

# Prevalence and Impact of Type I Sensitization to Alpha-Gal in Patients Consulting an Allergy Unit

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## Keywords

Galactose- $\alpha$ -1-3-galactose · Allergy care · Alpha-gal syndrome · Anaphylaxis · Tick bite · Epidemiology · Prevalence · Risk factors

## Abstract

**Background:** Alpha-gal syndrome is a complex allergy with high clinical relevance regarding mammalian-derived food and drugs and is characterized by the presence of IgE antibodies directed at the carbohydrate galactose- $\alpha$ -1,3-galactose. As not all alpha-gal sIgE-positive individuals present clinical symptoms upon consumption of mammalian meat, the diagnostic value of alpha-gal sIgE has yet to be clarified. **Objective:** To investigate the prevalence of alpha-gal sIgE positivity among allergy patients, examine the impact of tick bites as associated risk factors and determine the diagnostic value of alpha-gal sIgE positivity. **Methods:** A retrospective cross-sectional study evaluating patients in the Allergy Unit was performed. Alpha-gal sIgE levels were assessed by ImmunoCAP assay. Exposure to tick bites was assessed by a questionnaire. A receiver operating characteristics (ROC) curve analysis was performed to determine the diagnostic value of alpha-gal sIgE for the diagnosis of alpha-

gal syndrome. **Results:** In the study population ( $n = 1369$ ), the overall prevalence of alpha-gal sIgE-positive ( $\geq 0.10$  kU<sub>A</sub>/L) individuals was 19.9%, and the highest prevalence (30.2%) was found in patients with insect venom allergies. A reported tick bite within the 12 months prior to blood sampling significantly increased the risk of alpha-gal sIgE positivity (OR 2.084). The ROC curve analysis indicated alpha-gal sIgE  $\geq 0.54$  kU<sub>A</sub>/L as the optimal cutoff point for assessing the diagnostic value of alpha-gal syndrome in allergy patients. **Conclusions:** In allergy care settings, alpha-gal sIgE positivity is a common finding. Alpha-gal sIgE is a sensitive marker in the diagnosis of alpha-gal syndrome but has limited predictive value for the characteristics or severity of this allergy.

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## Introduction

A decade ago, a new worldwide allergy was discovered and named alpha-gal syndrome [1]. The target structure of this allergy is the carbohydrate galactose- $\alpha$ -1,3-ga-

Edited by: H.-U. Simon, Bern.

lactose (alpha-gal) [2]. During evolution, the ability to form this disaccharide on glycoproteins and glycolipids was lost in primates and humans but not in other mammals [3]. Therefore, this structure became immunogenic in humans and has long been known in transplantation medicine as a major immune barrier for xenotransplantation [4]. The fact that this structure is also an important allergen for IgE-mediated anaphylaxis has been understood only in recent years. Most often, alpha-gal syndrome manifests as food allergy after eating mammalian meat in alpha-gal sIgE-positive individuals [5]. The delayed onset of the allergic reaction 3–6 h after consumption is pathognomonic for this syndrome [6]. The content of alpha-gal in mammalian innards, such as pork kidney, is higher, and therefore anaphylaxis can manifest within 30–60 mins after consumption [7–9]. Thus, in accordance with the concept of food-dependent exercise-induced anaphylaxis, the presence of cofactors or augmentation factors (e.g., physical exercise, analgesics, or alcohol) may be needed to elicit the type I reactions [8, 10]. This results in high variability in the clinical expression of “red meat allergy” in alpha-gal syndrome. Complicating the diagnosis is the fact that alpha-gal sIgE-positive individuals, who eat mammalian meat daily and have never shown allergic reactions in their medical histories, are not uncommon [11]. Anaphylaxis to drugs of mammalian origin (e.g., cetuximab, snake antivenom, gelatin in colloids or vaccines) is another important aspect of alpha-gal syndrome [2, 12–18]. Since these drugs are given only for specific indications to a limited number of patients, allergic reactions to these medications are less frequently observed than allergic reactions to meat. Therefore, there is only limited knowledge on the potential risk of alpha-gal-containing drugs and the frequency of anaphylactic events in the general population [14, 16, 19]. Due to the parenteral route of administration, allergists consider alpha-gal-containing drugs even more dangerous for allergic patients than mammalian meat [20, 21]. Alpha-gal-sIgE positivity is considered an environmentally induced sensitization, and tick bites are considered a major source of sensitization to the alpha-gal epitope [22–27]. Several studies found atopy to be an associated risk factor for alpha-gal-sIgE positivity [11, 28, 29]. The proportion of atopic patients is expectedly high in an allergological practice. Therefore, the aim of the present study was to investigate the prevalence of alpha-gal-sIgE positivity among allergy patients, to examine the impact of tick bites as associated risk factors, and to determine the diagnostic value of alpha-gal-sIgE positivity.

## Methods

### *Concept of Alpha-Gal-Related Allergy Diagnostic and Care*

Patients presenting for allergy diagnosis due to suspected food allergy or anaphylaxis of unknown origin at the Allergy Unit of the Department of Dermatology were routinely serologically screened for alpha-gal sIgE independently from other allergy tests as part of regular medical care. In addition, alpha-gal sIgE together with other carbohydrate epitopes (horseradish peroxidase, MUXF3 from bromelain) were also determined in the diagnosis of insect allergy. The process of diagnostic procedure in case of detectable alpha-gal sIgE is presented as a flowchart in the supporting information (online suppl. repository E1; for all online suppl. material, see [www.karger.com/doi/10.1159/000503966](http://www.karger.com/doi/10.1159/000503966)). A diagnosis of alpha-gal syndrome was made if there was an allergic reaction after the consumption of mammalian meat or entrails, and/or the administration of alpha-gal-containing drugs (e.g., cetuximab, gelatin colloids) was reported in the patient's history, alpha-gal sIgE was detectable with the ImmunoCAP assay, and type I sensitization to the alpha-gal epitope was confirmed by skin testing. Avoidance of mammalian entrails and alpha-gal-containing drugs was recommended for all patients with alpha-gal syndrome. Some patients stopped the consumption of meat, and some continued the consumption with reduced amounts of meat. Therefore, patients diagnosed with alpha-gal syndrome were advised to undergo follow-up titer testing every 3–6 months to assess changes in alpha-gal-sIgE titers with a potential impact on the practiced allergy diet.

