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# Diagnostic Utility of Components in Allergy to *Anisakis simplex*

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## Abstract

*Background:* In our region, *Anisakis* allergy is responsible for 8% of acute urticarial reactions, 25% of which progress to anaphylactic shock. The poor specificity of skin tests and in vitro specific immunoglobulin (Ig) E means that *Anisakis* allergy is frequently overdiagnosed.
*Objective:* We studied the diagnostic value of 2 *Anisakis* allergens: rAni s 1 and rAni s 3.
*Methods:* Skin tests, the basophil activation test (BAT), and specific IgE determination were performed with rAni s 1 and 3 in 25 patients allergic to *Anisakis*, 17 atopic controls, and 10 controls with acute urticaria and positive skin test and sIgE results for *Anisakis*, but no allergy to *Anisakis*.
*Results:* For rAni s1, skin tests had a sensitivity and specificity of 100% and specific IgE had a sensitivity and specificity of 100% in the atopic control group and 90% in the urticaria control group. BAT had a sensitivity of 96.8% and a specificity of 100% in the atopic control group and 66.7% in the urticaria control group. For rAni s 3, only 1 patient had positive specific IgE results to rAni s 3. All other techniques gave negative results in patients and controls
*Conclusions:* rAni s 1 is the major allergen of *Anisakis* and the target allergen when diagnosing allergy to *Anisakis*. rAni s 3 is not relevant when attempting to explain false-positive results.

Key words: Allergy. *Anisakis*. Diagnosis. rAni s 1. rAni s 3.

## Resumen

*Antecedentes:* En nuestra región la alergia a *Anisakis* causa el 8% de las urticarias agudas, el 25% de las cuales cursan además con anafilaxia. Tanto las pruebas cutáneas como la IgE específica in vitro tienen una baja especificidad, lo que motiva un sobrediagnóstico de este proceso. Objetivos: Estudiar la utilidad diagnóstica de 2 alérgenos de *Anisakis*: rAni s 1 y r Ani s 3.
*Métodos:* Se realizaron pruebas cutáneas, test de activación de basófilos e IgE específica sérica con rAni s 1 y 3 en 25 pacientes con alergia a Anisakis. Se seleccionaron como controles 17 pacientes atópicos y 10 pacientes con urticaria aguda y test cutáneo e IgE específica positivos con Anisakis, pero sin alergia a Anisakis.
*Resultados:* Las pruebas cutáneas con rAni s1 tienen una sensibilidad y especificidad del 100%, la IgE específica tiene una sensibilidad y especificidad del 100% en el grupo de controles atópicos y del 90% en el grupo de pacientes con urticaria. El TAB tiene una sensibilidad del 96.8% y una especificidad del 100% en los controles atópicos y del 66.7% en el grupo control con urticaria. Con rAni s 3, solamente 1 paciente tuvo IgE específica in vitro positiva, en tanto que todas las demás técnicas diagnósticas fueron negativas en pacientes y controles con este alérgeno.
*Conclusiones:* El rAnis s 1 es el alérgeno mayor del *Anisakis* y por tanto, el principal alérgeno a la hora de diagnosticar la alergia a *Anisakis*. El rAni s 3 no es el alérgeno que pueda explicar la baja especificidad de los tests diagnósticos del *Anisakis*.

Palabras clave: Alergia. *Anisakis*. Diagnóstico. rAni s 1. rAni s 3.

## Introduction

*Anisakis simplex* is a nematode found in fish and cephalopods. In its adult phase, it can parasitize mammals. Ingestion of *A simplex* in its third larval stage through raw or undercooked fish can cause a disease in humans known as anisakiasis (for a review see Audicana and Kennedy [1]).

A study published 16 years ago presented preliminary data from patients allergic to *A simplex* [2], whose larvae remain alive in the gastric mucosa during an allergic reaction to *Anisakis* [3-5].

*A simplex* is responsible for 8% of all acute urticarial reactions treated by allergy services in our region [6]. Of these reactions, 25% progress to anaphylactic shock. Thus, this etiological agent is at least as important as the principal food groups responsible for allergic reactions.

