ORIGINAL ARTICLE

In Vivo and In Vitro Testing With rAni s 1 Can Facilitate Diagnosis of *Anisakis simplex* Allergy

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■ **Abstract**

Background: Traditional diagnostic tests such as skin prick tests (SPT) and specific IgE (sIgE) against whole *Anisakis simplex* extract have low specificity. Consequently, allergy to *A simplex* is overdiagnosed.
Objective: Our aim was to compare tests used in component-resolved diagnosis.
Methods: We evaluated 34 patients with allergy to *A simplex*, 15 patients with acute urticaria who were sensitized to *A simplex* but had no clinical history of allergy to *A simplex*, and 10 patients allergic to seafood. SPT, sIgE (ELISA and ISAC-112), and the basophil activation test (BAT) were performed with *A simplex* whole extract and the molecular components rAni s 1, rAni s 3, and nPen m 1. Sensitivity and specificity were calculated and compared with different cutoffs.
Results: With the *A simplex* whole extract, SPT, sIgE, and BAT yielded specificity values of 72%, 68%, and 70%, respectively, with a cutoff (wheal size) of 11.2 mm, an sIgE value of 7.9 kU_A/L, and a stimulation index of 1.9. Specificity increased to 100% using the molecular component rAni s 1 with SPT, sIgE by ELISA, and ISAC-112. Neither rAni s 3 sensitization nor cross-reactivity with Pen m 1 was observed in patients sensitized to *A simplex*.
Conclusion: rAni s 1 is recognized by 100% of our patients and is able to distinguish between patients allergic to A simplex and patients with acute urticaria who are sensitized to *A simplex* but have no clinical history of allergy to this parasite.

Key words: *Anisakis simplex* allergy. Acute urticaria. Ani s 1. Component-resolved diagnosis. In vitro test. BAT. ISAC. ELISA.

■ **Resumen**

Introducción: Las pruebas diagnósticas tradicionales como pruebas cutáneas (PC) e IgE específica (sIgE) con el extracto completo de *Anisakis simplex* tienen una baja especificidad. Esto conlleva a un sobrediagnóstico de alergia a *A simplex*.
Objetivo: Nuestro objetivo fue comparar diferentes pruebas de diagnóstico basado en componentes moleculares.
Métodos: Se estudiaron 34 pacientes con alergia a *A simplex*, 15 con urticaria aguda sensibilizados a *A simplex* pero sin historia clínica compatible con alergia a *A simplex* y 10 alérgicos a mariscos. A todos ellos se les realizaron PC, sIgE mediante ELISA e ISAC-112 y TAB con el extracto completo de *A simplex* y los componentes moleculares rAni s 1, rAni s 3 y nPen m 1. Se calculó y se comparó la sensibilidad y especificidad de cada prueba con diferentes puntos de corte.
Resultados: Las PC, la sIgE y el TAB con el extracto completo de *A simplex* mostraron una especificidad del 72%, 68% y 70% con un punto de corte de 11,2 mm de tamaño de pápula, 7,9 kU_A/L de sIgE y un índice de estimulación de 1,9, respectivamente. La especificidad incrementó al 100% utilizando el componente rAni s 1en PC e sIgE mediante ELISA e ISAC-112. No se observó sensibilización a rAni s 3 ni reactividad cruzada con nPen m 1 en los pacientes sensibilizados a *A simplex*.
Conclusión: el alérgeno rAni s 1 es reconocido por el 100% de nuestros pacientes y nos permite distinguir entre pacientes alérgicos a A simplex y pacientes con urticaria aguda sensibilizados a *A simplex* sin historia clínica de alergia a éste parásito.

Palabras clave: Alergia a *Anisakis simplex*. Urticaria aguda. Diagnóstico basado en componentes. Pruebas in vitro. TAB. ISAC. ELISA.

Introduction

Allergy to the nematode *Anisakis simplex* is highly prevalent in Spain owing to widespread consumption of fresh fish and marinated fresh fish [1-3]. Twenty-five percent of allergic reactions to *A simplex* are severe and can manifest as anaphylactic shock [4]. Furthermore, *A simplex* is also responsible for 10% of the most severe allergic reactions in our geographic area, with similar allergenic prevalence to that of the main food groups [1,5].

