



Allergologia et immunopathologia

Sociedad Española de Inmunología Clínica,
Alergología y Asma Pediátrica

www.elsevier.es/ai



ORIGINAL ARTICLE

Galactose- α -1,3-galactose (alpha-gal) allergy: first pediatric case in a series of patients in Spain



Martín-Lázaro J*, Núñez-Orjales R, González-Guzmán L.A., González M.T., Boquete M., Carballada F.

Allergy Section, Estructura Organizativa Integrada de Lugo, Cervo y Monforte. Lugo, Spain

Received 11 June 2019; accepted 26 July 2019
Available online 11 November 2019

KEYWORDS

Alpha-galactosidase;
Delayed meat allergy;
Anaphylaxis;
Cetuximab allergy;
Bovine amniotic fluid
allergy

Abstract

Introduction and Objectives: Allergy to galactose- α -1,3-galactose (alpha-gal) is a peculiar form of food allergy generally manifesting as an anaphylactic reaction hours after mammalian meat consumption, due to the presence of specific IgE against this oligosaccharide. In addition, immediate anaphylaxis may develop after exposure to other sources of alpha-gal, such as monoclonal antibody cetuximab, vaccines, plasma expanders or anti-snake venoms. Sensitization to alpha-gal has also been implicated in the rapid degeneration of biological valve implants, and recognized as a cause of occupational disease in cattle raisers. The implication of tick bites in this type of sensitization has been accepted by all the research groups dedicated to this disease.

Patients and method: The present study describes the clinical and sensitization characteristics of 39 patients diagnosed with alpha-gal allergy in the hospitals of our province (Lugo, Monforte de Lemos and Burela, Spain).

Results: Most patients were middle-age males. Of note, is the fact that the series includes the first pediatric patient reported in Spain to date. The predominant clinical manifestations were urticaria or delayed anaphylaxis after consumption of mammalian meat. Seventy-four percent of the patients reported having suffered a previous tick bite, and the clinical presentation of anaphylaxis was significantly more prevalent in those with a persistent local reaction following the bite than in those with no such reaction ($p=0.032$).

Conclusions: A review is also made of the disorder which, due to its variable clinical expression, is referred to as alpha-gal syndrome. The study concludes that a diagnosis of alpha-gal allergy should be considered in patients with urticaria-anaphylaxis of uncertain origin or manifesting after the administration of vaccines or products of bovine/porcine origin.

© 2019 SEICAP. Published by Elsevier España, S.L.U. All rights reserved.

* Corresponding author at: Hospital Universitario Lucus Augusti, Rúa Dr. Ulises Romero 1, 27003 Lugo, Spain.
E-mail address: joaquin.martin.lazaro@sergas.es (J. Martín-Lázaro).

Introduction

The irruption of alpha-gal allergy has questioned classical principles of IgE-mediated allergic reactions. The latency period of hours; the variability of the appearance of symptoms after exposure to the allergenic source; and the fact that the causal epitope is a carbohydrate are all features that challenge the dogmas of allergy, and define a unique disease condition that proves difficult to deal with.

Galactose- α -1,3-galactose (alpha-gal) is an oligosaccharide that glycosylates different proteins of non-primate mammals. Humans, apes and old-world monkeys do not express the alpha-gal epitope, since the α -1,3-galactosyltransferase gene is inactivated in these species, although they do produce IgM/IgG antibodies targeted to this carbohydrate and which are responsible for graft rejection of organs from pigs.¹

The first descriptions of alpha-gal disease correspond to Van Nunen² in Australia, who published cases of delayed allergy to meat in people with local reactions to tick bites. Previously, Adeyoyin³ reported that 31% of patients with cat allergy recognized cat IgA/IgM (IgA being Fel d5) – this sensitization depended on a carbohydrate. Concomitantly, O'Neil et al.⁴ reported an unexpected increase in the incidence of immediate reactions with the first infusion of cetuximab in the south-eastern United States, which Chung et al.⁵ attributed to preformed antibodies against the alpha-gal epitope, since cetuximab was also available without this epitope. Furthermore, Commins et al.⁶ related this phenomenon to late reactions to beef, and this in turn to tick bites.⁷ In 2011, the first five cases in Spain were reported in the region of Galicia.⁸

Specific IgE against alpha-gal is generally produced after tick bites, causing urticaria/delayed anaphylaxis (approximately 4–6 hours) after the consumption of mammalian meat.

The reactions occurring with cetuximab are immediate, severe and manifest with the first dose of the drug.⁴

Patients allergic to alpha-gal may suffer symptoms with sweets containing gelatin of porcine origin, such as Haribo gummy bears,⁹ as well as following the administration of intravenous plasma expanders (Gelofusine, Haemacel,¹⁰ Gelafundin⁹). Another female patient¹¹ suffered immediate anaphylaxis as a result of the application of an antifungal ovule containing alpha-gal in the capsule. Positive skin tests can also be observed with pancreatic extracts containing hydrolyzed bovine amylases, lipases, proteases or pepsin and blood proteins, as in the drugs Creon and Enzynorm f¹² (marketed as Kreon and Pankreoflat in Spain).

