

# IgE-Mediated Sensitization to Galactose- $\alpha$ -1,3-Galactose ( $\alpha$ -Gal) in Urticaria and Anaphylaxis in Spain: Geographical Variations and Risk Factors

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## ■ Abstract

**Background:** The objectives of this study were to investigate the prevalence of sIgE to galactose- $\alpha$ -1,3-galactose ( $\alpha$ -gal) in individuals with acute urticaria or anaphylaxis from different geographical areas of Spain and to evaluate the relevance of demographics and lifestyle as risk factors for this immune response.

**Methods:** Participants were recruited from allergy departments at 14 Spanish hospitals. Patients aged 18 years or older presenting with urticaria or anaphylaxis were enrolled into one of 2 arms: cases and controls. An interviewer-administered questionnaire collecting demographic data, lifestyle habits, and the presence of cofactors was obtained from each participant. sIgE to  $\alpha$ -gal and total IgE were determined using ImmunoCAP. sIgE levels  $\geq 0.35$  kU/L were considered a positive result.

**Results:** The study population comprised 160 cases and 126 controls. The median age was 44 years. The overall prevalence of a positive result of sIgE to  $\alpha$ -gal was 15.7%; this was higher in cases (26.3%) than in controls (2.4%). The sIgE anti- $\alpha$ -gal positivity rate ranged from 37.68% (rural) to 15.38% (semiurban), and 7.85% (urban). The rates of positivity were 46.32%, (Northern), 0.72% (Center), and 0% (Mediterranean). A positive result for sIgE to  $\alpha$ -gal was associated with a history of tick bites, participation in outdoor activities, pet ownership, and ingestion of mammalian meats or innards before the onset of symptoms. Only alcohol consumption could be implicated as a cofactor.

**Conclusion:** Sensitization to  $\alpha$ -gal in patients with urticaria or anaphylaxis differs considerably between the 3 geographical areas studied and is related to tick bites.

**Key words:**  $\alpha$ -Gal. Risk factors. Epidemiology. Outdoor activities. Tick bites. Cofactors.

## ■ Resumen

**Antecedentes:** Investigar la prevalencia de IgE específica a galactosa- $\alpha$ -1,3-galactosa ( $\alpha$ -gal) en sujetos con urticaria aguda o anafilaxia de diferentes zonas geográficas de España y evaluar la relevancia de factores demográficos y de estilo de vida como factores de riesgo para esta respuesta inmunológica.

**Métodos:** Pacientes de 18 años o mayores con urticaria o anafilaxia fueron reclutados en los Departamentos de Alergia de 14 hospitales españoles e incluidos en uno de dos grupos; casos o controles. Se recogieron datos demográficos, de estilo de vida y la presencia de cofactores. La IgE total e IgE específica a  $\alpha$ -gal se determinaron mediante ImmunoCAP. Niveles de IgE específica  $\geq 0,35$  kU/L fueron considerados como positivos.

**Resultados:** Se reclutaron 160 casos y 126 controles. La mediana de edad fue 44 años. La prevalencia global de positividad de IgE específica a  $\alpha$ -gal fue 15,7%; siendo mayor en casos (26,3%) que en controles (2,4%) y oscilando entre 37,68% (rural) a 15,38% (semiurbano) y 7,85% (urbano). Las frecuencias de positividad fueron 46,32%, (Norte), 0,72% (Centro), y 0% (Mediterráneo). La positividad de IgE específica a  $\alpha$ -gal se asoció a haber experimentado picadura de garrapata, participación en actividades de exterior, tenencia de mascotas e ingestión de carne de mamíferos o vísceras previo al inicio de los síntomas. Solo el consumo de alcohol podía ser implicado como cofactor.

**Conclusión:** La sensibilización a  $\alpha$ -gal en pacientes con urticaria o anafilaxia difiere considerablemente entre las tres zonas estudiadas y está relacionada con picadura de garrapata.

**Palabras clave:** Alfa-gal. Factores de riesgo. Epidemiología. Actividades de exterior. Picadura de garrapata. Cofactores.

