
Anisakis simplex-sensitized patients: should fish be excluded from their diet?

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Background: *Anisakis simplex* (A.s.) allergy is an emerging disease. The third-stage larvae of this nematode are a source of hidden allergens in fish. There are no clear guidelines concerning dietary restrictions for patients with serum-specific IgE to this parasite.

Objective: To follow up the clinical data and immunological parameters of patients sensitized to A.s. during 6 to 23 months.

Methods: The clinical symptoms and serologic status of 17 patients with specific IgE and positive skin prick test results to A.s. were studied prospectively. Six of these had anaphylaxis (ANA) attributed to A.s. and 11 patients experienced concomitant chronic urticaria (CU). All patients were advised not to eat fish for 6 months.

Results: Four patients from the ANA group excluded fish, and ANA did not recur. Two other patients with ANA refused to exclude fish; one remained free of symptoms and the other experienced several urticarial episodes. During this 6-month period total IgE levels decreased in all six ANA patients; specific IgE for A.s. decreased in four patients and increased in two. Two patients from the CU group did not exclude fish, and symptoms persisted in these two patients. Clinical improvement was observed in 78% of the patients with CU who excluded fish. Total and specific IgE levels decreased in all the patients with CU.

Conclusions: Because ANA symptoms are very severe, patients should always be advised to exclude fish until specific food allergens are identified. However, in patients with CU and specific IgE to A.s., only the clinical response to fish ingestion will determine the need for strict fish avoidance.

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INTRODUCTION

The genus *Anisakis simplex* (A.s.) belongs to the Nematode class and to the Ascaridae order.¹ This parasite is distributed worldwide and inhabits the stomach of marine cetaceans. The eggs of *Anisakis* are excreted with the feces (first-stage larvae). After maturation in water (second-stage larvae), they are ingested by tiny crustaceans (first-intermediate host). These crustaceans are

ingested by a second intermediate host (fish or shrimp), and the parasite continues its development to third-stage larva. Fish and cephalopods are ingested by large marine mammals (definitive host), and in these the life cycle of the parasite is completed (adult stage).

Man may become an accidental host of A.s. third-stage larvae by ingestion of parasitized raw or undercooked fish. The parasitic infection of the gastrointestinal tract in man causes anisakiasis with its diverse clinical features. It may induce an acute digestive disease (nausea, vomiting, stomach pain, intestinal obstruction, or appendicitis)^{2,3} or occasional chronic symptoms.⁴ If specific IgE antibodies have developed in the host, as a result of one or more

previous contacts with the parasite, patients may also experience immediate hypersensitivity reactions.^{5–7} Larvae may not necessarily be alive to elicit these reactions, because the allergens of this nematode have been shown to be thermostable.^{8,9} The ratio of infested fish in the North Atlantic ocean is high,^{10,11} and countries where this parasite has been investigated have reported high rates of fish infestation. In the Paris area, A.s. parasitizes mainly herring (*Clupea harengus*; 82.5%) and hake (*Merluccius merluccius*; 88.5%)¹²; in Spain, 88% of *Micromessitius potausou* and 45% of hake may be infested.^{13,14} Ten percent of salmon sushi samples and 5% of mackerel sushi samples were infested with A.s. in 32 of 50 restaurants in the Seattle, WA area in 1994.¹⁵ However, the prevalence of human infestation by this parasite is unknown.

Diagnosis of A.s. infestation (anisakiasis) is based on clinical data: endoscopy and radiographs with contrast medium can show either direct or indirect observations of the parasite.^{16,17} The pathognomonic endoscopic finding of A.s. infestation is the actual visualization of the larvae attached to the gastric mucosa. Edematous and hypertrophied folds have been commonly found.^{3,18,19} Further, small areas of hemorrhage and a swollen fold surrounding the point of entry of the parasite and diffusely thickened folds, consisting mostly of a thickened submucosal layer are also seen.^{20,21} Sometimes, infestation is detected by macro- or microscopic analysis of the section after surgery.²² On the other hand, a diagnosis of A.s. sensitization is based, at present, on an allergic reaction after eating fish but with negative prick test

