

ALLERGIES IN THE WORKPLACE

ALLSA RESEARCH AWARDS REPORT

APPROACHES TO DIAGNOSING ANISAKIS ALLERGY

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SUMMARY

Anisakis is a parasitic nematode which infects fish and can cause gastrointestinal disease if accidentally ingested. Infection can be accompanied by severe allergic reactions such as urticaria, angio-oedema and anaphylaxis. Furthermore, workers involved in fish processing can develop occupational allergy to *Anisakis*, including asthma, rhinoconjunctivitis and protein contact dermatitis. Diagnosis of allergy to *Anisakis* relies on skin-prick tests and the detection of specific IgE by ImmunoCAP. Since *Anisakis* infests fish, fish allergy should be investigated in symptomatic patients. *Anisakis* proteins also demonstrate considerable immunological cross-reactivity to proteins of related nematodes and other invertebrates such as house-dust mites and cockroaches; this needs to be borne in mind when the diagnosis is made. This review outlines the approaches that have been used to increase the specificity of *Anisakis* diagnosis, including the use of immunoblotting and the identification of *Anisakis* allergens.

ANISAKIS INFECTION

Anisakis species are marine roundworms which use sea mammals such as dolphins and whales as primary hosts. The stage 3 larval form (L3) of *Anisakis* (Fig. 1) infects fish and other seafood such as squid, and consequently humans may become accidental hosts for *Anisakis* if they consume raw or undercooked fish.¹ Infection is known as anisakiasis and is often associated with gastrointestinal symptoms such as abdominal pain, diarrhoea, nausea and vomiting. Patients' reactions range from being asymptomatic to requiring emergency room care. Since 1960 when anisakiasis was first described, thousands of cases have been reported from Japan and hundreds from Europe, the USA, and other parts of the world.²

The management of anisakiasis involves physically removing the larvae, if possible, or treating the patient with antihelmintics, anti-inflammatories and analgesics.^{3,4} The *Anisakis* larvae cannot survive or reproduce in humans, but if the larvae are not removed, the disease can become chronic as inflammatory cells surround the larval remains and lead to symptoms which can mimic dyspepsia, Crohn's syndrome, appendicitis, irritable bowel syndrome, diverticulitis, non-specific eosinophilic enteritis, or even gastric cancer.² Abdominal pain, nausea, vomiting and/or diarrhoea within 48 hours of consuming fresh seafood should indicate the possibility of *Anisakis* infection. As many cases of anisakiasis have occurred after consumption of freshly caught fish that appeared well-cooked but was not sufficiently heated through to kill larvae, ingestion of raw seafood should not be the only factor meriting further investigation. In order to kill larvae, fish should be frozen at -20°C for at least 24 hours or cooked so that all parts of the fish reach at least 60°C for 10-20 minutes.² Smoking fish or marinating it in lemon juice or vinegar does not kill *Anisakis*.

ANISAKIS ALLERGY

Of particular relevance to the physician is that *Anisakis* can also cause severe allergic reactions because of its ability to elicit strong Th2 responses.^{5,6} Many patients experience gastroallergic anisakiasis, in which infection



Fig. 1. *Anisakis* larvae removed from *Thyrsites atun* (snoek).

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is accompanied by allergic reactions such as urticaria, angio-oedema, bronchospasm and/or anaphylaxis.^{7,8} This allergic response can occur without gastrointestinal symptoms, leading to misdiagnosis of the reaction to *Anisakis* as fish allergy or idiopathic urticaria/anaphylaxis.⁵ Symptoms can begin anywhere between a few hours to more than a day after ingestion of the parasite, and patients may therefore not connect the ingestion of the fish to the symptoms. Although some patients tolerate dead larvae in frozen or cooked fish, others have symptoms after eating well-cooked or canned fish, indicating that both live and dead larvae and their proteins can cause allergic reactions.⁹⁻¹² A history of fish consumption prior to allergic symptoms and the absence of sensitisation to fish indicates the need to test for *Anisakis* allergy.