Diagnosis of allergy to *A simplex* is problematic, mainly because routine diagnostic tests in allergy departments such as skin-prick tests (SPT) and specific IgE (sIgE) against whole extract have low specificity values [6,7]. Consequently, allergy to *A simplex* is overdiagnosed [8].

In a recent study [9], we analyzed the diagnostic capacity of the molecular components of *A simplex*—Ani s 1 and Ani s 3—using SPT, the basophil activation test (BAT), and an ELISA-based in-house sIgE technique. Ani s 1 is the major allergen of *A simplex* and is recognized by 85% of patients allergic to this parasite [10,11]. Ani s 3 is a panallergen belonging to the tropomyosin family [12] and could be one of the proteins responsible for subclinical sensitization to *A simplex* [13].

In our previous study [9], we confirmed that both in vivo tests (SPT) and in vitro tests (determination of sIgE and BAT) with the recombinant form of Ani s 1 (rAni s 1) yield greater specificity in the diagnosis of allergy to *A simplex*.

Continuing this line of research, our objective in the present study was to evaluate the utility of a commercially available molecular diagnostics platform (ImmunoCap ISAC 112, Thermo Fisher) in the diagnosis of allergy to *A simplex* and to compare its diagnostic yield with the results obtained using in vivo methods (SPT) and in vitro methods (ELISA-based sIgE, BAT).

Materials and Methods

Patients

The patients were recruited from the Allergy Service of Hospital de Basurto, Bilbao, Spain. In this area, 99% of the adult population habitually consumes fish (89 g/person/day) [14], and contact with the *Anisakis* L3 larvae is frequent given the high levels of infestation by this parasite in fish in the Bay of Biscay [15,16]. The patients were seen in the Allergy Service within 6 months of symptom onset. All patients gave their written informed consent to participate in the study, whose protocol had been approved by the local ethics committee. Fifty-nine patients were recruited into 3 groups:

– Group A comprised 34 patients who were allergic to *A simplex* and had been diagnosed according to previously published criteria [9]. In brief, patients had to have experienced onset of symptoms, urticaria-angioedema, or anaphylaxis during the first 6-12 hours after ingestion of fresh fish, have a positive SPT result against *A simplex* whole extract (Bial-Aristegui) and sIgE (ImmunoCAP) against *A simplex* whole extract (>20 kU_A/L). In addition, they could not be sensitized to fish or foods or experience symptoms following ingestion of

fish frozen for at least 72 hours during the year following diagnosis.

– Group U consisted of 15 patients with acute urticaria who had a positive SPT result to *A simplex* whole extract. Allergy to this parasite was ruled out based on the clinical history, given that the symptoms were not related to prior consumption of fish and the patients had tolerated fresh fish regularly for at least 1 year following diagnosis. All patients consumed fresh fish.

– Group S comprised 10 patients with IgE-mediated shellfish allergy, urticaria, or anaphylaxis within 30 minutes of seafood intake, with a positive SPT result to shrimp and/or squid and a positive sIgE result (ImmunoCAP) to crustaceans and/or mollusks.

Allergens

A simplex whole extract obtained from stage L3 larvae, the purified recombinant allergens Ani s 1 and Ani s 3 (cloned in *Escherichia coli* and purified as previously described [17,18]), and the natural nPen m 1 allergen were provided by BIAL-Aristegui.

Skin Tests

All patients underwent an SPT against *A simplex* whole extract (1 and 5 mg/mL) and against the purified allergens rAni s 1, rAni s 3, and nPen m 1 (20 and 100 µg/mL). The results were considered positive when the wheals were greater than 3 mm in diameter.

Determination of Specific IgE Using ISAC 112

Serum from patients and controls was analyzed using an allergen microarray immunoassay (ImmunoCAP ISAC 112). sIgE was determined against 112 molecular components including the *A simplex* allergens rAni s 1 and rAni s 3, the tropomyosins nBla g 7, rDer p 10, and nPen m 1, and other allergic components of shrimp (nPen m 2 and nPen m 4) were determined. Briefly, 30 µL of serum was incubated on each microarray for 2 hours. The chips were then washed to eliminate IgE not bound to the allergens and then incubated for 30 minutes with 30 µL of the anti-IgE human detection antibody conjugated with fluorochrome. The chips were washed again to eliminate the unfixed antibody, and the fluorescence on each chip was read with a LuxScan 10K/A scanner (CapitalBio). The digitalized images were analyzed using Phadia Microarray Image Analyzer software (Thermo Fisher). This software converts the intensity of the fluorescence to sIgE values, which are expressed semiquantitatively as ISAC standard units (ISU). Values ≥0.30 ISU were considered positive.

