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Predictive values of alpha-gal IgE levels and alpha-gal IgE: Total IgE ratio and oral food challenge-proven meat allergy in a population with a high prevalence of reported red meat allergy

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Abstract

Background: Severe meat allergy with anaphylaxis may be caused by sensitization to alpha-gal. Levels of alpha-gal sensitization that correlate with high risk of meat allergy are currently unknown. We have identified an area with a high prevalence of reported red meat allergy which offered the opportunity to evaluate the diagnostic value of IgE antibody tests.

Methods: To determine levels of alpha-gal IgE and alpha-gal:total IgE ratio in a large cohort of subjects with challenge-proven meat allergy compared with control subjects from the same environment, we conducted fieldwork assessing 131 participants who reported adverse reactions to meat, and 26 control subjects, by questionnaires, IgE sensitization to alpha-gal and oral food challenge to beef sausage.

Results: Eighty-four participants were diagnosed with alpha-gal allergy. Alpha-gal IgE ranged between 0.7 and 344.5 kU/L. Alpha-gal:total IgE ratio ranged from 0.1% to 67.6%. Logistic regression analysis showed both alpha-gal IgE and alpha-gal:total IgE ratio strongly correlated with meat allergy, with AUC of 0.95. The values giving the best correct classification were IgE of 2.00 kU/L and ratio of 0.75%. The value above which there is a 95% probability of meat allergy is IgE>5.5 kU/L and ratio of 2.12%.

Conclusion: Alpha-gal allergy in a population with a high prevalence of reported red meat allergy showed a more rapid onset of symptoms than previously described and a high prevalence of isolated subjective gastrointestinal manifestations. Cutoff values are described for levels of sensitization to alpha-gal IgE and alpha-gal:total IgE ratio that are highly likely to result in clinically significant meat allergy.

KEYWORDS

Galactose-alpha-1,3-galactose, alpha-gal, anaphylaxis, cutoff values, food allergy, meat allergy, oral food challenges, predictive values, red meat allergy, ticks

1 | INTRODUCTION

Galactose-alpha-1,3-galactose (alpha-gal) has recently been identified as a novel food allergen.[1] The reaction has been described to either be (a) delayed, occurring 3 hours after ingestion of mammalian products (eg, beef, pork, and lamb) or (b) an immediate reaction after first exposure to cetuximab.[2,3]

This oligosaccharide is an antigen found in nonprimate mammalian cells.[4,5] Although it is well known that the carbohydrate moieties present in many plant foods can induce antiglycan IgE responses,

Abbreviations: Alpha-gal, Galactose-alpha-1,3-galactose; OFC, Oral food challenge.

the clinical significance of these cross-reactive carbohydrate determinants is unclear.[6-11] IgE to alpha-gal has been associated with food allergy that seems to occur in patients after being bitten by ticks.[1-3,12,13] Levels of alpha-gal sensitization that correlate with high risk of meat allergy are currently unknown. In addition, primary sensitization to cat IgA in some cat-allergic individuals may result in low-level detection of alpha-gal antibodies, not associated with meat allergy.[13,14] This hinders interpretation of IgE levels to alpha-gal.

There is an association between adverse reactions to red meat and alpha-gal allergy in areas of the Southern United States, Europe, and Australia with high exposure to hard ticks.[15-19] There are other possible sensitizing organisms for alpha-gal allergy. Oligosaccharides are well recognized as a target for antibody response to helminths, and both helminths and ectoparasites can give rise to oligosaccharide IgE responses.[20,21] Sera from patients with documented helminth infections, however, do not consistently contain IgE antibodies to alpha-gal.[22] Detection of anti-gal-binding sites in some strains of *Escherichia coli*, *Klebsiella*, and *Salmonella* suggests that this antigen could be present in the bacterial polysaccharides on the outer membranes of the bacterial flora in human intestines.[23,24]

Reports were received of high prevalence of adverse reactions to meat clustered in the Mqanduli district of the rural Eastern Cape Province of South Africa. This unusually large cluster of subjects offered the opportunity to assess whether participants reporting reactions to red meat were alpha-gal sensitized and the correlation between raised alpha-gal titers and challenge-proven meat allergy.

