

New Perspectives on the Diagnosis of Allergy to *Anisakis* spp.

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Abstract

Purpose of Review To compare the prevalence of sensitization in different countries based on specific IgE values and to evaluate the use of isolated native or recombinant allergens for diagnosis.

Recent Findings Isolated allergens help in the diagnosis of truly sensitized patients avoiding false positives due to cross-reactions. Their use is therefore highly recommended, especially when used as a combination of several relevant allergens.

Summary The use of purified allergens allows an accurate diagnosis and this has led to three important findings: (1) in addition to the digestive route of sensitization, occupational and non-digestive exposure seems to be clinically relevant. (2) The parasite appears as an important agent for chronic urticaria. And (3) in endemic countries, the amount of highly sensitized subjects in the general population could be as high as 7%. Adequate information to asymptomatic patients on fish consumption habits would avoid new contacts with parasite allergens and decrease their specific IgE levels and consequently the appearance of acute or chronic episodes induced by the parasite.

Keywords *Anisakis* · Parasite · Allergens · Prevalence of sensitization · Chronic urticaria · Specific IgE

Introduction

The description that a fish nematode (*Anisakis* spp.; Nematoda, Ascaridoidea) was the causal agent of a terminal ileitis in a patient was already published 50 years ago in The Netherlands by van Thiel and van Houten [1]. Soon, later cases of gastrointestinal infections (anisakiasis) were reported in many countries in the five continents. The adult worms of *Anisakis* spp. infect marine mammals. Several larval stages are found in intermediate and paratenic hosts [2]. Adult worms reside in the stomach of marine mammals embedded in the mucosa. Adult females produce unembryonated eggs that are expelled with the faeces. First- and second-stage larvae are formed in the eggs and hatch. Free-living stage 2 larvae are ingested by planktonic crustaceans (intermediate hosts). The ingested larvae develop into third-stage larvae that are infective to fish and cephalopods (paratenic hosts). When fish or cephalopods containing third-stage larvae are ingested by marine mammals, the larvae moult twice and develop into adult worms. The adult females produce eggs, closing the life cycle. Humans become infected by eating raw or undercooked fish or cephalopods containing the third-stage larvae. After ingestion, the *Anisakis* spp. larvae penetrate the gastric and intestinal mucosa, causing the symptoms of anisakiasis. The estimated frequencies of anisakiasis after raw or undercooked fish consumption have been reported in Japan (around 2000 cases/year), South Korea (200 cases/year) and some European countries (500 cases/year) [2, 3]. The increasing popularity of consuming raw fish in Western countries has led to an increase in the number of clinical reports of anisakiasis. Even though the reported cases indicate that this is a rare disease, the true

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incidence of the disease could be potentially higher than what is reported in the literature as cases can go undiagnosed [4].

Besides this gastrointestinal type of immune reaction to the parasite, Kasuya et al. described in 1990 that sensitization to *Anisakis simplex*, but not an allergy to fish, was the cause of some urticaria episodes present in Japanese patients who ate cooked mackerel [5]. Since the description in Spain of a case of recurrent anaphylaxis caused by *A. simplex* parasitizing fish, it became clear that both skin tests and specific IgE determination were useful tools in the diagnosis of this kind of sensitization [6]. *A. simplex* is, so far, the only described parasite associated with fishery products capable of causing allergic responses [2]. However, gastro-allergic reactions by *Anisakis pegreffii* have been recently reported in Italy [7].

The diagnostic procedure of the *Anisakis* spp. allergy begins with a medical history in order to link the symptoms with the consumption of raw or undercooked fish in the previous hours. A compatible history is further confirmed by a positive skin prick test or the detection of specific anti-*Anisakis* spp. IgE in the absence of IgE to the consumed fish by an ImmunoCAP system (Thermo Fisher Scientific, Phadia) [8]. Both confirmatory procedures use whole body antigen extracts from *Anisakis* spp. and they can produce false positive results due to homology between *Anisakis* spp. allergens and proteins in other nematodes, shellfish or insects [9, 10]. In consequence, many authors have reported the low specificity of the in vivo and in vitro tests, especially when subjects with other parasite infections are studied for *Anisakis* spp. allergy [11]. Several approaches have been developed to improve the anti-*Anisakis* spp. IgE determination.