### *Measurements of sIgE and tIgE Levels*

All sIgE measurements were performed on an ImmunoCAP 250 automated platform (Thermo Fisher Scientific, Uppsala, Sweden) according to the manufacturer's instructions. For testing anti-alpha-gal sIgE, an ImmunoCAP assay containing bovine thyroglobulin, which contains approximately 16 alpha-gal moieties, was used. Alpha-gal-sIgE levels and sIgE levels against other allergens (e.g., beef, pork, milk, cat dander, horseradish peroxidase, MUXF3 from bromelain) were quantified as  $\text{kU}_\text{A}/\text{L}$ , with a measurement range of 0.0–100  $\text{kU}_\text{A}/\text{L}$ . In clinical allergology, a cutoff of  $\geq 0.35$   $\text{kU}_\text{A}/\text{L}$  is typically used to indicate allergen-specific IgE positivity. However, the manufacturer of the ImmunoCAP assay considers values of  $\geq 0.10$   $\text{kU}_\text{A}/\text{L}$  to be positive. For epidemiological purposes, individuals with specific IgE levels of  $\geq 0.10$   $\text{kU}_\text{A}/\text{L}$  were considered to have positive reactivity. To evaluate atopy status, tIgE levels were determined in all collected sera using a tIgE ImmunoCAP assay (Thermo Fisher Scientific). Individuals with tIgE levels  $> 100$   $\text{kU}/\text{L}$  were considered to have a disposition for atopy.

### *Determination of the Prevalence of Alpha-Gal sIgE in Allergy Patients*

All patients from the Allergy Unit of the Department of Dermatology who had been probed for alpha-gal sIgE between January 1, 2012, and December 31, 2015, were included in this retrospective study to determine the prevalence of alpha-gal-sIgE positivity in an allergy practice. The patients were identified by a search with the statistical module of the laboratory information system (Roche Diagnostics IT Solutions, Berlin, Germany) of the Allergy Unit using the determination of alpha-gal sIgEs as the selector. The prevalence was stratified by sex and age. For the subanalysis of the prevalence of alpha-gal-sIgE positivity, the following six groups were defined based on the final allergy diagnoses: (1) alpha-gal syn-

drome, (2) other food allergies, (3) insect venom allergy (bee/wasp), (4) drug hypersensitivity (mainly non-IgE-mediated analgesic intolerance; including some testing prior to first administration of cetuximab), (5) urticaria (acute or chronic urticaria), and (6) others (no evidence for IgE-mediated or non-IgE-mediated hypersensitivity; e.g., irritable bowel disease and other unspecific symptoms). Physicians' notes, skin test protocols, laboratory findings, and other available information in the electronic medical file were used as source data for the assignment of the patients to the defined subgroups.

#### Determination of Tick Exposure in Allergy Patients

To assess a standardized history of tick exposure, vaccination status for tick-borne encephalitis, pet keeping, sports, and hobbies and the correlation of these variables with alpha-gal-sIgE positivity, a questionnaire was sent by mail to all identified patients probed for alpha-gal sIgE until the effective date August 15, 2014. The questionnaire was pseudonymized and linked to the serological findings, sex, and age. The participants of this survey returned the completed questionnaire together with a written informed consent form in a stamped, self-addressed envelope. Sports and leisure activities reported in the questionnaire were assigned to activities with and without vegetation contact for evaluation.

#### Ethical Approval

The retrospective analysis of the prevalence of alpha-gal-sIgE positivity in allergy practice as well as the questionnaire-based interview was approved by the Ethics Commission of the University Medical Faculty Tübingen (No. 382/2014/BO2).

#### Statistical Analysis

Qualitative variables are reported as proportions, and inter-group comparisons were made using  $\chi^2$  tests and Fisher's exact tests. Continuous variables were represented as medians and inter-quartile ranges and compared using the Mann-Whitney test and the Kruskal-Wallis test. Paired continuous variables were compared using the Wilcoxon test. Odds ratios (ORs) were calculated from contingency tables with a  $\chi^2$  test. Binary logistic regression models were fitted to assess the association of selected variables with positivity to alpha-gal-sIgE antibodies. Receiver operating characteristics (ROC) curve analysis was performed to determine the diagnostic value of alpha-gal sIgE for the diagnosis of alpha-gal syndrome. *p* values <0.05 were regarded as significant.

## Results

#### Description of Study Population and Prevalence of Alpha-Gal sIgE

Between January 1, 2012, and December 31, 2015, a total of 1,444 thyroglobulin ImmunoCAP measurements for clinical routine in 1,369 individuals were identified and considered in this study. From this total, 61 were archived blood samples collected between August 2005 and November 2011, and the other 1,308 samples were sampled within the period. The proportion of males was 40.0% ( $n = 547/1,369$ ), and the median age was 43.4 years

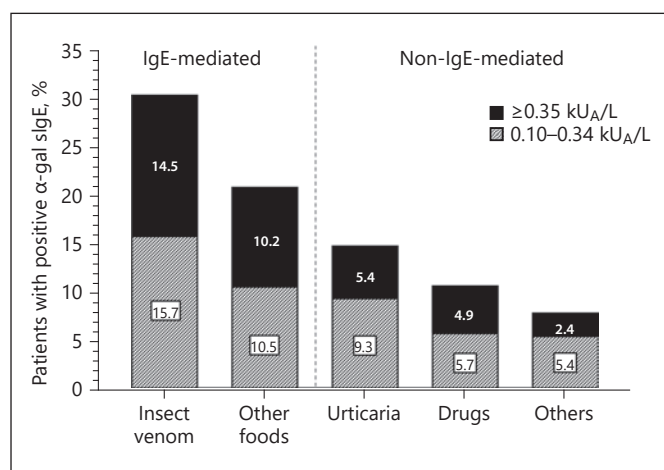


**Fig. 1.** Prevalence of alpha-gal sIgE in allergy patients not positively diagnosed for alpha-gal syndrome. In patients with confirmed food allergies, an alternative food allergen was identified for anaphylaxis in the medical history. Prevalence of alpha-gal sIgE in allergy patients depending on the time since the tick bites according to the questionnaire. Crosshatched: individuals with sensitization levels between 0.10 and 0.34 kU<sub>A</sub>/L; black: individuals with sensitization levels ≥ 0.35 kU<sub>A</sub>/L.