Determination of Specific IgE Using ELISA

sIgE values were determined against *A simplex* whole extract and against the purified allergens rAni s 1 and rAni s 3 in duplicate using ELISA, as previously described [9,19]. Optimal concentrations of *A simplex* whole extract (1.8 mg/mL) and recombinant allergens (0.1 mg/mL) were coupled to cyanogen bromide–activated paper discs. Bound IgE was determined using the Hytec specific IgE EIA test (HYCOR BioMedical). sIgE values ≥0.35 kU_A/L were considered positive.

### Determination of Total and Specific IgE Using a Fluoroenzyme Immunoassay

Total IgE values in serum (all patients) and sIgE values against shellfish (group S) were determined using the fluoroenzyme immunoassay ImmunoCAP (ThermoFisher). sIgE values ≥ 0.35 kU_A/L were considered positive.

Flow Cytometry Test

We performed a BAT as previously described by our group [20]. CD63 expression was analyzed on basophils from the 34 patients and 25 controls. Basophils were stimulated with whole *A simplex* extract (200, 20, and 2 ng/mL) and the allergens rAni s 1, rAni s 3, and nPen m 1 (20, 2, and 0.2 ng/mL). Basophils were stimulated with 1 μL/mL of monoclonal anti-IgE anti-receptor antibody (Bühlmann) as a positive control and with stimulation buffer as a negative control. The cells were labelled with anti IgE-FITC and anti-CD63-PE (Caltag Laboratories).

Finally, the cells were acquired with a FACSCanto II cytometer (Becton Dickinson) and analyzed with FACS Diva software (Becton Dickinson).
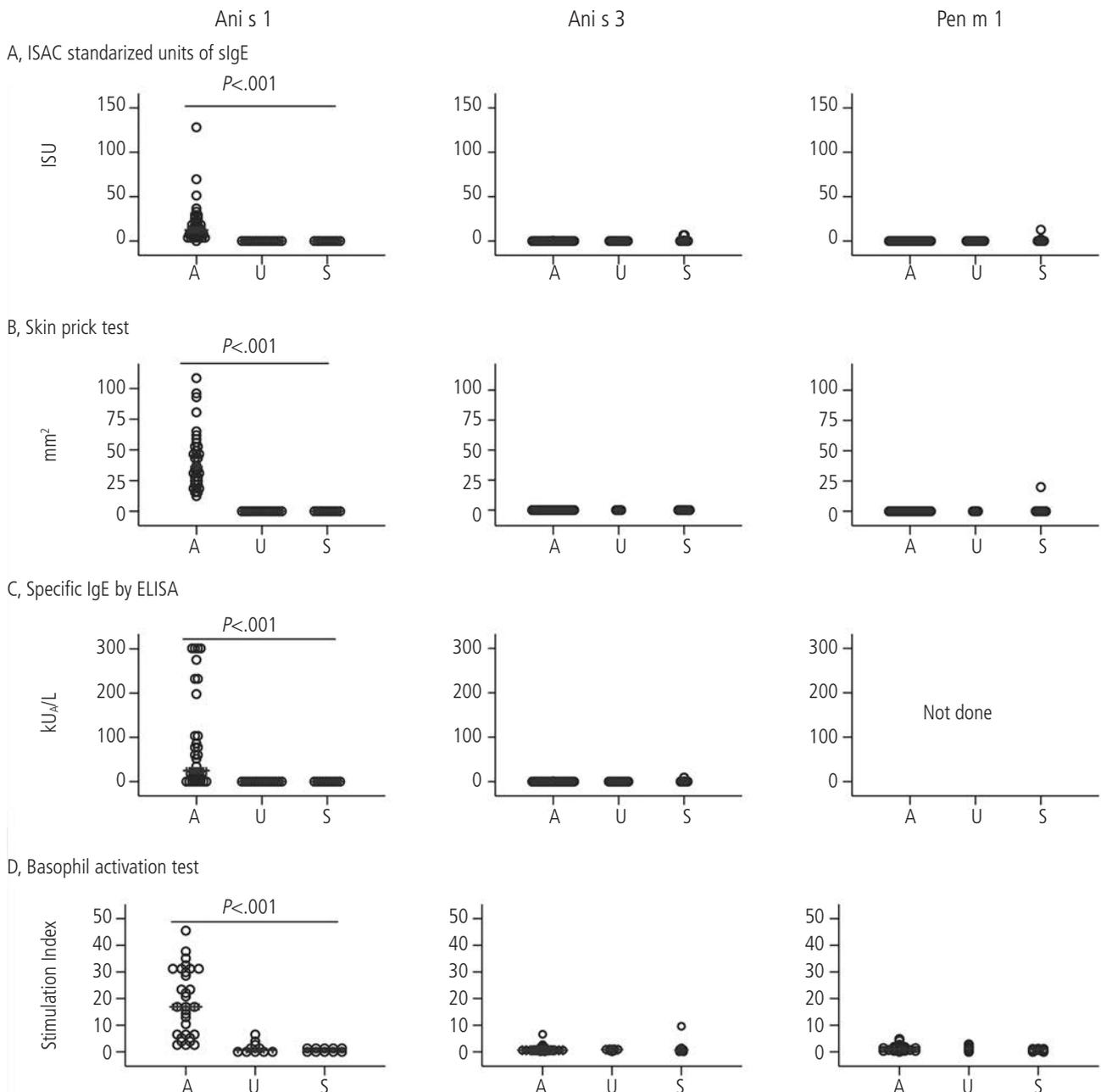
Figure 1. Results obtained in the molecular component-based diagnosis for patients allergic to *Anisakis simplex* (A, n=34), patients with acute urticaria (U, n=15), and patients allergic to shellfish (S, n=10). A, ISAC. B, Skin prick test. C, sIgE by ELISA. D, Basophil activation test.