***Anisakis* spp. Allergy Through non-oral Exposure**

As mentioned previously, the diagnosis of *Anisakis* spp. allergy starts with a medical history linking symptoms with fish consumption. However, symptoms can occur by exposure to parasite allergens through routes other than the digestive tract.

Symptoms have been reported when patients are exposed to *Anisakis* spp. proteins through the skin or respiratory tract as it occurs with other food allergies [12, 13]. Common symptoms are rhinoconjunctivitis asthma, anaphylaxis and dermatitis and they have been mainly reported in occupational settings, including fishery and aquaculture workers, cooks, fishmongers and anglers [14–16]. Cross-sectional epidemiological studies have shown a higher prevalence (8%) of sensitization to *Anisakis* spp. than sensitization to fish (6%) associated with dermatitis and non-specific bronchial hyperreactivity among fish processing workers in South Africa [17] and an IgE seroprevalence of 20% was detected in Sicilian workers in the fisheries sector [16]. The relevance of occupational exposure to *Anisakis* spp. allergens is also revealed in a study in

healthy Croatian coastal population where it has been found that occupational exposure is one of the risk factors associated with sensitization to the parasite (odds ratio = 8.89) [18•]. It is interesting to consider that, according to the Food and Agriculture Organization of the United Nations (FAO), 56.6 million people were engaged in the primary sector of capture fisheries and aquaculture in 2014 [19], besides people working in related sectors as animal feed and fish processing industries. To know the impact of *Anisakis* spp. in occupational allergy in this population would define the necessity of including this parasite in the medical surveillance programmes detecting early sensitization.

On the other hand, sensitized patients can report symptoms when they are exposed to *Anisakis* spp. allergens in non-occupational settings, i.e. during preparation or cooking fish at home or standing on the vicinity of fish markets. Barbarroja-Escudero et al. reported a case of anaphylaxis due to non-occupational airborne exposure to *Anisakis* spp. in a patient who had never experienced reactions after eating frozen, fresh or cooked seafood but reported several systemic reactions following different airborne exposures. Anaphylaxis was confirmed by a specific inhalation challenge [20•].

Sensitization to *Anisakis* spp. proteins in the absence of a previous infection is difficult to be demonstrated because fish consumption is probably frequent in patients who work in the fisheries industry. However, considering all reported cases and epidemiological studies of *Anisakis* spp. allergy by non-oral exposure, this possibility cannot be discarded, as suggested by Nieuwenhuizen and Lopata [21].

***Anisakis* spp.-Specific IgE Levels and Influence of Diet**

A putative factor influencing the *Anisakis* spp.-specific IgE levels is the exposure to allergens when sensitized subjects consume fishery products contaminated with parasite material. The general dietary advice for *Anisakis* spp. allergic subjects is to consume previously frozen or well-cooked fishery products, treatments aimed at killing the putative live larvae and preventing parasite infection, and these are the same recommendations as for the general population. Some authors indicate a strict diet with complete avoidance of fish for a few months in cases of severe reactions as anaphylaxis [22, 23], while other authors consider that for gastrointestinal allergy cases, fishery products may be consumed with no restriction if the parasite is dead based on the negative results in oral challenge test with dead *Anisakis* spp. larvae [24, 25]. In case of subjects who have a predilection for fresh fish, an alternative to the frozen products would be the consumption of aquaculture fish because the risk of exposure to *Anisakis* spp. larvae in farmed fish has been shown to be minimal, because of no *Anisakis* spp. larvae have been found in their

viscera or flesh [2, 26]. Several authors have followed up allergic patients after their acute episode of *Anisakis* spp. allergy for studying the evolution of symptoms and specific IgE levels and the influence of diet. Trujillo et al. evaluated the usefulness of different diets (fish-free, fish frozen and fresh fish diets) in preventing further reactions in patients allergic to *Anisakis* spp. None of the patients developed anaphylaxis. Urticaria appeared in 5.8% of patients not consuming fish, in 11.1% who ate frozen fish and in 33.3% of the subjects whose diet included fresh fish. This latter group of patients showed lower total and specific IgE decrease of the initial values when compared with patients whose diet excluded fish or ate only frozen fish [27]. The higher frequency of urticaria episodes and high IgE values of the patients eating fish suggest that in the absence of acute anisakiasis episodes, the immune response to dietary parasite allergens maintained the ongoing immune response. The influence of the diet was also investigated in several patients with gastro-allergic anisakiasis. These patients showed a negative oral challenge with dead larvae and were told to consume frozen seafood for 6 months. None of them reported any symptoms [28].