(min. 0.6 year/max. 89 year; quartiles 28.1 year/55.6 year). The proportion of minors (<18 years) was 7.8% ( $n = 107/1,369$ ).

The overall prevalence of alpha-gal-sIgE positivity (epidemiological threshold for positivity, ≥ 0.10 kU<sub>A</sub>/L) was 19.9% ( $n = 272/1,369$ ). The proportion of individuals with potentially clinically relevant type I sensitization to alpha-gal sIgE (classic threshold for positivity, ≥ 0.35 kU<sub>A</sub>/L) was 10.9%. The prevalence of alpha-gal-sIgE positivity (≥ 0.10 kU<sub>A</sub>/L) was higher in men, at 26.0% ( $n = 142/547$ ), than in women, at 15.8% ( $n = 130/822$ ). The proportion of minors with sIgE to alpha-gal was 18.7% ( $n = 20/107$ ), but none of the minors was diagnosed with alpha-gal syndrome.

Based on medical history, allergy testing, and optional oral challenge tests, alpha-gal syndrome was diagnosed in 4.2% of the cohort ( $n = 57/1,369$ ). A proportion of patients ( $n = 55$ ) showed urticaria or anaphylaxis after consumption of mammalian meat or entrails. The clinical characteristics of these patients have been published in two previous case series [8, 30]. Two patients developed anaphylaxis after the first application of cetuximab.

The prevalence of alpha-gal-sIgE positivity without obvious clinical impact in other subgroups of allergy unit patients is presented in Figure 1, and additional demographic information is provided in Table 1.

**Table 1.** Demographic data, total IgE (tIgE), and IgE specific for alpha-gal for the overall cohort and the six subgroups (1) alpha-gal syndrome, (2) other food allergies, (3) insect venom allergy (bee/wasp), (4) drug hypersensitivity (mainly non-IgE-mediated analgesic intolerance; including some testing prior to first administration of cetuximab), (5) urticaria (acute or chronic urticaria), and (6) others (no evidence for IgE-mediated or non-IgE-mediated hypersensitivity, e.g. irritable bowel disease and other unspecific symptoms)

|                                           | Total cohort  | Alpha-gal syndrome | Other food allergy | Insect venom allergy | Drug hypersensitivity | Urticaria    | Others       |
|-------------------------------------------|---------------|--------------------|--------------------|----------------------|-----------------------|--------------|--------------|
| Male gender                               | 547/1369 (40) | 37/57 (65)         | 103/266 (39)       | 116/255 (46)         | 50/123 (41)           | 114/258 (44) | 127/410 (31) |
| Age, years                                | 43 (28–56)    | 59 (50–70)         | 36 (23–51)         | 51 (39–60)           | 49 (31–64)            | 42 (27–54)   | 38 (25–50)   |
| Alpha-gal-sIgE                            |               |                    |                    |                      |                       |              |              |
| 0.10–0.34 kU <sub>A</sub> /L              | 123/1369 (9)  | 2/57 (4)           | 28/266 (11)        | 40/255 (16)          | 7/123 (6)             | 24/258 (9)   | 22/410 (5)   |
| ≥0.35 kU <sub>A</sub> /L                  | 149/1369 (11) | 55/57 (96)         | 27/266 (10)        | 37/255 (15)          | 6/123 (5)             | 14/258 (5)   | 10/410 (2)   |
| tIgE ≥100 kU <sub>A</sub> /L (proportion) | 668/1328 (50) | 43/55 (78)         | 196/265 (74)       | 117/252 (46)         | 45/107 (42)           | 125/251 (50) | 134/398 (34) |

The data are shown as absolute numbers (*n/N*) and percentages (within parentheses) or medians and interquartile ranges (within parentheses). Percentages were brought up/down to the next natural number.

**Table 2.** Binary logistic regression models were fitted to assess factors associated with the risk for tick bites within the previous 12 months and factors associated with sIgE to alpha-gal of patients presenting to an allergy unit

|                                                                                              | OR (95% CI)         | <i>p</i> value |
|----------------------------------------------------------------------------------------------|---------------------|----------------|
| <b>a Factors associated with the risk for tick bites within the previous 12 months</b>       |                     |                |
| Male gender                                                                                  | 1.240 (0.670–2.293) | 0.494          |
| Age (years)                                                                                  | 1.036 (1.006–1.068) | 0.019          |
| Professional outdoor working (yes)                                                           | 3.361 (1.527–7.398) | 0.003          |
| Leisure activities without vegetation contact (yes)                                          | 0.750 (0.406–1.385) | 0.357          |
| Leisure activities with vegetation contact (yes)                                             | 1.584 (0.671–3.735) | 0.294          |
| Private gardening (yes)                                                                      | 2.451 (1.161–5.171) | 0.019          |
| Pet keeping (yes)                                                                            | 1.363 (0.738–2.519) | 0.322          |
| <b>b Factors associated with sIgE to alpha-gal of patients presenting to an allergy unit</b> |                     |                |
| Male gender                                                                                  | 1.170 (0.746–1.834) | 0.494          |
| Age (years)                                                                                  | 1.026 (1.13–1.040)  | <0.001         |
| Total IgE (tIgE) ≥100 kU <sub>A</sub> /L (yes)                                               | 3.052 (1.851–5.033) | <0.001         |
| tIgE (kU/L)                                                                                  | 1.001 (1.000–1.001) | <0.001         |
| Tick bite within lifetime (yes)                                                              | 2.197 (1.221–3.953) | 0.009          |
| Tick bite within the last 12 months (yes)                                                    | 2.084 (1.220–3.561) | 0.007          |