## Introduction

Galactose- $\alpha$ -1,3-galactose ( $\alpha$ -gal) is an oligosaccharide that is abundantly expressed on the glycoconjugates of nonprimate mammal proteins. Humans, apes, and Old World monkeys do not express the  $\alpha$ -gal epitope as a consequence of inactivation of the enzyme 3Gal $\beta$ 1-4GlcNAc  $\alpha$ 1-3-galactosyltransferase. All immune-competent humans naturally produce IgG and IgM that are specific for  $\alpha$ -linked galactose. Human natural antialgal antibodies have been associated with xenotransplant rejection [1].

IgE antibodies against  $\alpha$ -gal were initially identified in patients who presented with anaphylactic reactions to their first treatment with cetuximab [2]. Such antibodies also bound to a range of mammalian proteins, including cat and dog extracts. Nevertheless, sIgE to  $\alpha$ -gal was more frequently detected among patients with idiopathic urticaria, angioedema, or anaphylaxis than among patients with asthma or other complaints [3]. The involvement of sIgE against  $\alpha$ -gal has been reported in patients who developed delayed allergic reactions after ingestion of mammalian meat [4], intravenous administration of gelatin colloids, and intake of foods containing gelatin of mammalian origin [5]. These antibodies have also been associated with allergic reactions to other products of mammalian origin, such as cardiac valves [6], Crotalidae antivenom [7], zoster vaccine [8], and fenticonazole vaginal capsules [9]. Bovine amniotic fluid has been proven as an occupational source of  $\alpha$ -gal [10].

Few epidemiological studies have evaluated the prevalence of sIgE to  $\alpha$ -gal and the factors associated with this response. Chung et al [2] reported marked differences in the rate of positivity of sIgE to cetuximab between different areas of the USA, where this finding was more common in the southeast.

In Europe, the frequency of positivity of sIgE to  $\alpha$ -gal has been reported to be 5.5% (Denmark) and 8.1% (Spain) [11]. Villalta et al [12] found the prevalence of sensitization to be 24.7% in a rural area in northeast Italy, which was significantly higher than in an urban area (1.2%). Stevens et al [13] reported a positivity rate of 7.2% in children in Ghana. IgE antibodies against  $\alpha$ -gal were found in 85% of parasite-infected individuals from rural Zimbabwe, leading the authors to suggest that their results supported the possibility that parasitic infections might induce IgE antibodies against  $\alpha$ -gal [14]. Intense geographical differences in the prevalence of sensitization to  $\alpha$ -gal were reported in Sweden, varying from

0.7% of teenagers in an area where tick bites were rare, to 10% of 143 healthy blood donors from the greater Stockholm area and 22% of patients with Lyme disease as a confirmed recently tick-bitten population [15].

Tick bites are the main factor involved in the development of IgE antibodies against  $\alpha$ -gal [11,12,15-18]. Therefore, activities that favor exposure to ticks could be associated with a higher prevalence of sensitization to  $\alpha$ -gal. German [17] and Spanish [18] authors have evaluated the prevalence of  $\alpha$ -gal sIgE in persons at risk of tick bites, namely, forest service employees and hunters in the German study and forest employees in the Spanish study. Both studies found a higher prevalence among at-risk subjects than among controls.

Data on the relationship between pet exposure and the prevalence of positive sIgE to  $\alpha$ -gal are scarce. Gonzalez-Quintela et al [11] reported an association between IgE to  $\alpha$ -gal and pet ownership in both a Spanish and a Danish series. The Danish series provided information about the type of pets at home and showed that having a cat was associated with sIgE to  $\alpha$ -gal. Nevertheless, in a German study, keeping a pet did not increase the risk of sIgE to  $\alpha$ -gal [17].

The structure of the epitope of  $\alpha$ -gal is similar to that of human blood group B [15]. It has been speculated that the presence of B-negative blood groups [15] and exposure to inhaled proteins of animal origin [19] are also related to production of IgE against  $\alpha$ -gal.

The aims of this study were to investigate the prevalence of sIgE to  $\alpha$ -gal and the factors associated with this finding in patients presenting with urticaria or anaphylaxis recruited from different areas of Spain.

## Material and Methods

Participants were enrolled by the allergy departments of 14 Spanish hospitals (Table S1 Supporting Information). The study was carried out from January 2015 to December 2016.