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results to fish, no specific IgE to fish, positive skin prick test (SPT) results to A.s., and specific IgE A.s. Standardized skin test preparations for A.s. (IPI and ALK-Abelló, Madrid, Spain) and specific IgE kits for A.s. by the CAP system (Pharmacia Diagnostics, Uppsala, Sweden) are available. There are no large studies regarding the reliability of these tests for A.s. and, as with other allergens, they just reflect the presence of specific IgE on mast cells or in the patient's serum.

Recently, there has been a progressive increase in the numbers of patients in whom allergy to A.s. has been diagnosed.^{6,22,23} We describe two different groups of patients with specific IgE to A.s. The first group includes patients who had anaphylaxis (ANA) after fish ingestion but tolerated fish on other occasions. They all had positive SPT results, elevated levels of specific IgE to A.s., and negative values to fish. Overall, the first group left little doubt as to the diagnosis. The second group includes patients with chronic urticaria (CU) in whom high levels of specific IgE to A.s. were detected. In Spain, 43% of patients with CU may have specific IgE to A.s.²⁴ However, the relationship between the clinical symptoms and A.s. are likely complex and to date unknown. We prospectively studied these patients for 6 months. To determine the actual importance of A.s. in the etiology, patients excluded fish from their diets in the CU group.

PATIENTS AND METHODS

Patients

A total of 17 patients with specific serum IgE for A.s. showed two different clinical pictures and were selected from our allergology clinic. The first group of six patients had suffered at least one anaphylactic episode associated with the ingestion of fish or shellfish; the second group of 11 patients had CU. Possible causes of symptoms, including sensitization to fish, were eliminated, and all the patients had negative skin test results and lacked specific IgE to fish.

Patients in the first group had ingested fish between 30 and 180 minutes before the anaphylactic episode. The protocol of the ANA study implemented at our clinic included: hemogram, biochemical analysis, 5-hydroxyindoloacetic acid levels, complement levels, total IgE, specific IgE against A.s., *Ascaris lumbricoides* (A.l.), and *Echinococcus granulosus* (E.g.), hydatidic serology, chest roentgenogram, and SPTs. In the second group, CU was studied using the same complementary exploratory techniques plus identification of the parasites in three samples of feces, levels of antinuclear antibodies, and serum immunocomplexes.

Skin Test

SPTs for A.s. (IPI, Madrid, Spain) for hake, cod, anchovy, prawns, and squid and for the fish implicated by each individual patient (C.B.F., S.A., LETI, Madrid, Spain) were performed on the volar side of the forearm in accordance with the procedure set by the subcommittee on skin test of the European Academy of Allergy and Clinical Immunology.²⁵ Saline solution and histamine hydrochloride (10 mg/mL) were used as negative and positive control tests, respectively. A SPT result was positive when the mean diameter of the wheal was 3 mm or larger than that observed with the negative control.

In Vitro Test

Total IgE was determined by CAP system IgE fluorescence enzyme immunoassay following the standard procedure (Pharmacia Diagnostics). Specific IgE against parasites (A.s., A.l., and E.g.) and the implicated seafood were measured by the CAP system. (Pharmacia Diagnostics) in accordance with the manufacturer's instructions. Results 0.35 kilounits (kU)/L were designated as positive.

Followup

Two visits were planned for each patient in the study. During the first visit, patients were advised to follow a fish-free and shellfish-free diet. The second visit was 6 months later and patients were clinically examined. Accurate in-

formation on each patient's current symptomatic status was obtained by a questionnaire used during an interview. Questionnaire items included compliance with fish avoidance and each patient's evaluation of their clinical condition regarding the intensity of their symptoms (asymptomatic, clinical improvement, or no improvement). On their second visit, serum specimens were again obtained, and levels of total and specific IgE were measured. Afterward, the patients returned to the clinic on a randomized basis during the following 6 to 23 months.