The lack of a diagnostic gold standard means that diagnosis of *Anisakis* allergy is based on clinical criteria (urticaria, angioedema, or anaphylaxis after ingesting fish in patients who may or may not be allergic to fish), positive skin prick test (SPT) results, or positive specific immunoglobulin (Ig) E for *Anisakis*. For ethical reasons, it is not possible to carry out an oral challenge test with the live parasite, although ingestion of an extract of the lyophilized parasite does not produce symptoms in allergic patients [7]. Additionally, the low specificity of skin tests and specific immunoglobulin (Ig) E tests [8,9] can lead to overdiagnosis.

Thus, in order to improve diagnosis in these patients, we selected a complete *Anisakis* extract and 2 recombinant *Anisakis* allergens, rAni s 1 (secretor allergen) and rAni s 3 (tropomyosin), in order to assess their diagnostic utility both in vivo (SPT) and in vitro (specific IgE and basophil activation test [BAT]).

## Patients and Methods

### Patients

The study sample comprised 25 patients (5 men, 20 women) who were allergic to *Anisakis* (13 with urticaria and 12 with anaphylaxis) and had been treated in the Allergy Service of Basurto Hospital, Bilbao, Spain. Median (IQR) age was 60 (52-64) years. Patients were considered to be allergic to *Anisakis* when they met all of the following inclusion criteria: *1)* Urticaria-angioedema or anaphylaxis in the first 6-12 hours after eating fresh fish; *2)* Positive SPT to Anisakis and specific IgE to *Anisakis* ≥20 $kU_A$/L (CAP-Phadia, Uppsala, Sweden); *3)* Negative skin prick and specific IgE tests to a battery of fish and other foods that the patient ingested prior to the reaction; *4)* Absence of prior allergy to foods or medicines of any type; *5)* Occurrence of the reaction during the 6 months before inclusion; *6)* Absence of symptoms after eating frozen fish (at least 72 hours) during the year following the initial reaction. Patients avoided eating fresh fish (including cephalopods). Other possible causes of urticaria were excluded by carrying out routine allergy tests (eg, SPTs, tests for food and drugs that typically induce allergies when necessary, and 3 separate analyses of feces). Patients with prior food or drug allergies of any type were excluded from the study, as were those with anaphylaxis and/or recurrent or chronic urticaria of any cause. Patients with dermatitis or dermographism, patients who were taking antihistamines (which interfere with the results or the interpretation of SPT results or in vitro tests), and pregnant women were excluded from the study.

As controls, we chose 17 atopic patients (11 men, 6 women) (median age, 38 years [IQR, 32-43 years]) who were not allergic to *Anisakis*, as demonstrated by skin tests to *Anisakis* and tolerance of fresh fish. Of the 17 controls, 11 had rhinitis and/or bronchial asthma due to sensitization to *Dermatophagoides pteronyssinus* and 6 were sensitized to grass pollens. We also selected 10 patients with acute urticaria (5 men, 5 women) (median age, 56 [46.5-67.8] years) with a positive SPT result to *Anisakis*, but whose symptoms were not related to the consumption of fish and who later tolerated fresh fish for at least 1 year. In these patients, the absence of intestinal parasites was verified by a series of 3 fecal analyses.

The local ethics committee approved the study protocol and patients and controls gave their informed consent to participate.

### Allergens

*A simplex* L3 larvae extracted from the viscera and body cavity of blue whiting (*Micromesistius poutassou*) were supplied by the Department of Microbiology and Parasitology of University of Santiago de Compostela, Santiago de Compostela, Spain. The larvae were washed in phosphate-buffered saline (PBS), snap-frozen in liquid nitrogen, and ground in a mortar. Proteins were then extracted by magnetic stirring (4 hours at 4ºC) in saline solution, clarified by centrifugation, dialyzed, and lyophilized [10]. Recombinant allergens (Ani s 1 and Ani s 3) were expressed and purified as previously described [11,12].