Currently, the diagnosis of *Anisakis* allergy relies on a clear history of potential exposure to *Anisakis* and symptoms of gastroallergic anisakiasis along with *Anisakis* specific IgE and positive *Anisakis* skin-prick tests (SPTs).^{5,7} However, because many allergens of *Anisakis* are heat stable, exposure to *Anisakis* proteins in fish on an ongoing basis can also cause symptoms such as chronic urticaria, protein contact dermatitis, asthma and rhinoconjunctivitis.¹³⁻¹⁹ In this case the clinical history may be less clear since patients may be exposed to many agents in their environment at the same time. The use of specific IgE alone to diagnose *Anisakis* allergy is confounded by the fact that even asymptomatic individuals can have *Anisakis* specific IgE because of cross-reactivity with other helminths (e.g. *Ascaris*) or invertebrates such as dust mites, cockroaches and shrimp.²⁰⁻²² Studies in Spain have found that a large number of asymptomatic individuals have *Anisakis* specific IgE, some related to subclinical sensitisation and others due to false-positive results as a result of cross-reactivity.²²⁻²⁴

The muscle protein tropomyosin is an important source of cross-reactivity with other invertebrates. Recently we showed by allergen microarray analysis that all patients with specific IgE antibodies to *Anisakis* tropomyosin (Ani s 3) also recognised tropomyosin of shrimp, dust mite, cockroach and snail (unpublished data). Whether *Anisakis* tropomyosin is a clinically relevant allergen is however controversial. Asturias *et al.*²⁰ have suggested that tropomyosin is not an important allergen as asymptomatic patients were sensitised to it whereas symptomatic patients were not. Other researchers suggest that *Anisakis* tropomyosin could play a role in eliciting food allergy after ingestion of cooked seafood, because it closely resembles the heat-stable shrimp tropomyosin, an important allergen in seafood allergy.²⁵

THE ROLE OF IMMUNOBLOTTING IN THE DIAGNOSIS OF ANISAKIS ALLERGY

Since cross-reactivity can cause false-positives in SPTs and specific IgE tests, some authors have used IgE immunoblotting to differentiate anisakiasis/*Anisakis* allergy from asymptomatic *Anisakis* sensitisation.^{24,26,27} One study found that patients with confirmed *Anisakis* allergy had IgE directed at several proteins of medium molecular weight as well as low-molecular-weight proteins, while patients with no allergy or doubtful symptoms were more likely to recognise either a single medium-molecular-weight protein of approximately 40 kDa (possibly *Anisakis* tropomyosin) or a few medium-molecular-weight proteins.²⁶ Another study also found that asymptomatic blood donors with specific IgE to *Anisakis* frequently detected a single protein of 42 kDa whereas truly sensitised patients recognised multiple allergens of the crude extract.²⁴

Only one case of food allergy to *Anisakis* has been documented in South Africa²⁸ despite the recent popularity of sushi, perhaps because the disease is largely unknown to physicians and may go undiagnosed. Recently, several case reports described adverse reactions to *Anisakis* in individuals handling fish or fishmeal, with symptoms ranging from conjunctivitis to allergic asthma.^{16,17,19} In an epidemiological study of two large fish-processing factories in St Helena Bay on the west coast of South Africa we found a prevalence of 8% sensitisation to *Anisakis* among the fish-processing workers,^{6,29} but only 1-3% had *Anisakis*-related allergic symptoms. The study also found that individuals with *Anisakis* sensitisation were twice as likely (OR = 2.24, CI: 1.01-4.97) to have high seafood intake as measured by elevated level of serum omega-3 fatty acids (eicosapentaenoic acid). We therefore decided to look at patterns of IgE-binding proteins recognised by our sensitised workers to compare them with patterns found in previous studies where patients had symptoms of gastroallergic anisakiasis.

Immunoblotting using serum from 15 workers who were ImmunoCAP or SPT positive to *Anisakis* (Table I) showed diverse patterns of IgE binding to *Anisakis* proteins (Fig. 2), as has been observed in previous studies.³⁰ Somatic *Anisakis* antigens were used for immunoblotting, as the workers were likely to be exposed to *Anisakis* through handling of fish, inhalation of vapours and consumption of cooked fish. Workers who were positive to *Anisakis* on ImmunoCAP were often also positive to *Ascaris lumbricoides*, a human roundworm, which is closely related to *Anisakis*.³¹ A subgroup analysis of sera (n = 129) demonstrated a very high correlation (r = 0.72, p < 0.001) between IgE reactivity to *Anisakis* and *Ascaris* (unpublished data). Immunoblotting against *Anisakis* extract may be less useful for diagnosis if the patient has a past *Ascaris* infection because of cross-reactivity between *Anisakis* and *Ascaris*.³² We therefore looked at patterns of IgE binding in workers who had a higher level of specific IgE to *Anisakis* than to *Ascaris*, similar levels of specific IgE to both worms, or a higher level of specific IgE to *Ascaris* than to *Anisakis*. Sera from three workers who were SPT positive but ImmunoCAP negative to *Anisakis* were also examined.

