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IgE-mediated Anisakis allergy in children

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Abstract
Anisakids are nematodes responsible for different clinical patterns in humans. The well-known human-infecting Anisakis species include members of the *Anisakis simplex* (AS) complex. Humans usually contract anisakiasis through ingestion of raw or undercooked seafood containing Anisakis larvae. Once Anisakis has been ingested, patients may develop disease driven directly by Anisakis larvae and/or by allergic reaction due to this nematode. The capability of inducing allergic reactions depends on the expression of specific antigens by nematodes and host factors. This study aims to resume actual knowledge about AS and Anisakiasis with regard to epidemiology, pathophysiology, clinical presentation, diagnosis, and treatment. Particular attention is paid to Anisakis allergens and their cross-reactivity on available diagnostic methods, and defining a diagnostic pathway for Anisakis allergy. Because only a few data are available in the literature about pediatric population, we focus on this group of patients specifically.

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Introduction

Anisakids are nematodes that belong to the family of *Anisakidae* and genus *Anisakis*. The known human-infecting anisakid species include members of the *Anisakis simplex* (AS) complex (including AS sensu stricto), the *Pseudoterranova decipiens* complex, and the *Contracecum osculatum* complex.[1] The life cycle of anisakids starts from its definitive host, the marine mammals. Female parasites produce unembryonated eggs inside the host's digestive tract, which are then released into the marine environment through their feces. Once released, eggs embryonate in water and the first-stage larvae develop inside them. The first-stage larvae hatch from eggs and mature into second-stage larvae (free-swimming larvae). Second-stage larvae are ingested by intermediate hosts (e.g., crustaceans) and mature into third-stage larvae. Intermediate hosts are predated by paratenic hosts (e.g., cephalopods and teleost fishes) with the third-stage larvae encysting on the intestines and visceral organs of the host. Paratenic hosts are eaten by definitive host marine mammals, with larvae moulting to fourth-stage larvae and finally to mature parasites, mating and releasing new eggs. Humans interpose in the life cycle as accidental hosts, eating raw or undercooked hosts and thereby ingesting the third-stage larvae[2] (Figure 1).

Anisakiasis refers to clinical patterns caused by pathogens of the genus *Anisakis* (Table 1).

This study aims to focus on allergic reactions mediated by AS sensu stricto in pediatric patients.

Epidemiology

Anisakis infection was first recognized in the 1960s,[5] but the first allergic reaction to Anisakis was reported only in 1990.[6] The estimated global incidence of anisakiasis is 0.32/100,000.[7] Japan has the highest incidence of anisakiasis, associated to the frequent consumption of traditional raw fish dishes.[1] In Europe, Spain is the leader, followed by Italy.[1] Interestingly, Herrador et al.[8] reported that the incidence of anisakidosis in Spain has increased in the last two decades. This increase might be explained by a major awareness of the disease and by an improvement in diagnostic tools as well as by the diffusion of the habit to eat raw seafood. Epidemiologic data on Anisakis sensitization vary depending on the population under investigation and on the diagnostic test applied: in their systematic review, Mazzucco et al.[9] evaluated 41 studies. Of these, only two had pediatric patients exclusively. Authors reported that in general population without clinical manifestations, sensitization to Anisakis was observed in 0.4–27.4% when investigated with specific immunoglobulin E (sIgE) detection and in 6.6–19.6% when investigated by skin prick test (SPT). On the other hand, in allergic patients (e.g., those with food allergy, respiratory allergy, chronic urticaria [CU]), sensitization to Anisakis was observed in up to 81.3% when investigated by sIgE detection and in 4.5–64% when investigated by SPT.

A study by Associazione Allergologi ed Immunologi Italiani Territoriali ed Ospedalieri – Federazione delle Società Italiane di Immunologia, Allergologia ed Immunologia Clinica (AAITO-IFIACI) Anisakis Consortium[10]