### Determination of Tick Exposure in the Study Population

A total of 563 completed questionnaires of the 1,018 mailed questionnaires were returned by the patients (55% recall rate). Compared to the total cohort, the patients participating in the survey were older (median age of 48.4 years), the proportion of males was slightly higher (42.3%), and the prevalence of alpha-gal-sIgE positivity (≥0.10 kU<sub>A</sub>/L) was 23.8% higher. The tick-borne encephalitis vaccination coverage for the survey participants was 51.5% and comparable to the reported vaccination coverage of 55.0% for the federal state of Baden-Württemberg

in 2014 (information kindly provided by Pfizer Deutschland GmbH, Karlsruhe, Germany). Of the participants, 73.2% reported that they had tick bites in their lifetimes. Tick bites within the last 10 years were reported by 61.5% of the participants. Regarding the number of tick bites, 47.1% of the participants remembered 1–5 tick bites and 14.4% remembered more than 5 tick bites. A tick bite within the 12-month period prior to blood sampling was remembered by 18.5% of the participants. A single tick bite was reported by 8.7% of the participants and more than one tick bite (range 2–30 tick bites, median 3 tick bites) by 9.4% of the participants.

To determine risk factors for tick bites within the 12 months prior to blood sampling, binary logistic regression conducted and sex, age, professional outdoor work, leisure activities without vegetation contact, leisure activities with vegetation contact, private gardening, and pet keeping were considered covariables in this model. A total of 318 questionnaires with complete data were considered in this model (Table. 2a). The covariables sex, leisure activities without vegetation contact, leisure activities with vegetation contact, and pet keeping did not significantly increase the risk of a tick bite within the 12 months prior to blood sampling. Age was found to be a relevant covariable and showed an increase in the risk for a tick bite by 3.6% per year (OR 1.036, CI 1.006–1.068;  $p < 0.019$ ). For the participants working outdoors, the OR for a tick bite was 3.361 (CI 1.527–7.398;  $p < 0.003$ ). The risk of a tick bite was significantly increased (OR 2.451, CI 1.161–5.171;  $p = 0.019$ ) by private gardening.

The prevalence of alpha-gal-sIgE positivity (Fig. 2) and alpha-gal sIgE (online suppl. repository E2) differed between participants remembering a tick bite within the 12 months prior to blood sampling, participants with no bites within the last 12 months but bites within the last 10 years and participants remembering no tick bites within 10 years. No differences were found between these subgroups in tIgE levels (data not shown).

#### Determination of Factors Associated with Alpha-Gal-sIgE Positivity

To investigate the possible impact of sex, age, tIgE levels, and tick bite experience across the lifetime and within the 12 months prior to blood sampling, binary logistic regression was performed with a cutoff of  $\geq 0.10$  kU<sub>A</sub>/L for positivity to alpha-gal sIgE, and 541 individuals with complete data were considered in this model (Table 2b). The covariable sex did not significantly increase the risk of alpha-gal-sIgE positivity. Advancing age was found to be a relevant covariable and showed an increase in the risk of alpha-gal-sIgE positivity by 2.6% per year (OR 1.026, CI 1.013–1.040;  $p < 0.001$ ). For the participants with tIgE levels  $> 100$  kU/L, the OR for alpha-gal-sIgE positivity was 3.052 (CI 1.851–5.033;  $p < 0.001$ ). The risk of alpha-gal-sIgE positivity was increased by 1% per 10 kU/L tIgE (OR 1.001, CI 1.000–1.001;  $p < 0.001$ ). The report of tick bites in their lifetime significantly increased the risk of alpha-gal-sIgE positivity (OR 2.197, CI 1.221–3.953;  $p = 0.009$ ), and a remembered event of at least one tick bite within the 12 months prior to blood sampling significantly increased the risk of alpha-gal-sIgE positivity (OR 2.084, CI 1.220–3.561;  $p = 0.007$ ).

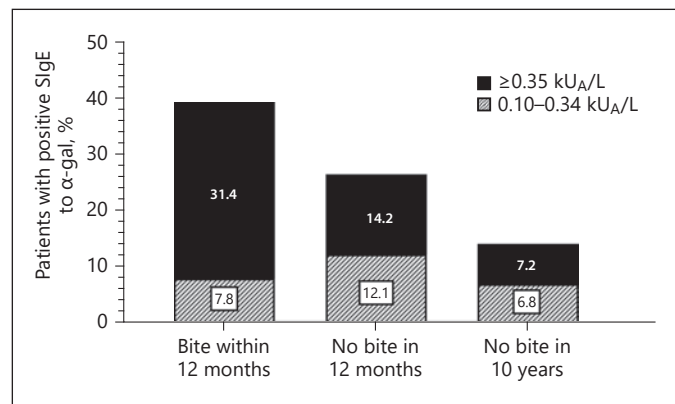


**Fig. 2.** Prevalence of alpha-gal sIgE in allergy patients depending on the time since the tick bites according to the questionnaire. Crosshatched: individuals with sensitization levels between 0.10 and 0.34 kU<sub>A</sub>/L; black: individuals with sensitization levels  $\geq 0.35$  kU<sub>A</sub>/L.

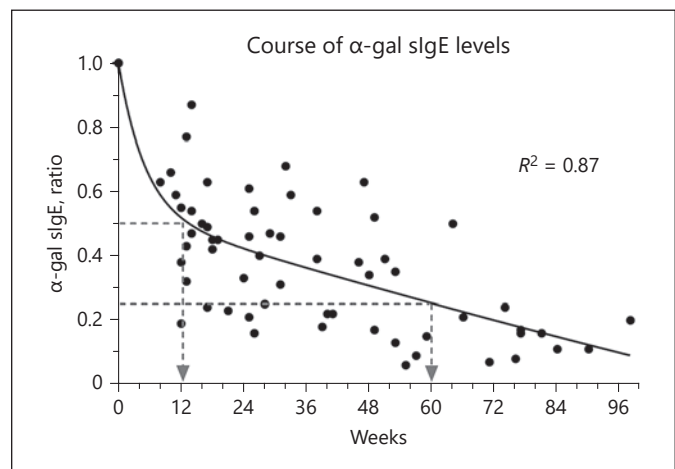


**Fig. 3.** Course of alpha-gal sIgE levels in 28 patients with alpha-gal syndrome revealed a rapid and continuous decline in the sIgE values as long as no events occurred. Based on a two-phase exponential decay equation model adjusted to the observations, the halving of the initial value can be expected after 12 weeks and a further halving after another 48 weeks.

