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Original Article

Follow-up of total and *Anisakis simplex*-specific IgE levels in patients with anaphylaxis or acute or chronic urticaria

The clinical course and the serum total IgE (t-IgE) and *Anisakis simplex*-specific IgE (AS-IgE) were assessed over two years at six-monthly intervals in 87 adult patients [16 with anaphylaxis (A), 22 with angioedema/acute urticaria (AU), 49 with chronic urticaria (CU)] in association to an initial diet devoid of any fish and shellfish and a final one devoid only of raw fish and shellfish. All 38 A or AU patients had a past history of fish and/or seafood ingestion prior to the manifestation of hypersensitivity, anchovies being implicated as the triggering factor in 37% of the cases. No obvious alimentary correlation was observable in the CU cases. There were no statistically significant differences in the initial t-IgE or AS-IgE levels between the A and AU cases. Both parameters evidenced a gradual decrease, which was greatest at the end of 12 months in the A patients and at the end of 18 months in the AU ones, at which time they were on a semi-restrictive diet allowing consumption of non-raw fish and seafood, with no symptomatic recurrences. The IgE levels evidenced almost no decrease in the CU patients, in whom the symptoms remit in about 50% of the cases with no relation to the diet. **Conclusions:** In the cases with acute manifestations, the decrease of the IgE levels is interpreted as indicative of the absence of new contact with the allergen (live *Anisakis simplex* larvae), which is prevented by not ingesting raw fish; we therefore come to the conclusion that patients with acute symptoms caused by *Anisakis simplex* sensitisation should avoid the ingestion exclusively of raw or undercooked fish and seafood. The clinical significance of the *Anisakis simplex* sensitisation in CU is still unknown, although it is surprising that this sensitisation is detected in ca. 50% of these patients.

Key words: *Anisakis simplex*. Sensitisation. Specific IgE.

Seguimiento de los niveles de IgE sérica total y específica a *Anisakis simplex* en pacientes con anafilaxia, urticaria aguda y urticaria crónica

Estudiamos la evolución clínica y la de los niveles de IgE sérica total (t-IgE) y específica para *Anisakis simplex* (AS-IgE) cada seis meses durante dos años en 87 pacientes adultos [16 con anafilaxia (A), 22 con urticaria aguda/angioedema (UA) y 49 con urticaria crónica (UC)], valorando la relación con una dieta

exenta totalmente de pescados y mariscos inicial, y de pescados y mariscos crudos final. Los 38 pacientes con A o UA tenían historia de ingestión de pescados-mariscos previa al comienzo del cuadro, estando implicado el boquerón en el 37%. No se apreció relación alimentaria en los casos con UC. No se objetiva diferencia estadísticamente significativa en los niveles iniciales de t-IgE ni AS-IgE entre A y UA. Ambos parámetros descienden paulatinamente, siendo este descenso máximo a los 12 meses en A y a los 18 meses en UA, época en la que los pacientes comían pescados y mariscos no crudos sin recidivas sintomáticas. Los niveles apenas disminuyen en los casos de UC, cuyos síntomas remiten en aproximadamente el 50% sin relación con la dieta. **Conclusiones:** En los casos con patología aguda, interpretamos el descenso de la IgE como ausencia de nuevos contactos con el antígeno (larva viva de *Anisakis simplex*), que se evita al no ingerir pescado crudo, y deducimos que los pacientes con síntomas agudos por sensibilización a *Anisakis simplex* deben evitar exclusivamente los pescados o mariscos crudos o poco cocinados. Desconocemos el significado clínico de la sensibilización a *Anisakis simplex* en la urticaria crónica, aunque sorprende que se detecte dicha sensibilización en cerca del 50% de estos casos.

Palabras clave *Anisakis simplex*. Sensibilización. IgE específica.

