IgE-mediated sensitization to galactose-alpha-1,3-galactose (α-gal) in urticaria and anaphylaxis in Spain: geographical variations and risk factors

Running title: Risk factors for IgE to galactose alpha 1,3 galactose in Spain

Mateo-Borrega MB[1], Garcia B[2], Hernando Larramendi C[3], Azofra J[4], González-Mancebo E[5], Alvarado MI[6], Alonso Díaz de Durana MD[7], Núñez-Orjales R[8], Diéguez MC[9], Guilarte M[10], Soriano-Galarraga AM[11], Sosa G[12], Ferrer A[13], García-Moral A[14], Beristain AM[15], Bartra J[16]

[1]GAI Guadalajara, Department of Allergy, Hospital Universitario de Guadalajara, Guadalajara, Spain.

[2]Department of Allergy, Complejo Hospitalario de Navarra, Pamplona, Spain.

[3]Allergy Section, Hospital Marina Baixa, Villajoyosa, Spain.

[4]Department of Allergy, Hospital Universitario Central de Asturias, Oviedo, Spain.

[5]Department of Allergy, Hospital Universitario de Fuenlabrada, Madrid, Spain.

[6]Department of Allergy, Hospital Virgen del Puerto, Plasencia, Spain.

[7]Department of Allergy, Hospital Universitario Fundación de Alcorcón, Madrid, Spain.

[8]Department of Allergy, Hospital Universitario Lucus Augusti, Lugo, Spain.

[9]Department of Allergy, Research Institute Hospital 12 de Octubre (i+12), Madrid, Spain.

[10]Department of Allergy, Hospital Universitari de la Vall d´Hebron, Barcelona, Spain.

[11]Department of Allergy, Hospital de Basurto, Bilbao, Spain.

[12]Allergy, Hospital La Zarzuela, Madrid, Spain.

[13]Department of Allergy, Hospital Vega Baja, Orihuela, Spain.

[14]Department of Allergy, Hospital de la Santa Creu i Sant Pau, Barcelona, Spain.

[15]Department of Allergy, Hospital Universitario Central de Asturias, Oviedo, Spain.

[16]Allergy Unit, Pneumology Department, Hospital Clinic, Barcelona, Spain

Corresponding autor

María Belén Mateo-Borrega

Sección de Alergia. GAI Guadalajara

Hospital Universitario de Guadalajara

C/ Donantes de Sangre, s/n.

19002 Guadalajara (Spain)

E-mail: mbmateo@gmail.com

Word count: 3473.
Number of tables: 3
Number of figures: 4

Conflicts of Interest Statement. The authors declare that they have no conflicts of interest.

ABSTRACT

Background: The aims of this study were to investigate the prevalence of sIgE to galactose-alpha-1,3-galactose (α-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 two 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 α-gal and total IgE were determined using ImmunoCAP®. sIgE levels ≥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 α-gal was 15.7%; this was higher in cases (26.3%) than in controls (2.4%). The sIgE anti α-gal positivity rate ranged from 37.68% (rural) to 15.38% (semi-urban) and 7.85% (urban). The rates of positivity were 46.32%, (Northern), 0.72% (Center), and 0% (Mediterranean). A positive result of sIgE to α-gal associated with 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 α-gal in patients with urticaria or anaphylaxis differs considerably between the three geographical areas studied and is related to tick bites.

KEY WORDS. Alpha-Gal, Risk Factors, Epidemiology, Outdoor Activities, Tick Bites, Cofactors.

RESUMEN

Antecedentes.: Objetivos: investigar la prevalencia de IgE específica a galactosa, α 1,3 galactosa (α-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 especifica a α-gal se determinaron mediante ImmunoCAP®. Niveles de IgE específica ≥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 α-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 α-gal se asoció a haber experimentado picadura de garrapata, participación en actividades de exterior, tenencia de mascotas e ingestion de carne de mamíferos o visceras previo al inicio de los síntomas. Solo el consumo de alcohol podía ser implicado como cofactor.