Results were considered positive when the stimulation index (SI) was >2 and the percentage of basophils activated (%CD63+) in the presence of the antigen was greater than 5% [9]. Patients whose percentage of activation in response to anti-IgE was <15% were classified as nonresponders to anti-IgE.

Statistical Analysis

Qualitative variables are expressed as totals and their percentages and quantitative variables as median and interquartile range (IQR). Quantitative values were compared using the Mann-Whitney or Kruskal-Wallis test depending on whether comparisons were made between 2 or more groups. Receiver operating characteristic (ROC) curves were plotted, and the results from the different diagnostic methods were compared using the chi-square test. The diagnostic utility of the Ani s 1 allergen in the different tools used was evaluated in terms of sensitivity and specificity according to the cutoff points established for each technique. Furthermore, the Youden index was calculated, as was the diagnostic efficacy of each method used. These values are shown with their 95% CI [21-23]. The diagnostic efficacy was calculated on the basis of a 3% prevalence of allergy to *A simplex* in the Spanish population, as established in the 2005 Alergológica Survey [24]. All statistical analyses were performed with STATA 12.0 for Windows software (StataCorp).

Results

Patient Characteristics and Results With Anisakis Whole Extract

Patient characteristics and the medians of the results of total IgE, SPT, sIgE, and BAT with the *A simplex* whole extract are summarized in Table 1. There were no statistically significant differences in total IgE levels between any of the patient groups. The SPT against *A simplex* whole extract was positive in all patients from groups A and U, as stipulated in the inclusion criteria, and negative in patients from group S. Determinations of sIgE against the extract were positive in all patients from group A (100%), in 14 from group U (93.3%), and in 3 from group S (30%). sIgE levels against the extract were significantly higher in group A than in group U (P<.01) and group S (P<.001). The BAT results could not be evaluated in 3 patients from group A and 5 patients from group U, since

the patients did not respond to the positive control. We obtained positive results against the whole extract in 28 of 31 patients from group A, 5 of 10 patients from group U, and none from group S. The differences between the %CD63+ and SI of patients from group A and groups U and S were statistically significant (P<.0001).

Determination of Specific IgE Using ISAC 112

The results obtained with the ISAC 112 show that all 34 patients in group A were sensitized to Ani s 1, with a median sIgE of 12.4 (6.2-25.4) ISU. Only 1 patient in this group had a positive result to the tropomyosin Ani s 3. Furthermore, 2 of these patients were sensitized to dog dander, 3 to pollens, 3 to dust mites, and 15 to hymenoptera venom.