#### Diagnostic Value of Alpha-Gal-sIgE Positivity

At the initial diagnosis, patients with alpha-gal syndrome ( $n = 57$ ), considered as a group, showed high titers for alpha-gal sIgE (median 14.5 kU<sub>A</sub>/L, IQR 2.70–47.05 kU<sub>A</sub>/L), but at the individual level, alpha-gal sIgE titers were distributed over a wide range (min. 0.12 kU<sub>A</sub>/L, max. 230 kU<sub>A</sub>/L). The follow-up with 28 patients to redetermine titer levels for up to 98 weeks revealed a rapid and continuous decline in the sIgE values as long as no events

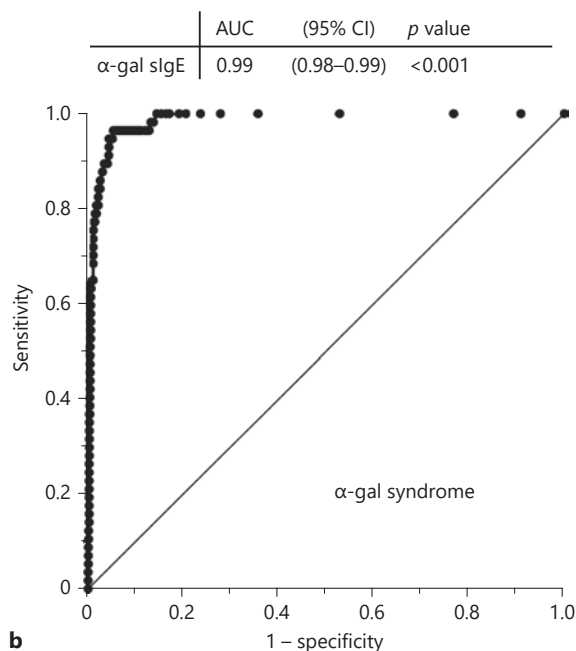
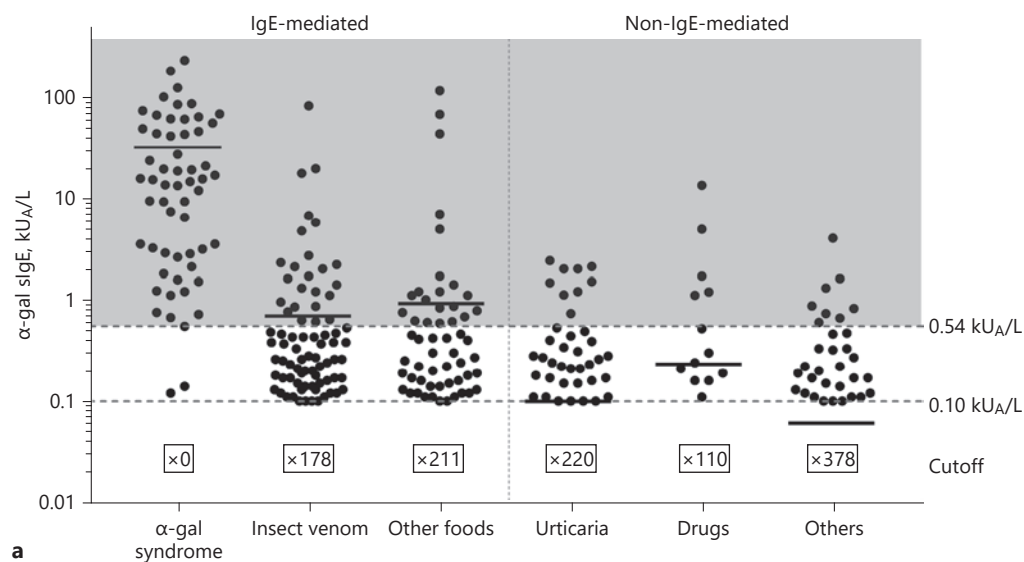


**Fig. 4. a** For an analysis of the diagnostic value of alpha-gal-sIgE positivity, alpha-gal sIgE levels in patients with alpha-gal syndrome were compared with levels in patients with other allergic and nonallergic diseases. Each dot indicates the measured sIgE levels from an individual. The boxes indicate the number of individuals with alpha-gal sIgE < 0.10 kU<sub>A</sub>/L. The gray box contains the

measured sIgE levels above the cutoff point suggested by the ROC curve analysis. **b** The ROC curve analysis indicated alpha-gal sIgE levels of 0.54 kU<sub>A</sub>/L as a cutoff point for assessing the diagnostic value of alpha-gal syndrome in allergy patients, resulting in a sensitivity of 96.5% and a specificity of 94.9%.