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

INTRODUCTION

Galactose-alpha-1,3-galactose (α-gal) is an oligosaccharide that is abundantly expressed on the glycoconjugates of non-primate mammal proteins. Humans, apes, and Old World Monkeys do not express the α-gal epitope as a consequence of inactivation of the enzyme 3Galβ1-4GlcNAc α1-3-galactosyltransferase. All immune-competent humans naturally produce IgG and IgM that have a specificity for α-linked galactose. Human natural anti-gal antibodies have been associated with xenotransplant rejection [1].

IgE antibodies against α-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 α-gal was more frequently detected among patients with otherwise idiopathic urticaria, angioedema, or anaphylaxis than among patients with asthma or other complaints [3]. The involvement of sIgE against α-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 a fenticonazole vaginal capsule [9]. Bovine amniotic fluid has been proven as an occupational source of α-gal [10].

Few epidemiological studies have evaluated the prevalence of sIgE to α-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 α-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

α-gal were found in 85% of parasite-infected individuals from rural Zimbabwe, suggesting the authors that their results supported the fact that parasitic infections might induce IgE antibodies against α-gal [14]. Intense geographical differences in the prevalence of α-gal sensitization were reported in Sweden, varying from 0.7% of teenagers in an area where ticks 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 α-gal [11, 12,-15, 16, 17, 18]. Therefore, activities that favor exposure to ticks could be associated with a higher prevalence of sensitization to α-gal. German [17] and Spanish [18] authors have evaluated the prevalence of α-gal sIgE in subjects at risk of suffering tick bites; 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 compared with controls.

Data on the relationship between pet exposure and the prevalence of positive sIgE to α-gal are scarce. Gonzalez Quintela et al [11] reported that this finding was associated with pet ownership in both Spanish and 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 α-gal. Nevertheless, in a German study, keeping a pet did not increase the risk of sIgE to α-gal [17].

The structure of the epitope of α-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 α-gal.

The aims of this study were to investigate the prevalence of sIgE to α-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 three areas: Northern, Mediterranean, and Center (Table S1, Supporting Information). Residence was divided into three classes; urban (>15,000 residents), semi-urban (2,500-15,000 residents), and rural (<2,500 residents).

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

Written informed consent was obtained from all participants. Patients aged 18 years or older were enrolled in one of two arms: cases and 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 NSAIDS), tick bites before the reaction, and exposure to cetuximab, gelatins, and mammalian meat or innards. Blood samples were obtained and stored at $-20°C$ until laboratory determinations were performed.

Total IgE, specific IgE, and tryptase were determined using an automated ImmunoCAP platform (Thermo Fisher Scientific, Uppsala, Sweden) according to the manufacturer's instructions. sIgE levels were quantified as kU/L, with a measurement range of 0.0-100 kU_A/L. sIgE levels ≥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., Armonk, New York, USA), and GraphPad Prism, version 4 (GraphPad Software, La Jolla, California, USA). Proportions were used to report categorical variables; intergroup comparisons were made using a chi-square test or Fisher's exact test when applicable. Continuous variables were presented as medians and interquartile range (IQR) and compared using the Mann-Whitney test. Spearman's rho was used to assess correlations between continuous variables. Multivariate logistic regression analysis was performed. Statistical significance was set at $p<0.05$.

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 age of the study population was 44 years (IQR: 33-53). 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).

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<0.001).

Trigger factors in cases and controls. Distribution of cases and controls according to the etiologies enumerated in 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 category among cases from the Center and Mediterranean area, whereas ingestion of mammalian meat or innards clearly predominated in Northern patients (p< 0.001). Only one patient reported symptoms (anaphylaxis) after a tick bite.

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

When food was the trigger, the latency period was longer in cases (median, 240 minutes [IQR, 120-360]) than in controls (median, 30 minutes [IQR, 10-60]) (p<0.001). 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 co-factors (Figure 3).

In vitro study

The overall prevalence of a positive result for sIgE to α-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<0.001).