In contrast, in the 15 sera from the patients in group U, positive levels of Ani s 1 sIgE were only found in 1 patient (0.56 ISU), whereas 4 patients were sensitized to this allergen with very low sIgE levels (<0.30 ISU). Another patient from group U had positive results to the tropomyosins Bla g 7 and Der p 10, although the result for Ani s 3 was negative. Furthermore, 3 patients were sensitized to dust mite proteins, 5 to pollens, and 2 to hymenoptera venom, whereas 4 patients from this group showed no sensitization to any of the ISAC 112 components.

sIgE to Ani s 1 was not detected in the sera from patients in group S (Figure 1A). However, we did detect sIgE to Ani s 3 in 3 of 10 sera (6.6 [6.7-0.6] ISU). Positive values of sIgE were also detected against the tropomyosins Pen m 1, Der p 10, and Bla g 7 in these 3 sera; therefore, we can consider these patients to be allergic to shellfish via sensitization to tropomyosin. Seven patients from this group (including the 3 patients sensitized to tropomyosins) were sensitized to a molecular component of dust mites, and 4 patients were also sensitized to pollen components. The other 3 patients in this group were not sensitized to any of the proteins present in the microarray. Furthermore, we did not detect sIgE against the shrimp proteins arginine kinase (Pen m 2) or sarcoplasmic calcium-binding protein (Pen m 4).

Component-Resolved Diagnosis Using Skin Prick Test

The result of the SPT with rAni s 1 was positive in all the patients from group A at the 2 concentrations used, with a median wheal size of 71.4 (40-99.5) mm^2 for 100 µg/mL

Table 1. Patient Characteristics and Results of Total Ige, Skin Prick Test, Specific IgE and Basophil Activation Test Against Anisakis Simplex Whole Extract

Group	Age[a]	Gender, %M/%F	Total IgE, kU$_A$/L[b]	*Anisakis simplex* Whole Extract		
				SPT, mm^2[b]	sIgE kU$_A$/L[b]	BAT %CD63[b]
Anisakis	60 (40-77)	24/76	422 (120-698)	29.9 (21.4-47.0)	94.1 (78.5-300.0)	58.7 (24.9-75.4)
Urticaria	57 (20-81)	40/60	246 (128-467)	24 (15.0-39.3)	13.6 (2.2-78.5)[c]	4.1 (1.3-12.8)[c]
Shellfish	37 (18-25)	60/40	239 (160-356)	0 (0.0-0.0)[c]	0 (0.0-0.4)[c]	1.5 (0.9-8.2)[c]

Abbreviations: BAT, basophil activation test; sIgE, specific IgE; SPT, skin prick test.
[a]Mean (min-max)
[b]Median (interquartile range)
[c]Statistically significant differences with respect to *Anisakis* group (Kruskal-Wallis rank test, P<.001).

and 36.4 (24-52) mm^2 for 20 μg/mL. The SPT results with Ani s 1 were negative in all the patients from groups U and S. The SPT results with the tropomyosins rAni s 3 and nPen m 1 were negative in all patients, except 2 patients in group S (1 positive to Ani s 3 and 1 to Pen m 1) (Figure 1B).

Determination of Specific IgE Using ELISA

The results of ELISA-based determination of sIgE against rAni s 1 were positive in all patients from group A, with a median of 24.9 (5.6-100) kU$_A$/L. sIgE was also positive in one patient from group U (0.6 kU$_A$/L) and for another from group S (0.4 kU$_A$/L). sIgE against rAni s 3 was detected in 3 patients from group A (0.4 [0.4-0.6] kU$_A$/L) and in 4 from group S (4.5 [0.5-8.3] kU$_A$/L) (Figure 1C).

Component-Resolved Diagnosis Using the Basophil Activation Test

BAT results were positive to rAni s 1 in 30 of 31 patients from group A, 3 of 10 from group U, and 1 of 10 from group S. BAT was positive to rAni s 3 in 2 patients from group A and 3 patients from group S and negative against nPen m 1 in 3 patients from group A (Figure 1D).

Diagnostic Capacity of the Techniques

The diagnostic capacity of the rAni s 1 allergen in ISAC 112 and the in vivo test (SPT) and other in vitro tests (ELISA, BAT) at the cutoff points established for each technique is shown in Table 2. In terms of diagnostic capacity, rAni s 1 provides a sensitivity of 100% with ISAC 112, SPT, and ELISA and 97% with BAT. The specificity of these 4 techniques was also high for Ani s 1, but was highest with SPT (100%), followed by sIgE using ISAC (96.1%), ELISA (92.2%), and BAT (90.2%).