Most of the workers recognised a variety of medium-molecular-weight proteins ranging from about 33 to 75 kDa, including the workers who were primarily sensitised to *Ascaris*. Some also recognised low-molecular-weight proteins, reportedly an indication of real exposure to *Anisakis* rather than cross-reactivity to other invertebrates.²⁶ The IgE-binding pattern was more variable in the workers who had higher specific IgE to *Anisakis* than to *Ascaris*. One of these workers had IgE against only two proteins of approximately 52 and 75 kDa (with fainter binding at 37 kDa) and another recognised only a single band at about 42 kDa. A third was strongly sensitised to a cluster of proteins between 43 and 49 kDa.

Some of the IgE-binding proteins identified in our study have not yet been characterised or identified as allergens. Previous studies have also detected IgE-binding proteins different to the known allergens by immunoblot analysis.^{23,30,33,34} Furthermore, up to the present allergen characterisation has used sera from patients with gastroallergic anisakiasis, and it is possible that different proteins may be involved in occupational sensitisation through inhalation or skin contact. Allergen recognition is thought to vary significantly from patient to patient in *Anisakis* allergy, and patients may also recognise cross-reactive proteins from other invertebrates.^{23,30,33,34} Originally, authors used immuno-

Table I. Descriptive data of Anisakis-sensitised workers whose sera were investigated by immunoblotting

| Worker | Symptoms | Non-specific broncho-hyper responsiveness (NSBH) | Anisakis ImmunoCAP (kU/l) | Anisakis SPT | Ascaris ImmunoCAP (kU/l) | Sensitisation to Anisakis tropomyosin (Ani s 3) on microarray* | Other sensitisations (microarray, ImmunoCAP* or SPT) |
|--------|---|--|---------------------------|--------------|--------------------------|--|--|
| 1 | None | No | 1.4 | - | 0 | - | None |
| 2 | None | No | 10.4 | + | 0.4 | Not tested | ImmunoCAP: lobster, pilchard |
| 3 | None | No | 3.6 | + | 0.5 | - | ImmunoCAP: latex, lobster SPT: HDM, dog, cockroach Microarray: Api m 1, Cup a 1, Lol p 1 |
| 4 | None | Not tested | 2.4 | + | 0 | - | SPT: HDM |
| 5 | Work-related chest and skin symptoms | Yes | 3.6 | - | 4 | - | SPT: HDM |
| 6 | Work-related ocular-nasal symptoms Seafood allergy (lobster, mussels), reactions after ingestion | Yes | 2.8 | + | 5.1 | + | ImmunoCAP: lobster SPT: HDM, cockroach Microarray: Api m 1, Gal d 4, Pen i 1, Pen m 1, Per a 7, Der p 10, Hel as 1 |
| 7 | Work-related chest symptoms | Yes | 4.8 | + | 8 | - | SPT: HDM, cockroach |
| 8 | Work-related ocular-nasal symptoms | No | 2.5 | + | 1.9 | - | ImmunoCAP: lobster (low - 0.58kU/L) |
| 9 | No | Yes | 2.1 | + | 4.2 | - | ImmunoCAP: latex, lobster, anchovy, pilchard SPT: cockroach Microarray: Api m 1, Cup a 1, Lol p 1, Ole e 1 |
| 10 | Work-related chest symptoms | Not tested | 2 | + | 4.1 | + | ImmunoCAP: latex, lobster SPT: HDM, cockroach, ryegrass, raw lobster, Aspergillus Microarray: Api m 1, Lol p 1, Pen i 1, Per a 7, Pen m 1, Phl p 1, Der p 10, Hel as 1 |
| 11 | No | Yes | 2.4 | + | 23 | - | Microarray: Bos d 7, Der f 2 |
| 12 | Work-related ocular-nasal and chest symptoms | No | 3.7 | + | 61.3 | - | ImmunoCAP: lobster SPT: HDM |
| 13 | No | Yes | 0 | + | 0 | - | None |
| 14 | Work-related chest symptoms | No | 0 | + | 0.9 | - | SPT: cat, dog |
| 15 | Work-related chest symptoms | Yes | 0 | + | 0 | - | None |

* A value greater than 0.35 kU/l was considered positive.
SPT - skin-prick test, HDM - house-dust mite.