The detection of positive specific IgE antibodies to *Anisakis simplex* (AS) both *in vitro* and *in vivo* (by means of skin tests) is considered to represent one of the main parameters, together with the history of having eaten fish in the foregoing hours, for establishing the diagnosis of allergic reaction induced by this parasite. We first began encountering a considerable number of cases of anaphylaxis and acute and chronic urticaria/angioedema back in the early months of 1995, subsequent to the publication of the first cases to be observed in Spain by the Allergology Service of the "Santiago Apóstol" Hospital¹. The number of sensitisations detected has progressively increased, although it is at times rather difficult to establish a cause-effect relationship on the basis of the anamnesis in order to establish a definitive diagnosis. In those cases with acute symptoms, the patient frequently does not remember what food or foods he/she ate previously, and often attributes the episode to some other mo-

re striking cause, such as for instance the foregoing administration of a drug. In chronic urticaria, the patient usually denies having observed any worsening after the ingestion of seafood in general.

This frequency of sensitisation surprised us in the early months after beginning these studies, and for this reason we decided to ascertain its prevalence among the adult population attending our Outpatient Clinic. We therefore carried out skin tests with an *Anisakis simplex* extract (I.P.I. Laboratories) and specific *Anisakis simplex* IgE quantitation (CAP System, Pharmacia, Uppsala, Sweden) on the first 87 new patients who came to our Outpatient Clinic in a given week, regardless of the actual cause of the consultation. Among these 87 patients, 16% evidenced positive skin tests and 22% positive specific IgE to *Anisakis simplex*, but only 9% of the cases had coincident positivity in both determinations (resulting sensitisation frequency, 28.8%). When analysing as a group the patients who consulted because of anaphylaxis episodes, acute urticaria/angioedema recurrent acute urticaria and chronic urticaria (18.3% of the total sample), the percentage of cases with positive specific IgE increased to 45%. However, the proportion of patients who were finally diagnosed of *Anisakis* allergy because they reported a suggestive clinical history was only 5.7%. In the urticaria/angioedema or anaphylaxis subgroup these patients represented 25% of the sample, while the remaining 23% were diagnosed as evidencing a subclinical sensitisation².

In the light of these previous data, we decided to analyse the levels of serum total and specific *Anisakis simplex* IgE antibodies and their evolution after suppression of all marine foodstuffs from the diet, in order to ascertain whether this observation might be of value for the clinical diagnosis.

MATERIAL AND METHODS

We have assessed the clinical and laboratory evolution of 87 adult patients presenting with anaphylaxis (16 cases, 7 of them recurrent), acute urticaria/angioedema (22 cases, 4 of them recurrent) or chronic urticaria (49 cases) in whom the serum levels of specific *Anisakis simplex* antibodies were higher than 0.75 kU/l (CAP System, Pharmacia) (Fig. 1). The gender and mean age distribution in each of the three groups is reflected in Table I.

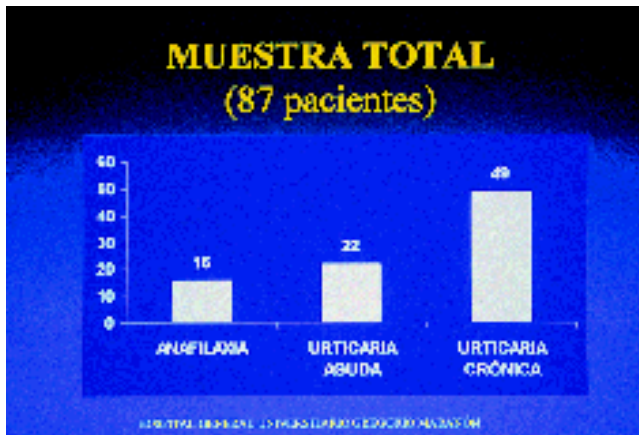


Fig. 1. Distribution of the diagnoses among 87 studied patients with *Anisakis simplex* sensitisation.

Table I. Demographic data of the 87 patients investigated for *Anisakis simplex* sensitisation.

MUESTRA TOTAL (87 pacientes)			
	ANAFILAXIA	URTICARIA AGUDA	URTICARIA CRÓNICA
n	15	22	49
EDAD	48 ± 12	47 ± 13	47 ± 14
FEDELES	81,20%	90,18%	88,16%

Table II. Foodstuffs involved in 49 acute episodes.