### In Vivo Tests

SPTs were carried out using routine techniques with whole *A simplex* extract (BIAL-Arístegui, Bilbao, Spain) at 3 concentrations (1, 5, and 10 mg/mL), rAni s1 at 3 concentrations (4, 20, and 100 μg/mL), and rAni s 3 at 3 concentrations (4, 20, and 100 μg/mL). In a similar manner, SPTs were administered using commercially available extracts of cod, roosterfish, hake, and sardine (BIAL-Arístegui) and with the foods ingested along with the fish before the allergic reaction. The results of the SPTs were recorded on film for subsequent planimetry.

### In Vitro Tests

Specific IgE to cod, hake, sardine, and roosterfish and to the foods ingested with the fish before the reaction were carried out using CAP (Phadia). Results ≥0.35 $kU_A$/L were considered positive. Patients also underwent an A simplex-specific IgE test (CAP-Phadia) before inclusion in the study. Values ≥20 $kU_A$/L were considered positive.

Specific IgE levels against *A simplex* extract and Ani s 1 and Ani s 3 purified allergens were evaluated in duplicate using an enzyme allergosorbent test. After preliminary testing, optimal concentrations of *A simplex* extract and purified proteins (Ani s 1

 

and Ani s 3, at 1.8 mg/mL for whole extract and 0.1 mg/mL for purified proteins, respectively) were coupled to cyanogen bromide–activated paper discs. Bound IgE was determined using the Hytec specific IgE EIA test as described by the manufacturer (Hycor Biomedical, Kassel, Germany).

The BAT was performed as previously described [13,14]. After separation of blood cells, 50 μL of the cell suspension was incubated with 50 μL of 5 final concentrations of the tested samples: 200 ng/mL, 20 ng/mL, 2 ng/mL, 0.2 ng/mL, and 0.02 ng/mL for *Anisakis* whole extract, rAni s 1, and rAni s 3 (BIAL Arístegui). In order to evaluate background baseline values without stimulation (negative control), 50 μL of stimulation buffer (N-2 hydroxyethylpeperazine-N-2-ethanesulphonic acid [HEPES] 20 mM, NaCl 133 mM, KCl 5 mM, CaCl2 7 mM, MgCl2 3.5 mM, bovine serum albumin 1 mg/mL, pH 7.4) containing interleukin (IL) 3 (2 ng/mL) and heparin (10 μL) (5000 IU/mL; ROVI, Madrid, Spain) was added to the cell suspension in another well. As a positive control, a monoclonal anti-IgE receptor antibody (Bühlmann, Allschwil, Switzerland) was used at a final concentration of 1 μL/mL.

The optimal cutoff point, calculated using receiver operating characteristic (ROC) curves, combines sensitivity and specificity and is the point of the curve furthest from the diagonal. From this, we determined the cutoff point by preferentially selecting the optimal values for specificity over sensitivity, as *Anisakis* allergy is a low-prevalence condition. On that basis, results indicating a percentage of basophil activation >5% with a stimulation index (SI: test value/background value) >2 were considered positive for the BAT. Nonresponders were those patients whose BAT results demonstrated basophil activation <15% in response to anti-IgE.

### *Statistical Analysis*

Quantitative variables were described as median (IQR) and qualitative variables as percentages. Quantitative variable averages were compared using the Mann-Whitney test. All comparisons were 2-tailed, and $P<.05$ was considered statistically significant. The analysis was performed using SPSS, version 12.0 (SPSS Inc, Chicago, Illinois, USA). The clinical validity of the different diagnostic methods was determined by calculating sensitivity and specificity.