occurred. Based on a two-phase exponential decay equation model (Fig. 3) adjusted to the observations, a decline of 50% from the initial value can be expected after 12 weeks and a further decline of 50% is observed after another 48 weeks. Boosted alpha-gal sIgE was observed in the same individuals after further tick bites (online suppl. repository E3). In contrast, alpha-gal-sIgE-positive allergy patients not diagnosed with alpha-gal syndrome ( $n = 215/1312$ ; Fig. 4a) exhibited alpha-gal sIgE with a median of  $0.27 \text{ kU}_A/\text{L}$  (IQR  $0.16\text{--}0.76 \text{ kU}_A/\text{L}$ ; min.  $0.10 \text{ kU}_A/\text{L}$ , max.  $115 \text{ kU}_A/\text{L}$ ). The ROC curve analysis (Fig. 4b) indicated a sIgE against alpha-gal level of  $0.54 \text{ kU}_A/\text{L}$  as the optimal cutoff point for assessing the diagnostic value of alpha-gal syndrome in allergy patients. The area under the curve was  $0.99$  (CI  $0.98\text{--}0.99$ ;  $p < 0.001$ ), with a sensitivity of  $96.5\%$  and a specificity of  $94.9\%$  for the cutoff value of  $\geq 0.54 \text{ kU}_A/\text{L}$  required for diagnosis of alpha-gal syndrome. Next, prick-to-prick testing with mammalian meat and intradermal testing with gelatin-derived colloid was examined to support the differentiation between alpha-gal syndrome and silent type I sensitization to alpha-gal. Retrospectively reviewing patients' medical histories, a total of 38 skin tests of alpha-gal syndrome patients and 38 skin tests of patients with well-documented tolerance of mammalian meat and alpha-gal sIgE were identified and fulfilled the criteria for comparison. Skin tests of 4 additional individuals with no sensitization to alpha-gal served as controls. None of these controls reacted to mammalian meat preparations or intradermal testing with gelatin. Prick-to-prick testing with pork kidney and intradermal testing with gelatin revealed positive skin tests in patients with alpha-gal syndrome as well in individuals with asymptomatic type I sensitizations to alpha-gal (online suppl. repository E4). The frequency of positive test results seemed to be associated with the alpha-gal sIgE levels. A further reliable differentiation in cases of alpha-gal-sIgE positivity between alpha-gal syndrome and silent type I sensitization to alpha-gal was not possible with prick-to-prick testing with mammalian meat preparations or intradermal testing with gelatin.

## Discussion

In this study, we showed that alpha-gal-sIgE positivity is a common finding among patients presenting to an allergy unit. Several studies have found that elevated tIgE associated with alpha-gal-sIgE positivity [11, 28, 29]. Since allergic diseases (e.g., allergic rhinitis, atopic eczema) are often associated with elevated tIgE levels, a high-

er prevalence of alpha-gal-sIgE positivity has to be expected among patients presenting to an allergy unit than in the general population. In our opinion, it is important that allergists feel confident in the differentiation between symptomatic allergy and sensitization without symptoms in the case of alpha-gal-sIgE detection. According to the current state of knowledge, alpha-gal sIgE has to be considered an environmentally induced type I sensitization, and tick bites are a major source [31, 32]. In accordance with this concept, alpha-gal-sIgE positivity was high in individuals reporting a tick bite within the last 12 months. In addition to professional outdoor working, we identified private gardening as a leisure activity associated with an elevated risk for tick bites. Interestingly, the alpha-gal-sIgE positivity rate in insect venom allergy patients was similar to hunter and forest workers in our previous epidemiological study [15]. We assume that the relation between insect venom allergy and alpha-gal-sIgE positivity is individuals being in an environment with a higher risk for insect stings as well as tick bites. Retitering of alpha-gal sIgE showed an unusual dynamic of sIgE titers that has not yet been observed for food allergies. Without further immunological boost, alpha-gal sIgE can decline rapidly within weeks and can continue to decline over a long period of time. We have also observed that high alpha-gal sIgE titers can rebound rapidly after an additional tick bite. All the patients with alpha-gal syndrome we have diagnosed to date have been middle-aged or older adults. In contrast to studies from the United States, we have never encountered children or young adults with confirmed alpha-gal syndrome [33, 34]. This is a surprising observation because the prevalence of alpha-gal-sIgE positivity seems to be comparable between minors ( $<18$  years) and adults. The determination of alpha-gal sIgE using a bovine thyroglobulin-containing ImmunoCAP is the most feasible and sensitive marker as a diagnostic, but interpretation is not without potential pitfalls. As indicated by the ROC analysis, already low levels of alpha-gal sIgE within the class 1 range ( $0.35\text{--}0.70 \text{ kU}_A/\text{L}$ ) should be cause for extended examination by an allergist. If there is a clinical history suggestive of anaphylaxis after the consumption of mammalian meat or the administration of alpha-gal-containing drugs, the detection of alpha-gal sIgE strongly supports the diagnosis of alpha-gal syndrome. Without anaphylaxis in the patients' history, reported regular meat consumption and no medical condition in history with the need for the administration of alpha-gal-containing drugs, the diagnostic value of alpha-gal sIgE is limited. It remains unclear whether these alpha-gal sIgE-sensitized

individuals can become allergic upon sustained tick exposure or upon administration of alpha-gal via the parenteral route (e.g., gelatin-containing vaccines or infusion solutions). Therefore, medical advice from an allergist should also be given to alpha-gal sIgE-sensitized individuals. As described by previous studies, prick-to-prick or intradermal testing only confirms the serological finding of the alpha-gal sensitization by another allergological method [8]. Unfortunately, skin testing does not allow differentiation between different mammalian species, the clinical characteristics of alpha-gal-associated meat allergy (e.g., entrails vs. muscular meat), or the clinical impact in general. Therefore, hazardous oral challenge tests, potentially with regard to cofactors for anaphylaxis and entrails, are needed for clarification in particular clinical situations. Recently, a study described a basophile activation test as a possible diagnostic method that enables differentiation between alpha-gal syndrome and silent sensitization [35].

In conclusion, we found that alpha-gal-sIgE positivity is a common finding in allergy care. Alpha-gal sIgE is a sensitive marker in the diagnosis of alpha-gal syndrome, but due to its rapid changes, the measured levels are of low predictive value for the clinical characteristics of this allergy. Further research is needed to understand the differences between patients with alpha-gal syndrome and individuals with sIgE against alpha-gal who tolerate mammalian meat.

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## Acknowledgment

The authors thank Selma Cetinkaya, Elisabeth Merkle, Jutta Schatz, Catrin Walker, Bettina Keller, Uta Hamacher, Cornelia Grimm for their excellent technical assistance.

## Statement of Ethics

This study complies with the guidelines for human studies and was conducted ethically in accordance with the World Medical Association Declaration of Helsinki.

## Disclosure Statement

The authors declare no conflicts of interest.

## Funding Sources

This work was supported by a funding from the Deutsche Forschungsgemeinschaft (DFG), project FI 2226/2-1.

