

Usefulness of early serial measurement of specific and total immunoglobulin E in the diagnosis of gastro-allergic anisakiasis

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Summary

Background Sensitization to *Anisakis simplex* (*A. simplex*) has been documented to produce severe allergic reactions following ingestion of mainly raw or under-cooked parasitized fish. False positive skin prick tests (SPT) or specific IgE against this nematode and cross-reactivity restricts diagnosis. Gastric anisakiasis and gastro-allergic anisakiasis occur if fish is parasitized by live *A. simplex*.

Objective To investigate if serial serological analysis could be useful in the diagnosis of acute parasitization by this nematode.

Methods We included 41 patients who experienced an allergic reaction and/or abdominal symptoms after ingestion of raw or undercooked fish and displayed specific IgE against *A. simplex*. Total and specific IgE were determined two times: in the 24-h period after onset of clinical symptoms and after 1 month. SPTs were performed against *A. simplex* and implicated fish. A fibre optic gastroscopy was performed in 22 patients.

Results Median total IgE was 80.0 (Interquartile range [IQR] 41.5–186.5) kU/L in the first evaluation and 247.0 (IQR 96.5–649.5) kU/L after 1 month. Median specific IgE against *A. simplex* was 11.4 (IQR 7.1–33.5) kU/L in the first 24 h and 36.8 (IQR 19.5–79.5) kU/L after 1 month. A rise of total IgE was observed in 35 of 41 patients ($P < 0.00001$) and a rise in specific IgE against *A. simplex* in 37 of 41 patients ($P < 0.00001$). Mean percentage increment was 392% (215–571%; 95% confidence interval [CI] for total IgE and 339% (177–502%; 95% CI) for specific IgE. In nine of 22 gastroscopic examinations at least one larva, identified as *A. simplex*, could be detected by our microbiology service. In this group ($n = 9$) a rise of total and specific IgE was detected in eight patients (89%) ($P = 0.02$).

Conclusions We consider a rise of total and specific IgE in the first month after an allergic reaction as a useful tool in the diagnosis of gastro-allergic anisakiasis (together with patient's history), even if the parasite cannot be seen with fibre optic gastroscopy. The important rise of total and specific IgE against *A. simplex* can be considered as a reaction induced by the live parasitizing larva in the context of a polyclonal immunological stimulation.

Keywords: anaphylaxis, angioedema, *Anisakis simplex*, food allergy, gastric anisakiasis, nematode, parasitization, polyclonal stimulation, specific IgE, urticaria

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Introduction

Gastric anisakiasis is probably the most frequent clinical entity caused by the nematode *Anisakis simplex*. It has been

documented world-wide since the early 60s [1–6] and is caused by the third-stage larva of *A. simplex*, acquired by eating raw or under-cooked seafood. Clinical symptoms include epigastric pain, nausea, vomiting and sometimes diarrhoea. In Japan about 1000 cases per year are diagnosed and urticaria has been reported in about 10% of cases [7,8].

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In recent years the allergenic potential of *A. simplex* has been pointed out and numerous articles have been published on this topic [9–11]. The involvement of an IgE-mediated response has been proven by means of skin prick tests (SPTs) and specific IgE against this nematode. So *A. simplex* allergy has been described as a different clinical entity independent of a parasitosis [9,10].

'False positive' SPT and specific IgE against *A. simplex* can be observed in control subjects, and the apparent lack of specificity of these test has been explained by a high number of sensitized patients due to the high fish consumption in Spain [11,12]. Cross-reactivity has also been reported with other nematodes and antigens [13].

So other diagnostic tools like IgE immunoblotting have been proposed for the diagnosis of *A. simplex* allergy [11].

We have already reported on gastroallergic anisakiasis, where acute gastric parasitosis by *A. simplex* is accompanied by allergic hypersensitivity symptoms and where SPTs and specific IgE against this nematode could be detected [14–17]. We performed this study to investigate if serial serological analysis could be useful in the diagnosis of acute parasitosis by this nematode.

Methods

Patients were selected as follows: 84 patients with suspected gastric or gastro-allergic anisakiasis were seen in the emergency room in a 9-month-period and evaluated by an allergist within 24 h. All patients had gastric and/or allergic (hypersensitivity) symptoms and had eaten raw or undercooked fish in the 24 h prior to onset of symptoms. Of these patients, 71 showed positive specific IgE against *A. simplex*. Of these, only 41 patients could be followed for complete serological evaluation. Of these 41, 22 were male. Mean age was 45.5 years (SD 28.4–62.7). Mean time interval between ingestion of fish and onset of allergic symptoms was 4.9 h (SD $\pm$ 5.3) and mean time interval between ingestion and onset of gastric symptoms was 4.3 h (SD $\pm$ 3.1).