## Results (Table)

### *Skin Tests*

All of the patients presented positive SPT results against *Anisakis* whole extract at 10 mg/mL (median wheal size, 30.3 [26-46] $mm^2$) and 5 mg/mL (median wheal size, 26.5 [20.9-34.5] $mm^2$) and against rAni s 1 at 20 μg/mL (median wheal size, 43.9 [23-52] $mm^2$) and 100 μg/mL (median wheal size, 75.1 [36.7-103.2] $mm^2$). Therefore, sensitivity to rAni s 1 at these concentrations was 100%. A positive SPT result was recorded with *Anisakis* extract at a concentration of 1 mg/mL (median wheal size, 20.6 [14.2-27.6] $mm^2$) in 24 patients and with rAni s 1 at a concentration of 4 μg/mL (median wheal size, 2.6 [12.9-36.1] $mm^2$) in 21 patients (sensitivity to rAni s 1 at 4 μg/mL, 84%). None of the patients allergic to *Anisakis* had a positive SPT result to rAni s 3.

All of the controls with acute urticaria presented positive SPTs with the 2 highest concentrations of the whole *Anisakis* extract: 10 mg/mL (median wheal size, 30.7 [25.4-55.5] $mm^2$) and 5 mg/mL (median wheal size, 19.9 [12.8-32] $mm^2$), and 7 at a concentration of 1 mg/mL (median wheal size, 7.15 [0-15.6] $mm^2$). The size of the wheal produced in the skin test with Anisakis extract was larger in patients than in the controls with urticaria, even at the lowest concentration of 1 mg/mL ($P<.005$). None of the controls with urticaria had a positive SPT result to rAni s 1 (specificity in this group, 100%) or rAni s 3 at any concentration used.

None of the 17 atopic controls presented a positive SPT result to the whole *Anisakis* extract, to rAni s 1 (specificity, 100%), or to rAni s 3.

### *Specific IgE*

All of the *Anisakis*-allergic patients showed positive specific IgE results to rAni s 1 (median, 15.9 [3.7-148.1] $kU_A/L$; sensitivity 100%), whereas only 1 of the controls with urticaria demonstrated positive specific IgE (0.6 $kU_A/L$) to rAni s 1 (specificity in the urticaria group, 90%). All of the atopic controls had negative specific IgE to rAni s 1 (specificity in the atopic group, 100%; global specificity, 96.3%).

All of the controls with urticaria presented positive values for specific IgE against *Anisakis* extract (median, 8.6 [2.13-32.75] $kU_A/L$; specificity in the urticaria group, 0%). IgE values for *Anisakis* were significantly higher in patients (patient median, 100 [15.2-296.5] $kU_A/L$) than in controls with urticaria ($P<.001$). Two atopic controls presented positive specific IgE results to *Anisakis* (0.40 $kU_A/L$; specificity in the atopic group, 88.2%; global specificity, 55.6%).

All patients and controls had negative specific IgE to rAni s 3.

### *Basophil Activation Test*

In 1 of the patients, the BAT could not be interpreted, as no response was observed in the positive control with anti-IgE. Of the 24 remaining patients, the BAT was positive in 22 patients against *A simplex* whole extract (BAT patient sensitivity interpretable at 91.7%) and in 23 patients with rAni s 1 (BAT patient sensitivity interpretable at 95.8%).

As for the diagnostic yield of the BAT test for the different allergens studied, the result was positive for *Anisakis* at a concentration of 200 ng/mL in 21 patients, and at 2 ng/mL in 17 patients, where the remaining concentrations used were less effective (data not shown), such that 22 positive cases were detected using these 2 concentrations only. As for the BAT results with rAni s 1, concentrations of 200 and 2 ng/mL revealed 23 and 22 of the positive cases, respectively. The remaining concentrations of rAni s 1 detected lower percentages (data not shown).

With these same 2 concentrations of *Anisakis* and rAni s 1, none of the 17 atopic controls presented positive results, and the results were interpretable in all 17 cases (specificity, 100%).