ALIMENTOS IMPLICADOS (49 cuadros agudos)			
	Anisakis	Unidentified	TOTAL
Baquerosa Ahogado	7	0	7
Baquerosa Frito	2	0	2
Marisco - Crustáceos	3	4	7
Pescado	1	4	5
Gallo	0	4	4
Pescado general	1	3	4
Pescado + Marisco	2	1	3
Calamar cocinado	1	1	2
Salmón	1	0	1
Cangrejo	1	0	1
Almeja	0	1	1

The 38 patients with acute symptoms (anaphylaxis or urticaria) reported having ingested fish or seafoods in the hours preceding the apparition of the symptoms; the time range was between 5 min and 2 h in most cases, although in some cases the ingestion had occurred up to 6 h earlier. The 22 patients with urticaria had evidenced a total number of 26 episodes, with gastrointestinal symptoms in 54% of them; however, the diagnosis of anaphylaxis was not established, as we considered that the digestive symptoms might have been attributable to contact with the parasite. In the anaphylaxis group there were three cases of anaphylactic shock, the remainder having evidenced arterial hypotension and/or dizziness and/or urticaria, with accompanying dyspnoea in some of the cases. Gastrointestinal symptoms were also recorded in a high proportion of the cases. The fish and/or seafoods apparently involved in the 49 acute episodes are summarised in Table II.

The patients diagnosed of chronic urticaria did not report any correlation between their symptoms and the ingestion of fish or seafoods, nor any digestive symptoms associated to such ingestion.

Blood samples were collected from all patients for serum total and specific *Anisakis simplex* IgE quantification on the day in which the anamnesis was recorded, a mean of three months (range, 15 days to 8 months) after the last symptomatic episode in the cases with acute urticaria. Once the –positive– results were known, the patients were advised to delete all types of fish or seafood from their diets. They were thereafter reviewed at six-monthly intervals over two years, and at each review visit blood samples were again obtained for repeat total and specific IgE determinations, at the same time recording the clinical evolution and the diet being adhered to. Deep-frozen fish was usually reintroduced to the diet six months after the diagnosis had been established, and fully cooked fresh fish a further six months later, but they were expressly told never again to consume raw fish or seafoods. The time periods stated have not been fully adhered to; despite our advice, some patients in the acute group did reintroduce raw fish into their diet after one year, and one half of those with chronic urticaria never complied with the dietetic advice from the start. Three patients with previous anaphylaxis episodes refused eating fish ever again.

RESULTS

No statistically significant differences were observed between the three groups in the age and gender parameters. There is a slight predominance of the female gender in all three groups (Table I).

The antecedent of anchovy ingestion in a sizeable proportion of the (acute) cases for which data were available is striking (Table II). No patient had eaten deep-frozen fish in any of these cases.

The laboratory and clinical evolution data will be addressed and analysed separately for the patients with an acute diagnosis (acute urticaria or anaphylaxis) and for those with chronic urticaria.

Anaphylaxis and acute urticaria

Initial results: Figure 2 shows the results of the total

serum IgE assessment in the two groups. There is a considerable variation in the levels, the mean value being $1,122 \pm 1,616$ kU/l in the anaphylaxis cases and $782 \pm 1,094$ kU/l in the acute urticaria ones, without statistically significant difference between the two. Similarly, there was no significant difference between the specific *Anisakis simplex* levels of the two groups (mean, 61 ± 34.66 kU/l in the anaphylaxis cases and 44.7 ± 40.65 kU/l in the acute urticaria ones; Fig. 3). Both in the cases of anaphylaxis (Fig. 4) and in those of acute urticaria (Fig. 5), a correlation was observed between the total serum IgE and the specific *Anisakis simplex* IgE levels.

The data from a previous study³ showed that no correlation existed between the specific *Anisakis simplex* IgE levels and the time elapsed between the ingestion of the fish involved and the onset of the symptoms (Fig. 6).