Although the aim was to study the evolution of serological parameters in allergic reactions by this nematode, we also included three patients who had only abdominal symptoms. Here we suspected gastric anisakiasis. Clinical data about abdominal and allergic symptoms were collected in this group. In nine of 22 gastroscopic examinations an acute parasitosis with *A. simplex* could be confirmed.

All ($n=41$) patients were skin prick tested against *A. simplex* and implicated fish, and total IgE as well as specific IgE to *A. simplex* was determined within 24 h of onset of symptoms (day 0) and after 1 month (day 30). They were put on a fish-avoidance diet.

Skin prick tests were performed with *A. simplex* antigen (Lab. IPI, Madrid, Spain) and with implicated fish (ALK-Abelló, Madrid, Spain). SPTs were considered positive with

a mean weal diameter of $\geq 3 \times 3$ mm. Histamine at 1% concentration and saline solution 0.9% (NaCl) were positive and negative controls.

Total IgE was assessed by IMx-Method (Abbot Diagnostics, Chicago, IL, USA). Specific IgE against *A. simplex* was assessed by CAP-FEIA (Pharmacia, Uppsala, Sweden). Specific IgE was considered positive with results >0.35 kU/L.

Statistics

Statistical analysis was performed using SPSSV Program (SPSS for Windows Student Version 6.0.1, SPSS Inc., 1989–93). Median values and interquartile ranges (IQRs) were calculated for total and specific IgE at day 0 and day 30. Wilcoxon matched-pairs signed rank test was used for comparison of total and specific IgE at day 0 and day 30.

Mean percentage variation of increment was calculated for total and specific IgE by one-sample *t*-test. Only those samples with increment were included in this analysis.

The same statistical analysis was performed for the whole group ($n=41$) as well as for the limited group of patients with gastroscopic confirmation of acute gastro-allergic anisakiasis ($n=9$).

Results

Of the 41 patients urticaria could be observed in 27, angioedema in 16, asthma in three, erythema in three, conjunctivitis in one and anaphylaxis in seven patients. Abdominal symptoms were as follows: nausea in 27, abdominal pain in 24, vomiting in 17 and diarrhea in six patients. Three patients had only abdominal symptoms and no allergic symptoms and seven patients had only allergic but no abdominal symptoms.

Median values for the group with confirmed gastro-allergic anisakiasis ($n=9$) total and specific IgE at day 0 and day 30 can be seen in Table 1.

Total IgE augmented in eight patients and decreased in one patient ($P<0.02$). Specific IgE augmented in eight and decreased in one patient ($P<0.015$) (see Fig. 1).

Percentage increment between day 0 and day 30 values was 601% (95% CI: 240–960, $P<0.006$) for total IgE and 339% (95% CI: –196–1291%, $P<0.126$) for specific IgE.

Median values for the whole group ($n=41$) total and specific IgE at day 0 and day 30 can be seen in Table 1.

Total IgE augmented in 37 and decreased in four patients ($P<0.00001$). Specific IgE augmented in 35 patients, decreased in five and was found equal in one patient ($P<0.00001$).

Percentage increment between day 0 and day 30 values, was 392% (95% CI: 215.0–570.7, $P<0.0001$) for total IgE and 339% (95% CI: 176.6–501.6%, $P<0.0001$) for specific IgE.