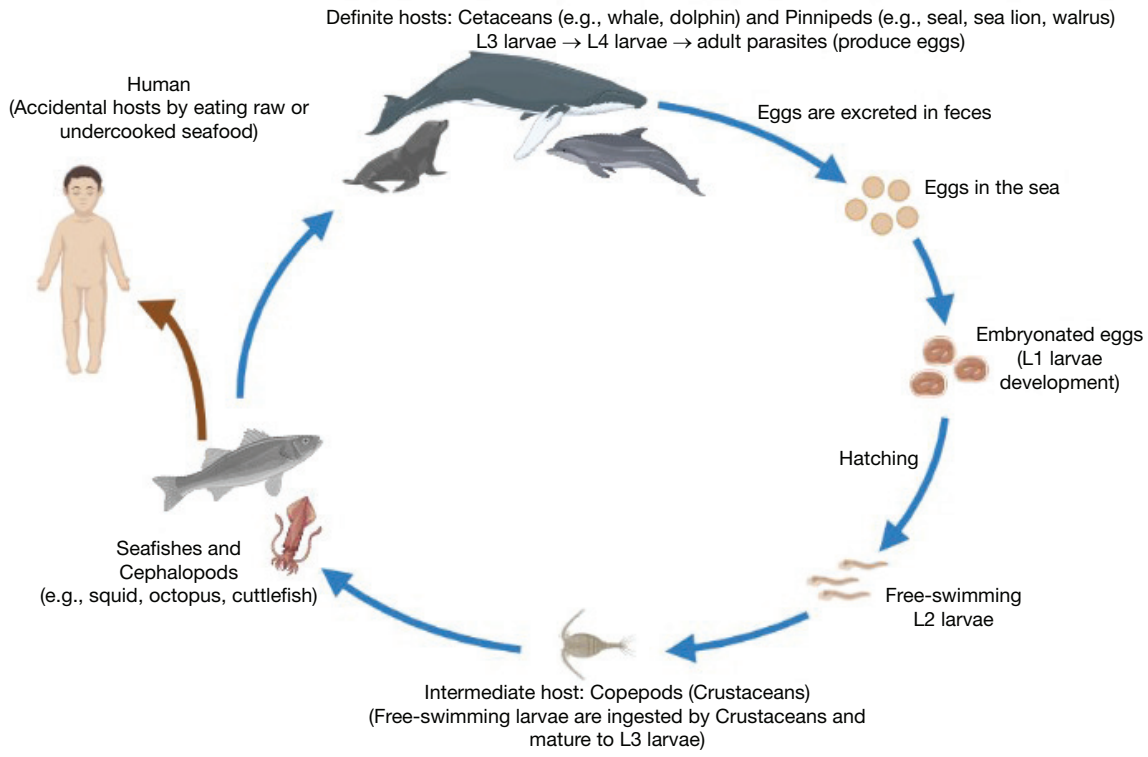

Figure 1 Life cycle of *Anisakis simplex*. Created with BioRender.com.

Table 1 Anisakiasis including clinical patterns.[3,4]

	Typical onset after contact	Clinical presentation	Mechanism
Gastric anisakiasis	1–12 hours	Epigastric pain Nausea Vomiting	Direct damage by Anisakis
Intestinal anisakiasis	5–7 days	- Acute presentation: Severe abdominal pain mimicking acute abdomen muco-hematic diarrhea - Chronic presentation: Granuloma Abscess	Direct damage by Anisakis
Ectopic anisakiasis (or extraintestinal anisakiasis)	Variable	Variable, depending on the anatomical site involved: For example, peritoneal and pleural cavities, mesentery, liver, pancreas, ovary, and subcutaneous tissues	Penetration of Anisakis larvae through the gastric or intestinal wall
Gastroallergic anisakiasis	Variable	Combination of signs and symptoms found in gastrointestinal and allergic anisakiasis	Direct damage by Anisakis + Immune-mediated, IgE-mediated
Allergic anisakiasis	Variable	Urticaria Angioedema Anaphylaxis	Immune-mediated, IgE-mediated
Occupational anisakiasis	During occupational exposure to allergens, with improvement when not working	Asthma Dermatitis Conjunctivitis	Immune-mediated, IgE-mediated

reported a prevalence of AS sensitization of 4.5% in 10,570 consecutive subjects across Italy, with just 14% of the sensitized patients reporting a history of clinically relevant allergy. Patients involved in the study came from 34 different centers located across Italy. Notably the authors described a higher prevalence of Anisakis hypersensitivity among people living along Italian Adriatic, and Tyrrhenian coasts, which was due to frequent consumption of marinated anchovies.

Furthermore, AS sensitized patients are often cosensitized to house dust mites (HDM) (>40%); however, this proportion drops to 26% when considering clinical allergy to AS.[10] This suggests that, in some cases, Anisakis sensitization could be the result of cross-sensitization between AS and HDM allergens. This cross-sensitization could be motivated by tropomyosin, which is a thermostable protein.[11] While tropomyosins were identified as animal food allergens in, for example, crustaceans, molluscs, and AS,[12] they are also known as respiratory allergens in arthropods (e.g., mites, cockroaches).[12] Cross-sensitization between tropomyosin-including allergen sources is explained by the highly conserved tropomyosin sequences.[13] In addition, paramyosin, which is a myosin filament-related protein, is responsible for cross-sensitization as well: recent studies have reported that Ani s 2 (paramyosin) and Ani s 3 (tropomyosin) allergens have similar homology and strong cross-sensitization to HDM and crustacean homologues, for example.[1,14]

De Corres et al.[15] conducted a multicenter study among 868 Spanish subjects to determine the prevalence of AS hypersensitivity: 38.1% of the subjects with a previous episode of urticaria or angioedema and 13.1% of people without previous allergic reactions resulted in being sensitized to AS, respectively. The median age of the patients was 38 years, with only one pediatric allergy unit participating in the study. The authors explained that the high prevalence was registered as a result of either high rate of contamination among consumed fish or cross-reactivity among AS and other parasites. Even in this work, the major frequency of sensitization had been found in the area of the country where fish was more commonly consumed, underlining the dietary influence on AS sensitization.