Follow-up: The evolution of the total serum IgE le-

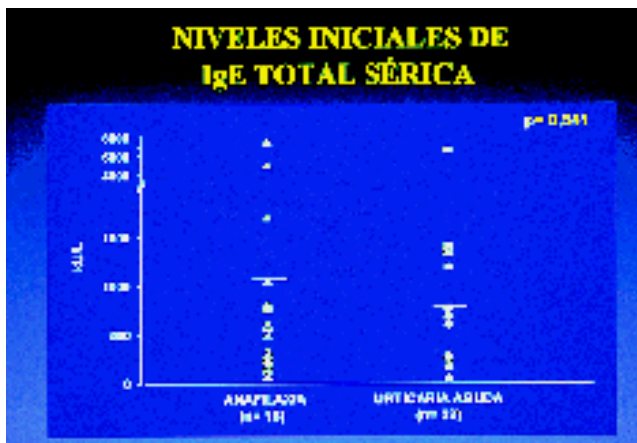


Fig. 2. Total serum IgE levels in the anaphylaxis and acute urticaria groups.



Fig. 3. Specific serum *Anisakis simplex* IgE levels in the anaphylaxis and acute urticaria groups.

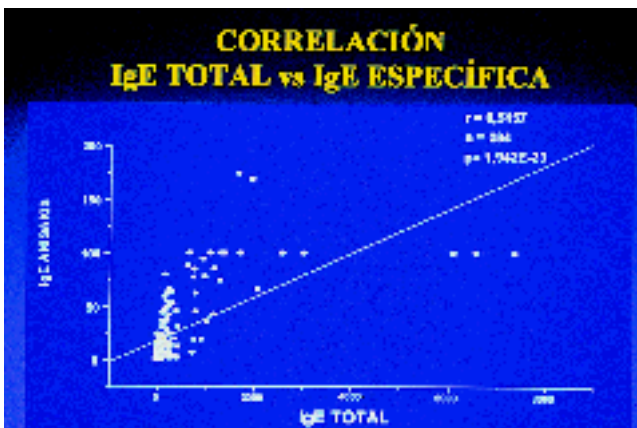


Fig. 4. Correlation between the serum levels of total and specific *Anisakis simplex* IgE in the anaphylaxis cases.

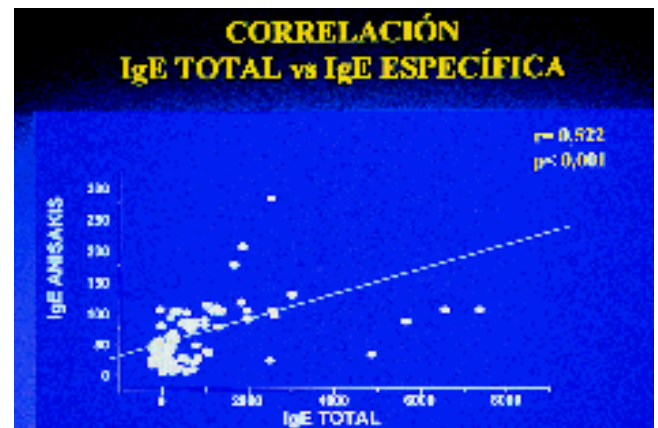


Fig. 5. Correlation between the serum levels of total and specific *Anisakis simplex* IgE in the acute urticaria cases.

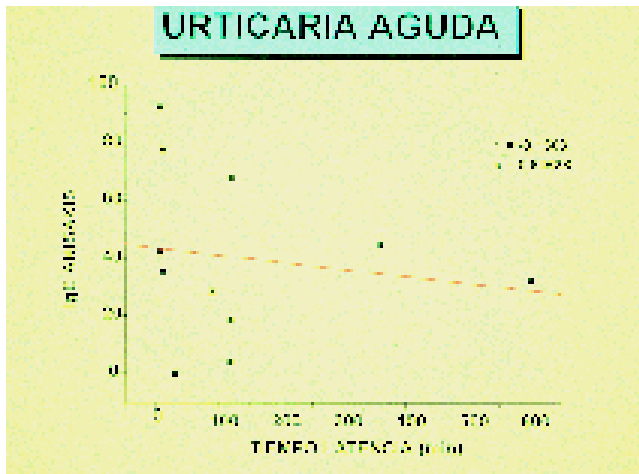


Fig. 6. Correlation between the serum *Anisakis simplex* IgE levels and the time elapsed between the ingestion of the implicated fish and the onset of symptoms.

vels is shown for the anaphylaxis cases in Figure 7, and for those with acute urticaria in Figure 8. Both evidence a gradual decrease achieving its nadir at the end of 12 months in the first case and at the end of 18 months in the second. A slight increase appears to occur by the end of the two-year follow-up period. It should be remembered that most patients were consuming deep-frozen fish by the end of the first year, and fully cooked fresh fish after 18 months of follow-up.