In 4 of the 10 controls with urticaria, the BAT could not be evaluated owing to a lack of response to anti-IgE

**Table.** Patients and Controls: In Vivo and In Vitro Data

| | | | Skin Test, $mm^2$ | | | | Specific IgE, $KU_A/L$ | | Basophil Activation Test | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | *Anisakis* extract, mg/mL | | rAni s 1, mg/mL | | | | | | *Anisakis* extract, ng/mL | | rAni s 1, ng/mL | |
| N | Sex | Age | 5 | 10 | 20 | 100 | *Anisakis* extract | rAni s 1 | Baseline | Anti-IgE | 200 | 2 | 200 | 2 |
| Patients | | | | | | | | | | | | | | |
| 1 | M | 64 | 16.2 | 21.1 | 11.2 | 19.9 | 293.0 | 33.0 | 3.9 | 65.6 | 10.2 | 0.8 | 42.7 | 12.4 |
| 2 | F | 60 | 20.9 | 44.7 | 26.8 | 47.5 | 188.1 | 51.4 | 3.8 | 63.9 | 54.8 | 29.3 | 56.3 | 76.5 |
| 3 | F | 64 | 35.6 | 46.1 | 24.4 | 59.6 | 88.2 | 5.6 | 11.2 | 80.4 | 76.9 | 53.6 | 70.7 | 60.9 |
| 4 | M | 60 | 21.4 | 34.4 | 52.3 | 67.6 | 78.5 | 13.9 | 1.5 | 15.2 | 2.1 | 0.0 | 0.0 | 0.0 |
| 5 | F | 61 | 26.5 | 30.3 | 19.5 | 25.0 | 300.0 | 234.0 | 7.4 | 76.6 | 67.4 | 28.1 | 52.0 | 78.7 |
| 6 | M | 42 | 33.9 | 45.9 | 43.9 | 88.8 | 300.0 | 300.0 | 2.2 | 54.8 | 21.1 | 0.0 | 48.8 | 28.2 |
| 7 | F | 49 | 18.7 | 27.6 | 21.3 | 29.4 | 300.0 | 100.0 | 4.5 | 70.6 | 33.3 | 21.4 | 15.4 | 50.0 |
| 8 | F | 65 | 35.0 | 47.0 | 94.5 | 109.4 | 279.0 | 78.5 | 1.8 | 72.8 | 51.3 | 12.9 | 78.3 | 77.8 |
| 9 | F | 52 | 30.2 | 21.8 | 54.7 | 99.5 | 13.1 | 6.7 | 1.4 | 41.6 | 7.1 | 1.5 | 33.7 | 24.6 |
| 10 | F | 54 | 13.5 | 16.3 | 26.4 | 50.2 | 78.5 | 15.9 | 3.1 | 72.7 | 1.8 | 6.2 | 46.1 | 53.2 |
| 11 | F | 60 | 22.1 | 29.4 | 31.4 | 37.6 | 15.8 | 1.2 | 1 | 72 | 12.1 | 8.1 | 74.6 | 70.1 |
| 12 | F | 60 | 42.0 | 53.7 | 46.3 | 80.5 | 100.0 | 3.6 | 2.3 | 59.2 | 26.3 | 4.6 | 46.8 | 46.1 |
| 13 | F | 55 | 217.0 | 160.5 | 51.7 | 162.7 | 300.0 | 57 | 5.4 | 50 | 73.1 | 55.9 | 91.7 | 91.5 |
| 14 | F | 51 | 21.3 | 56.7 | 64.3 | 76.2 | 88.2 | 6.5 | 2.1 | 62.9 | 70.4 | 47.5 | 72.9 | 80.6 |