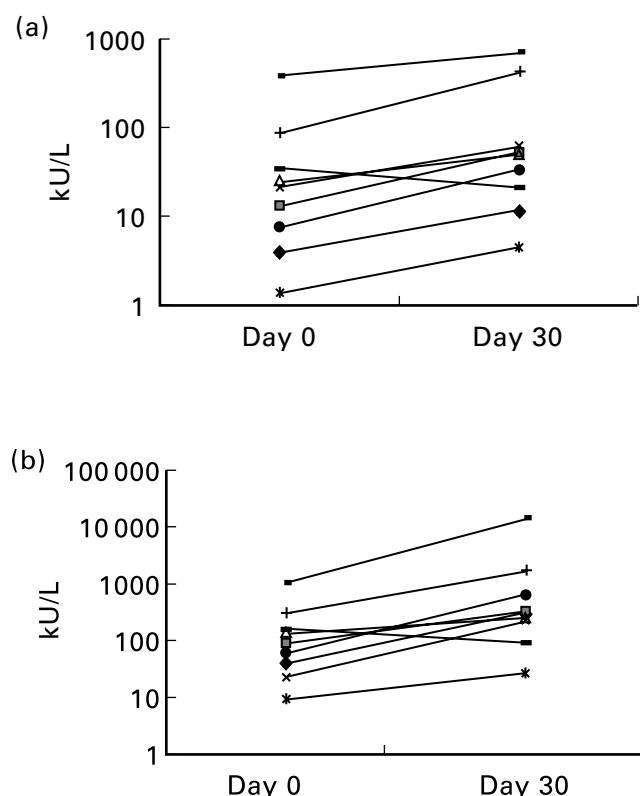


Fig. 1. Evolution of specific (a) and total (b) IgE. Total IgE and specific IgE against *A. simplex* was determined within 24 h after onset of symptoms (day 0) and after 30 days (day 30). In all nine patients acute parasitisation was diagnosed by fibre optic gastroscopy.

Discussion

Although an allergic mechanism was already suspected as a possible explanation in the pathogenesis of gastric anisakiasis, it is in the last 3 years that allergy to *A. simplex* has widely been studied, mainly in Spain, where fish consumption is high [9,10,18–20]. Allergy to *A. simplex* has been reported as allergic type I hypersensitivity reaction following

ingestion of infested fish not necessarily implicating a true parasitisation by this nematode. Thermostability of *A. simplex* antigens has been reported, so hypersensitivity reactions are thought to be possible even if fish was well-cooked or frozen [21].

On the other hand acute gastric anisakiasis can be accompanied by allergic symptoms. From Japan, where this entity is endemic because of the high raw fish consumption, we know that about 10% of cases are accompanied by urticaria [7,8]. We already reported gastro-allergic anisakiasis as an acute parasitisation of *A. simplex* with both gastric as well as allergic symptoms [14–17]. Here allergic symptoms can also occur without abdominal symptoms. When allergic (and abdominal) symptoms are followed by intake of raw or undercooked fish, this entity can be suspected, even if the time interval between ingestion of fish and clinical onset of symptoms is more than 12 h. An early gastroscopic examination can be useful for diagnosis and extraction of the live nematode, thus improving clinical outcome and preventing some rare complications like chronic anisakiasis [22–24].

Diagnosis of acute parasitisation vs allergic reaction is important as treatments differ. Usually patients with diagnosed *A. simplex* allergy are set on a fish and sea-fish avoidance diet to prevent contact with thermostable proteins (thought to be allergens) from *A. simplex* larvae. If allergic reactions could be ascribed to an acute parasitisation, with the live nematode producing an allergic reaction probably by the excretory–secretory proteins, dietary advice would only be directed towards raw or undercooked fish [19,25].

For a better comprehension Table 2 resumes the three clinical entities mentioned.

In our experience true *A. simplex* allergy seems to be rare and can't be diagnosed without a provocation test. Gastro-allergic anisakiasis seems to be more frequent: we were able to follow 41 patients with gastroscopic confirmation or high suspicion of acute parasitisation and determined total IgE and specific IgE against *A. simplex* in the acute phase and after 1 month.

The analysis of the nine patients with gastroscopic

Table 1. Median and interquartile ranges for total and specific IgE at day 0 and day 30

	Whole group*		Subset (<i>n</i> = 9)	
	Total IgE (kU/L)	Specific IgE (kU/L)	Total IgE (kU/L)	Specific IgE (kU/L)
Day 0	80 (41.5–186.5)	11.4 (7.1–33.5)	87 (31–226.5)	13.2 (3–60.6)
Day 30	247 (96.5–649.5)	36.8 (19.5–79.5)	303 (150–1193.5)	50.2 (31–226.5)

* Statistical analysis of whole group (*n* = 41) and subset of patients with confirmed Gastro-allergic anisakiasis (*n* = 9).