Based on geographical and climatic conditions, the participating centers were grouped into 3 areas: Northern, Mediterranean, and Center (Table S1, Supporting Information). Residence was divided into 3 classes; urban (>15 000 residents), semiurban (2500-15 000 residents), and rural (<2500 residents).

This study was approved by the Ethics Committees of the University Hospitals of Guadalajara and Navarra, Spain.

Table 1. Group Criteria

Cases	Controls
- Acute urticaria and/or anaphylaxis of unknown origin	- Acute urticaria and/or anaphylaxis of known etiology excluding foods of mammalian origin, cetuximab infusion, colloid-based gelatin administration, and tick bites
- Acute urticaria and/or anaphylaxis after eating mammalian meat or innards	
- Acute urticaria and/or anaphylaxis related to administration of mammalian gelatin	
- Acute urticaria and/or anaphylaxis after cetuximab infusion	
- Acute urticaria and/or anaphylaxis related to tick bites	

Written informed consent was obtained from all participants. Patients aged 18 years or older were enrolled as cases or controls. The inclusion criteria are presented in Table 1. An interviewer-administered questionnaire was used to collect the following data: sex, age, place of residence, blood group, atopy, history of tick bites, participation in outdoor activities (hunting, mountaineering/trekking, forest service, farm activity), exposure to pets, clinical presentation (urticaria/anaphylaxis), suspected trigger agent, latency of symptoms, presence of cofactors (alcohol intake, exercise, and/or nonsteroidal anti-inflammatory drugs [NSAIDs]), tick bites before the reaction, and exposure to cetuximab, gelatins, and mammalian meat or innards. Blood samples were obtained and stored at  $-20^{\circ}\text{C}$  until laboratory determinations were performed.

Total IgE, specific IgE, and tryptase were determined using an automated ImmunoCAP platform (Thermo Fisher Scientific) according to the manufacturer's instructions. sIgE levels were quantified as kU/L, with a measurement range of 0.0-100 kU/L. sIgE levels  $\geq 0.35$  kU/L were considered a positive result. Values above 100 kU/L were classed as 100 kU/L.

### Statistical Analysis

The statistical analyses were performed with SPSS, version 20 (IBM Corp.), and GraphPad Prism, version 4 (GraphPad Software). Proportions were used to report categorical variables; intergroup comparisons were made using the  $\chi^2$  test or Fisher exact test when applicable. Continuous variables were presented as median (IQR) and compared using the Mann-Whitney test. The Spearman rho was used to assess correlations between continuous variables. Multivariate logistic regression analysis was performed. Statistical significance was set at  $P < .05$ .

### Results

The study population comprised 286 participants (160 cases and 126 controls). Demographic and other epidemiological data obtained through the questionnaire are summarized in Table 2

The median (IQR) age of the study population was 44 (33-53) years. There were no significant differences between cases and controls.

Most patients were from the Center, followed by the Northern and Mediterranean areas (Table 2).

The proportion of cases in Northern centers was higher than in the other geographical areas, although the difference did not reach statistical significance.

Men and women were almost equally represented (50.35% male), with a similar distribution between cases and controls.

Urban residence predominated in all geographical areas, with a more intense representation in the Center and Mediterranean area (76.09 and 77.36%, respectively) than in the Northern area (47.37%) (Table S2 Supporting Information).