## Author Contributions

J. Fischer and A.S. Yazdi conceived, designed and supervised the study. J. Fischer and S. Forchhammer examined the patients in the Allergy Unit. H.-N. Huynh and J. Hebsaker collected and analyzed the data as part of their doctoral theses. J. Fischer wrote the paper in close collaboration with all the authors.

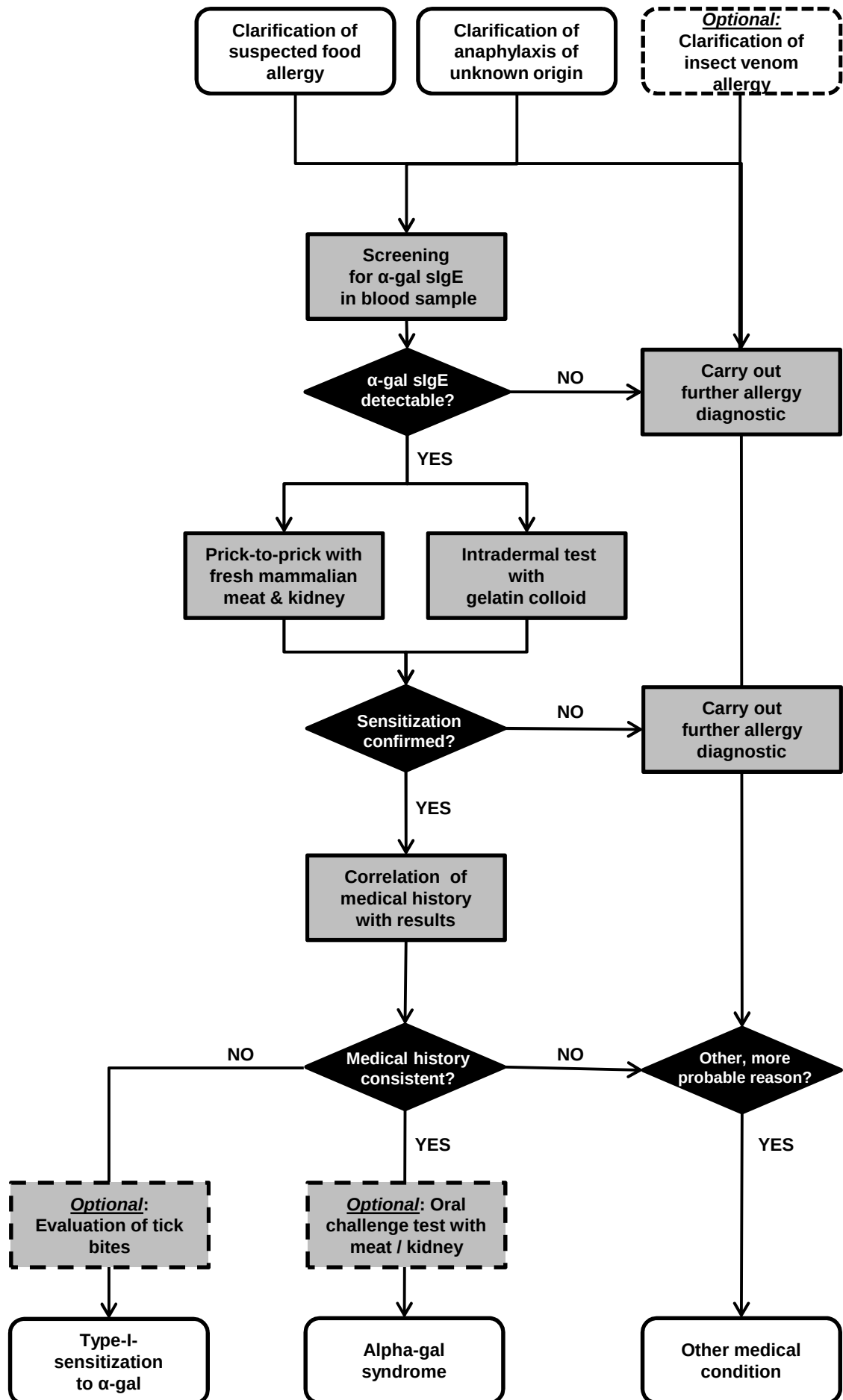
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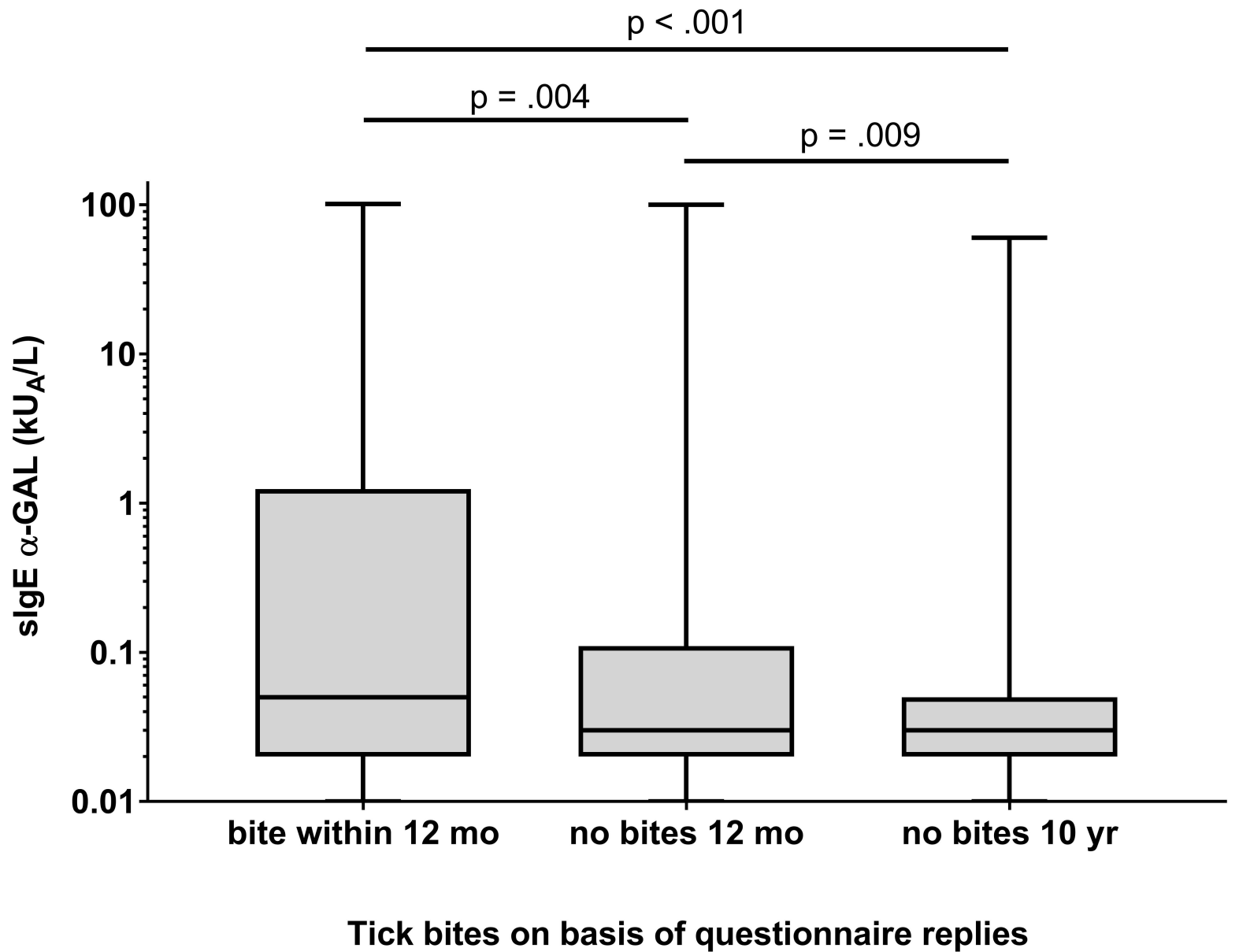
**Online Suppl. Figure E1.** Patients presenting for allergy diagnosis due to suspected food allergy or anaphylaxis of unknown origin at the Allergy Unit of the Department of Dermatology were routinely serologically screened for nonobvious food allergies that were independent from other allergy tests as part of regular medical care. In the case of suspected alpha-gal syndrome, prick-to-prick tests using raw and cooked muscular meat and kidney from pork and beef were performed as advanced diagnoses to determine the individual's sensitivity to the alpha-gal epitope. This testing was combined with an intradermal testing with gelatin-derived colloid (Gelafundin 4%, B. Braun Melsungen, Germany). Histamine was used as a positive control, and the diluent was used as a negative control. Reactions producing wheals with a diameter of >3 mm on the prick-to-prick test and wheals with a diameter of >5 mm on the intradermal test were considered positive results. Further optional diagnostic oral challenge tests with pork and beef were performed as previously described. A diagnosis of alpha-gal syndrome was made if there was an allergic reaction after consumption of mammalian meat or entrails and/or administration of alpha-gal-containing drugs (e.g., cetuximab, gelatin colloids) was reported in the patient's history, alpha-gal sIgE was detectable with the ImmunoCAP assay and type I sensitization to the alpha-gal epitope was confirmed in skin testing.