Descriptive data for *in vitro* testing are shown in Table 3. Although sIgE to α-gal ranged from 0 kU/l to >100 kU/l, many negative or low results were obtained in both cases and controls. 3 patients showed a value >100 kU/l and in 5 patients the result was100 kU/l.Even if median values in both groups were 0.00 kU/l (IQR 0.00-0.69 in cases and 0.00-0.01 in controls), levels of sIgE to α-gal were significantly higher (p<0.001) in cases (arithmetic mean, 10.47; SD, 25.69)

than in controls (arithmetic mean, 0.25; SD, 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 α-gal (p=0.009) were observed among patients presenting with anaphylaxis (arithmetic mean, 10.5; SD, 27.4; median, 0.01 [IQR 0.00-0.10]) than among those with acute urticaria (arithmetic mean [SD], 4.44 [15.4]; median [IQR], 0.00 [0.00-0.28]). 27 patients with anaphylaxis and 18 patients with urticaria displayed a positive result. 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 α-gal was more frequently observed among men than women (24.31% vs. 7.04%; p<0.001).

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<0.001) (Table 3).

As Figure 4 shows, several circumstances were associated with differences in the prevalence of sensitization to α-gal. Data correspond to the overall group. When data correspond to cases the quote epigraph (cases) has been added. Wide differences in sensitization to α-gal were observed according to place of residence. Positive sIgE to α-gal was more frequent in patients from rural areas than in those from the other two types of residence, especially urban areas, with a gradient of positivity of 37.68% (rural), 15.38% (semi-urban), and 7.85% (urban). The finding of a positive result in sIgE to α-gal was associated with 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 α-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 one 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 α-gal and atopy, cetuximab infusion, or blood group.

The three controls with a positive result of sIgE to α-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 α-gal (Spearman rho, 0.41 and 0.24, respectively). Proportion of positive and negative results of sIgE to α-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 sIgE positivity to α-gal. Although the model's stability seems to be weak considering the amplitude of the confidence interval, the odds ratio for IgE to α-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.

DISCUSSION

We performed a prospective study to investigate the prevalence of sIgE to α-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 α-gal in patients with urticaria or anaphylaxis was 15.7% (Table 3). Positive sIgE to α-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. The higher

prevalence of sIgE to α-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 α-gal.

We found a higher prevalence of IgE to α-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, similar to the results reported by Fischer at al. [17], our multivariate analysis showed that neither gender nor pet ownership were 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).

In our study, patients with a positive sIgE result to α-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 the majority of patients allergic to red meat who likely had IgE specific to α-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 α-gal, since this finding was found almost exclusively in the Northern area. Moreover, α-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 α-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. No genetic factors 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 this 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 α-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 one patient had sIgE to α-gal (1.4%).

The multivariate analysis showed that a history of tick bites was the main factor associated with a positive result of sIgE to α-gal in patients from the Northern area. The association between tick bites and the production of IgE antibodies to α-gal has been previously demonstrated [11, 12, 15, 16, 17, 18]

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

The higher prevalence in the Northern area in our study could be explained by several reasons. Firstly, it could be due to the fact that lifestyle factors that favour 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 α-gal.

Different tick species have been involved with the development of red meat allergy related to α-gal sensitization in different countries [21] and regional differences in the distribution of tick species have been reported in Spain, with *I. ricinus* and *Hyalomma lusitanicum* being the predominant species in the Northern area and Center, respectively [22]. Few reports have evaluated sIgE to α-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 α-gal (0.65 kU/L). More intense IgE positivity to α-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 α-gal (0.01 kU/L). None of the three patients had allergic symptoms after eating mammalian meat.