Two patients with biological aortic valve prostheses developed alpha-gal allergy,¹³ with rapid implant degeneration requiring replacement with mechanical prostheses. There have also been descriptions of cases of perioperative allergic reactions in bovine or porcine valve replacement surgery¹⁴ in patients with elevated alpha-gal specific IgE titers.

Anti-snake venoms also contain alpha-gal,¹⁵ and a reaction to such antidotes has been reported.¹⁶ Similar phenomena could also be expected with other antidotes (e.g., Spiders, Jellyfish, Scorpions).

Our group has recently described three cases of urticaria and anaphylaxis secondary to contact with cow amniotic fluid in cattle raisers presenting alpha-gal allergy,¹⁷ with confirmation of the presence of alpha-gal in the amniotic fluid. One of these cases was recognized as an occupational disease by the corresponding official organisms.

With regard to the impact of alpha-gal disease in pediatric patients, 47 children with alpha-gal positive IgE titers and symptoms of recurrent urticaria, anaphylaxis or angioedema have been reported in the United States.¹⁸ A five-year-old child with alpha-gal allergy suffered immediate anaphylaxis¹⁹ on receiving three vaccines: measles-mumps-rubella (MMR), varicella and diphtheria-tetanus-pertussis-polio (DTaP/IPV), with positive testing to MMR, varicella and gelatin. A previous report²⁰ described an immediate systemic reaction in an adult woman with alpha-gal allergy following herpes zoster vaccination. No previous pediatric cases of the disease have been reported in Spain.

Material and methods

The study involved 39 patients referred to the Allergy Units of the three hospitals of our province (Lugo, Monforte de Lemos and Burela, Spain) because of episodes of urticaria/delayed anaphylaxis and diagnosed with allergy to mammalian meat due to sensitization to alpha-gal between the years 2010 and 2018. The clinical characteristics of the patients and the allergological tests made, along with other parameters such as a history of tick bites are detailed in Table 1.

The skin prick tests were made using a standard battery (Laboratorio Alk-Abelló, Madrid, Spain) of aeroallergens (*Dermatophagoides pteronyssinus*, *Lepidoglyphus destructor*, *Tyrophagus putrescentiae*, *Chortoglyphus arcuatus*, *Glycyphagus domesticus*, *Alternaria*, *Cladosporium*, *Aspergillus fumigatus*, *Betula verrucosa*, *Cupressus arizonica*, *Lolium perenne*, *Phleum pratense*, *Plantago*, *Parietaria judaica*, profilin, dog, cat, cow, horse and rabbit) and food allergens (milk, egg, walnut, peanut, lentil, soybean, gliadin, white fish, wheat flour, *Anisakis*, shrimp, apple, peach LTP, kiwi, latex and beef) routinely employed in our Units. As positive control we used histamine 10 mg/ml, with saline solution as negative control. Wheal size was measured 20 min after the test, and the result was considered positive when the size of the wheal induced by the allergen was at least equal to the diameter of the wheal induced by histamine.

In addition, 14 patients underwent skin prick tests with 5 mg/ml of cetuximab (Erbitux, Merck S.L., Madrid, Spain). If the prick test proved negative, intradermal skin testing (0.5 mg/ml) was performed following the instructions of the European Committee²¹ on the volar surface of the forearm. The result was considered positive when at least one wheal measuring 5 mm in diameter was obtained, with erythema, 20 min after the test.

Total and specific IgE against alpha-gal were determined. Specific IgE against other meats was tested in some patients. The ImmunoCAP technique (Thermo Fischer Scientific, Uppsala, Sweden) was used for both total IgE and specific IgE.

In patients with the implication of non-steroidal anti-inflammatory drugs (NSAIDs) as possible cofactors,

Table 1 Description of the patient characteristics.