Polimeno et al. performed a study on intestinal permeability, measured by means of lactulose/mannitol ratio in the urine of *Anisakis* spp.-sensitized patients. They observed a correlation between the intestinal permeability and a graded symptom scale. When allergic patients showing increased permeability followed a raw fish-free diet, a significant decrease until normal values was observed [29]. Another study aimed at analysing the changes in IgE sensitization to *Anisakis* spp. allergens in allergic patients showed the influence of the consumption of fishery products in the specific IgE levels. All patients were advised at the first visit to avoid the

consumption of raw and undercooked fish and to eat farmed fish and deep-frozen fishery products. Specific IgE and analysis of the allergen profile by Western blot were repeatedly performed over several years of follow-up. Among subjects following the dietary recommendations, there were 4/12 cases whose IgE levels increased at some time points during follow-up coincident with the reintroduction of fish to the diet [22].

These data suggest that allergen contamination of fishery products could play a role in the ongoing of *Anisakis* spp.-specific IgE response and contribute to the presence of positive-specific IgE in asymptomatic subjects.

Use of Excretory/Secretory Extracts for Diagnosis

The use of excretory/secretory antigens has been shown to have a good diagnostic efficiency in several parasite infections [30, 31]. In the case of anisakiasis, excretory/secretory products contain proteins with higher allergenic potential than somatic components, so that these products can improve the sensitivity of the test used for the *Anisakis* spp. allergy diagnosis [24, 32]. The excretory/secretory proteins include most of the allergens identified to date as Ani s 1, Ani s 4–9 and Ani s 13 (Table 1). Another diagnostic advantage of using *Anisakis* spp. excretory/secretory allergens is that these protein fractions do not contain the somatic allergens Ani s 2 (paramyosin) and Ani s 3 (tropomyosin) that would represent a putative source of cross-reactions with the invertebrates (dust mites, crustaceans, molluscs, midges and cockroaches) IgE-targeted paramyosin and tropomyosin [33•].

Table 1 *Anisakis simplex* allergens approved by the WHO/IUIS Allergen Nomenclature Sub-Committee

Name	Type	MW (kDa)	IgE prevalence (%)	Other characteristics	Reference
Ani s 1	E/S	24	85	Similar to Kunitz serine protease inhibitors. Thermo-stable	[60]
Ani s 2	Somatic	97	88	Paramyosin. Responsible for cross-reactions	[34]
Ani s 3	Somatic	41	13	Tropomyosin. Responsible for cross-reactions. Thermo-stable	[35]
Ani s 4	E/S	9	27	Cysteine protease inhibitor. Stable to heat and pepsin digestion	[36]
Ani s 5	E/S	15	25	SXP-RAL-2 family. Thermo-stable	[37]
Ani s 6	E/S	15	18	Serine protease inhibitor	[37]
Ani s 7	E/S	139	94	Unknown function	[53]
Ani s 8	E/S	15	25	SXP-RAL-2 family. 35% of homologous sequence with Ani s 5. Thermo-stable	[38]
Ani s 9	E/S	14	14	SXP-RAL-2 family. Thermo-stable	[39]
Ani s 10	Somatic	21	39	Unknown function. Thermo-stable	[40]
Ani s 11	Unknown	27	50	Unknown function	[41]
Ani s 12	Unknown	31	57	Unknown function	[41]
Ani s 13	E/S	37	64	Haemoglobin	[42]
Ani s 14	Unknown	24	54	Unknown function	[43]

E/S excretory/secretory allergens

Purified or Enriched Native Allergens as an Alternative for Diagnosis

Other *Anisakis* spp. protein sources assessed in the diagnosis of *Anisakis* spp. allergy are purified or semi-purified allergens. An easy, rapid and low-cost procedure for obtaining enriched allergen preparations is the differential ethanolic precipitation of *Anisakis* spp. crude extract [44]. The 50–66% ethanolic fraction mainly contains the Ani s 1 and Ani s 4 allergens and shows the highest diagnostic sensitivity when compared with the crude and excretory/secretory extracts, certainly due to the antigen concentration achieved during the process. Allergens such as Ani s 5, Ani s 9 and Ani s 10 among others, are obtained by a subsequent 66–90% ethanolic fractionation, thus, allowing a high yield recovery of the semi-purified and concentrated native allergens. These steps are especially useful for further immunoblotting procedures since allergen identification is straightforward by their electrophoretic mobility (Fig. 1).