2 | METHODS

2.1 | Participants and controls

This was a cross-sectional study, approved by Human Research Ethics Committee of the University of Cape Town (174/2017), and informed consent, parental consent, and assent were obtained from all participants. A total of 131 participants with a history of symptoms of adverse reaction to red meat and without poorly controlled chronic medical conditions were enrolled. Moreover, 26 participants with no history of adverse reaction to red meat and who were regularly consuming meat served as controls. To control for environmental influences, controls were selected from the family living in the same household as allergic participants wherever possible.

2.2 | Questionnaire

A questionnaire was completed with the research team member, assessing demographics of the participants, clinical symptoms (onset of reaction, symptoms experienced, type of red meat ingested), and a history of exposure to tick bite, scabies, or parasite infection.

2.3 | IgE sensitization

Blood samples were collected from all participants to test total IgE and specific IgE antibodies (ImmunoCAP® Phadia) to beef, pork, lamb, alpha-gal antigen, cat serum albumin, cat dander, and *Ascaris*. Serum was analyzed using Immunocap100 instrument (Thermo Fisher), and the results were expressed as international units per milliliter. Alpha-gal-specific IgE of >the lower detectable level of 0.10 kU/L was interpreted as sensitized.

2.4 | Oral Food Challenge (OFC)

Participants with a history of adverse reactions to meat and who were sensitized to alpha-gal were invited for an OFC to cooked beef sausage performed at Zithulele Hospital. Participants ingested 1 beef sausage (63 g) for body weight of <30 kg; 2 beef sausages (126 g) for body weight 30-60 kg; and 3 beef sausages (189 g) for body weight >60 kg. Each beef sausage contained 756 kilojoules of energy, 8.3 g protein, 13.5 g fat, 21 mg cholesterol, 0.6 g fiber, and 431 mg sodium. Participants were medically fit on the day of the challenge and had not ingested antihistamines, steroids, or leukotriene receptor antagonists. Participants were allowed to drink water but only allowed to consume other food after 5 hours into the challenge. No exercise was allowed. Vital signs were checked prior to the challenge, and at half-hourly intervals throughout the challenge, on appearance of any clinical signs and after any reaction resolved. Participants were observed for at least 8 hours from ingestion, and if a reaction occurred, for at least 2 hours after the reaction resolved.

2.5 | Statistical analysis

Data were entered in Microsoft Access and exported for statistical analysis using STATA version 14.1 (Texas, USA). Results were described using proportions for categorical variables and medians (with ranges and interquartile ranges) for numerical variables if the data were skewed. Associations between categorical variables or categorical and numerical variables were performed using chi-square/Fisher's exact test and Mann-Whitney *U* tests, respectively. To assess the association between alpha-gal sensitization and meat allergy, a logistic regression for prediction model was used. A *P*-value of less than or equal to 0.05 was considered to be statistically significant.

3 | RESULTS

3.1 | Alpha-gal allergy and control subjects

Two participants had beef allergy, diagnosed from a history of immediate reaction to beef but no other mammalian meats with raised beef IgE levels and no IgE (in one case, far lower IgE) against alpha-gal. One participant had pork allergy, diagnosed by a history of immediate reaction to pork alone but no other meats and a raised pork IgE and negative alpha-gal IgE. Three participants out of those positive for IgE to alpha-gal were excluded from challenge because of a very high alpha-gal IgE and alpha-gal: total IgE ratio and recent (within 2 years), severe symptoms and 114 participants underwent an OFC (Figure 1). During OFC, 81 (71.1%) had