#Patient	Gender	Age	1st reaction	Symptoms	Tick bite	Tick local reaction	Prick beef	Cut test cetuximab	Total IgE	a-Gal IgE	#React before diagn	#React after diagn
1	M	24		Urticaria	NO	?	POS		350	22.8	3	0
2	M	47		Urticaria	YES	YES	POS		198	55.4	3	4
3	M	83		Anaphylaxis	YES	YES	POS		64	30	3	1
4	M	46		Anaphylaxis	YES	YES	POS	PRICK	2080	100	4	0
5	F	91		Anaphylaxis	NO	?	POS	PRICK	248	22.3	1	0
6	M	31		Urticaria	YES	YES	POS	PRICK	291	61.5	3	0
7	M	49		Anaphylaxis	YES	?	POS	PRICK	607	100	5	1
8	M	29		Anaphylaxis	YES	?	POS		695	100	3	2
9	F	60		Anaphylaxis	YES	NO	POS	PRICK	98.7	6.37	1	13
10	M	34		Anaphylaxis	YES	NO	POS	PRICK	597	100	2	1
11	F	56		Urticaria	YES	NO	POS		106	2.69	6	2
12	M	55		Anaphylaxis	YES	YES	POS		464	100	1	1
13	M	26		Urticaria	YES	NO	NEG		926	100	3	0
14	M	62		Anaphylaxis	YES	YES	NEG		494	81.5	4	2
15	M	56		Anaphylaxis	?	?	POS	PRICK	346	51.1	3	0
16	M	44		Urticaria	NO	?	POS		239	21.4	3	0
17	M	61		Urticaria	YES	?	POS	PRICK	1441	377	5	0
18	M	67		Anaphylaxis	YES	NO	POS		336	100	8	3
19	M	59		Anaphylaxis	YES	?	NEG	ID	219	3.01	1	0
20	M	74		Anaphylaxis	?	?	POS	PRICK	98	60.7	2	0
21	F	56		Anaphylaxis	NO	?	NEG	PRICK	44.5	21.7	1	0
22	M	63		Urticaria	YES	NO	NEG		575	26.2	5	1
23	M	45		Anaphylaxis	YES	YES	POS		447	88.8	4	2
24	M	73		Anaphylaxis	NO	NO	POS	PRICK	32.1	1.42	1	0
25	F	36		Urticaria	YES	YES	POS		25.9	4.21	20	0
26	F	81		Anaphylaxis	NO	?	NEG	ID	202	1.11	2	0
27	F	64		Urticaria	NO	?	POS		457	13.2	2	0
28	M	13		Urticaria	YES	?	POS		70.9	1.04	1	0
29	M	43		Anaphylaxis	YES	?	POS	PRICK	280	100	3	1
30	M	62		Anaphylaxis	YES	YES	NEG		39	14.2	3	1
31	M	40		Anaphylaxis	YES	YES	POS		136	4.07	3	1
32	F	60		Anaphylaxis	YES	YES	POS		106	24	3	0
33	M	75		Urticaria	YES	NO	NEG		132	1.58	1	0
34	F	31		Anaphylaxis	YES	YES	POS	PRICK	1082	26.3	1	0
35	M	41		Anaphylaxis	YES	YES	POS	PRICK	541	21.9	6	0
36	M	28		Urticaria	YES	YES	NEG	ID	123	1.03	1	0
37	F	72		Anaphylaxis	YES	YES	POS	PRICK	112	10.9	3	0
38	M	73		Anaphylaxis	?	NO	NEG	ID	1670	11.6	1	0
39	M	67		Urticaria	YES	YES	POS	PRICK	45	2.89	1	0

controlled exposure testing was carried out with progressively increasing doses until the final therapeutic dose was reached.

Statistical analysis

The chi-squared test, Student t-test, Kruskal-Wallis test, Mann-Whitney U-test and Wilcoxon test were used to analyze the results obtained. The SPSS version 25.0 statistical package was used throughout.

Results

All the patients were Caucasians. Twenty-nine of the 39 patients were males (74%). There were no statistically significant differences in the presence of urticaria or anaphylaxis between males and females ($p=0.652$). The mean patient age was 53 years (range 13–91), and a full 69% of the patients were between 40–75 years of age. The mean age of the patients with urticaria was 45.3 years, versus 57.68 years among those with anaphylaxis – the difference between groups being statistically significant ($p=0.0043$). The present study reports a delayed reaction to pork in a 13-year-old boy, which constitutes the first pediatric case published in Spain to date.

All the patients were referred by their doctors due to symptoms hours after exposure to meat, except two: one male diagnosed with an immediate reaction to cetuximab, and a female diagnosed with an intraoperative immediate reaction related to the use of Gelaspan (plasma expander).

Twenty-five of the patients (64%) presented anaphylaxis with the rest presenting urticaria. The mean latency between meat consumption and the appearance of symptoms was five hours (range 2–10). The types of meat implicated in the reactions were beef, pork and lamb. One patient also developed symptoms in relation to wild boar meat. Two patients with symptoms in response to beef experienced faster reactions (two hours) on consuming kidney.

Among the patients with anaphylaxis, 13 suffered at least one further reaction after the diagnosis (52%). Before the diagnosis the patients experienced an average of 2.76 episodes, versus 1.16 episodes after the diagnosis – the difference was statistically significant ($p=0.000$).

Of the patients with urticaria, three suffered at least one further reaction after the diagnosis (25%). Before the diagnosis the patients experienced an average of 4.07 episodes, versus 0.5 episodes after the diagnosis – the difference was statistically significant ($p=0.002$).

With regard to tick bites, 29 patients recalled having been bitten (74%), three did not remember, and seven denied having been bitten by ticks (18%). There was no statistically significant association between tick bites and the severity of the symptoms, i.e., the presence of urticaria or anaphylaxis ($p=0.390$). Of the patients who had been bitten by ticks, 58% developed a local reaction as a result, and this reaction could last for months. In the patients with urticaria, the bites caused a local reaction in 45.45% of the cases, versus in 61.1% of the patients with anaphylaxis. The Kruskal-Wallis test showed older age and the presence of a local reaction to tick bites to be associated to an increased risk of anaphylaxis ($p=0.032$) – a situation not seen in the patients with

urticaria ($p=0.815$). The reaction to meat manifested weeks or months after the bite.