Other authors have used *Anisakis* spp. antigens purified by affinity chromatography with the IgG fraction from rabbits immunized with *Anisakis* spp. This procedure decreases the cross-reactions with *Ascaris suum* and *Toxocara canis* [45]. The purification to homogeneity of native parasite allergens is another alternative for the *Anisakis* spp. allergy diagnosis, but it would require a purification method able to provide sufficient amount of allergen. Of the allergens described to date, purification methods have been reported for native Ani s 1, a major allergen of *A. simplex*. These procedures are time-consuming and relatively complicated because they involve several chromatographic steps and the yield of native allergen is low (40–50 µg/g larvae) [46–48]. On the other hand, since the false positive results in the diagnostic tests can also be due to the presence of glycoproteins, Lorenzo et al. proposed the use of deglycosylated allergens for anti-*Anisakis* spp. IgE detection [49].

Some authors have applied thermal treatments to different parasite extracts to characterize the response to frozen or

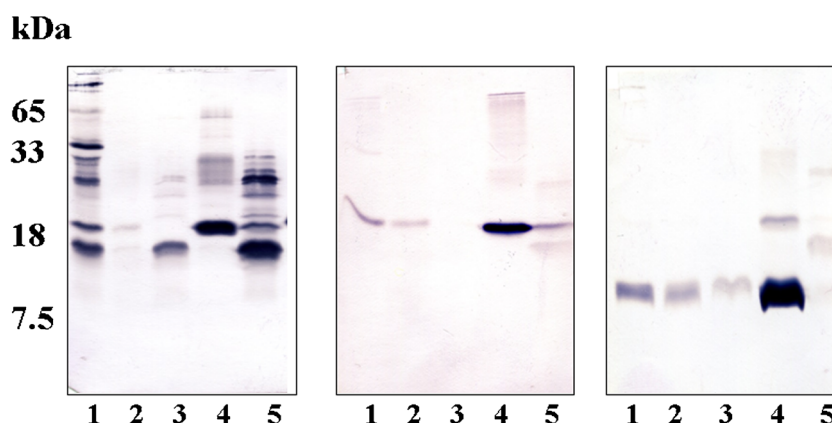
heated parasite allergens. The reason is that allergic symptoms can occur when sensitized patients are exposed to *Anisakis* spp. proteins without the presence of live larvae [2]. The antigenic potential of heat-stable parasite proteins has been studied since some relevant *Anisakis* spp. allergens retain their capacity to bind IgE and activate basophils after being subjected to autoclaving [50]. Furthermore, crude extract boiled for 30 min is able to induce a cellular immune response similar to that observed with untreated extract [51].

Recombinant Allergens in the Diagnosis of *Anisakis* Allergy

The cross-reactions that can interfere with the accuracy of the in vitro tests for *Anisakis* spp. allergy based on crude parasite extracts can be avoided with the use of single native or recombinant allergens or a cocktail of them. The production of recombinant allergens would have the advantage over crude extracts, antigens fractions or isolated native antigens that they can be purified to homogeneity, they can be produced in unlimited quantity and they represent standardized reagents which would allow a more accurate diagnosis. In addition, they can provide information on the different antigenic profile detected by allergic patients. On the contrary, the use of a recombinant allergen may be of limited use if it does not contain all IgE-binding epitopes of the native allergen due to problems in protein folding or post-translational modifications. A second problem in the use of recombinant allergens for diagnosis is the frequent isoforms presence and some epitopes cannot be detected when a single isoform is used as a diagnostic reagent [52].