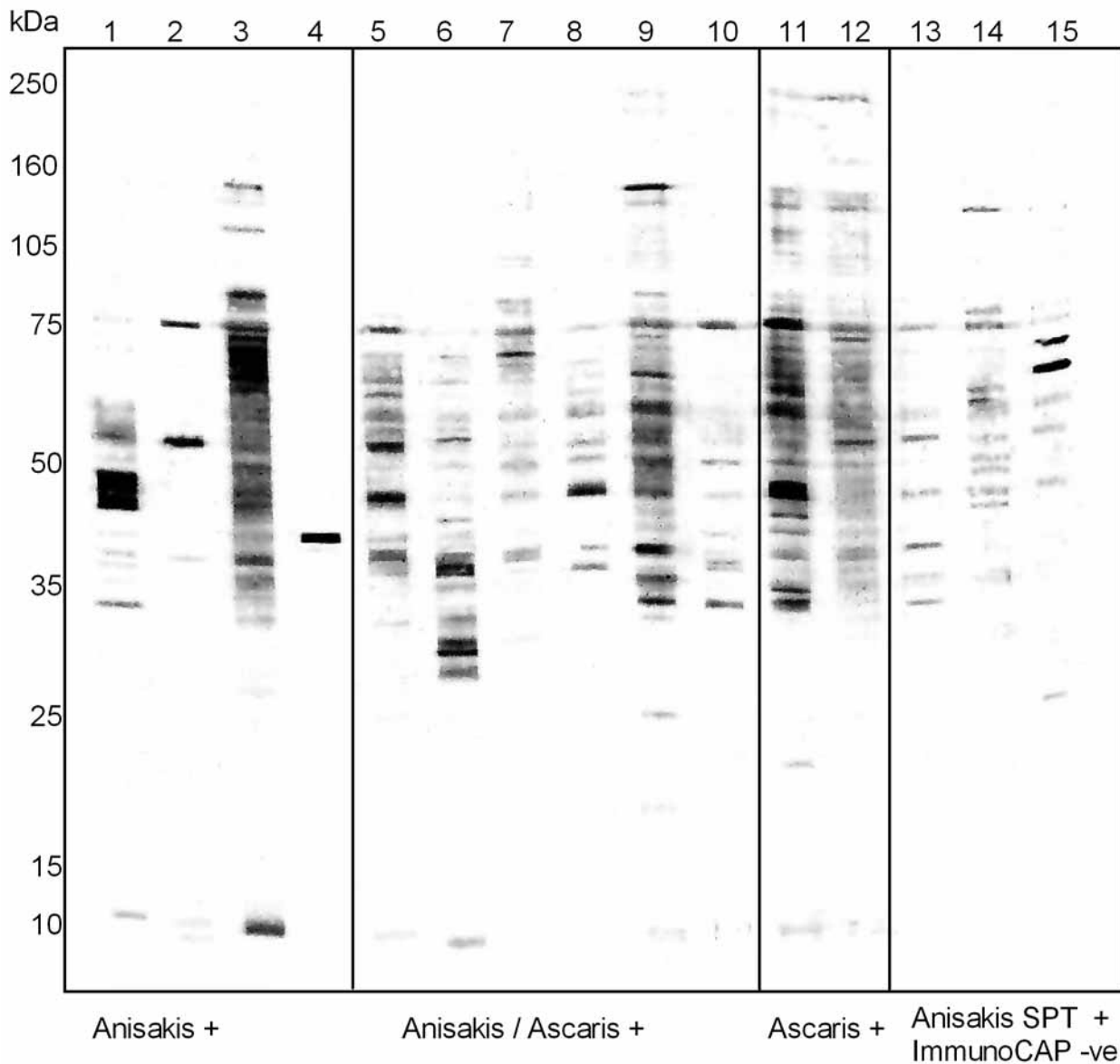


Fig. 2. IgE immunoblotting against *Anisakis* antigens using sera from 15 *Anisakis*-sensitised fish-processing workers. Workers 1-4 had higher specific IgE to *Anisakis* than to *Ascaris*, workers 5-10 had specific IgE to both *Anisakis* and *Ascaris*, workers 11-12 had higher levels of specific IgE to *Ascaris* than to *Anisakis* and workers 13-15 were SPT positive to *Anisakis* but negative on ImmunoCAP tests.

blotting with deglycosylated *Anisakis* proteins or excretory-secretory (ES) proteins to increase the specificity of *Anisakis* diagnosis.^{24,33} However, to avoid misdiagnosis due to cross-reactivity, it is ideally better to use purified or recombinant allergens that are specific for *Anisakis*-allergic patients.³⁴ The identification of specific *Anisakis* allergens which could be used in tests such as ImmunoCAP, SPT, allergen microarray or immunoblotting will in the long term increase the specificity of diagnosis.