There is no sufficient data available in the literature about the epidemiology of AS sensitization in children. Bernardini et al.[16] reported a prevalence of 6.1% in a population of 805 pediatric patients attending their hospital in Florence, Italy. Sensitized children have never been investigated for allergic signs and symptoms in response to AS, and sensitization was significantly associated with HDM *(Dermatophagoides pteronyssinus)* and other allergens such as cod and soya.[17]

Verga et al.[11] assessed the prevalence of sensitization to AS in children sensitized and/or allergic to HDM in a population of 294. Data showed that the prevalence of AS sensitization was 13.4% among children sensitized to HDM and 3.8% among children not sensitized to HDM; when considering children allergic to HDM, 15.3% were sensitized against AS, while the prevalence dropped to only 7.1% among those not allergic to HDM. Of note, patients sensitized to AS have never manifested allergic reaction to AS, suggesting that sensitization often does not play a clinically relevant

role in the pediatric population and that AS sensitization might be connected to cross-sensitization with HDM allergens. Gonzalèz de Olano et al.[18] evaluated the rate of sensitization to Anisakis by measuring sIgE in a population of 210 patients affected by mastocytosis, 47 of whom were children: none of the children resulted being sensitized to Anisakis.

Pathophysiology

Allergic anisakiasis (AA) is mediated by the immune system. According to type I hypersensitivity, the first contact with AS allergens leads to presentation of AS antigens to $CD4^+$ T lymphocytes (TH), driven by antigen-presenting cells. In atopic patients, $CD4^+$ T lymphocytes are polarized to TH_2 cells, producing cytokines (e.g., IL-4, IL-5, IL-13) and inducing isotypic switch of B lymphocytes, with the consequent release of immunoglobulins E (IgE). Circulating IgE bind to their receptors (FcεRI), for example, on mast cells and basophils, finalizing the sensitization process. On contact with AS allergens, antigens bind IgE–FcεRI complexes, causing basophils and mastocytes activation with the release of allergic reaction mediators (e.g., histamine, proteases, cytokines). These mediators act on several targets, for example: blood vessels, dilatation and permeabilization; airways, constriction; digestive tract, increase of motility.[19] Notably, allergic reactions, often including nausea and diarrhea, allow the ejection of AS from the host's intestine, thereby acting as a defensive mechanism.[20]

Gastroallergic anisakiasis (GAA) combines acute parasitism and immune-mediated, IgE-mediated reactions.[21]

Allergens and Cross-reactivity

Anisakis simplex has been recognized as the parasite with the highest number of known allergens.[22] AS allergens may be divided into two categories[23]: somatic allergens derived from dead or live larvae, and excretory–secretory (ES) antigens released when larvae are expelled from the host digestive tract or surgically removed.

To date, 14 allergens of AS have been described; some of them—Ani s 1, Ani s 4, Ani s 5, Ani s 8, Ani s 9, Ani s 10—have been reported to be thermostable, giving the possibility to develop an allergic reaction even after ingesting dead AS larvae.[24-29] Ani s 4 and Ani s 6 are resistant to gastric pepsin.[4] Ani s 1, Ani s 2, Ani s 7, Ani s 12, Ani s 13, and Ani s 14 are defined as "major allergens"[30-35]. Ani s 2 and Ani s 3 are identified as pan-allergens, which are involved in cross-reactivity with other food and inhalant sources.[12,36,37]

Ani s 1 is considered to be a major allergen of AS. It is a serine protease inhibitor, and shows homology to serine protease inhibitors from *Caenorhabditis elegans*.[2] Among allergic patients, 86% demonstrated IgE against Ani s 1 and 29% demonstrated IgG against Ani s 1.[38]

Ani s 2 is a paramyosin, and Ani s 3 is a tropomyosin. These two somatic allergens play a major role in AS cross-reactivity: AS paramyosin is closely related to, for example, mites and insects, while AS tropomyosin is closely related to, for example, crustaceans, insects, and mites.[36,39]

Ani s 4 is a cysteine protease inhibitor produced in the secretory gland and the basal cuticle layer of third-stage larvae.[38] Two isoforms of Ani s 4 have been described: one containing leucine at the third position of the mature protein, and the other containing proline in the mentioned position; the former isoform has been demonstrated to be more allergenic compared to the latter.[40] Moreover, it seems to play a central role in eliciting anaphylaxis.[38]