| 15 | F | 72 | 26.5 | 28.5 | 58.8 | 77.4 | 13.7 | 3.1 | 1.1 | 64.1 | 88.0 | 53.0 | 78.6 | 83.6 |
| 16 | F | 63 | 45.7 | 51.2 | 37.9 | 106.9 | 88.2 | 2.5 | 4.8 | 15.5 | 29.2 | 9.4 | 21.0 | 19.5 |
| 17 | F | 73 | 47.0 | 43.9 | 109.4 | 164.3 | 9.5 | 1.6 | 3.2 | 76.9 | 29.1 | 8.9 | 54.0 | 54.3 |
| 18 | M | 64 | 22.9 | 22.2 | 47.0 | 109.0 | 14.6 | 2.8 | 1.5 | 38.9 | 22.4 | 5.2 | 80.4 | 79.4 |
| 19 | F | 70 | 14.6 | 19.4 | 15.4 | 28.8 | 279 | 279.0 | 3.7 | 73.5 | 46.3 | 7.5 | 88.5 | 88.6 |
| 20 | F | 40 | 30.8 | 35.6 | 49.9 | 215.7 | 11.6 | 3.8 | 2 | 87.1 | 89.6 | 75.4 | 91.3 | 90.2 |
| 21 | F | 44 | 26.5 | 30.0 | 31.7 | 75.6 | 300.0 | 300.0 | 2.7 | 84.1 | 82.7 | 68.2 | 84.7 | 89.1 |
| 22 | F | 68 | 25.0 | 29.7 | 21.5 | 30.6 | 300.0 | 234.0 | 1.8 | 91.8 | 52.7 | 25.6 | 94.9 | 66.7 |
| 23 | F | 58 | 29.6 | 35.9 | 45.2 | 75.1 | 193.0 | 63.4 | 1.1 | 0.6 | 1.8 | 0.4 | 0.8 | 0.8 |
| 24 | M | 66 | 20.8 | 27.2 | 19.4 | 41.2 | 195.6 | 196.2 | 9.1 | 66.6 | 31.7 | 16.9 | 51.0 | 25.0 |
| 25 | F | 45 | 18.3 | 24.8 | 44.2 | 35.8 | 11.2 | 6.5 | 3.2 | 59.4 | 1.2 | 3.3 | 6.9 | 3.0 |
| Patients With Urticaria | | | | | | | | | | | | | | |
| 26 | F | 76 | 18.4 | 34.1 | 0 | 0 | 2.2 | 0.0 | 1.05 | 15.2 | 1.6 | 2.9 | 2.8 | 0.6 |
| 27 | F | 54 | 20.3 | 31.9 | 0 | 0 | 5.4 | 0.0 | 1.49 | 17.4 | 1.1 | 1.9 | 1.4 | 2.25 |
| 28 | M | 47 | 43.6 | 55.3 | 0 | 0 | 2.8 | 0.0 | 2.50 | 31.8 | 0.0 | 0.0 | 0.0 | 1.3 |
| 29 | M | 56 | 10.8 | 27.5 | 0 | 0 | 16.7 | 0.0 | 1.4 | 0.6 | 0.5 | 0.2 | 0.0 | 0.7 |
| 30 | M | 81 | 29.6 | 55.9 | 0 | 0 | 78.5 | 0.0 | 3.6 | 0.9 | 0.0 | 1.5 | 0.0 | 1.2 |
| 31 | M | 63 | 9.5 | 14.7 | 0 | 0 | 17.5 | 0.6 | 4.7 | 2.6 | 2.2 | 9.0 | 4.8 | 4.3 |
| 32 | M | 56 | 27.3 | 29.4 | 0 | 0 | 13.6 | 0.0 | 2.5 | 58.3 | 1.7 | 9.1 | 5.6 | 5.3 |
| 33 | F | 20 | 39.3 | 80.0 | 0 | 0 | 3.5 | 0.0 | 1.9 | 30.5 | 0.6 | 1.2 | 1.0 | 1.2 |
| 34 | F | 45 | 19.5 | 29.1 | 0 | 0 | 90.7 | 0.0 | 2.3 | 1.2 | 1.9 | 1.0 | 2.4 | 1.3 |
| 35 | F | 65 | 13.4 | 19.1 | 0 | 0 | 1.9 | 0.0 | 1.1 | 41.2 | 27.4 | 30.7 | 34.6 | 36.2 |