The total IgE titers ranged between 25–2080 IU/ml, with an arithmetic mean of 403 IU/ml (after excluding the patients that yielded values of 2080, 1670 and 1441 IU/ml, the mean total IgE titer was 293 IU/ml) and a median of 248 IU/ml. There were no statistically significant differences between the patients with urticaria and those who suffered anaphylaxis ($p=0.621$).

The specific IgE titers against alpha-gal ranged between 1.03 and >100 kU/l, with a mean of 47.97 kU/l. In seven patients the titers exceeded 100 kU/l, which is the upper limit of detection in our laboratory. There were no statistically significant differences between the patients with urticaria and those who suffered anaphylaxis ($p=0.157$).

The 14 patients subjected to skin testing with cetuximab all yielded positive results (12 with prick testing and two with the intradermal test). The only patient in our series who had been referred due to anaphylaxis with the first cetuximab dose had never experienced problems with meat, and the intradermal test proved positive.

In five of the patients in our series (three of them have been described in a recent article published by our group¹⁷), a diagnosis of contact urticaria or anaphylaxis due to cow amniotic fluid allergy was established, demonstrating the presence of alpha-gal in the amniotic fluid.

The 26 patients whose blood group was recorded all had groups other than group B.

Of the 39 patients, none reported symptoms caused by milk or dairy products, or gelatins. Cured ham was tolerated by all the patients.

Skin testing with commercial beef extract proved positive in 29 patients (74%) and negative in 10 (26%). A positive test was obtained in 71.43% of the patients with urticaria and in 76% of those with anaphylaxis – the difference was not significant ($p=0.753$).

Specific IgE against rabbit, lamb, pork and beef was positive in 19 of the 23 patients in whom such testing was requested (83%). The IgE titers corresponding to bovine serum albumin (BSA) were generally low. Specific IgE against chicken was negative in all cases.

Before the diagnosis, the patients had experienced between 1–20 episodes, with an average of 3.2 episodes per patient (after excluding the patients with 20 episodes of recurrent urticaria, the mean was 2.7 episodes per patient). Following the diagnosis, the patients experienced between 0–13 episodes, with an average of 0.92 episodes per patient (after excluding the single patient with 13 episodes, the mean was 0.6 episodes per patient).

With regard to atopy (defined as a positive skin test for some aeroallergen), 12 of the total patients (30%) yielded positive skin tests: 10 to dust mites, one to pollen and one to dust mites/pollen. An additional patient had positive skin tests for LTP, and another one for bee venom. The tryptase values were within normal ranges.

Controlled exposure to NSAIDs was well tolerated by four (ibuprofen in three and naproxen in one) of the six patients in whom those drugs possibly intervene as a cofactor; two patients tolerated alternatives and rejected testing.

Lastly, physical exercise and alcohol consumption were evaluated as possible cofactors in the respective reactions.

Posterior exposure to these two cofactors caused no adverse reaction in any of the patients.

Discussion

Galactose- α -1,3-galactose (alpha-gal) syndrome is a disorder that has become known during the last decade. In 2011, our group reported the first five cases in Spain, and the present study describes the data corresponding to 39 diagnosed patients.

Carbohydrates as such were not regarded as allergens, and although the confounding effect they produced in the context of in vitro allergy testing was known, it was not considered to be of clinical relevance.^{22–24}

Alpha-gal was known to be responsible for hyperacute porcine transplant rejection in humans, although in that context the reaction is mediated by IgM/IgG antibodies. It is present in the tissues of mammals (except humans, apes and old-world monkeys). The existing evidence indicates that the development of specific IgE against alpha-gal occurs as a result of tick bites, and does not seem to be caused by food.⁷ The disorder is characterized by urticaria/delayed anaphylaxis (3–6 hours) following the consumption of non-primate mammalian meat. The symptoms develop faster with the consumption of viscera, particularly kidney.²⁵ It is not clear why these reactions occur, although the way in which the meat is prepared for consumption appears to play a role. Nevertheless, this epitope is known to be resistant to both heat²⁶ and pepsin-mediated lysis²⁷; as a result, the symptoms do not manifest every time the patient consumes meat – a fact that complicates clinical suspicion and diagnosis. Some authors¹⁸ have offered hypotheses attempting to explain why the consumption of mammalian meat containing alpha-gal does not always produce an allergic reaction, including variations in the metabolic process (digestion and absorption) of carbohydrates, in the amount of alpha-gal that reaches the bloodstream, the amount of alpha-gal found in different foods, the food dose or portion consumed, preparation of the food, and the latency period from the last mammalian meat intake.

Once this particular antigen started to be taken into account, the diagnosis of idiopathic anaphylaxis in regions of high alpha-gal intake such as Tennessee (USA) decreased from 59% to 35%. In effect, while alpha-gal allergy was once practically unknown, it now represents the most commonly identified cause of anaphylaxis in patients of this kind.²⁸ A link has even been established between specific IgE against alpha-gal in people from Virginia (USA) and an increased atheroma and unstable plaque burden – thus representing a possible new risk factor for coronary artery disease.²⁹

Tick bites can produce a local reaction or cause anaphylaxis secondary to sensitization to the tick salivary proteins.³⁰ Alternatively, some time after the bite, sensitization to alpha-gal may develop, producing the syndrome addressed in our study.