Ani s 6 is a serine protease inhibitor, which shows homology to other serine protease inhibitors, for example, from *Boophilus microplus* (cattle thick), *Anopheles stephensi* (mosquito), *Glossina morsitans* (tse-tse fly), and *Apis mellifera* (honeybee) allergen Api m 6.[2]

Ani s 7 is a serine protease inhibitor for which IgE has been identified in almost 100% of the allergic patients. This protein's particular structural features might explain this. Even if commonly targeted by IgE, no experimental proof of its allergenic activity has been reported yet.[38]

Ani s 5, Ani s 8, and Ani s 9 belong to SPX/RAL-2 family and share protein sequence homology. Moreover, Ani s 9 shows homology with antigens from *Ascaris suum* and *Acanthocheilonema viteae*.[28] Notably, cross-reactivity between Ani s 9 and wasp venom allergens has been reported.[41]

Ani s 10 is a protein with unknown function. It is composed of seven amino acid repeats, each containing theoretical cleavage sites for trypsin and pepsin, leading to think that Ani s 10 is cleaved in seven active peptides in the digestive tract.[29]

Ani s 11 and Ani s 12 are proteins of unknown function described by Kobayashi et al.[33] Ani s 11 is characterized by five or six types of short repetitive sequences comprising 6–15 amino acids, and Ani s 12 by tandem motif with four cysteine residues.

Ani s 13 represents AS hemoglobin, and it has been described as one of the major AS allergens.[34] Surprisingly, even if it is a part of a conserved protein family, it does not show cross-reactivity.[42]

Ani s 14 is a protein with an unknown function. It is characterized by two homologous regions, similar to sequences found in Ani s 7 and Ani s 12, and redundant sequences, possibly acting as IgE binding site.[35]

Table 2 summarizes the characteristics of AS allergens registered in the Allergen Nomenclature Sub-Committee of the World Health Organization (WHO) and the International Union of Immunological Societies (IUIS).

Clinical Presentation

Allergic reactions to AS include several different conditions (Table 1):

1. Allergic anisakiasis (AA) develops as a consequence of ingestion of seafood parasitized by AS larvae, leading to sensitization in susceptible individuals. Once a patient comes in contact with AS larvae again—through ingestion of parasitized food—AA usually occurs in a few hours following ingestion, presenting with classical

Table 2 WHO/IUIS-registered *Anisakis simplex* allergens.

Allergen	Biochemical function	Molecular weight (kDa)	Compartment S = Somatic ES = Excretory-Secretory	IgE reactivity (%)	Type of allergen	Resistance properties	Authors
Ani s 1	Kunitz-type serine protease inhibitors	24	ES	85	Major allergen	Thermostable	(Moneo et al., 2000)
Ani s 2	Paramyosin	97	S	88	Major allergen Pan-allergen		(Pérez-Pérez et al., 2000)
Ani s 3	Tropomyosin	41	S	4-?	Minor allergen Pan-allergen		(Asturias et al., 2000)
Ani s 4	Cysteine protease inhibitor	9	ES	27	Minor allergen	Thermostable Resistant to pepsin	(Moneo et al., 2005; Rodriguez-Mahillo et al., 2007)
Ani s 5	SXP/RAL-2 family protein	15	ES	25–49	Minor allergen	Thermostable	(Kobayashi et al., 2007)
Ani s 6	Serine protease inhibitor	7	ES	18	Minor allergen	Resistant to pepsin	(Kobayashi et al., 2007)
Ani s 7	Serine protease inhibitor	139	ES	83–100	Major allergen		(Rodríguez et al., 2008)
Ani s 8	SXP/RAL-2 family protein	15	ES	25	Minor allergen	Thermostable	(Kobayashi et al., 2007)
Ani s 9	SXP/RAL-2 family protein	14	ES	13	Minor allergen	Thermostable	(Rodriguez-Perez et al., 2008)
Ani s 10	-	21	S	39	Minor allergen	Thermostable	(Caballero et al., 2011)
Ani s 11	-	27	-	47	Minor allergen		(Kobayashi et al., 2011)
Ani s 12	-	31	-	57	Major allergen		(Kobayashi et al., 2011)
Ani s 13	Hemoglobin	37	-	64	Major allergen		(González-Fernández et al., 2015)
Ani s 14	-	24	-	54	Major allergen		(Kobayashi et al., 2015)

IgE reactivity: Percentage of patients exhibiting sIgE against the allergen of a selected group of sensitized patients; Pan-allergen: Highly conserved allergen explaining cross-reactivity

features of allergic reactions: for example, urticaria, angioedema, or anaphylaxis. Notably, it is still not clear if AA is initiated by dead or live AS larvae: it seems that the sensitization process needs live larvae to take place—through raw or undercooked fish—while allergic reaction might take place in response to both live or dead larvae (Table 2).[4,37]