RESULTS

ANA Group

ANA caused by A.s. was diagnosed in six patients in this group (four women and two men). In 50% of the cases, the fish consumed was pickled anchovies. The patients ages ranged from 31 to 68 years (mean: 49 years). The complementary tests described showed negative or normal results. The SPT results were negative for the fish species and positive for A.s. The median value of total IgE at the beginning of the study was 1,443.5 kU/L (range: 252 to 5,040), and the median value of specific IgE for A.s. was 125.75 kU/L (range: 13.7 to 237). When the study began, fish was withdrawn from the diet. Table 1 shows the total and specific IgE levels to A.s. when the study began and after 6 months of fish avoidance. The specific IgE levels for A.l. and E.g. are also shown.

Despite initial medical advice, only four of these patients with ANA totally excluded fish from their diets over times ranging from 9 to 23 months. ANA symptoms did not recur in any of these four patients. After 6 months, the total IgE had decreased in all six patients by a mean of 52.3% (range: 49.9 to 1,378 kU/L). Specific IgE levels for A.s. decreased in three patients by 67.6% (range: 55.2 to 87.5), and in one patient, it increased by 59% (Fig 1). The evolution of patient 6 was remarkable. This patient had totally excluded fish from his diet and a diagnosis was

Table 1. Total and Specific IgE Values to Parasites in 17 Patients Sensitized to A.s.

Patient no.	Initial total IgE, kU/L	% variation of total IgE to A.s. after 6 mos	Initial A.s. IgE, kU/L	% variation* of specific IgE to A.s. after 6 Mos	<i>Ascaris</i> IgE, kU/L	<i>Echinococcus</i> IgE, kU/L	Fish avoidance
ANA							
1	2,600	-61.5	222	-61.5	6.25	<0.35	No
2*	1,193	-67.3†	77.5	+38†	8.19	0.37	No
3*	89.9	-44.4	11.2	-60.3	0.81	ND	Yes
4*	6,625	-77.8	237	+59.4	10	2.62	Yes
5*	252	-44.6	13.7	-87.5	0.49	<0.35	Yes
6*	1,694	-18.6	174	-55.2	1.25	ND	Yes
CU							
7*	253	-63.6	38.3	-64.2	0.52	0.38	Yes
8*	62.2	-33.3	13.8	-28.3	<0.35	ND	Yes
9*	96.4	-56.4	16.1	-51.2	1.08	<0.35	Yes
10*	265	-57.5	30.4	-71.4	<0.35	<0.35	Yes
11*	64	-25.1	1.36	-58	<0.35	ND	Yes
12	276	-34.4	41.4	-46.1	1.42	ND	Yes
13	68.7	-24.4	2.3	-33	0.46	ND	Yes
14	2,100	-32.8	164	-43.5	10	ND	Yes
15	567.5	-26.5	100	-64.9	<0.35	0.52	Yes
16	523.5	-41.7	57.5	-76.1	<0.35	<0.35	No
17	599	-70.5	97.5	-54.9	8.34	<0.35	No

* Asymptomatic after 6 months.

† Data 23 months later.

first made after an anaphylactic episode with simultaneous detection of A.s. in his stomach by endoscopy. One month later, his total and specific IgE levels increased by 30% but decreased again later. This patient excluded fish from his diet for 9 months and then

started to eat frozen fish and experienced no cutaneous or systemic symptoms. When he resumed eating fish, his total and specific IgE levels increased slightly during the first few months. However, these values decreased again during the next 3 months (Fig 1).

ANA symptoms did not recur in the two patients who did not exclude fish from their diets, although one of these (patient 1) did have sporadic symptoms of recurring urticaria. Later, this patient strictly excluded fish for 5 months but continued to have this symptoms. His total and specific IgE values decreased during the months without fish. The other patient remained asymptomatic, although he included many kinds of fish during the time of the study (he also ate pickled anchovies, the presumed causal agent of the ANA). His total IgE level decreased in a fashion similar to that recorded in the patients, but his specific IgE levels to A.s. increased by 38% 18 months after the ANA episode (Fig 1).