Table 2. Questionnaire Data

	Cases	Controls	P
Male sex, No. (%)	83 (51.88%)	61 (48.41%)	NS
Median (IQR) age, y	44 (33-55)	44 (33-52)	NS
Recruitment area (N=286)			
Northern	59 (36.9%) <sup>a</sup> (62.1%) <sup>b</sup>	36 (28.6%) <sup>a</sup> (38%) <sup>b</sup>	NS
Mediterranean	29 (18.1%) <sup>a</sup> (54.7%) <sup>b</sup>	24 (19%) <sup>a</sup> (45.3%) <sup>b</sup>	NS
Center	72 (45%) <sup>a</sup> (52.2%) <sup>b</sup>	66 (52.4%) <sup>a</sup> (47.8%) <sup>b</sup>	NS
Place of residence			
Urban	105 (65.6%) <sup>a</sup>	86 (68.2%) <sup>a</sup>	NS
Semiurban	14 (8.7%) <sup>a</sup>	12 (9.5%) <sup>a</sup>	
Rural	44 (27.5%) <sup>a</sup>	25 (19.8%) <sup>a</sup>	
Clinical picture			
Urticaria	85 (53.13%) <sup>a</sup>	63 (50%) <sup>a</sup>	NS
Anaphylaxis	75 (46.88%) <sup>a</sup>	63 (50%) <sup>a</sup>	
Atopy (N=286)	66 (41.25%) <sup>a</sup>	60 (47.62%) <sup>a</sup>	NS
Outdoor activities <sup>c</sup>	70 (46.36%) <sup>a</sup>	34 (27.2%) <sup>a</sup>	$P < .01$
Pet ownership <sup>d</sup>	67 (43.79%) <sup>a</sup>	47 (37.6%) <sup>a</sup>	NS
B-negative blood group <sup>e</sup>	54 (85.71%) <sup>a</sup>	35 (83.33%) <sup>a</sup>	NS
History of tick bites <sup>f</sup>	53 (33.3%) <sup>a</sup>	6 (4.6%) <sup>a</sup>	$P < .001$

<sup>a</sup>Percentage refers to total patients by columns (cases or controls).

<sup>b</sup>Percentage refers to total by area.

<sup>c</sup>Available data from N=276 (151 cases, 125 controls).

<sup>d</sup>Available data from N=278 (153 cases, 125 controls).

<sup>e</sup>Available data from N=105 (63 cases, 42 controls).

<sup>f</sup>Available data from N=285 (159 cases, 126 controls).

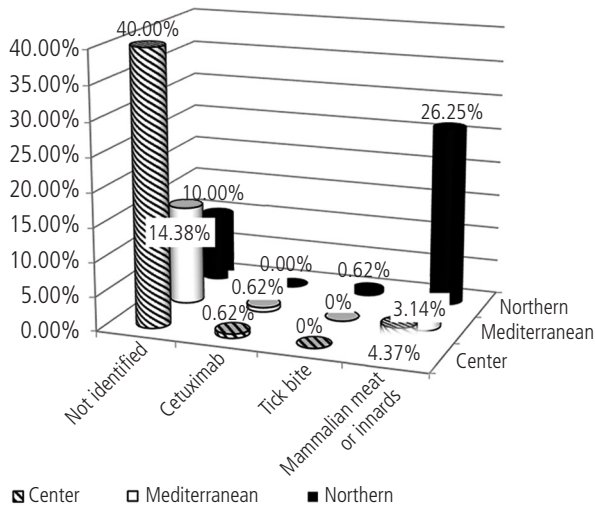


Figure 1. Trigger factors in cases.

More patients from Northern centers participated in outdoor activities than those from the other geographical areas. Mountaineering/trekking was the most frequently reported activity in all areas; 12.32%, 18.87%, and 42.35% in the Center, Mediterranean and Northern area respectively.

Women more frequently reported not participating in outdoor activities. A history of tick bites was more frequently reported by men (Table S3 Supporting Information). Patients who participated in outdoor activities reported tick bites more frequently ( $P < .001$ ).

**Trigger Factors in Cases and Controls**

The distribution of cases and controls according to the etiology (Table 1) are shown in Figures 1 and 2, respectively. No patients presented with symptoms after exposure to gelatin. "Not identified" was the most frequently recorded

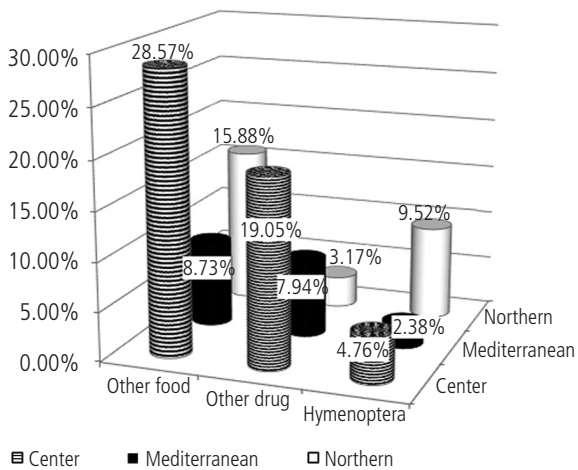


Figure 2. Trigger factors in controls.