**Online Suppl. Figure E2.** Alpha-gal sIgE levels in allergy patients depending on the time since the tick bites according to the questionnaire.

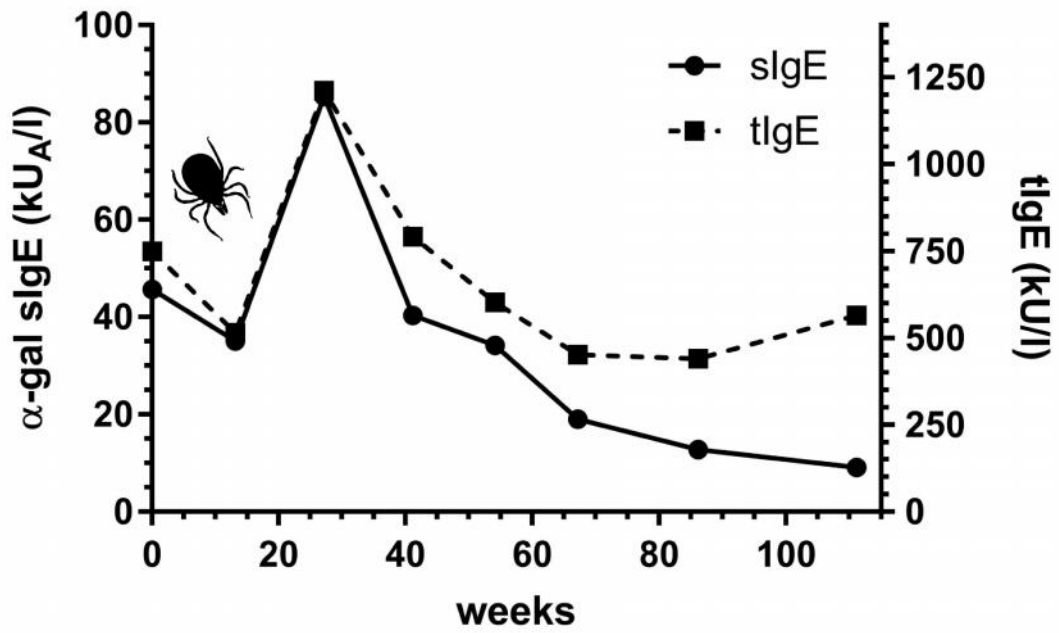
**Online Suppl. Figure E3.** Visualization of the course of alpha-gal sIgE and tIgE levels in two selected patients with alpha-gal syndrome after tick bites.

**Online Suppl. Figure E4.** Proportion of positive prick-to-prick testing with mammalian kidney/meat and intradermal testing with gelatin in patients with no tolerance of mammalian meat in their medical history (patients with alpha-gal syndrome) in comparison to allergy patients with tolerance of mammalian meat (silent type I sensitization to alpha-gal).





### F-071



### F-013