ROC Curves

We plotted the ROC curves for the results obtained with the different techniques (Figure 2A). We observed significant differences (P=.03) between the diagnostic capacity of SPT at 1 mg/mL and 5 mg/mL (AUC, 0.79-0.78, respectively) and between determination of sIgE (AUC, 0.91) and of BAT (AUC, 0.88). The SPT yielded a specificity of 72% with 1 mg/mL and 52% with 5 mg/mL of extract, with a cutoff wheal size of 11.2 mm^2 and 13.5 mm^2, respectively. sIgE yielded a specificity

of 68% with a cutoff of 7.9 kU$_A$/L, and the BAT a specificity of 70% with a cutoff of 1.9 ISU.

When we plotted the ROC curves with the results for rAni s 1 (Figure 2B), we obtained an AUC of 1 for the SPT (in both concentrations of rAni s 1 tested) and sIgE and of 0.98 for the BAT. The SPT yielded a sensitivity and specificity of 100%, as did sIgE using ISAC and ELISA with a cutoff of 1.1 ISU and 1 kU$_A$/L, respectively. No significant differences were observed in the diagnostic capacity of these 4 techniques for Ani s 1 (P=.19). However, when we compared diagnostic efficacy using ROC curves, we obtained significant differences for the efficacy of SPT (P=.004) and sIgE (P=.01) using the molecular component rAni s 1 with respect to the *A simplex* whole extract.

Discussion

Allergy to *A simplex* is highly prevalent and one of the most frequent causes of anaphylaxis in our region of Spain [1]. In clinical practice, the diagnosis of allergy to *A simplex* is difficult owing to the low specificity of traditional diagnostic methods such as skin tests and determination of sIgE with *A simplex* whole extract [8,11,25,26]. In addition, a compatible clinical history is critical for a correct diagnosis [27]. Of the 12 *A simplex* allergens identified, Ani s 1 and Ani s 7 are considered to be the major allergens [9,28,29]. In this study, we did not analyze Ani s 7, as it is not covered by ISAC 112, which we compared with other diagnostic techniques. Moreover, ISAC 112 is the only system on the market that enables detection of sIgE against the molecular components of *A simplex*. Furthermore, we had previously shown the diagnostic usefulness of analyzing sensitization to Ani s 1 in our geographic area [9].

The specificity of the whole extract was low for both SPT and sIgE [6]. Although increasing the cutoffs against whole extract improves specificity in quantitative terms (72% and 68% in SPT and sIgE, respectively), these techniques still overdiagnosed about 50% of patients from group U. The specificity obtained with BAT was similar to that obtained with SPT but lower than that previously obtained by González-Muñoz et al [30].

The results obtained with the allergen rAni s 1 in the present study enable the diagnosis of allergy to *A simplex* to be improved using molecular components, as reported elsewhere [9,31]. Similarly, in vivo diagnosis (SPT) with the

Table 2. Diagnostic Values Obtained With the rAni s 1 Allergen at the Cutoff Points Recommended for Each Technique[a]

	Sensitivity, % (95% CI) [P/TP]	Specificity, % (95% CI) [N/TN]	Youden Index	Diagnostic Accuracy, % (95% CI)
ISAC	100 (89.8-100) [34/34]	96.0 (80.5-99.3) [24/25]	0.96 (0.77-0.99)	96.1 (77.1-99.5)
SPT	100 (89.6-100) [34/34]	100 (86.7-100) [25/25]	1.00 (0.87-1.00)	100 (84.0-100.0)
ELISA	100 (89.8-100) [34/34]	92.0 (75.0-97.8) [23/25]	0.92 (0.72-0.98)	92.2 (73.8-98.0)
BAT	100 (89.0-100) [31/31]	90.5 (77.9-96.2) [18/20]	0.90 (0.74-0.96)	84.5 (65.3-94.0)

