significant difference found in 1:100 and 1:10 concentrations.

ns: no significance. **: p<.00
Table I. Baseline demographic and clinical characteristics stratified by groups: sensitized-non-allergic (S-nA), sensitized-allergic (S-A), control sensitized to *Dermatophagoides pteronyssinus* (control-DPT+) and control-non-atopic.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>S-nA</th>
<th>S-A</th>
<th>Control-DPT+</th>
<th>Control-non atopic</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Participants number:</strong></td>
<td>21 (39.6%)</td>
<td>22 (41.0%)</td>
<td>5 (9.4%)</td>
<td>5 (9.4%)</td>
</tr>
<tr>
<td>**Sex: Females absolute (percentage)</td>
<td>10 (47.6%)</td>
<td>10 (45.4%)</td>
<td>3 (60.0%)</td>
<td>3 (60.0%)</td>
</tr>
<tr>
<td><strong>Shrimp sensitization absolute (percentage)</strong></td>
<td>21 (100%)</td>
<td>22 (100%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td><strong>DPT sensitization absolute (percentage)</strong></td>
<td>18 (86%)</td>
<td>19 (86%)</td>
<td>5 (100%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td><strong>Anaphylaxis after shrimp intake absolute (percentage)</strong>*</td>
<td>0 (0%)</td>
<td>15 (68%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td><strong>Clinical manifestations referred in recruitment absolute (percentage)</strong></td>
<td>6 (28%)</td>
<td>22 (100%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td><strong>Tolerance referred in recruitment absolute (percentage)</strong></td>
<td>15 (71%)</td>
<td>0 (0%)</td>
<td>5 (100%)</td>
<td>5 (100%)</td>
</tr>
<tr>
<td><strong>OFC (DBPCFC)</strong> Positive/Realized.</td>
<td>0 / 6</td>
<td>7 / 7</td>
<td>0 / 0</td>
<td>0 / 0</td>
</tr>
</tbody>
</table>

Oral food challenge: OFC. Double-blind, placebo-controlled food challenge: DBPCFC.
<table>
<thead>
<tr>
<th>S-nA</th>
<th>PDVol2</th>
<th>VAS</th>
<th>NAPT result</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Final V</td>
<td>Class</td>
<td>Final V</td>
</tr>
<tr>
<td>S-nA 1</td>
<td>15.2%</td>
<td>N</td>
<td>15</td>
</tr>
<tr>
<td>S-nA 2</td>
<td>23.7%</td>
<td>N</td>
<td>0</td>
</tr>
<tr>
<td>S-nA 3</td>
<td>3.1%</td>
<td>N</td>
<td>0</td>
</tr>
<tr>
<td>S-nA 4</td>
<td>13.8%</td>
<td>N</td>
<td>14</td>
</tr>
<tr>
<td>S-nA 5</td>
<td>4.9%</td>
<td>N</td>
<td>0</td>
</tr>
<tr>
<td>S-nA 6</td>
<td>+2.8%</td>
<td>N</td>
<td>0</td>
</tr>
<tr>
<td>S-nA 7</td>
<td>11%</td>
<td>N</td>
<td>0</td>
</tr>
<tr>
<td>S-nA 8</td>
<td>+28%</td>
<td>N</td>
<td>0</td>
</tr>
<tr>
<td>S-nA 9</td>
<td>1%</td>
<td>N</td>
<td>0</td>
</tr>
<tr>
<td>S-nA 10</td>
<td>27.6%</td>
<td>MP</td>
<td>33</td>
</tr>
<tr>
<td>S-nA 11</td>
<td>12.5%</td>
<td>N</td>
<td>12</td>
</tr>
<tr>
<td>S-nA 12</td>
<td>11.6%</td>
<td>N</td>
<td>0</td>
</tr>
<tr>
<td>S-nA 16</td>
<td>34.3%</td>
<td>MP</td>
<td>9</td>
</tr>
<tr>
<td>S-nA 17</td>
<td>+11.5</td>
<td>N</td>
<td>17</td>
</tr>
<tr>
<td>S-nA 18</td>
<td>43.2%</td>
<td>CP</td>
<td>24</td>
</tr>
<tr>
<td>S-nA 19</td>
<td>+0.1%</td>
<td>N</td>
<td>0</td>
</tr>
<tr>
<td>S-nA 20</td>
<td>16.6%</td>
<td>N</td>
<td>09</td>
</tr>
<tr>
<td>S-nA 21</td>
<td>23.2%</td>
<td>N</td>
<td>8</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>S-A</th>
<th>PDVol2</th>
<th>VAS</th>
<th>NAPT result</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Final V</td>
<td>Class</td>
<td>Final V</td>
</tr>
<tr>
<td>S-A 1</td>
<td>49.6%</td>
<td>CP</td>
<td>29</td>
</tr>
<tr>
<td>S-A 2</td>
<td>51.7%</td>
<td>CP</td>
<td>43</td>
</tr>
<tr>
<td>S-A 3</td>
<td>41.5%</td>
<td>CP</td>
<td>74</td>
</tr>
<tr>
<td>S-A 4</td>
<td>37.7%</td>
<td>CP</td>
<td>73</td>
</tr>
<tr>
<td>S-A 5</td>
<td>27.4%</td>
<td>MP</td>
<td>33</td>
</tr>
<tr>
<td>S-A 6</td>
<td>36.2%</td>
<td>MP</td>
<td>57</td>
</tr>
<tr>
<td>S-A 7</td>
<td>32.7%</td>
<td>MP</td>
<td>25</td>
</tr>
<tr>
<td>S-A 8</td>
<td>34.3%</td>
<td>MP</td>
<td>31</td>
</tr>
<tr>
<td>S-A 9</td>
<td>37.7%</td>
<td>MP</td>
<td>24</td>
</tr>
<tr>
<td>S-A 10</td>
<td>27.8%</td>
<td>MP</td>
<td>32</td>
</tr>
<tr>
<td>S-A 11</td>
<td>11.4%</td>
<td>N</td>
<td>0</td>
</tr>
<tr>
<td>S-A 12</td>
<td>32.1%</td>
<td>MP</td>
<td>25</td>
</tr>
<tr>
<td>S-A 13</td>
<td>45.5%</td>
<td>CP</td>
<td>36</td>
</tr>
<tr>
<td>S-A 14</td>
<td>18.1%</td>
<td>N</td>
<td>53</td>
</tr>
<tr>
<td>S-A 15</td>
<td>65%</td>
<td>CP</td>
<td>69</td>
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<tr>
<td>S-A 16</td>
<td>24.1%</td>
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<td>33</td>
</tr>
<tr>
<td>S-A 17</td>
<td>28.8%</td>
<td>MP</td>
<td>43</td>
</tr>
<tr>
<td>S-A 18</td>
<td>27.5%</td>
<td>MP</td>
<td>53</td>
</tr>
<tr>
<td>S-A 19</td>
<td>57%</td>
<td>CP</td>
<td>36</td>
</tr>
<tr>
<td>S-A 20</td>
<td>45%</td>
<td>CP</td>
<td>29</td>
</tr>
</tbody>
</table>
Table II. Results of nasal allergen provocation test.

Values of the Objective measurements and the Subjective measurements following the EAACI task group criteria[30] obtained in control group, sensitized non allergic group (S-nA), and sensitized-allergic group (S-A).


The subject ID is the result of the acronym of the group to which it belongs plus the subject order number. Subjects S-nA14, S-nA15, S-A7, and S-A22, were removed because of hyperreactive response with saline solution. Subject S-nA13 was removed because of SPT negative with shrimp extract preparation.