The tick *Amblyomma sculptum* contains alpha-gal in its saliva,³¹ and *Ixodes ricinus* contains alpha-gal in its intestine,³² although the literature does not specify whether alpha-gal was endogenous to the tick itself or exogenous (i.e., originating from the ingested mammalian blood). In one study,⁷ the bites of *Amblyomma americanum* (lone star

tick) induced both IgE against tick proteins and IgE against alpha-gal, yet this was not seen in the case of *Dermacentor variabilis* (brown dog tick).

Since *Ixodes ricinus* is the most common tick in our geographical setting,³³ we presume that it is the species that bites and sensitizes our patients.

A recent study has shown that the tick *Ixodes scapularis* activates the genes that produce alpha-gal in response to infection by *Anaplasma phagocytophilum*; as a result, the ticks most infected with that type of *Rickettsia* generate more alpha-gal. These findings could help explain the geographical differences in the prevalence of alpha-gal allergy.³⁴

It has been shown that tick bites induce an increase in the titers of specific IgE against alpha-gal³⁵ and that the levels are higher in patients that have received multiple bites.⁷ It has been ruled out that persistent local reactions at the bite site are secondary to infection with *Borrelia burgdorferi*.³⁶

Tick bites were implicated in most of our patients (70%), but not in all of them. This raises the question as to whether there may be other sensitization routes. Over half of our patients developed local reactions to the bite (52%), and these could persist for weeks or months – being considered as a risk factor for the development of symptoms in relation to alpha-gal. A total of 45.45% of the cases of urticaria were associated to local reactions to tick bites, versus 61.1% of the cases of anaphylaxis. We found that the most severe cases (anaphylaxis) corresponded to males of older age and with local reactions to tick bites.

In Spain, in the region of La Rioja,³⁷ 15% of the exposed population (forestry workers) had high titers of IgE against alpha-gal, compared with 4% of the controls (although none of them presented symptoms with the consumption of meat). These data can be compared with those obtained in Germany,³⁸ where the prevalence of positive IgE against alpha-gal in hunters and forestry workers was 35% (using CAP > 0.1 kU/l as cut-off point) or 19.3% (using CAP > 0.35 kU/l as cut-off point), but only 8.6% of the latter showed delayed anaphylaxis in response to beef.

The sources of exposure in alpha-gal syndrome are highly varied, with cases having been related to cetuximab, gelatins, plasma expanders, meat/innards, vaccines, sweets, drug capsules, anti-snake venom sera and heart valves. In our setting, the presentation is typically characterized by anaphylaxis (in up to 65% of the patients) occurring in the early morning hours, between 2 and 10 hours after consuming beef or pork. The condition is more often seen in males of about 50 years of age. This is probably because such individuals are more exposed to tick bites (cattle raisers, forestry workers, outdoor sports, hunters, hikers, or subjects involved in walking or bicycling activities). Of note is the fact that our series includes the first case in Spain of a 13-year-old boy with delayed anaphylaxis due to pork, and with positive beef skin test results and specific IgE against alpha-gal.

On the other hand, the condition may also constitute an occupational disease, as in reactions caused by exposure to cow amniotic fluid at calving. We have already documented five such cases to date. Some patients also develop skin symptoms on the arms on kneading raw meat for the preparation of sausages. None of our patients reported symptoms associated with milk or dairy products.

Six patients had taken NSAIDs on the day of the reaction. Controlled exposure testing was thus performed to four and was well tolerated in all of them. The drug possibly may have acted as a cofactor, though in our patients this does not seem likely, since the medication failed to shorten the reaction latency period.

In our setting, the meats most often implicated in alpha-gal syndrome are beef, pork and lamb, although other types of mammal meats can be involved – including wild boar, as in one of our cases. The consumption of viscera such as kidney – which contains abundant alpha-gal^{26,29} – is associated with shorter latency reactions.

It should be noted that in the subgroup of patients with anaphylaxis, 60% suffered another reaction after the diagnosis of the syndrome. In this regard, we believe that patients find it hard to accept complete avoidance of meat, because they do not trust the diagnosis (e.g., they sometimes eat meat without suffering symptoms); or due to cultural/diet reasons. In this context it is essential to have an epinephrine self-injector available in the event of an emergency. On the other hand, before being diagnosed, the patients had suffered an average of 3.5 episodes each – although one individual had experienced 20 previous reactions. Following the diagnosis, the average dropped to 1.6 episodes per patient, with statistically significant differences in both the patients with urticaria and in those with anaphylaxis – this demonstrates improvement in patient quality of life and prognosis. Furthermore, all the patients were seen to tolerate cured ham, which thus allowed them to eat at least one pork product without problems.