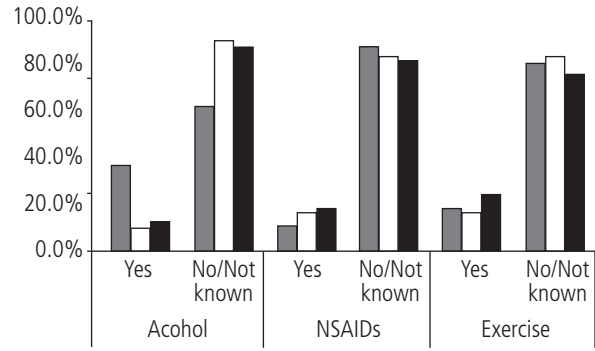


Figure 3. Association between cofactors and triggers. Abbreviation: NSAIDs indicates nonsteroidal anti-inflammatory drugs.

category among cases from the Center and Mediterranean area, whereas ingestion of mammalian meat or innards clearly predominated in Northern patients ( $P < .001$ ). Only 1 patient reported symptoms (anaphylaxis) after a tick bite.

"Other foods" was the most frequently reported trigger in controls (Figure 2).

When food was the trigger, the median latency period was longer in cases than in controls (240 [120-360] vs 30 [10-60] minutes) ( $P < .001$ ). A significant association was observed between alcohol intake and mammalian meat or innards as the trigger. No association was observed for NSAID intake or exercise as potential cofactors (Figure 3).

**In vitro Study**

The overall prevalence of a positive result for sIgE to  $\alpha$ -gal was 15.7% (95%CI, 11.5-20.0), with a significantly higher prevalence among cases (26.3% [95%CI, 19.4-33.1]) than among controls (2.4% [95%CI, 0.0-5.1]) ( $P < .001$ ).

Descriptive data for in vitro testing are shown in Table 3. Although sIgE to  $\alpha$ -gal ranged from 0 kU/L to >100 kU/L, many negative or low results were obtained in both cases and controls. Three patients had a value >100 kU/L, and in 5 patients the result was 100 kU/L. Even if median values in both groups were 0.00 kU/L (0.00-0.69 in cases and 0.00-0.01 in controls), levels of sIgE to  $\alpha$ -gal were significantly higher ( $P < .001$ ) in cases (arithmetic mean [SD], 10.47 [25.69]) than in controls (arithmetic mean, 0.25 [2.13]). The geometric mean of the positive results was 17.4 kU/L, 18.9 kU/L, and 3.6 kU/L in the whole group, cases and controls, respectively.

As for the clinical picture (urticaria/anaphylaxis), higher levels of sIgE to  $\alpha$ -gal ( $P = .009$ ) were observed among patients presenting with anaphylaxis (arithmetic mean, 10.5 [27.4]; median, 0.01 [0.00-0.10]) than among those with acute urticaria (arithmetic mean, 4.44 [15.4]; median, 0.00 [0.00-0.28]). A positive result was recorded in 27 patients with anaphylaxis and 18 patients with urticaria. The values obtained for the geometric mean of the positive results were 19 kU/L in patients presenting with anaphylaxis and 14.4 kU/L in patients with urticaria.

A positive result for sIgE to  $\alpha$ -gal was more frequently observed among men than women (24.31% vs 7.04%;  $P < .001$ ).

Table 3. Laboratory Tests

	sIgE $\alpha$ -Gal, kU/L <sup>a</sup>	Tryptase, $\mu$ g/L	Total IgE, IU/mL (N=213)	sIgE <i>Ascaris</i> , kU/L <sup>b</sup> (N=199)	sIgE <i>Anisakis</i> , kU/L <sup>c</sup>
Median	0	4.5	128	0.02	0.03
Mean	Overall 5.96 North 17.86 Center 0.06 Mediterranean 0.02	5.24	275.87	Overall 0.33 North 0.73 Center 0.17 Mediterranean 0.09	Overall 2.71 North 5.24 Center 1.86 Mediterranean 1.16
Range	0-100	1-25.9	2.63-5470	0-22.1	0-100
95%CI	3.649-8.282	4.795-5.682	201.69-350.06	0.131-0.538	1.095-3.965
Positive results	No. (%) <sup>d</sup>			No. (%) <sup>d</sup>	No. (%) <sup>d</sup>
Overall	45 (15.7%)			36 (14.81%)	60 (23.35%)
North	44 (46.32%)			18 (23.38%)	18 (24%)
Center	1 (0.72%)			15 (12.61%)	35 (26.32%)
Mediterranean	0 (0%)			3 (6.38%)	7 (14.29%)

<sup>a</sup>Overall 286; North 95; Center 138; Mediterranean 53.