Abbreviations: BAT, basophil activation test; N, negative results; P, positive results; SPT, skin prick test; TN, true negative; TP, true positive.
[a]Recommended cutoff point: ISAC, 0.30 ISU; SPT, 3 mm^2; ELISA, 0.35 kU$_A$/L; and BAT, SI=2.
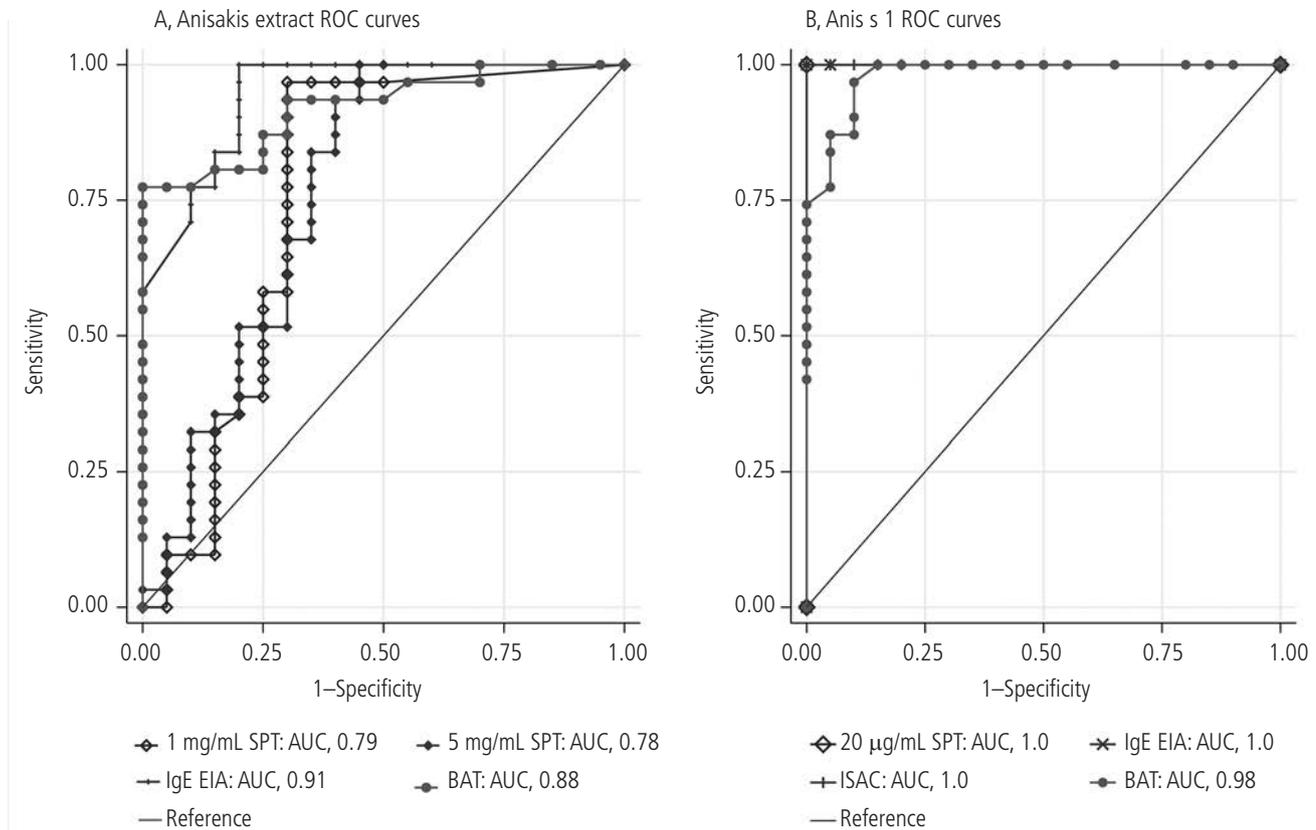
Figure 2. ROC curves and AUC values obtained with *Anisakis simplex* whole extract (A) and with rAni s 1 (B) using SPT, sIgE (ELISA-based and ISAC), and the BAT. ROC indicates receiver operating characteristic; AUC, area under the curve; BAT, basophil activation test.

rAni s 1 allergen yields a diagnostic accuracy of 100%, as does determination of sIgE using ISAC and ELISA, with a cutoff point of 1.1 ISU and 1 kU$_A$/L, respectively.