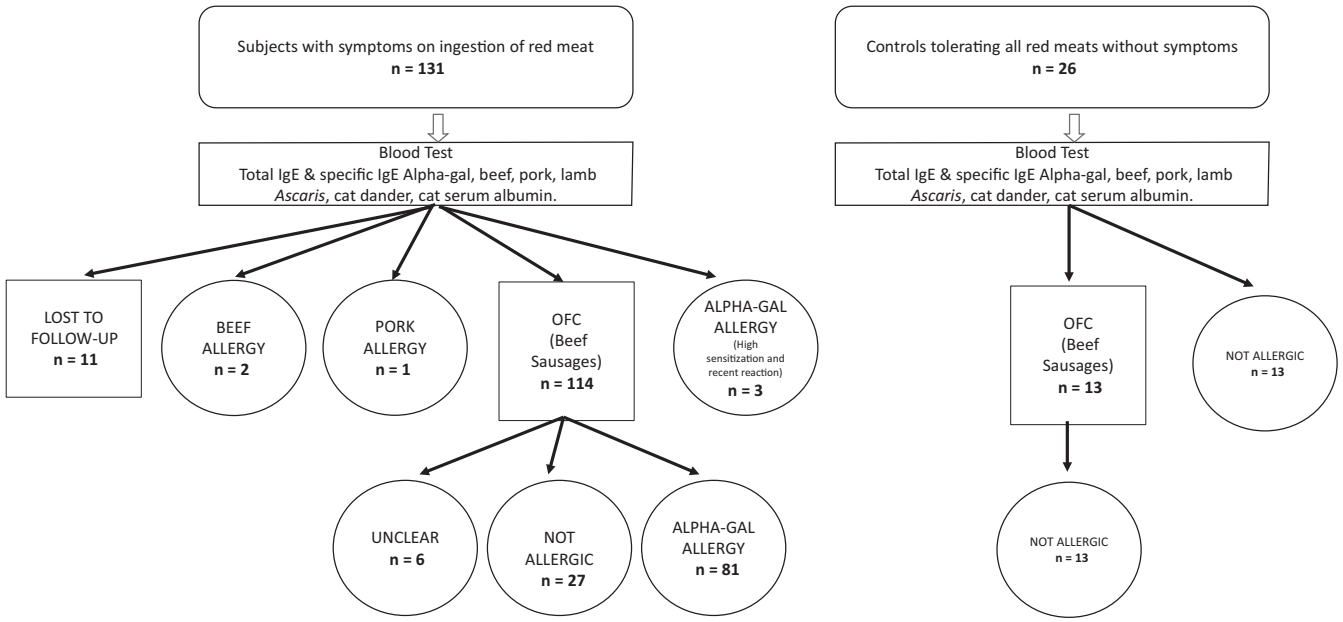

FIGURE 1 Flowchart of participants

a reaction and were diagnosed with alpha-gal allergy, 27 (23.7%) did not have any adverse reactions and 6 (5.3%) were unclear. Participants experienced reactions between 45 and 375 minutes (median 105; IQR 85-135) after ingestion. Eleven participants did not complete the study.

Treatment of mild-to-moderate skin or abdominal symptoms was oral antihistamine (cetirizine 5-10 mg). Participants were observed for 8 hours in hospital and followed up by telephone 24 hours after the challenge.

Twenty-six control participants who were regularly consuming red meat underwent the same questionnaire and tests for sensitization. Half (13) of them had no alpha-gal sensitization and did not undergo a food challenge. The other 13 showed no response to an oral food challenge performed as above.

There were more women than men in both the control and alpha-gal groups, but the two cohorts did not differ from each other in the gender distribution. Participants with alpha-gal allergy were younger (median 12 years; IQR 8-25.5) than controls (23.5 years; IQR13-34). 55 (65.5%) were 13 years or younger, 4 (4.8%) were aged 14-18, and 25 (29.8%) were aged above 18. Participants with alpha-gal allergy and controls did not recall different exposures to ticks, scabies, worms, and schistosomiasis (Table 1). The alpha-gal IgE and alpha-gal:total IgE ratio differed significantly between cases and controls (Table 1, logarithms displayed in Figure 2).

In participants diagnosed with alpha-gal allergy, 90.5% reported multiple reactions and 7.1% a dose response. The median delay from 1st symptoms to diagnosis was 5.5 years (Range: 0-65; IQR 2.5-8). The most recent reaction prior to diagnosis was experienced at a median of 1 year (range: 0-43; IQR 0-4.5) prior to diagnosis.

TABLE 1 Description of control vs alpha-gal allergy participants

Category	Control n = 26	Alpha-gal allergy n = 84	*P*-value**
Gender, n (%)			
Female	20 (76.9)	56 (66.7)	0.48*
Male	6 (23.1)	28 (33.3)	
Age years, median (range; IQR)	23.5 (6-42; 13-34)	12 (4-65; 8-25.5)	<0.01**
Exposure, n (%)			
Tick bite	1 (3.9)	9 (10.7)	0.29*
Scabies	14 (53.9)	39 (46.4)	0.65*
Worms	8 (30.8)	22 (26.2)	0.63*
Bilharzia	2 (7.7)	8 (9.5)	1.00*
Alpha-gal IgE kU/L, median (IQR)	0.5 (0-1.2)	12.0 (4.2-33.4)	<0.01**
Alpha-gal IgE: total IgE ratio, median (IQR)	0.1 (0-0.4)	4.2 (1.9-11.0)	<0.01**

*Fisher's exact.
***P*-value calculated using Mann-Whitney test.