A greater tendency to bite humans of definite tick species that are more frequent in the Northern area could also explain our results. A greater aggressiveness of the genus Ixodes towards humans has been reported [25] and I ricinus is the tick species that more frequently bites humans in the Northern area of Spain [26]. Nevertheless, during the period 2009-2014 there were more reported cases of Mediterranean Spotted Fever, a tick-borne disease in which another tick species (Riphicephalus sanguineus) had been involved as 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 have not detected patients sensitized to α-gal in the Mediterranean area suggests a tick species restriction for the development of 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 α-gal. The case that residual mammalian glycoproteins are present in the tick from a previous blood meal is one of the proposed theories to explain how tick bites spark off an IgE response to α-gal [29]. Nevertheless, the origin of α-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, 31, 32]. It would be of great interest to evaluate the presence and amount of α-gal in other tick species and the factors involved in the development of α-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 α-gal and Fischer et al. reported that not all tick bites lead to increased α-gal-sIgE levels [17].

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 co-factor in hypersensitivity reactions to meat or innards that are dependent on sensitization to α-gal. Alcohol intake, exercise, and NSAID intake have been reported to be co-factors in food allergy [33]. Co-factors of anaphylaxis were identified in 81% of German patients sensitized to α-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 co-factor could be a distinctive feature of meat allergy secondary to a-gal sensitization, since NSAIDs and exercise are the main co-factors in other food allergies, such as those associated with sensitization to lipid transfer protein or ω-5-gliadin [33].

CONCLUSION

The overall prevalence of positive sIgE to α-gal was15.7%. We recorded marked geographical differences in prevalence rates, with IgE to α-gal being much more prevalent in the Northern area of Spain. Outdoor activities, male sex, pet ownership, and living in a rural area are risk factors for developing sIgE to α-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.

REFERENCES

1. Macher BA, Galili U. The Galα1,3Galβ1,4GlcNAc-R (α-Gal) epitope: a carbohydrate of unique evolution and clinical relevance. Biochim Biophys Acta 2008; 1780: 75-88.

2. Chung CH, Mirakhur 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: 1109-17.

3. Commins SP, Kelly LA, Rönmark E, James HR, Pochan SL, Peters EJ et al. Galactose-α-1,3-galactose-specific IgE is associated with anaphylaxis but not asthma. Am J Respir Crit Care Med 2012; 185:723-30.

4. 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: 426-33.

5. Mullins RJ, James H, Platts-Mills TA, Commins S. Relationship between red meat allergy and sensitization to gelatin and galactose-alpha-1,3-galactose. J Allergy Clin Immunol 2012; 129:1334-1342.

6. Mozzicato SM, Tripathi A, Posthumus JB, Platts-Mills TA, 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.

7. 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. 2017; 55:668-669.

8. Stone CA Jr, Hemler JA, Commins SP, Schuyler AJ, Phillips EJ, Peebles RS Jr et al. Anaphylaxis after zoster vaccine: Implicating alpha-gal allergy as a possible mechanism. J Allergy Clin Immunol. 2017; 139:1710-1713.

9. Vidal C, Méndez-Brea P, López-Freire S, González-Vidal T. Vaginal Capsules: An Unsuspected Probable Source of Exposure to α-Gal. J Investig Allergol Clin Immunol. 2016; 26:388-389.

10. Nuñez-Orjales R, Martin-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;27:313-314.

11. 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; 44:1061-8.

12. Villalta D, Pantarotto L, Da Re M, Conte M, Sjolander S, Borres MP et al. High prevalence of sIgE to Galactose-α-1,3-galactose in rural pre-Alps area: a cross-sectional study. Clin Exp Allergy 2016; 46:377-80.

13. Stevens W, Addo-Yobo E, Roper J, Woodcock A, James H, Platts-Mills T et al. Differences in both prevalence and titre of specific immunoglobulin E among children with asthma in affluent and poor communities within a large town in Ghana. Clin Exp Allergy 2011;41:1587-94.

14. Arkestål K, Sibanda E, Thors C, Troye-Blomberg M, Mduluza T, Valenta R et al. Impaired allergy diagnostics among parasite-infected patients caused by IgE antibodies to the carbohydrate epitope galactose-α 1,3-galactose. J Allergy Clin Immunol 2011; 127:1024-8.