Our results show that the high diagnostic yield of recombinant allergens means that they could be used in skin tests as part of daily practice in allergology departments. However, recombinant allergens have limited clinical use for biosafety reasons. Nevertheless, in vitro techniques are highly reliable, and ISAC 112 has achieved an accuracy of 96.1%, which is higher than that obtained with other in vitro diagnostic techniques, such as determination of IgE using ELISA or BAT, which have also proven useful in the diagnosis of allergy to *A simplex* [9,11,29]. The sensitivity obtained with the rAni s 1 allergen in determination of sIgE (100% in ELISA and ISAC) is higher than that obtained by Cuéllar et al [29] and Caballero et al [11], who identified approximately 85% of patients allergic to *A simplex* using sensitization to Ani s 1. This finding could be explained by the inclusion criteria applied to our patients, all of whom had an sIgE value of >20 kU$_A$/L against *A simplex*. We used this IgE value to ensure that our patients were really allergic and not merely sensitized to *A simplex*. When trying to validate a technique or diagnostic antigen, the ideal situation would be to have a gold standard against which results can be compared. However, since such a gold standard does not exist in *A simplex* allergy, we are obliged to adopt strict clinical

selection and analytical criteria, even though in daily practice there may well be patients who are really allergic to *A simplex* with lower sIgE levels. High levels of sIgE (ImmunoCAP Class 3) have been detected in up to 50% of patients sensitized to *A simplex* with no symptoms on ingestion of fish [11] or with chronic urticaria [29], as was the case in group U (median *A simplex* sIgE, 13.6 kU$_A$/L). The differences in the percentage of sensitization to Ani s 1 could be due both to the inclusion criteria for our group (elevated sIgE) and to the high consumption of fresh fish or the high levels of *A simplex* infestation in fish in our area. The origin of the patients could also play a role [32]. Furthermore, we did not observe sensitization to tropomyosins in patients who were allergic to *A simplex* or in patients who were sensitized without clinical symptoms (group U), as suggested elsewhere [33,34]. Only 1 patient in group A was positive for Ani s 3, and 1 patient in group U was positive for Bla g 7 and Der p 10. Therefore, we can confirm that this panallergen is not the main cause of sensitization to *A simplex* in these patients [13]. Moreover, with the techniques used against Ani s 3, we only obtained positive results in 5 out of 10 crustacean-allergic patients, and no sensitization to any of the other allergens in the microarray for shellfish (Pen m 2, Pen m 4) was detected. Furthermore, all the tests performed against Ani s 3 were positive in only 1 patient. This leads us to believe that these allergens have low sensitivity or that tropomyosins are not the proteins

responsible for allergy to shellfish [34-36]. Nevertheless, this issue needs to be investigated further in specifically designed studies.

Four of the patients with acute urticaria were not sensitized to the allergens covered by the ISAC 112 microarray, although 8 of them were sensitive to aeroallergens (dust mites, pollen, and animal dander). Another 2 patients were sensitized to hymenoptera venom, which could be the cause of cross-reactivity with *A simplex* [37]. Furthermore, 4 of these patients (26%) presented sIgE to Ani s 1 (3 with IgE <0.30 ISU) and another patient (6.6%) presented sIgE to tropomyosins (although not Ani s 3), as observed previously in asymptomatic patients from Italy and Spain, respectively [11,32]. Furthermore, asymptomatic sensitization to *A simplex* has also been attributed to reaction with cross-reactive carbohydrate determinants [38]. However, in our case, we observed no sensitization to the cross-reactive carbohydrate determinants or MUXF3 present in the ISAC 112 kit. Sensitization to *A simplex* in these patients could be due to previous contact with the larvae of this or other nematodes [39]. Further studies with a greater number of molecular components, including the major allergens of *A simplex*, would be necessary to confirm this hypothesis.

In conclusion, our study shows that Ani s 1 is the major allergen of *A simplex* and that it was recognized by 100% of the patients tested. In addition, the use of the recombinant form makes it possible to distinguish between patients allergic to *A simplex* and patients with acute urticaria who are sensitized to *A simplex* but whose symptoms were unrelated to ingestion of fresh fish. To our knowledge, this is the first study to analyze the diagnostic utility of the ISAC 112 allergen microarray immunoassay in sensitization to *A simplex*. This approach yielded 100% sensitivity and 96% specificity in a heterogeneous group of patients. We only obtained better results (100% specificity) using the SPT with the rAni s 1 allergen, whose use is limited for reasons of biosafety.

Acknowledgments

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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