CU Group

There were 11 patients in the CU (seven women and four men) between 31 and 65 years of age (mean: 49 years). The general complementary tests and skin test results described previously showed normal or negative results. The SPT findings for hake, cod, an-

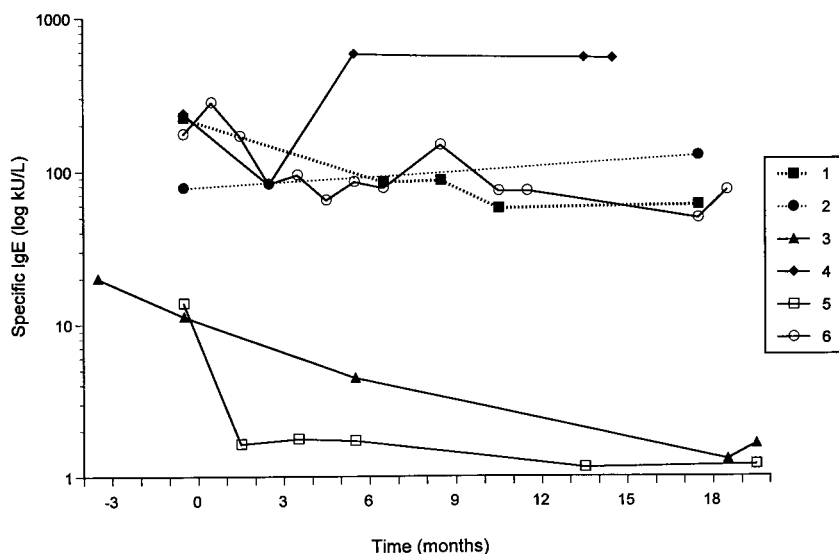


Figure 1. Levels of specific IgE to A.s. in patients with ANA during 6 to 21 months. Solid line, Patients who excluded fish from their diets; dotted line, patients who did not exclude fish.

chovy, prawn, and squid were all negative and the skin test result for *A.s.* was positive. The median value of total IgE at the beginning of the study was 265 kU/L (range: 62.2 to 1,396) and specific IgE was 35.1 kU/L (range: 1.36 to 196). This value was lower than that from patients in the ANA group. CU and sensitization to *A.s.* were diagnosed in the CU group.

In the CU group, nine patients totally excluded fish from their diets for 6 to 16 months. Hives disappeared in five of these patients and the symptoms persisted in another four. Two of the latter four showed clinical improvement with regard to frequency and intensity of CU symptoms and the need for medication (12 and 14, respectively). Total and specific IgE levels decreased after 6 months in all patients in a similar way (Fig 2).

In the CU group, the two patients who, despite medical advice, did not exclude fish from their diets did not show any change in symptoms. Six months later their total and specific IgE levels decreased and showed a pattern similar to that for the rest of the patients in the CU group who followed a fish-free diet (Fig 2). The values of total IgE and specific IgE for *A.s.*, *A.l.*, and *E.g.* are shown in Table 1.

The variation in total IgE values was proportional to the variation in *A.s.*-

specific IgE in the ANA and the CU groups. A statistical correlation was found between both parameters (Pearson's correlation: $r = 0.86$, $P < 0.05$). This value was calculated using the results of all patients ($n = 69$ data pairs), but excluding those of patient 4 (in the ANA group) who showed large oscillations in specific IgE serum levels (Fig 1). When this patient's data were included, the r value decreased to 0.59.

Reintroduction of Fish to the Diet in the CU Group

After 6 months, frozen or fresh fish ingestion was variable in all patients. Only six patients with CU (patients 9 and 11 to 15) began consuming fish again after several months of total avoidance. Patient 11 began to eat frozen fish after 6 months and fresh fish 2 months later but did not resume raw fish ingestion. As shown in Figure 3, the total and specific IgE levels of this patient decreased progressively until they were finally undetectable. However, this patient's symptoms returned 4 months after the reintroduction of fish and coincided with increased total IgE level and slightly increased specific IgE (0.45 kU/L). He excluded fish from his diet for a second time and became asymptomatic 2 months later. This was followed by a decrease in specific IgE to undetectable levels.