ANISAKIS ALLERGENS

Currently nine allergens of *Anisakis simplex* have been identified, most of which exist in recombinant form. Patients may be exposed primarily to somatic antigens from dead larvae in food, ES antigens when there is expulsion or surgical removal of the intact larvae, or both, in cases where the larva penetrates the tissue, is killed by the host, and subsequently degenerates inside the host.³⁵ Many allergens of *Anisakis* are heat and/or pepsin resistant^{9,36,37} and most of them are present in ES products.

The major allergens of *Anisakis* (recognised by more than 50% of patients) are considered to be Ani s 1 and Ani s 7,³⁸ although in one study Ani s 5 was recognised by 49% of patients (41/84). The 24 kDa Ani s 1 is recognised by 67-87% of patients with gastroallergic anisakiasis and is not detected by asymptomatic individuals.^{23,39} This allergen is secreted by the worm and shows homology to serine protease inhibitors. A 21 kDa isoform of Ani s 1 also exists.³⁹ Ani s 1 is heat stable and can act as a food allergen, causing reactions after ingestion of cooked fish. The other major allergen, Ani s 7, is also an ES product of 139 kDa and is a novel glycoprotein.⁴⁰ It was recognised by 100% of patients with *Anisakis* allergy.⁴⁰ However, Ani s 7 has cross-reactive O-glycans and is better for diagnostic tests when deglycosylated.⁴¹

Another important allergen is Ani s 4, a heat-stable nematode cystatin that is recognised by only 27-30% of patients but appears to be particularly important in eliciting anaphylaxis.⁹ Heat-stable allergens such as Ani s 4 are important even if they are classified as minor allergens as a result of their frequency of recognition, because these allergens are associated with allergic

Table II. *Anisakis* allergens

| Allergen | Molecular weight | Description | Location | Recognition in <i>Anisakis</i> -sensitised patients | Recombinant protein exists | References |
|------------------------|--------------------------|--|---|---|--|---|
| Ani s 1 | 24 kDa 21 kDa isoform | Kunitz-type serine protease inhibitor like Heat stable | Excretory gland, secreted (ES products) | 86% (42/49) 88% (7/8) 67% (56/84) | Yes | Moneo <i>et al.</i> , ²³ Caballero <i>et al.</i> , ³⁶ Shimakura <i>et al.</i> ³⁹ |
| Ani s 2 | 100 kDa | Paramyosin | Muscle | 88% (23/26) 23% (6/26) (r Ani s 2) | Yes | Perez-Perez <i>et al.</i> ⁴⁴ |
| Ani s 3 | 41 kDa | Tropomyosin | Muscle | 13% (8/62) patients with specific IgE 0% (0/10) patients with true <i>Anisakis</i> allergy | Yes | Asturias <i>et al.</i> ²⁰ |
| Ani s 4 | 9 kDa | Nematode cystatin (cysteine protease inhibitor) Heat-stable | Excretory gland and underneath the cuticle in L3 ES product | 27% (8/30) 22% (6/27) 30% (25/84) | Yes | Moneo <i>et al.</i> ⁹ Caballero <i>et al.</i> , ³⁶ Caballero & Moneo, ³⁷ Roderiguez-Mahillo <i>et al.</i> ⁴⁶ |
| Ani s 5 | 15 kDa | Homologous with nematode proteins in the SXP/RAL-2 family Heat resistant | Excretory gland, ventriculus and luminal surface of the intestinal epithelium ES protein | 49% (41/84) 25% (7/28) to r Ani s 5 | Yes | Kobayashi <i>et al.</i> , ³⁴ Caballero <i>et al.</i> , ³⁶ |
| Ani s 6 | 7 kDa | Serine protein inhibitor | ES products | 18% (5/28) to rAni s 6 | Yes | Kobayashi <i>et al.</i> ³⁴ |
| Ani s 7 | 139 kDa | Novel protein. (glycoprotein) | ES products | 100% (60/60) | No – but a recombinant fragment exists | Anadon <i>et al.</i> , ³⁸ Rodriguez <i>et al.</i> ⁴⁰ |
| Ani s 8 | 15 kDa | Heat stable SPX/RAL protein Homologous with proteins in the SXP/RAL-2 family, including Ani s 5 | ES products | 25% (7/28) | Yes | Kobayashi <i>et al.</i> ⁴³ |
| Ani s 9 | 14 kDa | Belongs to SXP/RAL-2 family Heat and pepsin resistant | ES products and crude extract | 13.8% (5/36) | Yes | Rodriguez-Perez <i>et al.</i> ⁴² |
| Troponin-like allergen | 21 kDa | Homology to nematode troponins | Muscle | 20% | Yes | Arrieta <i>et al.</i> ⁴⁵ |