3.2 | Predictive value of alpha-gal IgE levels and alpha-gal:total IgE ratio

A logistic regression model was used to assess the relationship between alpha-gal sensitization and meat allergy. The model was also used to predict the probability of a positive outcome for every value of alpha-gal sensitization. Alpha-gal IgE was a significant predictor of meat allergy with an odds ratio (for every 1kU/L increase) of 2.12 (CI 1.39-3.21) $P < 0.001$. Alpha-gal:total IgE ratio was a significant

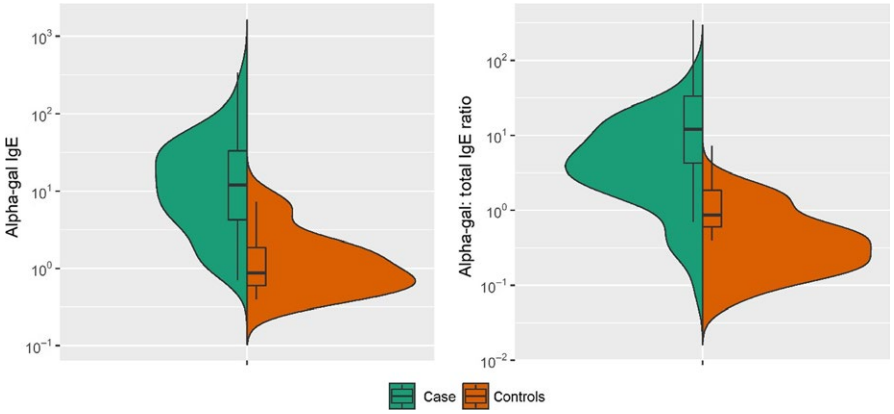

FIGURE 2 Log alpha-gal IgE and log alpha-gal:total IgE ratio in alpha-gal allergy cases and controls

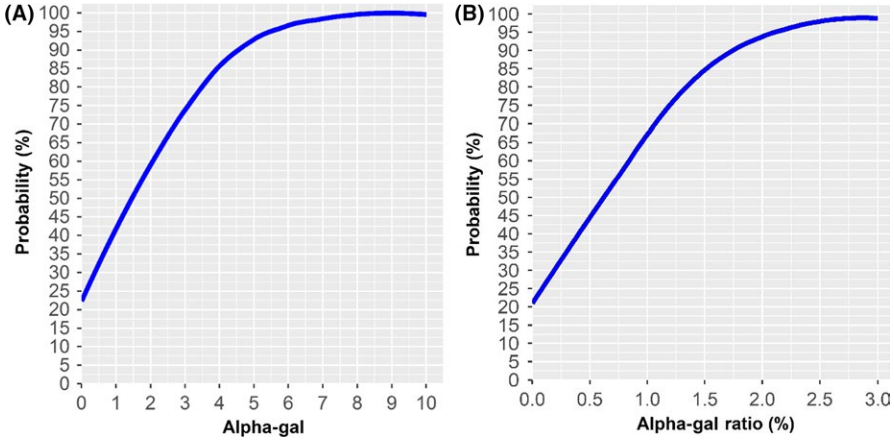

FIGURE 3 Probability of meat allergy with increasing IgE to alpha-gal (A) and alpha-gal:total IgE ratio (B)

predictor of meat allergy with an odds ratio (for every 1% increase in ratio) of 7.32 (CI 2.62-20.42) $P < 0.001$.

ROC analysis estimated the sensitivity, specificity, and correctly classified cases. ROC AUC for both alpha-gal IgE and alpha-gal:total IgE ratio was 0.95. The values above which there is a 95% probability of having meat allergy are alpha-gal IgE = 5.5 kU/L and alpha-gal:total IgE ratio of 2.1% (Figure 3). This is, however, biased toward having meat allergy as the sensitivity and NPV are low (Table 2).

The cutoff values associated with a PPV of >95% are alpha-gal IgE of 2.00 kU/L and alpha-gal:total IgE ratio of 0.75%. These values coincide with the intersection of sensitivity and specificity giving the best value associated with correct classification (Figure 4).