Two patients with CU (12 and 14) showed clinical improvement with avoidance of fish began eating fish again after 6 and 16 months, respectively. Nine and 4 months later, respectively, their symptoms remained unchanged, and no significant changes in their total or specific IgE levels were observed. (Fig 2). Patients 13 and 15 continued with the same symptoms despite total avoidance of fish. Patient 13 showed an increase in total IgE level (27%) after 12 months of a fish-free diet but a decrease in specific IgE level for *A.s.* Later, after following an unrestricted diet for 3 months, both parameters decreased (Fig 2), and the symptoms remained unchanged in both of patients. After 12 months, another asymptomatic (patient 9) resumed eating fish, and 3 months later he was still asymptomatic. The remaining three (patients 7, 8, and 10) did not resume eating fish and remained asymptomatic for times up to 23 months. Two other patients (patients 16 and 17) did not avoid fish ingestion.

DISCUSSION

The existence of the fish parasite *A.s.* has been known for many years, although its association with immediate hypersensitivity reactions is relatively recent.^{5,7,26} In the context of allergology, the importance of *A.s.* is still unknown, although it is currently under investigation. Infestation in man occurs with ingestion of fish that contain third-stage larvae of this parasite. These larvae are highly resistant to eradication states and are only destroyed by industrial freezing²⁷ or heating above 60°C.²⁸ Further, they are not killed by traditional preservation techniques such as soaking in vinegar, salting, or marinating. We analyzed the clinical and serologic data of 17 patients who initially had high levels of specific IgE against *A.s.*, and a positive skin test to *A.s.* but were not sensitive to fish. All 17 were included in a clinical and immunologic followup after excluding fish from their diets.

The first group contained patients with a symptoms of ANA due to *A.s.* In an earlier report, pickled anchovies were considered to be responsible for

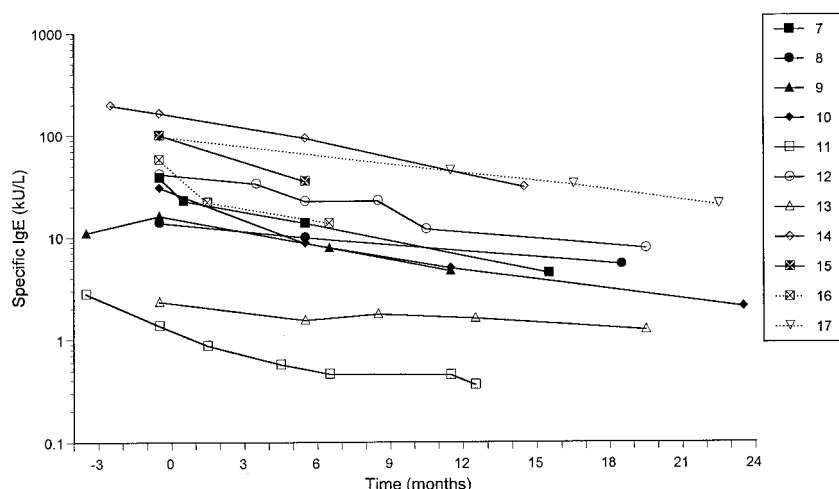


Figure 2. Levels of specific IgE to *A.s.* in patients with CU during 6 to 23 months. Solid line, patients who excluded fish from their diets; dotted line, patients who did not exclude fish.