Most of the 14 *A. simplex* allergens approved so far by the World Health Organization/International Union of Immunological Societies (WHO/IUIS) Allergen Nomenclature Sub-Committee (<http://www.allergen.org/index.php>; Table 1) have been expressed as recombinant proteins to analyse their molecular characteristics and frequency

Fig. 1 Representative IgE immunoblotting patterns of sensitized patients (panels from left to right): From left to right patient detecting multiple IgE-binding proteins, patient monosensitized to Ani s 1, patient mainly sensitized to Ani s 4. 1 crude extract, 2 excretory/secretory antigens, 3 heated crude extract, 4 50–66% ethanol fractionation, 5 66–90% ethanol fractionation. Copyright Springer Nature, London, UK, reproduced with permission [44]



of detection in *Anisakis* spp.-sensitized subjects. Recombinant *Anisakis* spp. allergens have been produced in bacteria- or yeast-based heterologous expression systems. The production in bacteria requires a renaturation step when the protein is found in inclusion bodies [47]. Renaturation of the allergen obtained from inclusion bodies is an essential step in obtaining a properly folded allergen that contains all the allergenic epitopes of the native counterpart and a partially efficient refolding can explain some discrepancies in the seroprevalence results [48, 53].

Only recombinants Ani s 1, Ani s 3 and Ani s 7 are commercially available for use in diagnosis [54, 55], therefore, some laboratories have produced their own recombinant allergens and developed in-house diagnostic methods for *Anisakis* spp. allergy [56–58].

Recombinant Ani s 1 is the allergen most widely used in *Anisakis* spp. allergy diagnosis [48, 54, 56, 57, 59]. It has been produced in both bacteria and yeasts and is detected by a similar frequency of the *Anisakis* spp.-allergic patients than the native protein (>80%) [48, 60]. The frequency increased to 100% when the patients were selected with specific anti-*Anisakis* spp. IgE values ≥ 20 kU/L [59]. The results obtained with individual recombinant allergens demonstrate that they are useful for diagnosis with high specificity but the use of a combination of selected allergenic components further increases the sensitivity of the diagnosis test for *Anisakis* spp. allergy [53, 57, 58].

In addition to their utility in diagnosis, *Anisakis* spp. recombinant allergens have also been used in studies on pathogenic mechanisms in gastro-allergic anisakiasis and *Anisakis* spp.-sensitization-associated chronic urticaria [61, 62] and follow-up of the IgE sensitization [22, 53]. Recombinant Ani s 1 has been evaluated in patients with gastro-allergic anisakiasis (82.1% positives) and in subjects with chronic urticaria (42.5% positives). On the contrary, Ani s 7 was positive in more than 90% of both samples, that is, it is a major allergen (>50% positives) in both entities [61]. Therefore, Ani s 7 could be considered to have a better diagnostic capacity, but these differences in prevalence could also reflect the special clinical characteristics of chronic urticaria or the response to parasites other than *Anisakis* spp. [63, 64].

Chronic Urticaria and *Anisakis* spp.

Lopez-Saez et al. found positive *Anisakis*-ImmunoCAP values in 55% of 101 patients with chronic urticaria. The clinical evolution and variations in specific IgE values were not statistically different among the patients eating a normal diet or those who avoided fish, a fact that suggests that these ImmunoCAP values lacked any clinical significance [63]. These results differ from those obtained in further studies. Daschner et al. reported a 52.6% of sensitization to *Anisakis*

spp. in chronic urticaria patients that is in concordance with the data previously reported [63, 65]. Of the 65 sensitized patients, 52 experienced clinical improvement after diet, compared with 3 out of 11 patients without sensitization. The authors conclude that *Anisakis* spp. are possibly widespread etiologic agents able to induce chronic urticaria. In a study performed in Italy, Frezzolini et al. found a significant association between chronic urticaria and *Anisakis* spp. sensitization, with an overall prevalence of 75.4% in patients with chronic urticaria, compared with 18% in atopic subjects and 10% of the controls. After 6 months of fish-free diet, clinical improvement was seen in all patients and specific IgE decreased in 60% of them. The percentage of *Anisakis* spp.-induced basophil activation decreased in 100% of the patients during this period [66]. This study confirmed again the relevance of *Anisakis* spp. in chronic urticaria. Ventura et al. studied a total of 213 patients with chronic urticaria and 106 (49.7%) of them were sensitized to *Anisakis* spp. All were regular consumers of marinated fish. After a 6-month period of fish-free diet, urticaria disappeared in 77% of the cases and relapsed in 88% of those who had reintroduced raw fish versus 14% of those who were still on the diet. The authors conclude that *Anisakis* spp. hypersensitivity is a frequent cause of chronic urticaria in areas where raw or marinated fish is frequently eaten [67].