The levels of specific *Anisakis simplex* IgE also show a decrease, which is slower and more sustained than that of the total serum IgE levels, both in the anaphylaxis (Fig. 9) and in the acute urticaria patients (Fig. 10).

However, and as usual in any allergic condition, there

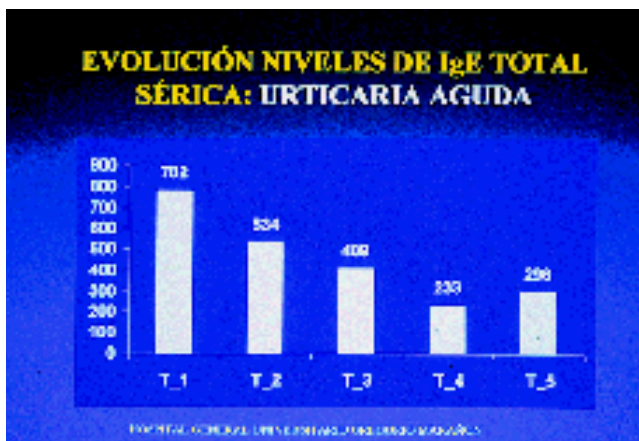


Fig. 8. Evolution over time of the serum total IgE levels in cases of acute urticaria after implementation of dietetic restrictions. T1 = at diagnosis; T2 = after 6 months; T3 = after 12 months; T4 = after 18 months; T5 = after 24 months (see text).



Fig. 7. Evolution over time of the serum total IgE levels in cases of anaphylaxis after implementation of dietetic restrictions. T1 = at diagnosis; T2 = after 6 months; T3 = after 12 months; T4 = after 18 months; T5 = after 24 months (see text).

are considerable individual variations; two examples of this are illustrated. One case is that of a 31-year-old male; in the second anaphylaxis episode he evidenced gastric endoscopy was performed and four *Anisakis* larvae attached to the gastric wall were removed, the symptoms subsiding almost at once. A blood sample was collected on the next day and then at shorter intervals than in the other cases, the follow-up continuing for three years. Figure 11 shows that the total and specific IgE levels increased some 15 days after the acute episode and then decreased. This patient complied correctly with the dietary instructions for about 18 months, but he then reintroduced undercooked fish (which he very much likes), without having again evidenced either symptoms or increases in his IgE levels.

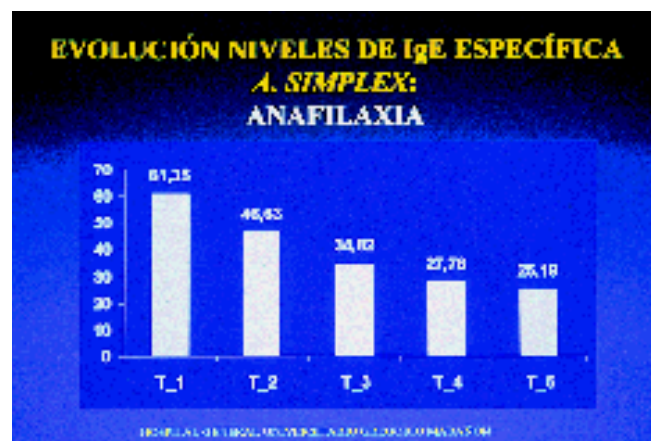


Fig. 9. Evolution over time of the serum specific *Anisakis simplex* IgE levels in cases of anaphylaxis after implementation of dietetic restrictions. T1 = at diagnosis; T2 = after 6 months; T3 = after 12 months; T4 = after 18 months; T5 = after 24 months (see text).