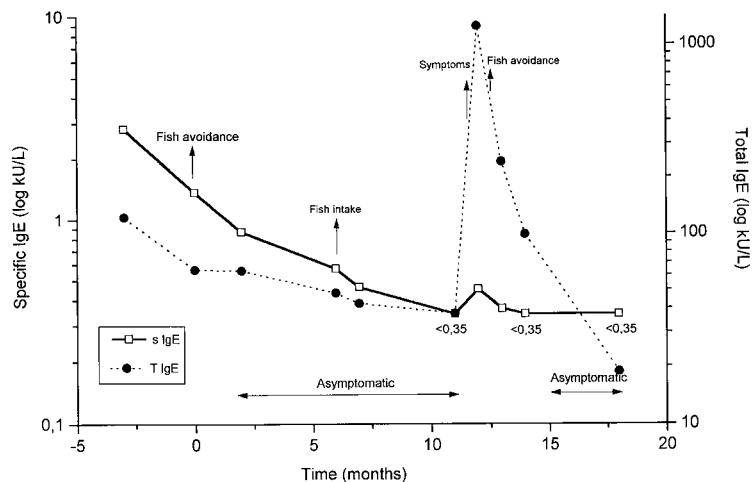


Figure 3. Levels of total (T) and specific (s) IgE to A.s. during 18 months in patient 11 (CU).

the anaphylactic reactions in a large proportion (50%) of patients.²⁹ This is a traditional Spanish dish in which the fish are not cooked but are marinated in vinegar for approximately 1 day. Contrary to medical advice, two patients in our study continued to eat fish, and their clinical reactivities were compared with those of the other four patients in this group who did not follow a fish-free diet. Remarkably, these two patients did not have new ANA episodes, despite the high percentage of fish infested with A.s. in our environment.^{13,14} These two patients did have generalized urticaria, angioedema, dyspnea, and throat swelling 30 to 60 minutes after eating pickled anchovies; their SPT results and specific IgE were positive to A.s. and negative to fish. For ethical reasons we did not perform oral challenges. The possibility that the anaphylactic episodes were not related to eating fish exists, but the clinical histories, SPT results, and elevated specific IgE levels were manifest. Perhaps real ingestion of the parasite is not frequent; perhaps the parasites are eliminated during washing or cooking or both. Alternatively, the antigens may not be as thermostable as it has been stated. The two patients who continued eating fish probably did not have contact with the parasite (ie, ate the tail). The larvae may need to be attached to the gastric

mucosa and thus contact the immune system, but this needs to be elucidated.

The total IgE levels decreased in all patients. The decrease in specific IgE was not homogeneous (Fig 1). However, most patients who totally avoided fish in their diets also showed a progressive decrease in this parameter. Only one patient's specific IgE level increased and showed unexpected large oscillations that did not correlate with any dietary habits (patient 4). Although this patient denied eating fish, unsuspected contact with A.s. may have been possible. Animals such as chickens that are fed fish flour may be a source of tiny amounts of this antigen. Eating chicken would therefore influence the specific IgE levels and eventually the clinical symptoms. One of the two patients, who continued to eat fish, also showed a decrease in specific IgE (patient 1), but the specific IgE increased in the other patient. Thus, the changes in the specific IgE may not be closely correlated with the clinical symptoms and fish consumption. Therefore, because of the difficulty in detecting this parasite in fish products and the severity of the symptoms, we still consider it advisable to avoid eating all kinds of fish or shellfish in patients with A.s.-induced ANA. In the future, other factors may be found that can predict the occur-

rence of allergic episodes on contact with the parasite.

The second group contained patients with CU of unknown etiology with a positive skin test result to A.s. and detectable specific IgE to A.s. Because they had negative results with the other allergens, CU and A.s. sensitization were diagnosed. No evidence of a causal relationship between these conditions has been reported. To study the relation between these two conditions (specific IgE to A.s. and CU), fish avoidance was started in this group. Five patients became asymptomatic, and symptoms subsided in two other patients (patients 12 and 14). However, the symptoms of two other patients who did not exclude fish from their diets remained unchanged. Although the group studied here is small, the clinical results suggest that sensitivity to A.s. may be related, as happens with other hidden antigens, to CU in some patients.³⁰ The CU may be associated with recurrent contacts with the parasite or with the presence of parasite remains forming granulomas in digestive mucosae for months and eventually causing appendicitis or bowel obstruction, as reported in the literature. Some authors have suggested that the parasite may remain alive as larva migrans for several weeks.^{8,31} In the CU group, A.s. has not always been found to be related to the symptoms. The followup of CU symptoms after fish avoidance is the only clue to evaluate the meaning of specific IgE in the context of this disease. Symptoms and parasite may be associated only if the symptoms disappear after fish avoidance. This association may never be proven unless patients follow a trial of fish exclusion for a few months.