Positive Tests and Asymptomatic Subjects, a Global Perspective

Asymptomatic subjects presenting positive skin tests or specific IgE values to *Anisakis* spp. constitute today a nightmare in the diagnosis of *Anisakis* spp. allergy. The same positive result that is found in patients that develop intense symptoms during an acute episode can be found in subjects who lack any clinical symptom and this fact questions the reliability of the diagnostic procedures in *Anisakis* spp. sensitivity. Some authors consider these apparent false positive results as a simple cross-reaction to other parasites or allergens. The presence of positive skin test and/or specific IgE values among asymptomatic individuals was found soon after the initial cases were described. In 1998, del Pozo obtained 27.3% of positive ImmunoCAP values in 150 patients with urticaria and in 15.3% of control subjects. These latter individuals were considered as having a “non-relevant clinical sensitization” or “asymptomatic sensitization” [68]. Other authors have estimated that this subclinical sensitization is present in the 23% of the patients attending an allergy service in Madrid [69]. The existence of all these confusing factors that rendered the clinical diagnosis many times difficult was discussed by E. Buendia who in 1997 said: “these conditions make it impossible to determine the actual prevalence of allergic reactions due to *A. simplex* sensitization” [70].

Anisakis spp. hypersensitivity has been difficult to measure and compare between countries because it has been estimated using different methods, many times employing in-house assays such as ELISA tests. This fact renders the comparison of the results often impossible. However, the use of the ImmunoCAP test, which is globally admitted as a highly sensitive method for *Anisakis* spp. allergy diagnosis facilitated a comparison of the asymptomatic sensitization frequency that, as said previously, was calculated initially to occur in Spain in 15.3% of samples from normal subjects. Specific IgE against *Anisakis* spp. was detected in 629 out of 2108 (29.8%) patients with initial diagnosis of urticaria or food allergy in a study carried out in Japan [71]. In Morocco, 283 samples obtained from a general laboratory and 50 from men working in the fishing industry were studied. The ImmunoCAP was positive in 5.1% of the samples. The highest values obtained in the ImmunoCAP were reached by four individuals (1.2%) who had class 3 ImmunoCAP values (3.5–17.5 kU/L) and in general terms, a correlation was found between band intensities on immunoblotting and specific IgE values. Furthermore, positive values were found to be dependent on the frequency of contacts with the parasite [72].

The possibility that positive ImmunoCAP values were influenced by cross-reactions having, therefore, questionable clinical interest has been recently reported. The diagnostic value of specific IgE to *Anisakis* spp. was assessed in a study with native Spanish residents and subjects coming from tropical and sub-tropical areas. The authors reported that increasing the cut-off value for the specific IgE to 0.71 kU/L and using the *Anisakis/Ascaris* IgE ratio the specificity of the test increases considerably in both study groups [73•]. In a wider study, Lin et al. found that only 0.4% of blood donors ($n = 993$) and 16.2% of 414 patients with total IgE levels >1000 kU/L were positive for *Anisakis* spp. when tested by the ImmunoCAP method. Furthermore, 0.0% of the blood donors and 0.2% of the high total IgE level patients gave positive results when tested with recombinant Ani s 1 and Ani s 7 by ELISA. The authors suspected that *Anisakis* spp. IgE-positive samples in Norway were probably false positive results due to a cross-reaction with mite or shrimp allergens [55]. These results differ from those obtained by Caballero ML et al. who found that 17% of their asymptomatic patients ($n = 99$) had IgE-positive values to *Anisakis* spp. and most of them (88%) detected at least one of the *Anisakis* spp. recombinant allergens tested [57]. These results suggest that specific IgE present in samples from asymptomatic subjects in Spain is in most cases directed to clinically relevant *Anisakis* spp. allergens. The highest frequency of ImmunoCAP-positive results was obtained by Rodero et al. in the study of 200 samples

from subjects with ages ranging from 30 to 60 years obtained from an epidemiological survey that represented the population of Madrid, Spain. The prevalence of samples with ImmunoCAP values 17.5 to 50.0 (class 4) was 3%, class 5 values (50.0–100.0 kU/L) were found in 2% and class 6 (higher than 100.0 kU/L) in an additional 2% of the samples. All these high positive samples had a strong IgE recognition of relevant allergens in the IgE immunoblotting to *Anisakis* spp. allergens [74]. It is worth mentioning that elevated *Anisakis* spp.-specific IgE values (>17.5 kU/L) have been found to be reliable indicators for the diagnosis of true sensitizations to *Anisakis* spp. with a sensitivity of 86.8% and a specificity of 82.4% compared with 88.2 and 69.6% of the skin tests using a crude extract [32].