Fig. 10. Evolution over time of the serum specific *Anisakis simplex* IgE levels in cases of acute urticaria after implementation of dietetic restrictions. T1 = at diagnosis; T2 = after 6 months; T3 = after 12 months; T4 = after 18 months; T5 = after 24 months (see text).

The other example was a 49-year-old male (Fig. 12) with acute urticaria. He fully complied with the eviction advice for one year, with gradual decrease of the total and specific IgE levels. He then began again eating vinegar-marinated anchovies frequently, without developing symptoms, but with a considerable increase in both the total and specific IgE levels.

One further patient with acute urticaria reintroduced raw fish into his diet, in this case with two further episodes of acute urticaria.

The remainder of the patients have never again developed symptoms and comply with the dietary advice, with the exception of three patients who steadfastly refuse to ever again eating fish or seafoods.

Chronic urticaria

In these patients, the initial observed IgE levels were

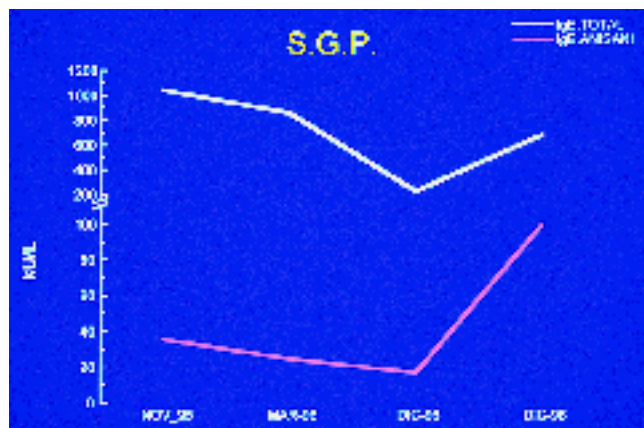


Fig. 12. Evolution of the serum total and specific *Anisakis simplex* IgE levels in one patient with acute urticaria who resumed consumption of raw fish one year after the studied episode.

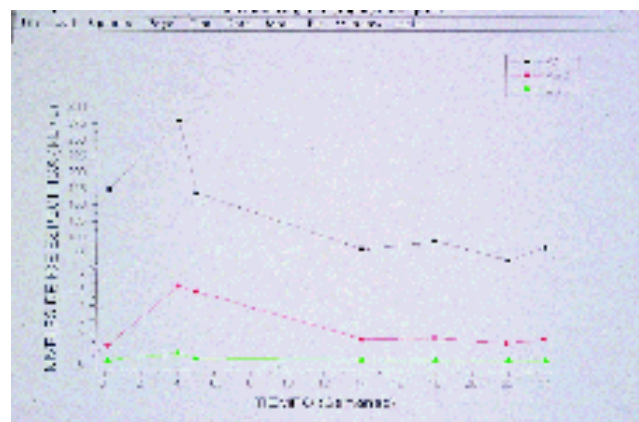


Fig. 11. Evolution of the serum total and specific *Anisakis simplex* IgE levels in the one anaphylaxis patient in whom *Anisakis simplex* larvae attached to the gastric mucosa were endoscopically extracted.

470.20 ± 943.39 kU/l for the total serum IgE and 24.07 ± 27.97 kU/l for the specific *Anisakis simplex* IgE. These patients were not as rigorous as the acute ones in their compliance with the dietetic recommendations, perhaps because chronic urticaria is not as alarming for the patient as anaphylaxis or explosive acute urticaria are, or perhaps because they did not fully trust the diagnosis. One half of these patients, approximately, continued consuming fish without any precautionary measure, although the remainder did comply with our advice. A number of them also failed to come for their scheduled review visits (particularly among those who continued to eat fish), so that laboratory data are not available for all of them, although their clinical evolution is known through telephone interviews. The duration of the follow-up did not exceed 18 months in any of these cases.

The clinical evolution was quite variable: in about 50% of the cases the urticaria subsided and they stopped taking antihistamines, but this evolution was independent of their eating fish or not.