2. Gastroallergic anisakiasis (GAA) represents an entity in which acute parasitism by AS is accompanied by an allergic reaction as a result of ingestion of live larvae. Clinically, it presents with gastrointestinal signs and symptoms, for example, abdominal pain, nausea, vomiting, and diarrhoea, and allergic clinical manifestations, for example, urticaria, angioedema, and anaphylaxis.[43] Chronic urticaria (CU) has been proven to be associated with AS hypersensitivity in area where raw or marinated fish consumption is frequent,[44,45] even if a clear causal relationship has not been demonstrated yet.[46–48] It has been demonstrated that patients affected by GAA do not present signs and symptoms again when consuming cooked or frozen fish, showing the need for live larvae to cause GAA.[21] Of note, GAA clinical presentation could be similar to food protein-induced enterocolitis syndrome (FPIES) caused by seafood,[49] indicating that GAA should be evaluated in the differential diagnosis of seafood FPIES.
3. Occupational anisakiasis (OA) is an allergic form of anisakiasis consequent to inhalation or direct contact with AS allergens. This condition is prevalent among seafood industry workers (e.g., fishermen, fishmongers), and it may present with general allergic clinical manifestations (e.g., dermatitis, asthma, conjunctivitis, rhinitis).[50–52]

Anisakis has been reported to cause unusual clinical presentations of anisakiasis, which we need to bear in mind.

Bhargava et al.[53] described the case of a 6-year-old Indian girl affected by recurrent tonsillitis and adenoiditis. The child underwent tonsillectomy and adenoidectomy, and histopathology revealed Anisakis larvae located at tonsillar lymphoid tissue. Remarkably, the authors concluded that chronic tonsillitis was probably an independent process, and the finding of parasite was incidental.

Centonze et al.[54] reported the case of a 8-year-old child presenting with anaphylaxis, showing respiratory signs and symptoms, and scrotum acutum, with right testicular pain, swelling of 1 cm, and later appearance of lymphadenitis and erythematous skin. The boy underwent surgical

excision of the right testicle, and the operatory specimen was infested with worms, with the histological exam confirming extraintestinal anisakiasis.

Cusì-Sànchez et al.[55] described the case of a 16-year-old male presenting at the emergency department with incarcerated epigastric hernia; hernial tissue was surgically removed, and histology showed larvae of *P. decipiens*, a nematode belonging to the *Anisakidae* family, responsible for diseases similar to anisakiasis. Interestingly, the patient was a regular consumer of home-made fish with vinegar.

Juric et al.[56] described the history of a 14-year-old boy who complained of sudden onset of widespread abdominal pain, nausea, and vomiting. He was diagnosed with small intestine obstruction and underwent appendectomy and extirpation of local lymph node. Histological exam showed infiltration of eosinophils in muscular layer and serosa of the appendiceal wall. Given this, authors went through more detailed clinical history, and discovered that the boy had eaten raw fish 3 days before. They performed specific sIgE against AS dosage and found an increase in allergen specific IgE. The diagnosis of intestinal anisakiasis was posed.

As unusual presentations, eosinophilic esophagitis,[57] nephrotic syndrome,[58] and dyspepsia[59] have also been reported as case reports in adults and have to be considered in pediatric patients as well.

Diagnosis

The gold standard for food allergy diagnosis is a challenge test, but due to ethical objections this test is obviously not possible in the case of suspected Anisakis allergy.[60] Given this, an alternative gold standard test for diagnosing AS allergy does not exist,[61] so diagnosis is mainly made by the concomitance of patient history, clinical manifestations, and *in vivo* and *in vitro* tests. One of the principal concerns about diagnosing AS allergy is to differentiate between allergy and sensitization without signs and symptoms. Indeed, healthy individuals can have high serum levels of sIgE anti-AS without clinical manifestations of allergy, while other individuals with low levels of specific IgE-antibodies may have relevant clinical manifestations.[62] Clinical history of signs and symptoms and their timing after AS exposition play a pivotal role in differentiating between allergy and sensitization. Cross-reactivity represents a problem, especially when evaluating patients affected by other parasite infections.[63] Differentiating between AS allergy and cross-reactivity with other organisms may be more challenging, but the use of native or recombinant source of allergens may support the process.

The first step in facing a patient with signs and symptoms resembling AS allergic reaction is to obtain a detailed and accurate clinical history. The professional must focus on the patient's food intake, especially for the last 72 hours before the potential allergic reaction, with attention to seafood consumption.

As previously claimed, oral ingestion is not the only route of exposure to AS allergens: for example, skin contact, manipulating seafood, or inhalation, smelling seafood, may cause an allergic reaction,[64] so the interviewer must query these circumstances.

