APPROACHES TO DIAGNOSING ANISAKIS ALLERGY

Natalie Nieuwenhuizen, BSc(Med) Hons, PhD
Mohamed Jeebhay, MBChB, DOH, MPhil (Epi), MPhil (Occ Med), PhD
Andreas L Lopata, MSc, PhD (Med Science)

1 Division of Immunology, Institute of Infectious Diseases and Molecular Medicine, Faculty of Health Science, University of Cape Town, South Africa
2 Centre for Occupational and Environmental Health Research, School of Public Health and Family Medicine, University of Cape Town, South Africa
3 Allergy Research Group, School of Applied Science, Royal Melbourne Institute of Technology, Bundoora West Campus, Melbourne, Australia

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. 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.

The management of anisakiasis involves physically removing the larvae, if possible, or treating the patient with antihelminthics, anti-inflammatories and analgesics. 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. 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. 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. Many patients experience gastroallergic anisakiasis, in which infection...
is accompanied by allergic reactions such as urticaria, angio-oedema, bronchospasm and/or anaphylaxis. This allergic response can occur without gastrointestinal symptoms, leading to misdiagnosis of the reaction to Anisakis as fish allergy or idiopathic urticaria/anaphylaxis. 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 the symptoms.

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). 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. 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. 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.

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. 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.

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. 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. 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. Only one case of food allergy to Anisakis has been documented in South Africa 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. 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, but only 1-3% had Anisakis-related allergic symptoms. The study 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. 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. 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. 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. 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. 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. Originally, authors used immuno-
Table I. Descriptive data of Anisakis-sensitised workers whose sera were investigated by immunoblotting

<table>
<thead>
<tr>
<th>Worker</th>
<th>Symptoms</th>
<th>Non-specific broncho-hyper responsiveness (NSBH)</th>
<th>Anisakis ImmunoCAP (kU/l)</th>
<th>Anisakis SPT</th>
<th>Ascaris ImmunoCAP (kU/l)</th>
<th>Sensitisation to Anisakis tropomyosin (Ani s 3) on microarray*</th>
<th>Other sensitisations (microarray, ImmunoCAP* or SPT)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>None</td>
<td>No</td>
<td>1.4</td>
<td>-</td>
<td>0</td>
<td>-</td>
<td>None</td>
</tr>
<tr>
<td>2</td>
<td>None</td>
<td>No</td>
<td>10.4</td>
<td>+</td>
<td>0.4</td>
<td>Not tested</td>
<td>ImmunoCAP: lobster, pilchard</td>
</tr>
<tr>
<td>3</td>
<td>None</td>
<td>No</td>
<td>3.6</td>
<td>+</td>
<td>0.5</td>
<td>-</td>
<td>ImmunoCAP: latex, lobster</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Microarray: lobster, pilchard, dog, cockroach</td>
<td>SPT: HDM</td>
</tr>
<tr>
<td>4</td>
<td>None</td>
<td>Not tested</td>
<td>2.4</td>
<td>+</td>
<td>0</td>
<td>-</td>
<td>SPT: HDM</td>
</tr>
<tr>
<td>5</td>
<td>Work-related chest and skin symptoms</td>
<td>Yes</td>
<td>3.6</td>
<td>-</td>
<td>4</td>
<td>-</td>
<td>SPT: HDM</td>
</tr>
<tr>
<td>6</td>
<td>Work-related ocular-nasal symptoms</td>
<td>Yes</td>
<td>2.8</td>
<td>+</td>
<td>5.1</td>
<td>+</td>
<td>ImmunoCAP: lobster, SPT: HDM, cockroach</td>
</tr>
<tr>
<td></td>
<td>Seafood allergy (lobster, mussels), reactions after ingestion</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Microarray: Api m 1, Gal d 4, Pen i 1, Pen m 1, Per a 7, Der p 10, Hel as 1</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>Work-related chest symptoms</td>
<td>Yes</td>
<td>4.8</td>
<td>+</td>
<td>8</td>
<td>-</td>
<td>SPT: HDM, cockroach</td>
</tr>
<tr>
<td>8</td>
<td>Work-related ocular-nasal symptoms</td>
<td>No</td>
<td>2.5</td>
<td>+</td>
<td>1.9</td>
<td>-</td>
<td>ImmunoCAP: lobster, low – 0.58kU/L</td>
</tr>
<tr>
<td>9</td>
<td>No</td>
<td>Yes</td>
<td>2.1</td>
<td>+</td>
<td>4.2</td>
<td>-</td>
<td>ImmunoCAP: latex, lobster, anchoy, pilchard, SPT: cockroach</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Microarray: Api m 1, Cup a 1, Lol p 1, Ole e 1</td>
<td>ImmunoCAP: latex, lobster</td>
</tr>
<tr>
<td>10</td>
<td>Work-related chest symptoms</td>
<td>Not tested</td>
<td>2.0</td>
<td>+</td>
<td>4.1</td>
<td>+</td>
<td>SPT: HDM, cockroach, ryegrass, raw lobster, Aspergillus</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Microarray: Api m 1, Lol p 1, Pen i 1, Pen m 1, Per a 7, Phi p 1, Der p 10, Hel as 1</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>No</td>
<td>Yes</td>
<td>2.4</td>
<td>+</td>
<td>23</td>
<td>-</td>
<td>Microarray: Bos d 7, Der f 2</td>
</tr>
<tr>
<td>12</td>
<td>Work-related ocular-nasal and chest symptoms</td>
<td>No</td>
<td>3.7</td>
<td>+</td>
<td>61.3</td>
<td>-</td>
<td>ImmunoCAP: lobster, SPT: HDM</td>
</tr>
<tr>
<td>13</td>
<td>No</td>
<td>Yes</td>
<td>0</td>
<td>+</td>
<td>0</td>
<td>-</td>
<td>None</td>
</tr>
<tr>
<td>14</td>
<td>Work-related chest symptoms</td>
<td>No</td>
<td>0</td>
<td>+</td>
<td>0.9</td>
<td>-</td>
<td>SPT: cat, dog</td>
</tr>
<tr>
<td>15</td>
<td>Work-related chest symptoms</td>
<td>Yes</td>
<td>0</td>
<td>+</td>
<td>0</td>
<td>-</td>
<td>None</td>
</tr>
</tbody>
</table>