In our patients, the mean total IgE titer was 369 IU/ml (median 248 IU/ml), and the mean titer corresponding to specific IgE against alpha-gal was 47.97 kU/l. The true figure was probably higher, because in seven patients the titer was >100 KU/l, which exceeds the upper detection limit of our laboratory; the real titer is therefore not known. The specific IgE titers ranged between 1.03 and 100 kU/l. Skin testing with beef only proved positive in 76% of the patients. Specific IgE was positive in all cases. When IgE against alpha-gal proves negative but clinical suspicion is high, skin testing with cetuximab can be carried out, with positive results in 100% of the cases, according to Martinez-Arcediano et al.⁴⁰ In patients with doubtful symptoms and positive specific IgE against alpha-gal, basophil activation testing can be performed, and a positive result would support the clinical suspicion.

Consistent clinical findings and positive IgE against alpha-gal suffice to establish the diagnosis of the disease. Provocation testing is normally not needed, in view of the hypothetical appearance of potentially serious symptoms, and in our opinion it makes no sense to increase the dose as in controlled exposure tests in protein allergies, since the reaction is delayed and does not always appear. If controlled exposure testing is considered essential (a practice we do not advise), it could be performed with viscera (kidney), gelatin, Gelaspan or even cetuximab.

In agreement with Kennedy,¹⁸ we do not consider it necessary to conduct testing of IgE against other meats. Likewise, it is of no practical interest to test specific IgE against cat antigens, since it will prove positive in these

patients, as shown by Gonzalez-Quintela et al.,³⁹ due to the Fel d5 allergen, which is cat IgA. In the same way, testing of cow's milk is of little use, since it usually proves positive (with negative results referred to the milk fractions), due to the presence of alpha-gal.¹⁸ If such patients develop respiratory symptoms as a consequence of exposure to cat dander, specific IgE against cat antigen could constitute a false-positive result; we thus recommend testing for specific IgE against Fel d1.

Due to the structural similarity between alpha-gal and the blood group B antigen, blood group B status was believed to afford protection against alpha-gal syndrome. However, the latest studies⁴¹ have concluded that there is no evidence that blood group B results in lessened susceptibility to sensitization to type I alpha-gal, lowered specific IgE against alpha-gal, or a lesser risk of developing alpha-gal syndrome. The absence of patients with blood group B is explained by the low prevalence of this blood group. In this regard, in our setting (Galicia, Spain), the prevalence of blood group B is 6.8%, and that of blood group AB 3%.

Alpha-gal syndrome does not appear to be more frequent in atopic patients than in non-atopic individuals. In our series, 36% of the patients were atopic – this being consistent with the data published by other authors.¹³

We believe that thanks to the diagnosis of alpha-gal allergy, panallergens and the study of cofactors, the diagnosis of idiopathic anaphylaxis has decreased drastically in recent years.

In relation to pediatric patients, the literature only reports one boy with a reaction to measles-mumps-rubella (MMR) – varicella vaccine and gelatin positivity,¹⁹ and the cases described by Kennedy et al.,¹⁸ comprising 51 pediatric patients between 4–17 years of age in the state of Virginia (USA) with a history of recurrent acute urticaria (not chronic urticaria), idiopathic anaphylaxis or angioedema – with positive IgE against alpha-gal in 47 cases. Most of the children had suffered persistent local reactions after tick bites (90% of the patients had suffered bites in the last year).

Lastly, in children suffering recurrent urticaria with no clear causal relationship, anaphylactic reactions (particularly delayed anaphylaxis) or urticaria-like reactions to vaccines and products of bovine or porcine origin, the possibility of alpha-gal allergy as the underlying cause should be evaluated.

Conflict of interest

The authors have no conflict of interest to declare.

Funding sources

This research did not receive any specific grant from funding agencies in the public, commercial or not-for-profit sectors.

Acknowledgements

The authors wish to thank Professor Constantino Arosa Gómez, Informatic Engineering PhD and Economics PhD for his valuable help in the statistical analysis.