Beef, pork, and lamb IgE were strongly correlated with alpha-gal IgE and each other. Although beef, pork, and lamb IgE were also raised in subjects with alpha-gal allergy, the values were lower than that of alpha-gal IgE. Beef IgE typically achieved values of 50%-60% of that of alpha-gal IgE, with the others being lower. Cat IgE was occasionally slightly raised.

3.3 | Phenotypes of alpha-gal allergy

A total of 17 (21.0%) of 81 OFC-proven alpha-gal allergy had abdominal symptoms manifesting as severe and persistent abdominal cramping only, without any objective symptoms such as vomiting, rash, or cardiorespiratory involvement. In addition, 64 (79.0%) had objective signs and manifested a combination of abdominal symptoms (abdominal pain, vomiting, diarrhea in 77.7%), skin reaction (scratching, hives, erythema, or angioedema in 53.1%), and severe reactions (respiratory symptoms or hypoperfusion in 4.9%).

Alpha-gal IgE of participants with abdominal symptoms only ranged between 1.2 and 35.0 kU/L (median 7.3, IQR 2.9-21.4), and alpha-gal:total IgE ratio ranged between 0.1% and 29.7% (median 2.6%, IQR 1.8%-5.5%) (Table 3). Time of reaction after ingestion of red meat ranged from 45 to 210 minutes (median 100 minutes, IQR 75-135).

Participants with alpha-gal allergy and objective signs had alpha-gal IgE of between 0.7 and 344.5 kU/L (median 12.0, IQR 4.5-33.4) and alpha-gal:total IgE ratio of between 0.1% and 67.6% (median 4.2%, IQR 2.0%-11.6%) (Table 3). Time of onset for reaction ranged from 45 to 375 minutes (median 107.5 minutes, IQR 85- 135). Twenty-three subjects (28.4%) reacted later than 120 minutes after ingestion.

The two phenotypes of alpha-gal allergy did not differ with respect to age, exposures to infectious agents (tick bite, scabies, worm, and bilharzia), time of onset of symptoms (Table 3), and sensitization to alpha-gal and alpha-gal:total IgE ratio (Figure 5); however, the proportion of women was higher in the group with abdominal symptoms only.

4 | DISCUSSION

In our cohort, alpha-gal allergy presented at a younger age and with a predominance of women, rather than the older, predominantly male cohorts described in the United States. This likely reflects exposure

TABLE 2 Classification function values of alpha-gal cutoff levels

Cutoff value	Probability of meat allergy[a]	Sensitivity[b](%)	Specificity[c] (%)	Positive Predictive Value[d] (%)	Negative Predictive Value[e] (%)	Correctly classified[f] (%)
Alpha-gal IgE						
≥0	≥0.236	100.00	0.00	76.36		76.36
≥1	≥0.396	95.24	73.08	91.95	82.61	90.00
≥2	**≥0.581**	**84.52**	**92.31**	**97.26**	**64.86**	**86.36**
≥3	≥0.746	80.95	92.31	97.14	60.00	83.64
≥4	≥0.861	76.19	92.31	96.97	54.55	80.00
≥5	≥0.929	70.24	92.31	96.72	48.98	75.45
≥5.5	**≥0.95**	**67.86**	**96.15**	**98.28**	**48.08**	**75.45**
≥6	≥0.965	66.67	96.15	98.25	47.17	73.64
≥7	≥0.983	60.71	96.15	98.08	43.10	69.09
≥8	≥0.992	57.14	100.00	100.00	41.94	67.27
≥9	≥0.996	57.14	100.00	100.00	41.94	67.27
≥10	≥0.998	55.95	100.00	100.00	41.27	66.36
Alpha-gal:total IgE ratio						
≥0.00	≥0.220	100.00	42.31	84.85		86.36
≥0.25	≥0.317	96.43	65.38	90.00	85.00	89.09
≥0.50	≥0.433	89.29	80.77	93.75	70.00	87.27
≥0.75	**≥0.556**	**88.10**	**88.46**	**96.10**	**69.70**	**88.18**
≥1.00	≥0.674	84.52	88.46	95.95	63.89	85.45
≥1.25	≥0.772	84.52	88.46	95.95	63.89	85.45
≥1.50	≥0.848	82.14	96.15	98.57	62.50	85.45
≥1.75	≥0.902	78.57	96.15	98.51	58.14	82.73
≥2.00	≥0.938	71.43	100.00	100.00	52.00	78.18
≥2.12	**≥0.95**	**69.05**	**100.00**	**100.00**	**50.00**	**76.36**
≥2.25	≥0.961	69.05	100.00	100.00	50.00	76.36
≥2.50	≥0.976	67.86	100.00	100.00	49.06	75.45
≥2.75	≥0.985	65.8	100.00	100.00	47.27	73.64
≥3.00	≥0.991	63.10	100.00	100.00	45.61	71.82