ES – excretory-secretory

reactions to cooked or canned fish.⁴² Therefore, frequency of recognition is not always equal to clinical relevance. Other minor allergens include Ani s 5 (15 kDa), Ani s 8 (15 kDa) and Ani s 9 (14 kDa), which share homology and are all members of the SPX/RAL-2 family, which is specific to nematodes. They are all heat-stable ES products, although Ani s 9 is reportedly more abundant in crude extract, and their biological function is unknown.^{36,42,43} Another minor allergen, Ani s 6 (7 kDa), is homologous with serine protease inhibitors, including the honeybee allergen Api m 6.³⁴

The remaining two allergens, Ani s 2 (41 kDa) and Ani s 3 (100 kDa) are the muscle proteins paramyosin and tropomyosin, respectively, and are thought to be primarily responsible for cross-reactivity between *Anisakis* and other invertebrates.^{20,25,44} They do not appear to be important in eliciting allergic reactions to *Anisakis*,^{20,38} but further studies are needed. A 21 kDa protein with homology to nematode troponin has also been identified as an allergen but has never been named.⁴⁵

Purified *Anisakis* allergens have proven useful in diagnosis, especially in combination. In one study, 95% of 64 *Anisakis*-allergic patients tested positive for Ani s 1 and/or Ani s 4 by immunoblotting⁴⁷ and in a follow-up study, only 12% of patients (10/84) did not recognise one or both of these allergens.³⁶ Including Ani s 5 to the panel of allergens tested raised the sensitivity to 94%, with 79/84 patients recognising one or more of the three allergens.

Table II lists the nine *Anisakis* allergens.

APPROACH TO THE DIAGNOSIS AND MANAGEMENT OF ANISAKIS ALLERGY

The ideal diagnostic test for *Anisakis* allergy should include all clinically relevant *Anisakis* allergens. Currently, CAP-RAST and SPTs use whole *Anisakis* extracts, while the latest allergen microarrays only contain Ani s 1 and Ani s 3. Once a patient has confirmed *Anisakis* allergy, after excluding fish allergy and taking into consideration cross-reactivity to other helminths (e.g. *Ascaris*) or invertebrates such as dust mites, cockroaches and shrimp, identifying which allergens are recognised by the patient will assist in making dietary recommendations.⁷ Many patients with *Anisakis* allergy are able to tolerate a diet of frozen or well-cooked fish,⁴⁸ but a small percentage of patients are particularly sensitised to heat-stable allergens such as Ani s 4 and react badly to cooked or canned fish.^{2,9,33} These patients should avoid marine fish altogether.

Declaration of conflict of interest

The authors declare no conflicts of interest.