* A value greater than 0.35 kU/l was considered positive.  
SPT – skin-prick test, HDM – house-dust mite.
blotting with deglycosylated Anisakis proteins or excre-
tory-secretory (ES) proteins to increase the specificity
due to cross-reactivity, it is ideally better to use puri-
ified or recombinant allergens that are specific for
Anisakis-allergic patients. The identification of specif-
ic Anisakis allergens which could be used in tests such
as ImmunoCAP, SPT, allergen microarray or immuno-
blotting 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. Many allergens of Anisakis are heat
and/or pepsin resistant 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, although in one study Ani s 5 was recognised
by 49% of patients. The 24 kDa Ani s 1 is recog-
nised by 67-87% of patients with gastroallergic anisaki-
asis and is not detected by asymptomatic individuals. This allergen is secreted by the worm and shows homology to serine protease inhibitors. A 21 kDa isoform of Ani s 1 also exists. 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. It was recognised by 100% of patients with Anisakis allergy. However, Ani s 7 has cross-
reactive O-glycans and is better for diagnostic tests
when deglycosylated.

Another important allergen is Ani s 4, a heat-stable
cystatin that is recognised by only 27-30% of patients but appears to be particularly important in eliciting anaphylaxis. 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>
<thead>
<tr>
<th>Allergen</th>
<th>Molecular weight</th>
<th>Description</th>
<th>Location</th>
<th>Recognition in Anisakis-sensitised patients</th>
<th>Recombinant protein exists</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ani s 1</td>
<td>24 kDa</td>
<td>Kunitz-type serine protease inhibitor like</td>
<td>Excretory gland, secreted</td>
<td>86% (42/49)</td>
<td>Yes</td>
<td>Moneo et al.23</td>
</tr>
<tr>
<td></td>
<td>21 kDa isoform</td>
<td>Heat stable</td>
<td>(ES products)</td>
<td>88% (7/8)</td>
<td></td>
<td>Caballero et al.36</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>67% (56/84)</td>
<td></td>
<td>Shimakura et al.39</td>
</tr>
<tr>
<td>Ani s 2</td>
<td>100 kDa</td>
<td>Paramyosin</td>
<td>Muscle</td>
<td>88% (23/26)</td>
<td>Yes</td>
<td>Perez-Perez et al.44</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Heat stable (ES products)</td>
<td></td>
<td>23% (6/26) (r Ani s 2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ani s 3</td>
<td>41 kDa</td>
<td>Tropomyosin</td>
<td>Muscle</td>
<td>13% (8/62) patients with specific IgE</td>
<td>Yes</td>
<td>Asturias et al.20</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0% (0/10) patients with true Anisakis allergy</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ani s 4</td>
<td>9 kDa</td>
<td>Nematode cystatin cysteine protease inhibitor</td>
<td>Excretory gland and underneath the cuticle in L3 ES product</td>
<td>27% (8/30)</td>
<td>Yes</td>
<td>Moneo et al.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Heat-stable</td>
<td></td>
<td>22% (6/27)</td>
<td></td>
<td>Caballero et al.36</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>30% (25/84)</td>
<td></td>
<td>Rodriguez-Mahillo et al.46</td>
</tr>