[a]Was estimated using the logistic regression model that was fitted.
[b]Proportion of subjects with a true meat allergy who were detected using this cutoff.
[c]Proportion of subjects with a true no meat allergy who were detected using this cutoff.
[d]Proportion of those who were detected meat allergic using this cutoff that were really meat allergic.
[e]Proportion of those who were not detected meat allergic using this cutoff that were really not meat allergic.
[f]Overall proportion of positives and negatives that were correctly classified. Note that this proportion is always biased toward the larger group.

to ticks that occurs in all individuals in rural Africa, rather than in the United States where adult men (hunters and hikers) are more commonly exposed to ticks. Participants experienced more rapid onset of symptoms to ingestion of red meat than previously described. Rapid onset of reaction within minutes has, however, been described for immediate-type reactions against intravenous cetuximab caused by cross-sensitization to alpha-gal.[3]

Possible reasons for the faster onset of meat allergy reactions may relate to the methodology by which we diagnosed alpha-gal allergy. Participants were under direct close observation, and the very first symptoms were noted by the investigator, in contrast to ingestion in real-world circumstances, where the participant is not being observed and does not expect to have a reaction, therefore perhaps only recognizing a reaction later in the onset of symptoms. Despite this, however, reactions occurred faster than studies diagnosing alpha-gal allergy by controlled food challenges.[25,26]

A strength of the study is the verification of disease state with oral food challenges. However, food challenges to mammalian meat are not well standardized, some patients may not react with every exposure, requiring cofactors to react, and some patients react to some types of meat but not others. In 6 subjects, subjective reactions not accompanied by any diarrhea, vomiting, or objective signs

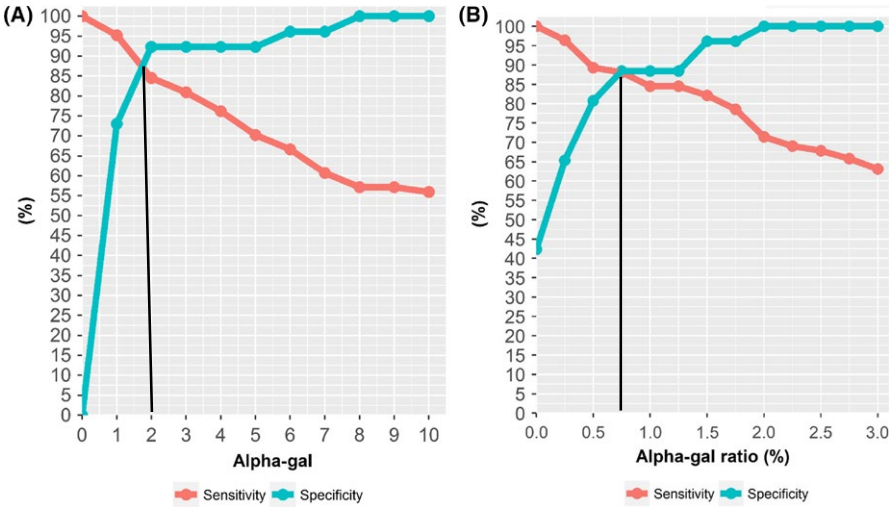

FIGURE 4 Intersection of PPV and NPV of alpha-gal IgE (A) and alpha-gal:total IgE ratio (B) cutoff levels