Regarding allergic reaction pathophysiology, a patient must be sensitized to AS allergens. Since subclinical parasitism may be very common among subjects regularly consuming seafood, many of them may not remember a previous contact with AS and a high prevalence of misdiagnosed AS contact is known to exist.[37]

Once patient history is collected, many techniques may be applied to diagnose AS allergy: *in vivo* test, such as SPT and *in vitro* test, such as sIgE and basophil activation test (BAT). Each of the aforementioned techniques consists of evaluating a patient's characteristics concerning AS allergens. Different forms of antigenic preparations are available for testing:

1. Whole-body antigen extracts represent the most straightforward preparation, but it is burdened by low specificity due to cross-reactivity between AS allergens and antigens of, for example, other nematodes, crustaceans, insects, and mites.[64] Furthermore, it cannot identify the causative allergens of allergic sensitization.[14] Armentia et al.[65] showed cross-reactivity between AS and *Eurygaster* and *Ephestia* grain pests. Johansson et al.[66] confirmed cross-reactivity with dust mites: *Acarus siro, Lepidoglyphus destructor, Tyrophagus putrescentiae, and D. pteronyssinus*. Iglesias et al.[67] demonstrated cross-reactivity with the nematodes *A. suum, Toxocara canis, Hysterothylacium aduncum, Trichinella spiralis,* and *Trichuris muris*. Pascual et al.[68] showed cross-reactivity between *Blattella germanica* and *Chironomus spp*.
2. ES antigen extracts: ES allergens have higher allergenicity compared to somatic (S) allergens usually included in whole-body antigen extracts. Indeed, ES allergens are able to bind more consistently to serum IgE and elicit a more extensive response to skin tests at the same protein concentration compared to S allergens, resulting in increased sensitivity of the diagnostic method.[69]
3. Purified or semi-purified antigens: Purification of native antigens (nAni) may be obtained by differential ethanolic precipitation, affinity chromatography, or purification to homogeneity.[64] Purified Ani s 13 has shown to improve sensitivity and specificity in AS allergy diagnosis.[42]
4. Recombinant allergens: Recombinant AS allergens (rAni) have been expressed in bacteria and yeast cultures. This type of allergens may be produced in industrial amounts and may be standardized. On the other hand, inadequate protein folding or posttranslational modifications, as existent on many native protein isoforms, may lead to the absence of epitopes commonly expressed in a native allergen, and possible false-negative results.[64] Ani s 1 helps to distinguish between allergic patients and sensitized patients.[70] Recently, it has been suggested that rAni s 1 may act as a potential biomarker in determining the risk of suffering from severe allergic reaction to AS, because the frequency of recognition of Ani s 1 is higher in patients who experienced severe reactions compared to those who suffered from moderate or mild reactions.[71]

Skin prick test

SPT was first described in 1924,[72] and since then, it has been routinely used for the diagnosis of allergies. SPT evaluates skin reaction to AS extracts 15 minutes after positioning them on the volar surface of the patient's forearm or on the patient's upper back. Histamine and normal saline are used as positive and negative controls, respectively.[73] Wheals equal to or greater than 3 mm in diameter are considered positive.[74,75] The advantages of this technique include low costs, rapidity, sensitivity, and the possibility to be run directly by a physician,[73,62] thus, it is helpful as a first-line diagnostic method. Tripodi et al.[76] evaluated the prevalence of AS in children living in endemic areas. In their study, they performed SPT with a commercial extract of AS in 443 children. To reduce the known risk of cross-reactivity, they performed SPT with extracts of HDM, codfish, shrimp, and cockroach as well and studied patients with unequivocal skin reactivity to Anisakis extract, in the absence of any skin reactivity to HDM, shrimp, or cockroach, in order to detect those who were genuinely sensitized to the helminth. They reported a prevalence of 4.5% among a population of 443 patients. Interestingly, AS-sensitized children were significantly older than controls, suggesting that the likelihood of sensitization increases over time, which could most likely be associated with a change in eating habits.

sIgE

AS allergy can be confirmed by the detection of serum sIgE against nematode allergens. Different diagnostic systems may be used to detect sIgE against AS, including:

1. ImmunoCAP (Thermo Fisher Scientific, Uppsala, Sweden) is one of the most commonly used tests and consists of a singleplex technique.[64] Many studies adopted 0.35 kU/l as cutoff for identifying sensitization to Anisakis,[18,60,77,78] while others adopted 0.7 kU/l.[75,79] Nonunique value had been described, but Carballeda-Sangiao et al.[63] proved that higher threshold drives to higher specificity (specificity = 84%, sensitivity = 99% for sIgE cutoff = 0.35 kU/L; specificity = 91%, sensitivity =98% for sIgE cut-off = 0.71 kU/l). Interestingly, Falcão et al.[80] claimed that the risk of acute relapsing urticaria in a population of 200 children allergic to AS varies according to sIgE levels: children with sIgE values > 0.7 kU/l had higher magnitude of association between urticaria and sensitization compared to those with sIgE > 0.35 kU/l, suggesting that sIgE levels may influence response to AS.
2. ImmunoCAP ISAC (Thermo Fisher Scientific, Uppsala, Sweden) is a microarray-based technique detecting reactivity to 112 common food allergens. Among these, rAni s1 and rAni s3 are included.