Figure 13 shows the evolution of the total serum IgE levels in this group of patients. Overall, a moderate decrease can be observed. In order to better analyse the laboratory follow-up, these patients were divided into two subgroups, according to whether they still ate fish or did not eat it. Six months after the initial study there was no significant difference between the two subgroups. The total serum IgE levels did show a significant difference ($p < 0.05$) between them at the 12-month follow-up visit but, surprisingly, they were higher in those patients who did not eat fresh fish. At the 18-month follow-up visit, there was again no statistically significant difference between the two subgroups.

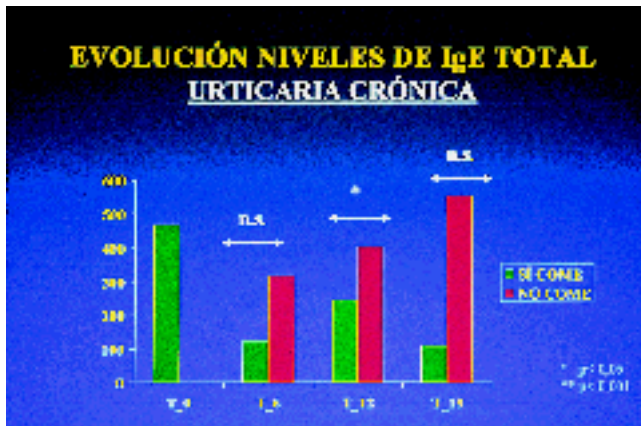


Fig. 13. Chronic urticaria. Evolution of the total serum IgE levels. The red bars represent the patients who did not comply with the dietary recommendations; the green ones represent those who did comply with them. T followed by a number indicates the months elapsed between the studied episode and the particular follow-up assessment.

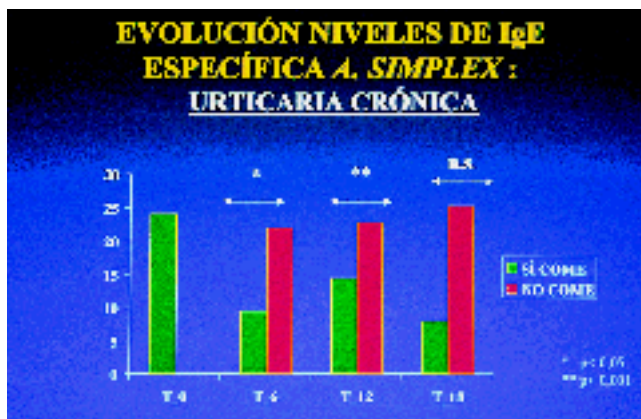


Fig. 14. Chronic urticaria. Evolution of the specific serum *Anisakis simplex* IgE levels. The green bars represent the patients who did not comply with the dietary recommendations; the red ones represent those who did comply with them. T followed by a number indicates the months elapsed between the studied episode and the particular follow-up assessment.

A similar course was observed for the specific *Anisakis simplex* IgE levels (Fig. 14), which at the end of one year were higher in those patients who still did not eat fresh fish.

DISCUSSION

La distribución de los pacientes por edad y sexo no The age and gender distribution of the patients is not different from those observed in other cases of anaphylaxis and acute or chronic urticaria of unknown origin in our experience³. The female gender predominates in these types of conditions, as already observed in the "Alergológica" epidemiologic study⁴.

From the aetiological point of view, the cases with acute clinical manifestation (anaphylaxis and acute urticaria) appear to be clearly related to contact with the live larvae of *Anisakis simplex*. The requirement for the larvae to be alive is underscored by the fact that vinegar-marinated anchovies –and thus raw fish, in which the larvae are assuredly alive– are the fish preparation most frequently implicated in our environment. Contact with the parasite is most probably through the attachment of the larva to the digestive tract mucosa, and the antigens penetrate into the organism through the eroded mucosa. We have had the opportunity to confirm this in the course of gastric endoscopy upon detecting the larvae attached to the gastric wall, an experience similar to that in other Hospitals.

In these acute cases, the levels of both total serum and specific IgE are increased at the time of diagnosis, with a high degree of variation as in any form of allergic condition. The gradual decrease in both parameters during the absolute exclusion diet appears logical, as possible contact with the antigen is suppressed. No new contact with the antigen appears to exist even when the patients reintroduce deep-frozen and later fresh but thoroughly cooked fish into their diets, in which the larvae would be expected to be dead if at all present, the latter being quite probable due to the high infestation rate of the fish.