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REFERENCES

- Sakanari JA, McKerrrow JH. Anisakiasis. *Clin Microbiol Rev* 1989; 2: 278-284.
- Audicana MT, Ansoategui U, de Corres LF, Kennedy MW. *Anisakis simplex*: dangerous – dead and alive? *Trends Parasitol* 2002; 18: 20-25.
- Moore DA, Girdwood RW, Chiodini PL. Treatment of anisakiasis with albendazole. *Lancet* 2002; 360: 54.
- Pacios E, Arias-Diaz J, Zuloaga J, Gonzalez-Armengol J, Villarreal P, Balibrea JL. Albendazole for the treatment of anisakiasis ileus. *Clin Infect Dis* 2005; 41: 1825-1826.
- Baeza ML, Zubeldia JM, Rubio M. *Anisakis simplex* allergy. *ACI International* 2001; 13: 242-249.
- Nieuwenhuizen N, Lopata AL, Jeebhay MF, Herbert DR, Robins TG, Brombacher F. Exposure to the fish parasite *Anisakis* causes allergic airway hyperreactivity and dermatitis. *J Allergy Clin Immunol* 2006; 117: 1098-1105.
- Daschner A, Alonso Giqu A, Cabanas R, Suarez-de-Parga JM, MC Liqm-S. Gastroallergic anisakiasis: borderline between food allergy and parasitic disease – clinical and allergologic evaluation of 20 patients with confirmed acute parasitism by *Anisakis simplex*. *J Allergy Clin Immunol* 2000; 105: 176-181.
- Audicana MT, Fernandez de Corres L, Munoz D, Fernandez E, Navarro JA, del Pozo MD. Recurrent anaphylaxis caused by *Anisakis simplex* parasitizing fish. *J Allergy Clin Immunol* 1995; 96: 558-560.
- Moneo I, Caballero ML, Gonzalez-Munoz M, Rodriguez-Mahillo AI, Rodriguez-Perez R, Silva A. Isolation of a heat-resistant allergen from the fish parasite *Anisakis simplex*. *Parasitol Res* 2005; 96: 285-289.
- Audicana L, Audicana MT, Fernandez de Corres L, Kennedy MW. Cooking and freezing may not protect against allergenic reactions to ingested *Anisakis simplex* antigens in humans. *Vet Rec* 1997; 140: 235.
- Fernandez de Corres L, Audicana M, et al. *Anisakis simplex* induces not only anisakiasis: report on 28 cases of allergy caused by this nematode. *J Investig Allergol Clin Immunol* 1996; 6: 315-319.
- Del Pozo MD, Audicana M, Diez JM, et al. *Anisakis simplex*, a relevant etiologic factor in acute urticaria. *Allergy* 1997; 52: 576-579.
- Montoro A, Perteguer MJ, Chivato T, Laguna R, Cuellar C. Recidivous acute urticaria caused by *Anisakis simplex*. *Allergy* 1997; 52: 985-991.
- Daschner A, Vega de la Osada F, Pascual CY. Allergy and parasites reevaluated: wide-scale induction of chronic urticaria by the ubiquitous fish-nematode *Anisakis simplex* in an endemic region. *Allergol Immunopathol (Madr)* 2005; 33: 31-37.
- Kasuya S, Hamano H, Izumi S. Mackerel-induced urticaria and *Anisakis*. *Lancet* 1990; 335: 665.
- Armentia A, Lombardero M, Callejo A, et al. Occupational asthma by *Anisakis simplex*. *J Allergy Clin Immunol* 1998; 102: 831-834.
- Scala E, Giani M, Pirrotta L, et al. Occupational generalised urticaria and allergic airborne asthma due to *Anisakis simplex*. *Eur J Dermatol* 2001; 11: 249-250.
- Carretero Anibarro P, Blanco Carmona J, Garcia Gonzalez F, et al. Protein contact dermatitis caused by *Anisakis simplex*. *Contact Dermatit* 1997; 37: 247.
- Anibarro B, Seoane FJ. Occupational conjunctivitis caused by sensitization to *Anisakis simplex*. *J Allergy Clin Immunol* 1998; 102: 331-332.
- Asturias JA, Eraso E, Moneo I, Martinez A. Is tropomyosin an allergen in *Anisakis*? *Allergy* 2000; 55: 898-899.
- Kennedy MW, Tierney J, Ye P, et al. The secreted and somatic antigens of the third stage larva of *Anisakis simplex*, and antigenic relationship with *Ascaris suum*, *Ascaris lumbricoides*, and *Toxocara canis*. *Mol Biochem Parasitol* 1988; 31: 35-46.
- Pascual CY, Crespo JF, San Martin S, et al. Cross-reactivity between IgE-binding proteins from *Anisakis*, German cockroach, and chironomids. *Allergy* 1997; 52: 514-520.
- Moneo I, Caballero ML, Gomez F, Ortega E, Alonso MJ. Isolation and characterization of a major allergen from the fish parasite *Anisakis simplex*. *J Allergy Clin Immunol* 2000; 106: 177-182.
- Moneo I, Audicana MT, Alday E, Curiel G, del Pozo MD, Garcia M. Periodate treatment of *Anisakis simplex* allergens. *Allergy* 1997; 52: 565-569.
- Guarneri F, Guarneri C, Benvenega S. Cross-reactivity of *Anisakis simplex*: possible role of Ani s 2 and Ani s 3. *Int J Dermatol* 2007; 46: 146-50.
- Garcia M, Moneo I, Audicana MT, et al. The use of IgE immunoblotting as a diagnostic tool in *Anisakis simplex* allergy. *J Allergy Clin Immunol* 1997; 99: 497-501.
- Del Pozo MD, Moneo I, de Corres LF, et al. Laboratory determinations in *Anisakis simplex* allergy. *J Allergy Clin Immunol* 1996; 97: 977-984.
- Du Plessis K, Lopata AL, Steinman H. Adverse reactions to fish. *Current Allergy & Clinical Immunology* 2004; 17: 4-8.
- Jeebhay MF, Robins TG, Miller ME, et al. Occupational allergy and asthma among salt water fish processing workers. *Am J Ind Med* 2008; 51: 899-910.
- Arlian LG, Morgan MS, Quirce S, Maranon F, Fernandez-Caldas E. Characterization of allergens of *Anisakis simplex*. *Allergy* 2003; 58: 1299-1303.
- Blaxter ML, de Ley P, Garey JR, et al. A molecular evolutionary framework for the phylum Nematoda. *Nature* 1998; 392: 71-75.
- Sakanari JA, Loinaz HM, Deardorff TL, Raybourne RB, McKerrrow JH, Frierson JG. Intestinal anisakiasis. A case diagnosed by morphologic and immunologic methods. *Am J Clin Pathol* 1988; 90: 107-113.