Finally, Carballeda-Sangiao et al.[63] showed that Anisakis/Ascaris sIgE ratio increases specificity in the diagnosis of AS allergy compared to Anisakis sIgE dosage alone for samples having sIgE to Ascaris ≥ 0.35 kU/l, in a population with a high rate of parasite infection (specificity: 77% vs 95%). The authors found that a value of ≥4.4 for Anisakis/Ascaris sIgE was the best cutoff to increase specificity. This result suggests that this ratio may be helpful in areas where infections with nematodes other than Anisakis are present, and cross-reactivity rates are high.

Basophil activation test

The BAT is based on the detection of basophil activation after exposure to allergens through flow cytometry; CD63, a plasma membrane protein expressed after activation, is the chosen marker of basophils activation. Gonzalez-Muñoz et al.[81] demonstrated the utility of BAT in diagnosing AS allergy. Notably, they applied a cutoff of 16% to differentiate between AS allergic patients versus healthy controls (sensitivity: 100%, specificity: 100%) and a cutoff of 21% to differentiate between AS allergic patients versus patients affected by urticaria or abdominal pain unrelated to fish ingestion (sensitivity: 100%, specificity: 96%). Brusca et al.[60] confirmed the high specificity of BAT in the detection of Anisakis sensitization, suggesting its use to confirm AS allergy in a patient with clinical history, SPT and sIgE indicative of AS allergy or to rule it out in a patient with high clinical history suspicion of AS allergy but negative SPT and sIgE. Frezzolini et al.[78] demonstrated the utility of BAT not only in diagnosing AS allergy in patients presenting with CU but also during follow-up. They applied a cutoff of 13% and showed that BAT had higher diagnostic accuracy compared to sIgE. They also proved that CD63 expression was significantly reduced when excluding seafood from the diet, in parallel with the clinical improvement observed in patients at the end of follow-up, with BAT demonstrating better association with clinical response compared with sIgE levels.

Figure 2 reports a suggested algorithm for the diagnosis of AS allergy in pediatric patients.

Management

AS allergy management consists of prevention of AS contact and treatment of clinical manifestations.

Prevention starts from fishing because fish must be immediately eviscerated to prevent larvae from migrating from fish viscera to flesh. Viscera removed must not be disposed of at sea to avoid larval comeback to the marine environment.[20]

Food prevention passes through the killing of AS larvae; this may be obtained by conserving fish at a temperature of −20°C for at least 168 hours or at −35°C for at least 15 hours or by cooking fish to a temperature of at least 63–74°C before consumption.[82] Nevertheless, it has to be borne in mind that GAA request live larvae infection to take place, while AA in sensitized patients may take place even in the presence of dead larvae. Moreover, some AS allergens are thermostable.[83] This means that previous methods avoid GAA,[37] but may not prevent an allergic reaction in sensitized patients. Removing Anisakis allergens from infected food requires several washing steps with water and strong buffers, which is an impractical procedure.[84] An alternative may be represented by the consumption of fish not parasitized by AS; for example,