References

- Macher BA, Galili U. The Gal α 1,3Gal β 1,4GlcNAc-R (alpha-Gal) epitope: a carbohydrate of unique evolution and clinical relevance. *Biochim Biophys Acta*. 2008;1780(2):75–88.
- Van Nunen SA, O'Connor KS, Clarke LR, Boyle RX, Fernando SL. An association between tick bite reactions and red meat allergy in humans. *Med J Aust*. 2009;190(9):510–1.
- Adédoyin J, Grönlund H, Oman H, Johansson SG, van Hage M. Cat IgA, representative of new carbohydrate cross-reactive allergens. *J Allergy Clin Immunol*. 2007;119(3):640–5.
- O'Neil BH, Allen R, Spigel DR, Stinchcombe TE, Moore DT, Berlin JD, et al. High incidence of cetuximab-related infusion reactions in Tennessee and North Carolina and the association with atopic history. *J Clin Oncol*. 2007;25(24):3644–8.
- Chung CH, Mirakhor B, Chan E, Le QT, Berlin J, Morse M, et al. Cetuximab-induced anaphylaxis and IgE specific for galactose-alpha-1,3-galactose. *N Engl J Med*. 2008;358(11):1109–17.
- Commins SP, Satinover SM, Hosen J, Mozena J, Borish L, Lewis BD, et al. Delayed anaphylaxis, angioedema, or urticaria after consumption of red meat in patients with IgE antibodies specific for galactose-alpha-1,3-galactose. *J Allergy Clin Immunol*. 2009;123(2):426–33.
- Commins SP, James HR, Kelly LA, Pochan SL, Workman LJ, Perzanowski MS, et al. The relevance of tick bites to the production of IgE antibodies to the mammalian oligosaccharide galactose- α -1,3-galactose. *J Allergy Clin Immunol*. 2011;127(5):1286–93.
- Núñez R, Carballada F, Gonzalez-Quintela A, Gomez-Rial J, Boquete M, Vidal C. Delayed mammalian meat-induced anaphylaxis due to galactose- α -1,3-galactose in 5 European patients. *J Allergy Clin Immunol*. 2011;128(5):1122–4.
- Caponetto P, Fischer J, Biedermann T. Gelatin-containing sweets can elicit anaphylaxis in a patient with sensitization to galactose- α -1,3-galactose. *J Allergy Clin Immunol Pract*. 2013;1(3):302–3.
- Mullins RJ, James H, Platts-Mills TA, Commins S. Relationship between red meat allergy and sensitization to gelatin and galactose- α -1,3-galactose. *J Allergy Clin Immunol*. 2012;129(5):1334–42.
- Vidal C, Méndez-Brea P, López-Freire S, González-Vidal T. Vaginal capsules: an unsuspected probable source of exposure to alpha-gal. *J Investig Allergol Clin Immunol*. 2016;26(6):388–9.
- Swiontek K, Morisset M, Codreanu-Morel F, Fischer J, Mehlich J, Darsow U, et al. Drugs of porcine origin - a risk for patients with α -Gal syndrome? *J Allergy Clin Immunol Pract*. 2018; pii: S2213-2198(18)30825-0. doi: 10.1016/j.jaip.2018.12.005. [Epub ahead of print] PubMed PMID: 30557715.
- Naso F, Gandaglia A, Bottio T, Tarzia V, Nottle MB, d'Apice AJ, et al. First quantification of alpha-Gal epitope in current glutaraldehyde-fixed heart valve bioprostheses. *Xenotransplantation*. 2013;20(4):252–61.
- Mozzicato SM, Tripathi A, Posthumus JB, Platts-Mills TAE, Commins SP. Porcine or bovine valve replacement in 3 patients with IgE antibodies to the mammalian oligosaccharide galactose-alpha-1,3-galactose. *J Allergy Clin Immunol Pract*. 2014;2:637–8.
- Fischer J, Eberlein B, Hilger C, Eyer F, Eyerich S, Ollert M, et al. Alpha-gal is a possible target of IgE-mediated reactivity to antivenom. *Allergy*. 2017;72(5):764–71.
- Rizer J, Brill K, Charlton N, King J. Acute hypersensitivity reaction to Crotalidae polyvalent immune Fab (CroFab) as initial presentation of galactose- α -1,3-galactose (α -gal) allergy. *Clin Toxicol (Phila)*. 2017;55(7):668–9.
- Núñez-Orjales R, Martín-Lazaro J, Lopez-Freire S, Galan-Nieto A, Lombardero-Vega M, Carballada-Gonzalez F. Bovine amniotic fluid: a new and occupational source of galactose- α -1,3-galactose. *J Investig Allergol Clin Immunol*. 2017;27(5):313–4.
- Kennedy JL, Stallings AP, Platts-Mills TA, Oliveira WM, Workman L, James HR, et al. Galactose- α -1,3-galactose and delayed anaphylaxis, angioedema, and urticaria in children. *Pediatrics*. 2013;131(5):e1545–52.
- Stone CA Jr, Commins SP, Choudhary S, Vethody C, Heavrin JL, Wingerter J, et al. Anaphylaxis after vaccination in a pediatric patient: further implicating alpha-gal allergy. *J Allergy Clin Immunol Pract*. 2018; pii: S2213-2198(18)30393-3.
- Stone CA Jr, Hemler JA, Commins SP, Schuyler AJ, Phillippe EJ, Peebles RE Jr, et al. Anaphylaxis after zoster vaccine: implicating alpha-gal allergy as a possible mechanism. *J Allergy Clin Immunol*. 2017;139(5):1710–3.
- Brockow K, Garvey LH, Aberer W, Atanaskovic-Markovic M, Barbaud A, Bilo MB, et al. Skin test concentrations for systemically administered drugs – an ENDA/EAACI Drug Allergy Interest Group position paper. *Allergy*. 2013;68(6):702–12.
- Commins SP, Platts-Mills TA. Allergenicity of carbohydrates and their role in anaphylactic events. *Curr Allergy Asthma Rep*. 2010;10(1):29–33.
- Homann A, Schramm G, Jappe U. Glycans and glycan-specific IgE in clinical and molecular allergology; sensitization, diagnostics, and clinical symptoms. *J Allergy Clin Immunol*. 2017;140(2):356–68.
- Gonzalez-Quintela A, Gomez-Rial J, Valcárcel C, Campos J, Sanz ML, Linneberg A, et al. Immunoglobulin-E reactivity to wine glycoproteins in heavy drinkers. *Alcohol*. 2011;45(2):113–22.
- Jappe U, Minge S, Kraft B, Ludwig A, Przybilla B, Walker A, et al. Meat allergy associated with galactosyl- α -(1,3)-galactose (α -Gal)-closing diagnostic gaps by anti- α -Gal IgE immune profiling. *Allergy*. 2018;73(1):93–105.
- Wilson JM, Schuyler AJ, Schroeder N, Platts-Mills TA. Galactose-alpha-1,3-galactose: atypical food allergen or model IgE hypersensitivity? *Curr Allergy Asthma Rep*. 2017;17(1):8.
- Apostolovic D, Krstic M, Mihailovic J, Starkhammar M, Cirkovic Velickovic T, Hamsten C, et al. Peptidomics of an in vitro digested α -Gal carrying protein revealed IgE-reactive peptides. *Sci Rep*. 2017;7(1):5201.
- Pattanaik D, Lieberman P, Lieberman J, Pongdee T, Keene AT. The changing face of anaphylaxis in adults and adolescents. *Ann Allergy Asthma Immunol*. 2018;121(5):594–7.
- Wilson JM, Nguyen AT, Schuyler AJ, Commins SP, Taylor AM, Platts-Mills TAE, et al. IgE to the mammalian oligosaccharide galactose- α -1,3-galactose is associated with increased atheroma volume and plaques with unstable characteristics-brief report. *Arterioscler Thromb Vasc Biol*. 2018;38(7):1665–9.
- Van Nunen SA. Tick-induced allergies: mammalian meat allergy and tick anaphylaxis. *Med J Aust*. 2018;208(7):316–21.
- Araujo RN, Franco PF, Rodrigues H, Santos LCB, McKay CS, Sanhueza CA, et al. *Amblyomma sculptum* tick saliva: α -Gal identification, antibody response and possible association with red meat allergy in Brazil. *Int J Parasitol*. 2016;46(3):213–20.
- Hamsten C, Starkhammar M, Tran TA, Johansson M, Bengtsson U, Ahlén G, et al. Identification of galactose- α -1,3-galactose in the gastrointestinal tract of the tick *Ixodes ricinus*; possible relationship with red meat allergy. *Allergy*. 2013;68(4):549–52.
- Vázquez L, Panadero R, Dacal V, Pato FJ, López C, Díaz P, et al. Tick infestation (Acari: Ixodidae) in roe deer (*Capreolus capreolus*) from northwestern Spain: population dynamics and risk stratification. *Exp Appl Acarol*. 2011;53(4):399–409.
- Cabezas-Cruz A, Espinosa PJ, Alberdi P, Šimo L, Valdés JJ, Mateos-Hernández L, et al. Tick galactosyltransferases are involved in α -Gal synthesis and play a role during *Anaplasma phagocytophilum* infection and *Ixodes scapularis* tick vector development. *Sci Rep*. 2018;8(1):14224.
- Commins SP, Platts-Mills TA. Tick bites and red meat allergy. *Curr Opin Allergy Clin Immunol*. 2013;13:354–9.