Excluding fish from the diet can be highly troublesome, both because of family-associated problems and also because of the dietary benefits of this food, particularly in patients with hyperlipemia. However, we advise our patients with CU and specific IgE against A.s. to follow a fish-free diet trial for a few months. This time would assure the absence of any parasite remains in the bowel and allow symp-

toms to evolve. After 3 months, we advise them to reintroduce fish and then we investigate each patient for any causal relation between these two parameters.

Serial determinations of total and specific IgE in serum do not appear to be the most suitable indicators for changes in symptoms in some patients. The total IgE decreased in all patients in the CU group and, similarly to the ANA group, the specific IgE values were not closely related to either the clinical evolution or to the avoidance of fish. Interestingly, in some patients, such as 11, clinical progression, patterns of fish ingestion, and changes in specific IgE all seem to be closely related. Nevertheless, in other patients with even higher serum IgE levels, the ingestion of fish and the levels of specific IgE for A.s. do not appear to be related. However, two aspects of this correlation are interesting. The patient started eating fish and began having symptoms 2 to 3 months later. Perhaps this delay occurred because the patient was not exposed to A.s. during that time. Perhaps the fish he ate may not have been infested or the patient did not eat the parts of the fish where the parasite was located. If a patient inspects the fish before eating he probably avoids some parasites, but he may still eat tiny, well-hidden parasites. It is impossible to verify, but if the patient starts to experience symptoms and his total IgE increases at the same time as the specific IgE to A.s. increases, we suspect that contact with the parasite has occurred. Remarkably, the increase in this patient's specific IgE (patient 11) after he included fish and developed symptoms was small (markedly below previous levels when he was asymptomatic). This patient always had low levels of specific IgE, and this observation corroborates that absolute amount of specific IgE levels do not always correlate well with the symptom severity. In some patients, the presence of specific IgE to A.s. may elicit clinical symptoms and in others it may be only a proof of latent sensitization. Neither clinical nor analytical factors that could predict these differ-

ent behavior patterns were found. Many other factors such as cross-reactivity among various parasites³²⁻³⁵ could also interfere with total and specific IgE values.

At the time of diagnosis, patients with very similar clinical pictures showed wide ranges in their total and specific IgE levels. Although these values in the ANA patient group were statistically higher than in those in the CU patient group, specific IgE levels overlapped between the two groups. Thus, specific IgE levels alone may not predict the severity of the reaction.

This type of food allergy is difficult to evaluate because the determinant factors (quantity and quality of the food allergen ingested) are often unknown. We also do not know whether part of the specific IgE provides a defense mechanism for the organism against the parasite, because this is one of the functions traditionally attributed to this Ig,³⁶ and another part may attach to other epitopes and be associated with allergic symptoms. Although some patients are highly sensitive to A.s., they still eat fish without any problem. Could this be due to the handling and washing of the fish? What conditions does this antigen require to relate to the immune system? Does the parasite need to attach itself to the mucosa to make the antigen accessible? Could tolerance against the antigens of this parasite be developed? Is there a determinant balance of IgE and other Igs for symptoms to appear? There are still many questions to be answered.

Until other important factors are described, we think it reasonable to recommend avoidance of fish and shellfish in patients with ANA induced by A.s. In the CU group, fish avoidance should be definitely advised only in patients in whom a causal relationship has been found. We recommend prospective studies to develop specific prevention and treatment protocols in patients with specific IgE against A.s.

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