15. Hamsten C, Tran TAT, Starkhammar M, Brauner A, Commins SP, Platts-Mills TAE et al. Red meat allergy in Sweden: association with tick sensitization and B-negative blood groups. J Allergy Clin Immunol 2013; 132: 1431-4.

16. 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: 1286-93.

17. 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: 1540-7.

18. Venturini M, Lobera T, Sebastián A, Portillo A, Oteo JA. IgE to α-Gal in Foresters and Forest Workers From La Rioja, North of Spain. J Investig Allergol Clin Immunol. 2018;28: 106-112.

19. Morisset M, Richard C, Astier C, Jacquenet S, Croizier A, Beaudouin E et al. Anaphylaxis to pork kidney is related to IgE antibodies specific for galactose-alpha-1,3-galactose. Allergy 2012; 67: 699-704.

20. Chinuki Y, Ishiwata K, Yamaji K, Takahashi H, Morita E. Haemaphysalis longicornis tick bites are a possible cause of red meat allergy in Japan. Allergy.2016;71: 421-5.

21. Kwak M, Somerville C, van Nunen S. A novel Australian tick *Ixodes* (*Endopalpiger*) *australiensis* inducing mammalian meat allergy after tick bite. Asia Pac Allergy. 2018 26;8(3):e31.

22. Barandika JF, Olmeda SA, Casado-Nistal MA, Hurtado A, Juste RA, Valcárcel F et al. Differences in questing tick species distribution between Atlantic and continental climate regions in Spain. J Med Entomol. 2011; 48: 13-9.

23. Sánchez M, Venturini M, Blasco A, Lobera T, Bartolome B, Oteo JA. Tick bite anaphylaxis in a patient allergic to bee venom. J Investig Allergol Clin Immunol 2014; 24:284-5.

24. Mateos-Hernández L, Villar M, Moral A, Rodríguez CG, Arias TA, de la Osa V et al. Tick-host conflict: immunoglobulin E antibodies to tick proteins in patients with anaphylaxis to tick bite. Oncotarget 2017; 8: 20630-20644.

25. Venturini Díaz M. Alergia a alfa-gal y picaduras de garrapata.
La perspectiva española. Retrieved from http://www.alergoaragon.org/2017/0101.pdf

26. Portillo A, Ruiz-Arrondo I, Oteo JA. Arthropods as vectors of transmisible diseases in Spain. Med Clin (Barc). 2018;151:450-459.

27. García-Magallón B, Cuenca-Torres M, Gimeno-Vilarrasa F, Guerrero-Espejo A. Mediterranean Spotted Fever: epidemiological Assessment in Spain during the Period 2009-2012. Rev Esp Salud Publica. 2015; 89:321-8.

28. Palau Miguel M, González Muñoz S, Aliaga García MJ. Indicadores de Salud y Cambio Climático 2016. Retrieved from
http://www.mscbs.gob.es/ciudadanos/saludAmbLaboral/docs/82_2016_INDICADORES.pdf

29. Steinke JW, Platts-Mills TA, Commins SP. The alpha-gal story: lessons learned from connecting the dots. J Allergy Clin Immunol.2015;135:589-96.

30. Cabezas-Cruz A, Espinosa PJ, Alberdi P, Šimo L, Valdés JJ, Mateos-Hernández L, Contreras M 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 21;8:14224.

31. 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):213-220.

32. 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; 68:549–52.

33. Cardona V, Luengo O, Garriga T, Labrador-Horrillo M, Sala-Cunill A, Izquierdo A et al. Co-factor-enhanced food allergy. Allergy 2012; 67:1316-8.

34. Fischer J, Hebsaker J, Caponetto P, Platts-Mills TA, Biedermann T. Galactose-alpha-1,3-galactose sensitization is a prerequisite for pork-kidney allergy and cofactor-related mammalian meat anaphylaxis. J Allergy Clin Immunol 2014; 134:755-9.