It thus seems fitting to establish a certain similitude between this particular form of allergic reaction and that occurring with Hymenoptera venoms. As in the latter case, the larva of *Anisakis simplex* would inject its "venom", the secretory antigen, into the patient, triggering the symptoms. However, and again in parallel to the case of Hymenoptera venoms, in some patients IgG-class blocking antibodies may develop (or some other sort of immunologic mechanism), so that the patient is "protected" in the case of a repeat inoculation; this happens in bee keepers and in patients allergic to such venoms in the course of immunotherapy. This would appear to have been the case in the one patient in our series who has continued to eat raw marinated anchovies and has had no further symptomatology despite evidencing an increase in the specific IgE levels as a reflection of new contacts with the antigen.

The cases of chronic urticaria are difficult to explain through these aetiological mechanisms. When we began encountering such cases, in 1995-96, we had hopes of achieving remission of their symptoms by excluding fish from their diets, on the assumption that repeated and frequent contact with the larva might be the cause of their urticaria, but this theory was abandoned in time upon observing the

patients' clinical evolution, in which symptom remission is independent of the diet. At present, the clinical significance of the presence of AS-IgE in about 50% of the chronic urticaria cases remains unknown. Sensitisation has occurred in almost one half of the cases at some unknown time, and we do not know whether it preceded or was coincident with the onset of the symptoms. It might have preceded the clinical condition, as we have detected positive anti-*Anisakis simplex* IgE in up to 23% of the asymptomatic cases we have studied². However, why is the frequency of sensitisation doubled among chronic urticaria patients? Two possibilities arise, in our opinion: The larva might somehow persist within the human organism, perhaps in the form of larva migrans or of eosinophilic granuloma; we would not be able to detect it in this form, but the antigenic presence would be maintained over long periods. Alternatively, the detected specific anti-*Anisakis* IgE may represent nothing else but the result of cross reactivity to some other unidentified parasite, different from *Ascaris suum* or *Echinococcus granulosus* –both of which were ruled out in our patients– which are the most frequent ones in our environment.

In the light of these experiences, our current attitude towards the exclusion diet has changed slightly. In the acute cases, we have shortened the period of total exclusion of fish and seafoods to two or three months if the patient has not consumed them since experiencing his/her last reaction (in many cases, this dietary recommendation is given at the Emergency Outpatient Clinic of our Hospital, before the patient is seen a short time later in our Allergy Outpatient Clinic), so as to observe the decrease of the specific parasite IgE levels, and then first deep-frozen and then fresh but thoroughly cooked fish are reintroduced with monthly intervals, raw and insufficiently cooked (grilled, marinated, microwave oven-prepared, etc.) fish and seafoods remaining expressly prohibited. When the patient has continued consuming fish for a number of months after his/her last reaction without having again developed symptomatology until being studied (because of the delay in the waiting lists), it does look quite unneces-

sary to completely forbid these foods, as this will not be of any use for observing IgE decreases or for suppressing any current manifestations. In such cases, only raw or undercooked fish and seafoods are prohibited, as stated above. We believe that a correct nutrition requires the intake of fish, particularly in middle-aged individuals who often evidence abnormalities in their cholesterol, urate, etc., levels, and who would be compelled to cover their caloric requirements mainly with meat and eggs.

In the case of chronic urticaria, we restrict ourselves to the recommendation not to eat raw fish, without at any time curtailing the use of deep-frozen or fresh but thoroughly cooked fish, as we do not believe that sporadic contact with the larvae might be the cause of their chronic condition. However, we do recommend avoidance of possible contacts with the live larvae, as we believe that those individuals in whom anti-*Anisakis simplex* IgE has been demonstrated (chronic urticaria and subclinical sensitisation) do present a higher risk for possibly severe acute reactions.

Many open questions still remain in the field of conditions related to or triggered by *Anisakis simplex*; it is our hope that these many questions will receive an answer from future investigations.

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