33. Baeza ML, Rodriguez A, Matheu V, *et al.* Characterization of allergens secreted by *Anisakis simplex* parasite: clinical relevance in comparison with somatic allergens. *Clin Exp Allergy* 2004; 34: 296-302.
34. Kobayashi Y, Ishizaki S, Shimakura K, Nagashima Y, Shiomi K. Molecular cloning and expression of two new allergens from *Anisakis simplex*. *Parasitol Res* 2007; 100: 1233-1241.
35. Audicana MT, Kennedy MW. *Anisakis simplex*: from obscure infectious worm to inducer of immune hypersensitivity. *Clin Microbiol Rev* 2008; 21: 360-379, table of contents.
36. Caballero ML, Moneo I, Gomez-Aguado F, Corcuera MT, Casado I, Rodriguez-Perez R. Isolation of Ani s 5, an excretory-secretory and highly heat-resistant allergen useful for the diagnosis of *Anisakis* larvae sensitization. *Parasitol Res* 2008; 103: 1231-1233.
37. Caballero ML, Moneo I. Several allergens from *Anisakis simplex* are highly resistant to heat and pepsin treatments. *Parasitol Res* 2004; 93: 248-251.
38. Anadon AM, Romaris F, Escalante M, *et al.* The *Anisakis simplex* Ani s 7 major allergen as an indicator of true *Anisakis* infections. *Clin Exp Immunol* 2009; 156: 471-478.
39. Shimakura K, Miura H, Ikeda K, *et al.* Purification and molecular cloning of a major allergen from *Anisakis simplex*. *Mol Biochem Parasitol* 2004; 135: 69-75.
40. Rodriguez E, Anadon AM, Garcia-Bodas E, *et al.* Novel sequences and epitopes of diagnostic value derived from the *Anisakis simplex* Ani s 7 major allergen. *Allergy* 2008; 63: 219-225.
41. Iglesias R, Leiro J, Santamarina MT, Sanmartin ML, Ubeira FM. Monoclonal antibodies against diagnostic *Anisakis simplex* antigens. *Parasitol Res* 1997; 83: 755-761.
42. Rodriguez-Perez R, Moneo I, Rodriguez-Mahillo A, Caballero ML. Cloning and expression of Ani s 9, a new *Anisakis simplex* allergen. *Mol Biochem Parasitol* 2008; 159: 92-97.
43. Kobayashi Y, Shimakura K, Ishizaki S, Nagashima Y, Shiomi K. Purification and cDNA cloning of a new heat-stable allergen from *Anisakis simplex*. *Mol Biochem Parasitol* 2007; 155: 138-145.
44. Perez-Perez J, Fernandez-Caldas E, Maranon F, *et al.* Molecular cloning of paramyosin, a new allergen of *Anisakis simplex*. *Int Arch Allergy Immunol* 2000; 123: 120-129.
45. Arrieta I, del Barrio M, Vidarte L, *et al.* Molecular cloning and characterization of an IgE-reactive protein from *Anisakis simplex*: Ani s 1. *Mol Biochem Parasitol* 2000; 107: 263-268.
46. Rodriguez-Mahillo AI, Gonzalez-Munoz M, Gomez-Aguado F, *et al.* Cloning and characterisation of the *Anisakis simplex* allergen Ani s 4 as a cysteine-protease inhibitor. *Int J Parasitol* 2007; 37: 907-917.
47. Moneo I, Caballero ML, Rodriguez-Perez R, Rodriguez-Mahillo AI, Gonzalez-Munoz M. Sensitization to the fish parasite *Anisakis simplex*: clinical and laboratory aspects. *Parasitol Res* 2007; 101: 1051-1055.
48. Garcia F, Blanco JG, Garces M, Juste S, Fuentes M, Herrero D. Freezing protects against allergy to *Anisakis simplex*. *J Investig Allergol Clin Immunol* 2001; 11: 49-52.



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