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	

Table 2. Questionnaire data		Cases	Controls	p (chi^2 test)
Male sex [n (%)]		83 (51.88%)	61 (48.41%)	NS
Age, Median (IQR)		44 (33-55)	44 (33-52)	NS
Recruitment area (N=286)	Northern	59 (36.9%)* (62.1%)**	36 (28.6 %)* (38%)**	NS
	Mediterranean	29 (18.1%)* (54.7%)**	24 (19%)* (45.3%)**	NS
	Center	72 (45%)* (52.2%)**	66 (52.4%)* (47.8%)**	NS
Place of residence	Urban	105 (65.6%)*	86 (68.2%)*	
	Semi-urban	14 (8.7%)*	12 (9.5%)*	NS
	Rural	44 (27.5%)*	25 (19.8%)*	
Clinical picture	Urticaria	85 (53.13%)*	63 (50%)*	NS
	Anaphylaxis	75 (46.88%)*	63 (50%)*	
Atopy (N=286)		66 (41.25%)*	60 (47.62%)*	NS
Outdoor activities #		70 (46.36%)*	34 (27.2%)*	p<0.01
Pet ownership $		67 (43.79%)*	47 (37.6%)*	NS
B-negative blood group &		54 (85.71%)*	35 (83.33%)*	NS
History of tick bites ¶		53 (33.3%)*	6 (4.6%)*	P<0.001

IQR: Interquartile range; * % refers to total subjects-by columns (cases or controls); ** % refers to total by area; # available data from N=276 (151 cases, 125 controls); $ available data from N=278 (153 cases, 125 controls); & available data from N=105 (63 cases, 42 controls); ¶ available data from N=285 (159 cases, 126 controls).

Table 3. Laboratory tests					
	sIgE α-Gal* kU/l	Tryptase (N 213) µg/l	Total IgE (N 199) IU/ml	sIgE *Ascaris* # kU/l	sIgE *Anisakis* ‡ kU/l
Median	0	4.5	128	0.02	0.03
Mean	Overall 5.96	5.24	275.87	Overall 0.33	Overall 2.71
	North 17.86			North 0.73	North 5.24
	Center 0.06			Center 0.17	Center 1.86
	Mediterranean 0.02			Mediterranean 0.09	Mediterranean 1.16
Range	0-100	1-25.9	2.63-5,470	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	¥ N (%)			¥ N (%)	¥ N (%)
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%)

Available data: *Overall 286; North 95; Center 138; Mediterranean 53. # Overall 243;

North 77; Center 119; Mediterranean 47. ‡Overall 257; North 75; Center 133;

Mediterranean 49.