<sup>b</sup>Overall 243; North 77; Center 119; Mediterranean 47.

<sup>c</sup>Overall 257; North 75; Center 133; Mediterranean 49.

<sup>d</sup>Positive result  $\geq 0.35$  kU/L

Strong geographical differences were also found, with nearly all of the positive results in the Northern area and rates of positivity of 46.32% (Northern), 0.72% (Center), and 0% (Mediterranean) ( $P < .001$ ) (Table 3).

As Figure 4 shows, several circumstances were associated with differences in the prevalence of sensitization to  $\alpha$ -gal. Data correspond to the overall group. When data correspond to cases, this is indicated in the figure. Wide differences in sensitization to  $\alpha$ -gal were observed according to place of residence. Positive sIgE to  $\alpha$ -gal was more frequent in patients from rural areas than in those from the other 2 types of

residence, especially urban areas, with a gradient of positivity of 37.68% (rural), 15.38% (semiurban), and 7.85% (urban). The finding of a positive result in sIgE to  $\alpha$ -gal was associated with a history of tick bites, participation in outdoor activities, pet exposure, and having eaten mammalian meats or innards in the 6 hours before onset of symptoms.

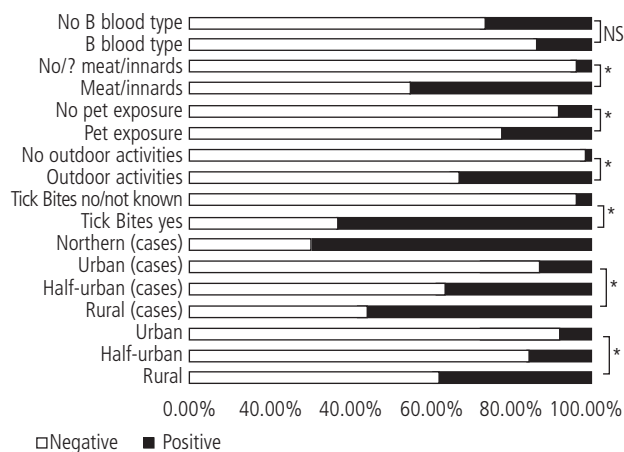
Overall, the prevalence of IgE sensitization to  $\alpha$ -gal was higher among patients who were exposed to mammals as pets than in those with no pets. Nevertheless, this finding was more frequently observed in participants exposed to several mammals (43.75%) or only dogs (23.08%) than in those with only cats (8.33%) and no pets at home (8.54%). The proportion of patients having more than 1 type of mammal as pets was higher in the Northern area (13.79%) than in the Center (2.9%) and Mediterranean area (0%).

No association was found between sensitization to  $\alpha$ -gal and atopy, cetuximab infusion, or blood group.

The 3 controls with a positive sIgE result to  $\alpha$ -gal lived in the Northern area and participated in outdoor activities. Two of them reported tick bites.

sIgE to *Ascaris* and *Anisakis* correlated with sIgE to  $\alpha$ -gal (Spearman  $\rho$ , 0.41 and 0.24, respectively). The proportion of positive and negative results of sIgE to  $\alpha$ -gal, *Ascaris*, and *Anisakis* are shown in Table S4 (Supporting information).

Multivariate analysis was performed for participants from the Northern area. After adjustment for sex, place of residence, pet ownership, and history of tick bites, only the last variable significantly increased the risk of positive sIgE to  $\alpha$ -gal. Although the stability of the model seems to be weak considering the amplitude of the confidence interval, the odds ratio for IgE to  $\alpha$ -gal is 32 times higher (95%CI, 8.43-122.63) in participants with a history of tick bites than in those with no history or who were not aware of having been exposed.