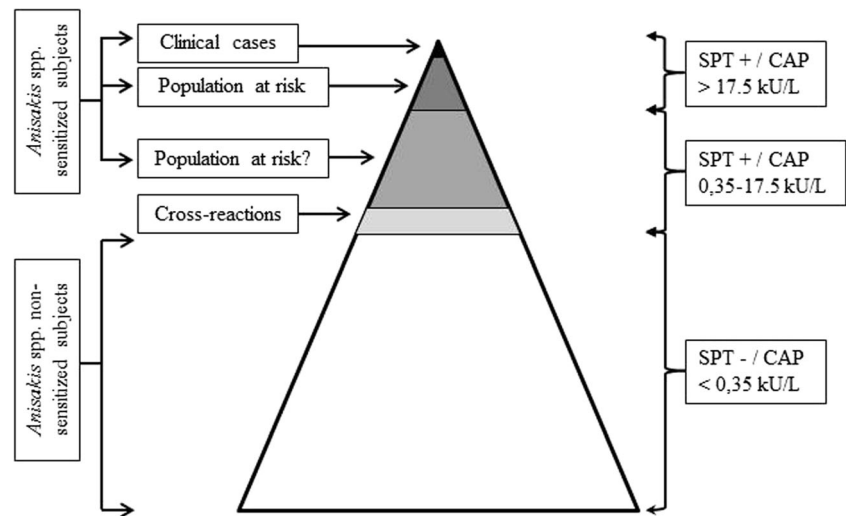
Taking all these facts into consideration, it becomes clear that the prevalence of positive results varies largely among the different countries and that the frequency of contacts with the parasite could be a risk factor for sensitization. The elevated total IgE levels could be the cause of a positive *Anisakis* spp. result due to cross-reactions, but the opposite is also true: in countries where raw fish is frequently consumed, a sensitization to *Anisakis* spp. can lead to elevated total IgE values even in the absence of clinical symptoms. In that case, acute clinical episodes would only constitute the tip of the iceberg and the presence of a high number of sensitized subjects among the normal population is then a real matter of concern (Fig. 2).

What Could Then Be the Number of Patients at Risk?

In a recent review, Nieuwenhuizen describes that infection with *Anisakis* spp. larvae can lead to four different clinical manifestations: gastric anisakiasis, intestinal anisakiasis, ectopic anisakiasis and gastro-allergic anisakiasis [75]. In view of the high prevalence of *Anisakis* spp. sensitivity in endemic regions previously published, a fifth clinical group can be added: sensitized asymptomatic patients. Specific IgE to *Anisakis* spp. has been detected at high frequency in otherwise healthy controls, and the possible reason for IgE detection in the healthy population is a subclinical or undiagnosed gastric anisakiasis without allergy [15]. In some studies, elevated specific IgE values have been considered as an alternative to identify real allergic patients and not merely sensitized to *Anisakis* spp. [32, 54, 59].

The group of patients having high titers of specific IgE (class 4 to class 6) seems to be astonishingly numerous in the Spain region if data obtained in the study performed in Madrid are extrapolated [74]. As stated earlier, it is important to mention that, those samples having high

Fig. 2 Suggested distribution of the *Anisakis* spp. sensitization in a general population from an endemic area. SPT skin prick test, CAP ImmunoCAP values for anti-*Anisakis* spp. IgE



specific IgE values by ImmunoCAP also detected relevant allergens by immunoblotting, using enriched parasite fractions that contained Ani s 1, Ani s 4 and Ani s 5 [74]. According to the Spanish National Statistics Institute (INE), the 30–60-year-old population of the town of Madrid comprised 1,517,458 persons in 2014. Taking into account the percentage of patients that had *Anisakis* spp.-specific IgE values higher than 17.5 kU/L (classes 4, 5 and 6), 106,222 subjects would represent the population at risk. It is hard to believe that such a number of patients can be underdiagnosed; however, several years ago, Baeza et al. stated that most patients allergic to *Anisakis* spp. report symptoms once or twice every year, despite the probably frequent contact with the parasite [24]. It must be borne in mind that the study by Rodero et al. [74] was performed before enforcing the obligation to freeze fish that will be consumed raw or undercooked. It would be interesting to know if the number of sensitized subjects has currently changed in this endemic area.