¥ positive result ≥0.35 kU/l

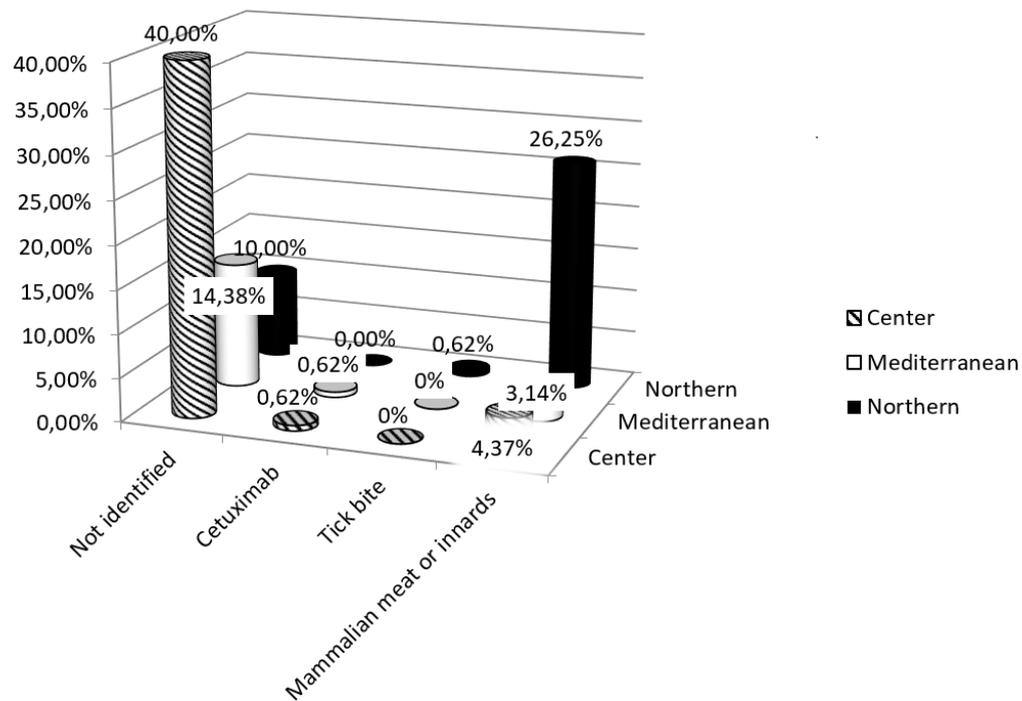**Figures**

Figure 1. Trigger factors in cases

Figure 1. Trigger factors in cases

Figure 2. Trigger factors in controls

Figure 2. Trigger factors in controls