Abbreviation: NS, nonsignificant.

Asterisks indicate  $P < .001$ ;

Figure 4. sIgE to  $\alpha$ -gal.

## Discussion

We performed a prospective study to investigate the prevalence of sIgE to  $\alpha$ -gal in cases and controls with acute urticaria or anaphylaxis from different geographical areas of Spain and to evaluate the relevance of demographic data and lifestyle as risk factors for the development of this immune response.

In our study, the overall prevalence of positive sIgE to  $\alpha$ -gal in patients with urticaria or anaphylaxis was 15.7% (Table 3). Positive sIgE to  $\alpha$ -gal was more frequent among men living in rural parts of the Northern area who participated in outdoor activities, had a history of tick bites, and were pet owners. An association was found between these variables. A higher prevalence of sIgE to  $\alpha$ -gal among patients living in rural areas (Figure 4) has also been found in other countries [12]. Consistent with results reported elsewhere [12,17,18], we found that participating in outdoor activities was also associated with type I sensitization to  $\alpha$ -gal.

We found a higher prevalence of IgE to  $\alpha$ -gal among men; the association was significant in the bivariate analysis. This finding is probably related to the fact that in our study, women less frequently participated in outdoor activities and were less frequently bitten by ticks than men (Table S3 Supporting Information). In fact, consistent with the results reported by Fischer et al [17], our multivariate analysis showed that neither sex nor pet ownership was a risk factor. Geographical differences were also observed when considering pet ownership and sex: in the Northern area, more patients had pets and fewer women had been recruited (Tables S2 and S3 Supporting Information).

We found that patients with a positive sIgE result to  $\alpha$ -gal more frequently had dogs. Our results differ from those reported by Gonzalez-Quintela et al [11], who found a relationship with cat ownership. In agreement with our results, Japanese authors found that most patients who were allergic to red meat and likely had specific IgE to  $\alpha$ -gal owned dogs as pets [20]. Outdoor activities, which are a risk factor for tick bites, may also have been involved in our study, as 10 out of 14 participants who hunted had dogs as pets.

Exposure to airborne mammalian proteins does not seem to play an important role in the development of type I sensitization to  $\alpha$ -gal, since this finding was found almost exclusively in the Northern area. Moreover,  $\alpha$ -gal was not detected as an aeroallergen in houses of pet owners by Commins et al [3].

We observed a much higher prevalence of positive sIgE to  $\alpha$ -gal in the Northern area. The fact that more patients who presented with symptoms after eating mammalian meat or innards were recruited in the Northern area seems to reflect a true higher prevalence of clinically relevant sensitization in this area. Genetic factors did not seem to be relevant in this observation, as we did not find an association with atopy or with blood group. Nevertheless, the fact that only 105 patients provided information on their blood group limits the validity of the finding.

Values of sIgE to *Ascaris* were higher and positivity more prevalent in the Northern area (Table 3), where nearly all the positive results for sIgE to  $\alpha$ -gal were observed. Nevertheless, exposure to parasites does not seem to play a relevant role in

this finding, since a prevalence of sensitization to *Ascaris* of 12.6% was observed in the Center, where only 1 patient had sIgE to  $\alpha$ -gal (1.4%).

The multivariate analysis showed that a history of tick bites was the main factor associated with a positive result of sIgE for  $\alpha$ -gal in patients from the Northern area. The association between tick bites and the production of IgE antibodies to  $\alpha$ -gal has previously been demonstrated [11,12,15-18].

Consistent with our results, other authors have reported considerable differences between areas of the USA [2] and Sweden [15].

The higher prevalence in the Northern area in our study could have several explanations. First, it could be due to the fact that lifestyle factors that favor exposure to tick bites were more frequent in this area.

The second possibility is that only specific tick species living and biting humans more frequently in the Northern area would be able to induce an IgE response to  $\alpha$ -gal.