In a recent paper, a quantitative risk assessment model was developed to estimate the risk of anisakiasis due to the consumption of anchovy. The results suggest that Spain has between 7700 and 8320 anisakiasis cases annually and that the estimates of anisakiasis in Europe are probably a considerable underestimation [76••]. Interestingly, asymptomatic intestinal anisakiasis [77] and symptomless clinical presentation [78] have been reported. Toro et al. suggested that only the most severe cases that require invasive techniques are diagnosed and that many of the mild cases remain underdiagnosed in Spain [79]. In the same sense, the EFSA panel on Biological Hazards suggests that gastro-allergic anisakiasis cases in Madrid (Spain) can be underestimated because many patients with mild symptoms were unlikely to seek the support of emergency medical or local primary health care provision [2]. On the other hand, we have evidence that asymptomatic patients show a

clear decrease in their specific IgE values when they follow a fish-free diet.

Useful Tips for Diagnosis

1. Remember that both skin prick tests with *Anisakis* spp. extracts and ImmunoCAP are highly sensitive diagnostic tools. However, they can produce false positive results, therefore,
2. Test for *A. simplex* and *Ascaris lumbricoides*, both available in the ImmunoCAP system in doubtful cases. The IgE values obtained in the *A. simplex* ImmunoCAP should be higher than those in the *A. lumbricoides*, otherwise, check for other parasitoses.
3. Measure the specific IgE value as close to the acute episode as possible. Repeat the test 1 month after this episode and check for a substantial rise of the initial value. This last result should then fall during the next months.
4. Perform new controls after 6 months, a year and yearly if necessary. If the values do not fall during the next year, reconsider the case and ask about the ingestion of undercooked fish. Some food like canned fresh anchovies is considered as a safe food by the patients in spite of the fact that these anchovies have not been heat-treated.
5. Measure specific IgE antibodies in the remaining members of the family once a patient has been diagnosed. You could often find unexpected high specific IgE values in subjects who do not present any clinical symptoms. Include these patients for routine controls in the future and make them aware about the risks of ingesting undercooked fish.
6. In case that an already diagnosed patient refers symptoms (even if minor) after fish ingestion, test again for specific IgE in order to exclude a reinfection that could increase the ImmunoCAP values to the initial results or even

higher. It is quite common that a diagnosed patient tries to eat raw fish thinking that this fact would not be dangerous.

Conclusions

The presence of cross-reactions between *Anisakis* spp. and crustaceans, insects and other organisms makes the use of purified native or recombinant allergens highly advisable to take advantage of their high specificity.

The role of *Anisakis* spp. as a relevant agent in chronic urticaria seems to be clear at present as published in several studies performed in different countries.

The occupational and environmental relevance of this parasite is increasing, and further studies are needed to obtain additional information about this global relevance as an occupational allergen.

The amount of high positive serological values suggests that in endemic areas, many asymptomatic patients remain underdiagnosed and that this group of patients must be the real concern of the sanitary authorities. It is the opinion of the authors of this review that in endemic regions, hypersensitivity to *Anisakis* spp. should not be considered as a rare disease that sometimes can lead to different clinical symptoms. It should be considered as a disease affecting a high number of individuals, most of them being asymptomatic, probably waiting only to a successful episode of parasitosis or an encounter with an undetermined amount of allergens, either by non-oral or by digestive exposures, to develop an acute or chronic disease. The correct management of this situation with appropriate information to the citizens would decrease the amount of sensitized asymptomatic population and in this way, the appearance of new clinical episodes.

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Compliance with Ethical Standards

Conflict of Interest Drs. Moneo, Carballeda-Sangiao, and González-Muñoz declare no conflicts of interest relevant to this manuscript.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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