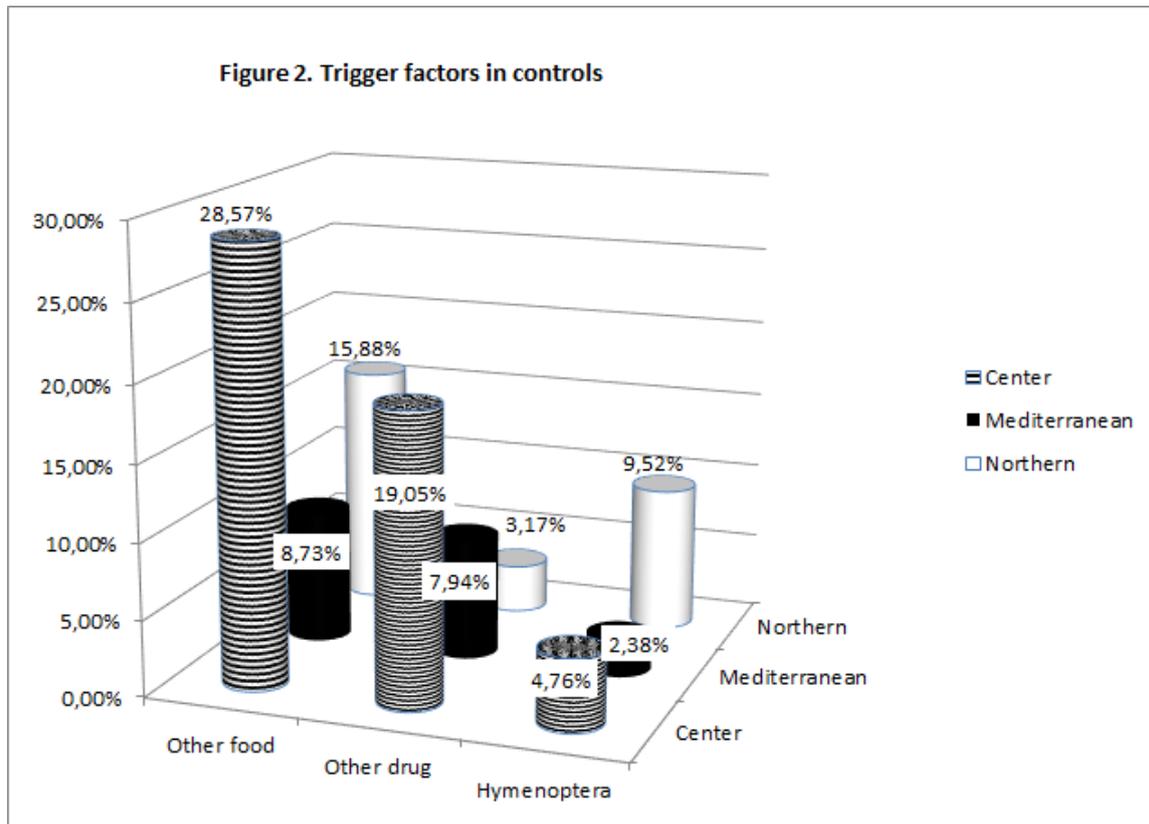
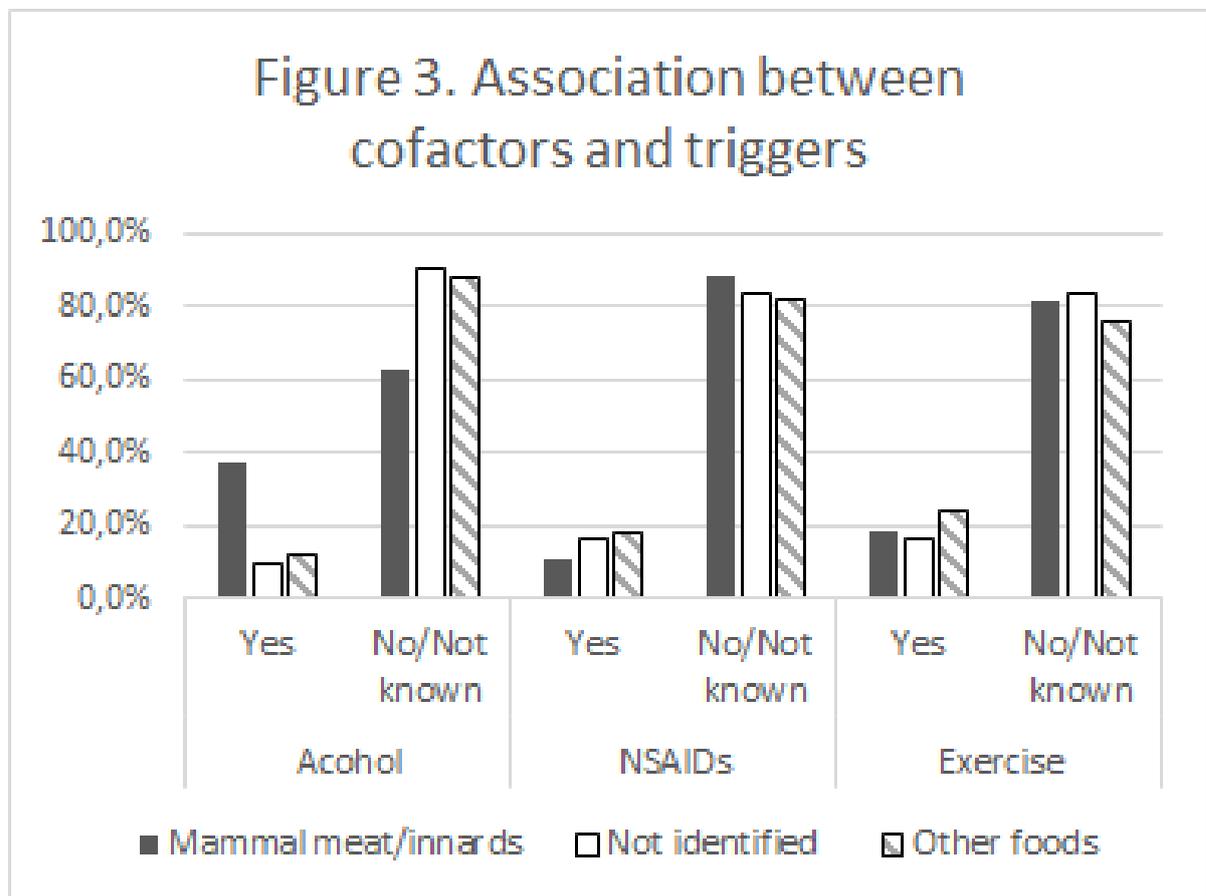Figure 3. Association between cofactors and triggers

Figure 3. Association between cofactors and triggers

Figure 4. sIgE to α-gal

Figure 4. sIgE to α-gal