TABLE 3 Different phenotypes of challenge-proven alpha-gal allergy

Characteristics	Subjective abdominal symptoms only n = 17	Objective abdominal, skin, or severe signs n = 64	*P* value**
Gender, n (%)			
Female	15 (89.2)	40 (62.5)	
Male	2 (11.8)	24 (37.5)	0.08*
Age yrs, median (range; IQR)	12 (6-57; 9-28)	12 (4-65;8-25)	0.71**
Exposures, n (%)			
Tick bite	4 (23.5)	5 (7.8)	0.09*
Scabies	9 (52.9)	28 (43.8)	0.59*
Worms	4 (23.5)	17 (26.6)	01.00*
Bilharzia	1 (5.9)	6 (9.4)	1.00*
Alpha-gal IgE kU/L, median (IQR)	7.3 (2.9-21.4)	12.0 (4.5-33.4)	0.24**
Alpha-gal: total IgE ratio, median (IQR)	2.6 (1.8-5.5)	4.2 (2.0-11.6)	0.16**
Onset of symptoms minutes, median (IQR)	100 (75-145)	107.5 (85- 135)	0.46**

*Fisher's exact.
**P-value calculated using Mann-Whitney test.

comprised only mild abdominal pain rather than severe cramping abdominal pain. As we could not confirm that these subjects were truly reacting, we classified their reactivity as uncertain and excluded them from further analysis.

Beef sausages were used in this cohort due to religious preferences of some subjects precluding the use of pork sausage. Prior research has used 150 g of pork sausage in adult participants, whereas we used 189 g of beef sausage.[26] Beef sausage has been reported to cause more severe reactions than pork sausage.[26] Our dose of beef sausage is therefore higher than the dose of pork sausage used in adult subjects in prior studies. Due to safety concerns, we used lower doses for young children of lower body weight; two-third of this dose for participants with a weight of 30-60 kg and one-third of the dose for children of less than 30 kg, to approximate the same dose/kg body weight and a weight-appropriate serving size. The sausages did not contain pork meat itself, but in addition to beef, meat contained wheat, salt, starch, spices, flavorings, and sodium sulfite, and was filled in hog casings. The ingestion of animal organs (including casings used in sausages) may cause more rapid onset of reactions.[25,27]

Logistic regression analysis showed that both alpha-gal IgE and the alpha-gal:total IgE ratio were strongly correlated with meat allergy, with an AUC for both of 0.95. In this study, levels of beef, pork, and lamb were also well correlated with alpha-gal allergy; however, two subjects with beef allergy and one with pork allergy were excluded from our ROC analyses, and these tests are typically of less utility in diagnosing alpha-gal allergy than alpha-gal antigen testing.[28]

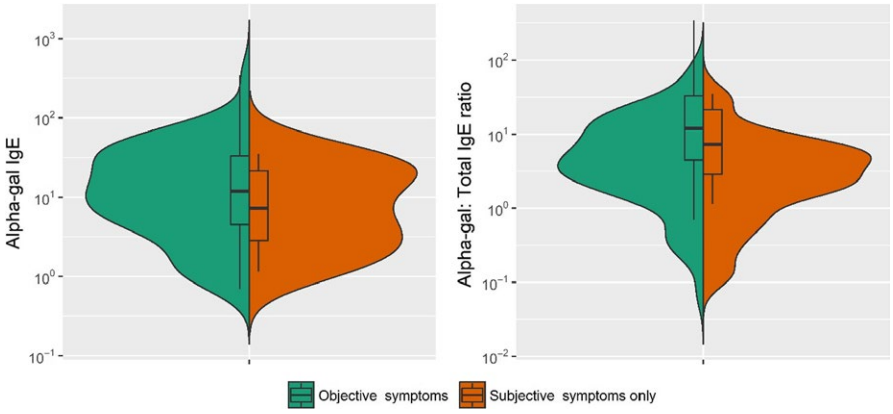

FIGURE 5 Log alpha-gal IgE and log alpha-gal:total IgE ratio in phenotypes of alpha-gal allergy

The values giving the best correct classification were alpha-gal IgE of 2.00 kU/L and alpha-gal:total IgE ratio of 0.75%; however, at these values the sensitivity is low and the percentage in the cohort identified with meat allergy is low. The value above which there is a 95% probability of having meat allergy is alpha-gal IgE of >5.5 kU/L and alpha-gal: total IgE ratio of 2.12%. At these values of the cutoffs, the sensitivity (69.05% vs 67.86%), specificity (100.00% vs 96.15%), PPV (100.00% vs 98.28%), NPV (50.00% vs 48.08%), and proportion correctly classified (76.36% vs 75.45%) are greater for alpha-gal:total IgE ratio than for alpha-gal IgE itself.