Table 2. Clinical entities of interest concerning allergy and *Anisakis simplex*

Clinical entity	Causing agent	Symptoms
Gastric anisakiasis	Acute parasitation with live nematode	Gastric symptoms Urticaria in 10% of cases* [7,8]
<i>Anisakis simplex</i> allergy	Cooked fish	Allergic symptoms [9,10]
Gastro-allergic anisakiasis	Live nematode	Allergic symptoms with or without gastric symptoms [14,17]

*Probably this subset of patients suffer of Gastro-allergic anisakiasis.

confirmation of acute parasitation confirms the suspected increase of total and specific IgE as a typical feature of parasitism. Median total IgE as well as median specific IgE were significantly higher (with a mean percentage increment of 601% and 339%, respectively) after 1 month with regard to the analysis at day 0. Maximum interassay coefficient is 11.4% for CAP-system [26] and 8.6% for IMx-method. Thus, high percentage increment in most patients rules out methodological error. But more striking is the fact that in the individual analysis eight of nine patients (89%) display a rise of specific IgE and total IgE after a 1-month period. High levels of IgE are typical in parasitic disease but the induction of an IgE response and an early rise of this immunoglobulin has only been showed in animal experiments. If we take into regard the whole group of 41 patients we find similar results with respect to the increment of total and specific IgE. Here too it is striking that 37 of 41 patients (90.2%) display a rise of specific IgE and 35 of 41 patients (85.4%) a rise of total IgE after a 1-month period. Even if we were not able to confirm acute parasitation in all patients, the suggestive history of the selected patients allows us to suspect acute gastro-allergic anisakiasis in all cases. The finding of increasing IgE values can be considered as a further argument.

To our knowledge this is the first report of a follow-up of this immunoglobulin after an acute parasitation. Most parasitic diseases are chronic and the moment of parasitation is not as clear as in acute anisakiasis. In our 'natural experiment', the moment of entry and contact of the nematode with human being can be determined by patient's history.

As specific and total IgE augmented in nearly all patients, we suppose that the sensitization of the 41 patients is induced by the infestation with *A. simplex* antigens. This is supported by the finding that rats inoculated with third stage larvae of *A. simplex* peak specific IgE against this larva 30 days after infection [25].

The high increment of specific IgE after 1 month encourages us to ascribe clinical and laboratory findings to an acute parasitation rather than a mere allergic reaction to thermostable proteins of *A. simplex*. We also show that a similar IgE response can be seen in three patients with

gastric anisakiasis, even if no allergic reaction was experienced. It is possible and even likely that the gastric symptoms are mainly caused by the infestation but not by allergic reaction. An IgE response in gastric anisakiasis has also already been reported [27,28]. So specific IgE response does not necessarily mean allergy and seems to be a typical response to parasitation. We suggest positive SPT or specific IgE to be marker of acute parasitation by this nematode against *A. simplex*. Thus it can be explained that specific IgE against *A. simplex* is a frequent finding in a raw fish-eating population. In the region studied (Madrid, Central Spain) the incidence of positive specific IgE against *A. simplex* in adult blood donors is 16% [29].

If allergic symptoms are due to an immediate hypersensitivity reaction (Coombs and Gell I), they should appear mostly within 1 h after intake [30]. In our patients symptoms often appear many hours after ingestion. If allergens are on the surface of the parasite, then an immediate reaction should be expected. But as allergic reaction can be triggered by excretory-secretory proteins of live larvae, these can be delivered by the live nematode at any time [31–33].

The amount of initial total or specific IgE is not a predictor of allergic symptoms and does not correlate with clinical features. In our experience patients who had an allergic reaction accompanying acute parasitation will again have an allergic reaction on re-exposure to live parasite. We are aware of some patients with repeated allergic reactions after fish intake, but only if it was eaten raw or undercooked, thus suggesting parasitation (data not shown). The usefulness of a diagnostic serological follow-up in the diagnosis of allergic reactions supposed to be due to *A. simplex* is as follows: in a next step patients with allergic reactions after intake of raw or undercooked fish could be diagnosed in a provocation test with freeze-dried noninfecting *Anisakis* antigens or frozen parasitized fish (up to 100% of fish is parasitized [34–37]) in order to rule out real sensitization to somatic *A. simplex* antigens. *A. simplex*-allergic patients set on a fish avoidance diet up to now could thus be told to only avoid ingestion of raw or undercooked fish. In conclusion we think that a significant rise of total and specific IgE of *A. simplex* after ingestion of raw or undercooked fish can be

a marker of acute gastro-allergic anisakiasis. As different raw or undercooked fish-eating habits are present all over the world, we encourage readers to search for this entity and take it into account if treating with acute allergic reactions.

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