Various tick species have been involved in the development of red meat allergy associated with sensitization to  $\alpha$ -gal in different countries [21], and regional differences in the distribution of tick species have been reported in Spain, with *Ixodes ricinus* and *Hyalomma lusitanicum* being the predominant species in the Northern area and Center, respectively [22]. Few reports have evaluated sIgE to  $\alpha$ -gal after bites by identified tick species in Spain. Sánchez et al [23] reported the case of a patient from northern Spain who developed anaphylaxis after being bitten by *Rhipicephalus bursa* and displayed a weak IgE response to  $\alpha$ -gal (0.65 kU/L). More intense IgE positivity to  $\alpha$ -gal (3.5 kU/L) was detected by Mateos-Hernández et al [24] in a patient living in central Spain who had also been bitten by *R. bursa*. Another patient from central Spain, who had presented with anaphylaxis after being bitten by *H. marginatum* [24], had negative IgE to  $\alpha$ -gal (0.01 kU/L). None of the 3 patients had allergic symptoms after eating mammalian meat.

Our results might also be explained by a greater tendency to bite humans in specific tick species that are more frequent in the Northern area. The genus *Ixodes* has been reported to be more aggressive towards humans [25], and *I. ricinus* is the tick species that most frequently bites humans in the Northern area of Spain [26]. Nevertheless, during the period 2009-2012 there were more reported cases of Mediterranean spotted fever, a tick-borne disease in which another tick species (*Rhipicephalus sanguineus*) was the vector [27], in the Mediterranean area than in the Northern area [28]. This finding implies that *Rh. sanguineus* (its main host is the domestic dog) can also be aggressive to humans. The fact that we did not detect patients sensitized to  $\alpha$ -gal in the Mediterranean area suggests that not all tick species cause this immune response.

Fischer et al [17] stated that any tick that takes blood meals from mammals should be considered a vector for transmission of  $\alpha$ -gal. The case that residual mammalian glycoproteins are present in the tick from a previous blood meal is one of the theories proposed to explain how tick bites induce an IgE response to  $\alpha$ -gal [29]. Nevertheless, the origin of  $\alpha$ -gal in ticks has remained uncharacterized [30], and the presence of this epitope has only been proven in a single tick species to date [20,24,30-32]. It would be of great interest to evaluate

the presence and amount of  $\alpha$ -gal in other tick species and the factors involved in the development of  $\alpha$ -gal sensitization after tick bites. Venturini et al [18] inferred that there was no relationship between having experienced bites by *Dermacentor* species and sensitization to  $\alpha$ -gal, and Fischer et al [17] reported that not all tick bites lead to increased  $\alpha$ -gal-sIgE levels.

In agreement with previous reports [4,5], we observed a longer latency period in patients who presented with symptoms after eating mammalian meat or innards than in controls who developed allergic reactions after eating other foods. Alcohol intake was more frequent in cases caused by meat intake. This finding could reflect both dietary habits and the role of alcohol as a cofactor in hypersensitivity reactions to meat or innards that are dependent on sensitization to  $\alpha$ -gal. Alcohol intake, exercise, and NSAID intake have been reported to be cofactors in food allergy [33]. Cofactors of anaphylaxis were identified in 81% of German patients sensitized to  $\alpha$ -gal who presented with allergic symptoms after eating pork. One patient presented with hypersensitivity symptoms only when alcohol, NSAIDs, and exercise were used together in the challenge test [34]. The importance of alcohol and not of NSAIDs or exercise as a cofactor could be a distinctive feature of meat allergy resulting from sensitization to  $\alpha$ -gal, since NSAIDs and exercise are the main cofactors in other food allergies, such as those associated with sensitization to lipid transfer protein or  $\omega$ -5 gliadin [33].

## Conclusion

The overall prevalence of positive sIgE to  $\alpha$ -gal was 15.7%. We recorded marked geographical differences in prevalence rates, with IgE to  $\alpha$ -gal being much more prevalent in the Northern area of Spain. Outdoor activities, male sex, pet ownership, and living in a rural setting are risk factors for developing sIgE to  $\alpha$ -gal in this geographical area. All of these risk factors are associated with the main risk factor, that is, having previously had a tick bite.

As this immune response is associated with severe allergic reactions to mammalian meat and innards, drugs such as cetuximab, and intravenous colloid gelatin, it is important for allergists, general practitioners, and emergency department physicians to be aware of it.

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## Conflicts of Interest

The authors declare that they have no conflicts of interest.

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