Utilizing odds ratios of a test may be an accurate means of estimating the individual's risk of a disease, taking into consideration the pretest probability of the condition occurring. PPVs are not an absolute "cutoff value," but are more commonly used to guide diagnosis. However, PPVs are often population specific, with the most important consideration being the prevalence of the condition in the population being tested. In this instance, the values were derived from a population with a high prevalence of the condition who were being screened for the concern that they might have alpha-gal-related meat allergy. This may be an advantage, as it is envisaged that this test would unlikely be performed as a random screening test; rather, it is more likely that the test would be performed for a subject who has a concern that meat allergy is being caused by alpha-gal sensitization.

In the setting of a screening test, sensitivity and specificity are more important, whereas for a confirmatory test specificity and PPV are more important. The higher cutoff levels of 5.5 kU/L and 2.12% perform better on specificity and PPV than the lower cutoff levels of 2.0 kU/L and 0.75% and may therefore be more useful when using the test as a confirmatory test rather than a screen. As the alpha-gal-specific IgE response is influenced by tick bites and perhaps other parasites the alpha-gal IgE may decline over time. A repeat alpha-gal IgE level may therefore have utility in guiding the timing of a rechallenge once the level is below 5.5 kU/L and 2.12% ratio.

The findings revealed two phenotypes of alpha-gal induced meat allergy: reactions with subjective abdominal symptoms alone; and reactions with objective gastrointestinal, skin, or severe signs. The phenotype presenting solely with rapid onset of subjective abdominal symptoms makes diagnosis challenging as this could be diagnosed as food poisoning if not investigated further. Other cases that are less obvious, with delayed reactions, no clear history of tick bites and gastrointestinal spasm alone or angioedema alone have been reported.[25,26,29] The second set of alpha-gal participants had skin signs with or without abdominal symptoms or severe reactions. The reason for the difference in characteristics is not known, as immunoassay testing did not reveal any significant difference. 90% of subjects with abdominal pain only were women, raising the question as to whether allergen-induced uterine cramping may be responsible for some of this manifestation. Data were not gathered on pubertal status. However, a large proportion (6 of 15) of women with isolated abdominal symptoms were at the age of 10 or below (a further three were aged 12) and only six were definitely postpubertal (aged 27 to 57), and the age range of subjects with abdominal symptoms only did not differ from that of subjects with objective symptoms.

In this study, four participants experienced symptoms that were classified as severe. The majority of these (3) had respiratory symptoms that improved rapidly on inhaled bronchodilator. Adrenaline was used on only one pediatric participant who had evidence of cardiac dysfunction manifesting as marked behavior change (abnormal stillness) and cool peripheries. Other colleagues' experience revealed that adults frequently presented with anaphylaxis, while the majority of children with this syndrome present with urticaria rather than acute episodes of delayed anaphylaxis.[15] On the contrary, in this study pediatric participants presented with a higher frequency of severe reactions than adult participants.

The generalizability of these data in a rural African population to other populations is not known, bearing in mind that no similar data exist. Our group has shown that black African subjects' total IgE levels do not vary according to ethnicity, provided that parasite infestation is accounted for.[30] In this study, *Ascaris*-specific IgE did not differ between cases and controls. Self-reported exposure to ticks was higher in participants with alpha-gal allergy than in controls, but the relevance of ticks is yet to be investigated in this setting. There was high self-reported exposure to scabies and worm infestation, and some exposure to bilharzia in both alpha-gal and control participants. *Ascaris*, mite, and alpha-gal sensitization has been described in rural villages in Ecuador,[31] but the relationship between sensitization

to *ascaris* and alpha-gal remains unclear. The importance of these exposures as possible sensitizing vectors for alpha-gal allergy is unknown.

5 | CONCLUSION

To the best of our knowledge, this cohort is the largest published challenge-proven alpha-gal cohort worldwide, providing an opportunity to propose decision points with predictive value for meat allergy based on alpha-gal IgE levels and/or alpha-gal:total IgE ratio. The discovery of different phenotypes of meat allergy that might mimic food poisoning in this rural community highlights the need for access to IgE testing in this setting. Understanding the causative factors of alpha-gal allergy in the rural population may provide insight in implementing programs to control exposure. The cohort presents a unique possibility of investigating the pathophysiological and mechanistic basis of alpha-gal sensitization and meat allergy.

CONFLICT OF INTERESTS

None.

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