36. Burke G, Wikel SK, Spielman A, Telford SR, McKay K, Krause PJ. Tick-borne Infection Study Group. Hypersensitivity to ticks and Lyme disease risk. *Emerg Infect Dis.* 2005;11(1):36–41.
37. Venturini M, Lobera T, Sebastian A, Portillo A, Oteo JA. IgE to alfa-tal in forestes. and forest workers from La Rioja, North of Spain. *J Investig Allergol Clin Immunol.* 2018;28(2): 106–12.
38. Fischer J, Lupberger E, Hebsaker J, Blumenstock G, Aichinger E, Yazdi AS, et al. Prevalence of type I sensitization to alpha-gal in forest service employees and hunters. *Allergy.* 2017;72(10):1540–7.
39. Gonzalez-Quintela A, Dam Laursen AS, Vidal C, Skaaby T, Gude F, Linneberg A. IgE antibodies to alpha-gal in the general adult population: relationship with tick bites, atopy, and cat ownership. *Clin Exp Allergy.* 2014;44(8):1061–8.
40. Martínez Arcediano A, Audicana Berasategui MT, Longo Areso N, Ibáñez EF, Balza de Vallejo OV, Velasco Azagra M, et al. Allergy to galactose-alpha-1,3-galactose: clinical features and the diagnostic value of cetuximab. *J Investig Allergol Clin Immunol.* 2014;24(6):450–2.
41. Cabezas-Cruz A, De la fuente J, Fischer J, Hebsaker J, Lupberger E, Blumensthck G, et al. Prevalence of type I sensitization to alpha-gal in forest service employees and hunters: is the blood type an overlooked risk factor in epidemiological studies of the alfa-gal syndrome? *Allergy.* 2017;72(12): 2044–7.