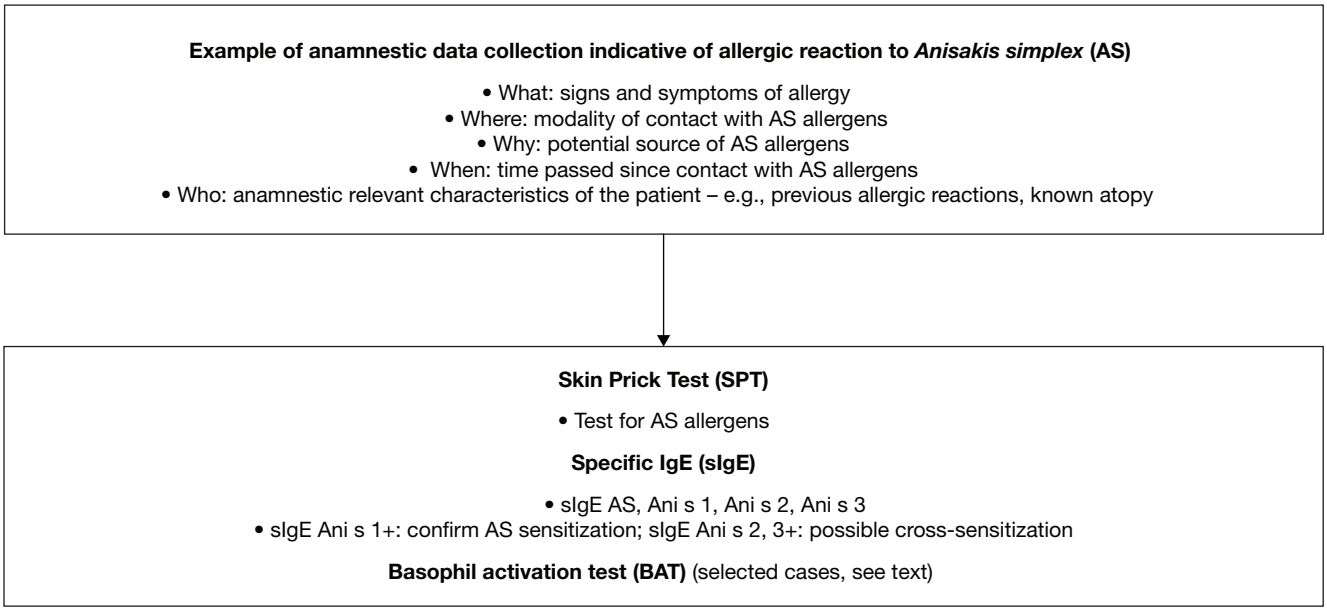

Figure 2 Algorithm for the diagnosis of AS allergy in pediatric patients.

aquacultured fish seems to reduce the risk of contamination by larvae,[85,86] suitable for AS allergic patients. Polimeno et al.[83] recently demonstrated that the presence of Ani s 4 in aquacultured fish may be secondary to feeding fish with parasitized poultry.

No clear dietary recommendations for AS allergic patients have been established.[87] Many authors recommend patients to avoid fish consumption, with the consequent risk of poor omega-3 fatty acids intake.[88] With regards to GAA patients, no seafood restriction may be followed, apart from adequately cooking or freezing.[87] Of note, Giuliano et al.[89] proved a statistically significant reduction in total IgE and sIgE counts among allergic patients following an 18-month-long fish-free diet. However, all the indications reported above should be extended to all the potentially parasitized seafood. Not much data in the literature focusing on pediatric patients' diets can be found.

Regarding treatment of clinical manifestations, in case of anaphylaxis in a pediatric patient, intramuscular adrenaline is injected. If auto-injectable adrenaline is the only form available, dosage must be adjusted.[90,91,92] If signs and symptoms do not improve, adrenaline must be repeated in 5–10 minutes; in case of refractory anaphylaxis, intravenous administration of adrenaline must be considered in an appropriate setting.[92] Anaphylaxis management should then follow an appropriate procedures, according to international guidelines. Before discharging patients from emergency room, adrenaline auto-injectors should be prescribed and patients must be trained on how to use these injectors.[93]

In patients presenting with mild allergic manifestations such as urticaria, administration of second generation H1-antihistamines allows to control signs and symptoms. A short-course of systemic corticosteroids may be required in severe cases or in patients with angioedema.[94] However, for the complete management of allergic reactions we refer the reader to relevant documents in the area.

Conclusion

Anisakidosis is a condition resulting from the consumption of raw seafood, which is parasitized with live larvae of the *Anisakidae* family. AS infects humans through its third-stage larvae and usually causes gastrointestinal signs and symptoms. However, allergic reactions ranging from urticaria and/or angioedema to anaphylaxis may occur. Most of the cases have been reported in Japan and Spain, while it is remarkable that there has been an increase in publications on AS allergy from many different parts of the world in recent years. In patients with suspected clinical history of Anisakis allergy, SPT and determination of sIgE levels to total extract or allergen components are useful in the confirmation of diagnosis. So far, 14 proteins of AS have been identified as allergens. Among them, Ani s 1 is suggested as a possible biomarker to identify patients at high risk for severe allergic reactions. Of note, some healthy subjects may have positive results to the AS allergens, which indicates only sensitization without any clinical reactivity.

Changes in the eating habits, introduction of culinary products from different cultures have resulted in increasing interest for the consumption of raw fish. Hence, knowledge and awareness of AS allergy among healthcare professionals need to be increased as many of these patients might be misdiagnosed if clinical history is not questioned elaborately. The data regarding the pediatric age group are very limited, and further studies are warranted. Indeed, we have a concern whether lack of data is due to underdiagnosis in children. In this sense, we recommend pediatricians and pediatric allergists to keep this type of allergy in mind and consider it in the differential workup and diagnosis.[4,71]

Confidentiality of Data

Not applicable.

Right to Privacy and Informed Consent

Not applicable.

Protection of Human Subjects and Animals in Research

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Contributors' Statement

MG and BB conceptualized and designed the work. MP acquired the data. MP, MG, ALL, and BB drafted the initial manuscript. MP, MG, SB, FM, EV, LG, CV, LDlV, CS, ALL, and BB analyzed the data and reviewed the manuscript. All authors approved the final manuscript as submitted and agreed to be accountable for all aspects of the work.

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