Table. Continued

| N | Sex | Age | Skin Test, mm² | | | | Specific IgE, $KU_A$/L | | Basophil Activation Test | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | *Anisakis* extract, mg/mL | | rAni s 1, mg/mL | | *Anisakis* extract | rAni s 1 | *Anisakis* extract, ng/mL | | | rAni s 1, ng/mL | |
| | | | 5 | 10 | 20 | 100 | | | Baseline | Anti-IgE | 200 | 2 | 200 | 2 |
| Atopic Controls | | | | | | | | | | | | | | |
| 36 | F | 35 | 0 | 0 | 0 | 0 | 0 | 0 | 3.3 | 83.5 | 1.8 | 0.7 | 1.4 | 1.5 |
| 37 | F | 52 | 0 | 0 | 0 | 0 | 0 | 0 | 1.5 | 78.2 | 0.8 | 0 | 0.8 | 0.8 |
| 38 | M | 30 | 0 | 0 | 0 | 0 | 0 | 0 | 6.7 | 27.2 | 3.3 | 4.4 | 4.1 | 0.5 |
| 39 | M | 40 | 0 | 0 | 0 | 0 | 0 | 0 | 2.8 | 43.4 | 1.4 | 1.9 | 1.9 | 2 |
| 40 | F | 35 | 0 | 0 | 0 | 0 | 0 | 0 | 2.4 | 55.6 | 0.6 | 3.3 | 3.8 | 4 |
| 41 | M | 33 | 0 | 0 | 0 | 0 | 0 | 0 | 8 | 15.7 | 0.9 | 0 | 1.5 | 0 |
| 42 | F | 33 | 0 | 0 | 0 | 0 | 0 | 0 | 17.1 | 93.4 | 12.5 | 14.1 | 8.2 | 4.1 |
| 43 | F | 31 | 0 | 0 | 0 | 0 | 0 | 0 | 6.9 | 22.5 | 9.4 | 6.4 | 12 | 6.4 |
| 44 | M | 38 | 0 | 0 | 0 | 0 | 0 | 0 | 5.2 | 61.8 | 0.7 | 3.7 | 5.7 | 4.9 |
| 45 | M | 24 | 0 | 0 | 0 | 0 | 0 | 0 | 17.7 | 52.1 | 19.7 | 9.4 | 14.4 | 14.2 |
| 46 | F | 39 | 0 | 0 | 0 | 0 | 0 | 0 | 5.1 | 88.9 | 2.3 | 3.5 | 5.9 | 2.5 |
| 47 | M | 56 | 0 | 0 | 0 | 0 | 0 | 0 | 2.7 | 84.5 | 3.7 | 3.5 | 3 | 1.9 |
| 48 | M | 39 | 0 | 0 | 0 | 0 | 0 | 0 | 12.7 | 62 | 12.4 | 11.2 | 8.3 | 5.6 |
| 49 | M | 42 | 0 | 0 | 0 | 0 | 0.4 | 0 | 5.1 | 27.8 | 3 | 4.2 | 2.1 | 1.8 |
| 50 | M | 44 | 0 | 0 | 0 | 0 | 0.4 | 0 | 1 | 23.2 | 1.9 | 2.5 | 1.1 | 2.5 |
| 51 | M | 29 | 0 | 0 | 0 | 0 | 0 | 0 | 19.1 | 79.6 | 16.2 | 13.5 | 13.7 | 15.1 |
| 52 | M | 45 | 0 | 0 | 0 | 0 | 0 | 0 | 5.7 | 84.8 | 6.2 | 1 | 3.4 | 0.5 |

(nonresponders). In the remaining 6 controls, 2 showed a positive BAT result with both whole *Anisakis* extract and rAni s 1, and in no cases was the BAT positive for rAni s 3. Specificity to both Ani s 1 and *Anisakis* extract was 66.7%.

The BAT was positive in only 1 of the patients with rAni s 3 and in none of the controls.

## Discussion

Allergy to *Anisakis* is one of the most frequent causes of anaphylaxis in our region [2,6]. The lack of a gold standard means that diagnosis is based on the patient's clinical history (ingestion of fresh fish in the hours prior to the reaction), positive skin test and/or specific IgE to *Anisakis* extract, and exclusion of other possible causes of anaphylaxis [7]. In clinical practice, diagnosis of allergy to *Anisakis* is difficult, owing to the low specificity of available diagnostic methods. Some studies indicate that 16%-22% of blood donors have positive specific IgE to *Anisakis* [15,16]. Approximately 24% of patients with acute urticaria present positive skin test and/or specific IgE results to *Anisakis*, although *Anisakis* is the causal agent in only 33% of these cases [6]. Consequently, allergy to *Anisakis* is frequently overdiagnosed.