<tr>
<td>Ani s 5</td>
<td>15 kDa</td>
<td>Homologous with nematode proteins in the SXP/RAL-2 family</td>
<td>Excretory gland, ventriculus and luminal surface of the intestinal epithelium ES protein</td>
<td>49% (41/84)</td>
<td>Yes</td>
<td>Kobayashi et al.34</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Heat resistant</td>
<td></td>
<td>25% (7/28) to r Ani s 5</td>
<td></td>
<td>Caballero et al.36</td>
</tr>
<tr>
<td>Ani s 6</td>
<td>7 kDa</td>
<td>Serine protein inhibitor</td>
<td>ES products</td>
<td>18% (5/28) to r Ani s 6</td>
<td>Yes</td>
<td>Kobayashi et al.34</td>
</tr>
<tr>
<td>Ani s 7</td>
<td>139 kDa</td>
<td>Novel protein. (glycoprotein)</td>
<td>ES products</td>
<td>100% (60/60)</td>
<td>No – but a recombinant fragment exists</td>
<td>Anadon et al.38</td>
</tr>
<tr>
<td>Ani s 8</td>
<td>15 kDa</td>
<td>Heat stable SPX/RAL protein</td>
<td>ES products</td>
<td>25% (7/28)</td>
<td>Yes</td>
<td>Rodriguez et al.40</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Homologous with proteins in the SXP/RAL-2 family, including Ani s 5</td>
<td></td>
<td></td>
<td></td>
<td>Kobayashi et al.43</td>
</tr>
<tr>
<td>Ani s 9</td>
<td>14 kDa</td>
<td>Belongs to SXP/RAL-2 family</td>
<td>ES products and crude extract</td>
<td>13.8% (5/36)</td>
<td>Yes</td>
<td>Rodriguez-Perez et al.48</td>
</tr>
<tr>
<td>Troponin-like allergen</td>
<td>21 kDa</td>
<td>Homology to nematode troponins</td>
<td>Muscle</td>
<td>20%</td>
<td>Yes</td>
<td>Arrieta et al.45</td>
</tr>
</tbody>
</table>
reactions to cooked or canned fish. 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. Another minor allergen, Ani s 6 (7 kDa), is homologous with serine protease inhibitors, including the honeybee allergen Api m 6.

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. They do not appear to be important in eliciting allergic reactions to Anisakis, but further studies are needed. A 21 kDa protein with homology to nematode tropomyosin has also been identified as an allergen but has never been named.

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 immunoblotting and in a follow-up study, only 12% of patients (10/84) did not recognise one or both of these allergens. 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 currently 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. Many patients with Anisakis allergy are able to tolerate a diet of frozen or well-cooked fish, 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. These patients should avoid marine fish altogether.

### Declaration of conflict of interest

The authors declare no conflicts of interest.

### Acknowledgements

This work was sponsored by the Medical Research Council (MRC) and National Research Foundation of South Africa and an Allergy Society of South Africa (ALLASA) research award.

### REFERENCES


**ALLSA CONGRESS 2010**

**ALLERGY IN THE BUSH**

**23 – 25 APRIL**

**LIMPOPO PROVINCE**

**FIRST ANNOUNCEMENT**

For further information contact:

The Congress Office
Sue McGuinness Communications & Event Management
PO Box 782243, Sandton, 2146,
Johannesburg South Africa
Telephone: +27 (0)11 447 3876
Fax: +27 (0)11 442 8094
Email: suemc@icon.co.za
www.allergyza.co.za