To date, 9 allergens [17] of *A simplex* have been identified. Of these, the secretor allergen Ani s 1 is considered the major allergen, as it is recognized by 86% of patients allergic to *A simplex* [10,18]. Therefore, we chose Ani s 1 as the potentially most useful allergen in the diagnosis of *Anisakis*-allergic patients. Purification of natural Ani s 1 is laborious and expensive, since larvae must be extracted manually from parasitized fish and the protein is present in very low quantities in the *A simplex* extract [11]. In order to overcome this problem, recombinant rAni s 1 expressed in *Escherichia coli* has been developed and is considered immunochemically equivalent to the natural counterpart [10]. Our results confirm that Ani s 1 is the principal allergen, since 100% of our *Anisakis*-allergic patients presented a positive SPT result to rAni s 1. Furthermore, it can discriminate allergic from nonallergic patients (specificity, 100%), including those with acute urticaria, based on positive SPT and specific IgE results against whole *A simplex* extract. Alternatively, current component-resolved diagnostic technology based on purified natural and recombinant allergens [19] could be used to diagnose specific *Anisakis* allergy. The results of specific IgE and the BAT are comparable to in vivo results. Specific IgE against *A simplex* cannot detect patients with urticaria sensitized to *Anisakis* who show a positive SPT result to this nematode (positive in 100%). However, specific IgE against rAni s 1 is negative in 90% of these patients, with the only positive serum having a very low IgE level (0.6 $kU_A$/L), which minimized false positives in this

group. Specific IgE to Ani s 1 had a specificity of 100% in the atopic controls, even though 64.7% were allergic to dust mites. These results are slightly higher than those of Caballero and Moneo [20] (sensitivity, 86%) and could be related to technical factors (using natural allergen and immunoblotting as detection techniques) and to increased requirements of the inclusion criteria of the patients in our series (positive SPT and specific IgE to *Anisakis*, 1-year asymptomatic period after ingestion of frozen fish).

The BAT results in this study are comparable to those of González-Muñoz et al [21], who found that the BAT with *A simplex* extract showed greater sensitivity and 100% specificity, although the control groups in the present study did include both atopic patients and patients with chronic urticaria or abdominal pain not related to the ingestion of fish, with no reference to skin test results. In our study, the BAT with whole *A simplex* extract and rAni s 1 showed a sensitivity >90% and specificity >80% in all of the groups. We believe that the disparity in specificity could stem from the fact that the patients in our study belonged to a population in which differentiation is more difficult, since they not only presented urticaria but also had positive skin test and specific IgE results against *Anisakis* extract. This was not the case in the study by González-Muñoz et al.

The data presented above prove that Ani s 1 is the major allergen in *Anisakis*-allergic patients, given that it is recognized in 100% of cases. Ani s 1 is the allergen of choice for the diagnosis of these patients, as sensitivity and specificity values are near 100% both in vivo and in vitro.

The presence of a high percentage of healthy individuals with positive skin test and/or specific IgE results has been attributed to cross-reactivity [22-24]. One of the causes of this cross-reactivity is the presence of a panallergen in *A simplex*, namely, a tropomyosin that can cause cross-reactivity in patients sensitized to arthropods. In our series, only 1 patient presented a positive BAT result with rAni s 3, and none of the other techniques, including skin tests and specific IgE, detected sensitivity in patients or controls. This finding supports our hypothesis that the high prevalence of sensitivity to *Anisakis* is not due to sensitivity to the tropomyosin, as suggested by other authors [25], especially given that 64.7% of our atopic controls were sensitized to dust mites.

The most important conclusion of our study is that rAni s 1 is currently the allergen of choice for diagnosing allergy to *A simplex*. Its sensitivity and specificity were 100% in skin tests and >90% in specific IgE and BAT. Furthermore, our study shows that the high sensitization to *Anisakis* in the general population does not seem to be due to tropomyosin.

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