

Gelatin-Containing Vaccines for Varicella, Zoster, Measles, Mumps, and Rubella Induce Basophil Activation in Patients with Alpha-Gal Syndrome

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Keywords

Alpha-gal syndrome · Anaphylaxis · Basophil activation test · Gelatin-containing vaccines

Abstract

Background: The alpha-gal syndrome (AGS) describes a new type I allergy entity to the carbohydrate epitope galactose- α -1,3-galactose (alpha-gal), which is mainly found in mammalian food products (e.g., beef, pork, and venison). Apart from meat products, alpha-gal can also be found in products containing gelatin of bovine or porcine origin. Recent case reports pointed to severe anaphylaxis in patients suffering from AGS after vaccination with vaccines containing hydrolyzed gelatin. It was the objective of this study to evaluate if basophil activation tests (BATs) performed with such vaccines were positive in patients with AGS. **Methods:** BAT was performed with different dilutions of a gelatin-containing measles, mumps, and rubella (MMR) live vaccine; an attenuated varicella (V) vaccine; an attenuated V-zoster (VZ) vaccine; a MMR live vaccine not containing gelatin (non-gelatin MMR vaccine) in 2 patients with confirmed AGS, 2 patients highly suspicious for AGS, and 2 healthy individuals without any previous medical history for allergies. **Results:** All patients showed strongly positive results for all gelatin-containing vaccines (MMR vaccine, V vaccine, and VZ vaccine).

Non-gelatin MMR vaccine was negative. The 2 healthy controls did not show any basophil activation. **Conclusions:** Gelatin-containing vaccines should be administered with caution or avoided in patients with AGS because of their high potential to activate basophils indicating a risk for anaphylaxis. Also, BAT is a useful additional tool when it comes to screening for potentially high-risk alpha-gal-containing drugs.

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Introduction

The alpha-gal syndrome (AGS), a new type I allergy entity to the carbohydrate epitope galactose- α -1,3-galactose (alpha-gal), was first described in 2009 [1]. High concentrations of alpha-gal are mainly found in mammalian food products (e.g., beef, pork, and venison), especially organ meats. Patients suffering from AGS tend to show anaphylactic symptoms 3–6 h after ingestion of red meat.

Apart from meat products, alpha-gal can also be found in medical products, for example, the EGFR an-

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

tibody cetuximab or volume expanders used in intensive care medicine. When alpha-gal is administered parenterally, affected patients suffer from immediate reactions [2–4].

In 2017, Stone et al. reported the case of a female patient with confirmed AGS suffering from severe anaphylaxis (dyspnea, flush, facial, oral, and uvular angioedema, bilateral conjunctival injections) upon receipt of attenuated live herpes zoster vaccine [5]. Only recently the same group of authors published a similar case of a 5-year-old boy who had been diagnosed with alpha-gal allergy only months earlier showing anaphylactic symptoms (shortness of breath, wheezing, disseminated urticaria, and angioedema) minutes after receiving measles, mumps, and rubella (MMR), varicella (V), and DTaP/ITV vaccines [6]. In both cases, the relatively high amount of hydrolyzed gelatin contained in the administered vaccines (especially MMR and V vaccines) on the one hand and low serum concentrations of gelatin sIgE on the other hand that was initially thought to be a possible elicitor made the authors conclude that sIgE toward alpha-gal must have been the main contributory mechanism for the observed anaphylactic reactions [5, 6].

Up-to-date diagnosis of AGS is mainly based on medical history, skin testing, sIgE levels, and oral provocation tests (OPTs). However, there are sensitized patients with elevated alpha-gal sIgE levels but no clinical manifestation of anaphylactic symptoms. Furthermore, a study by Mullins et al. found 30 of 40 patients with confirmed AGS positive in skin test with gelatin, but only 12 out of 40 showed symptoms in OPT with gelatin [3]. To discriminate between allergic patients and those only sensitized, several in vitro testing methods have emerged in recent years, giving additional information and making evaluation of clinical relevance easier. One of those tests is the basophil activation test (BAT). In a recent study by Mehlich et al., significant differences in the BAT were found between a group of patients with AGS and a group of individuals with asymptomatic alpha-gal sensitization [7].

Additionally, BAT has shown to be useful identifying alpha-gal-containing drugs such as cetuximab [8], anti-venoms [9], and pancreatic enzymes of porcine origin [10] by detecting basophil activation and therefore indicating potentially high-risk drugs for patients with AGS. It was the aim of this study to evaluate if BATs performed with vaccines containing presumably high concentrations of hydrolyzed gelatin were positive in patients with confirmed AGS and therefore might indicate patients at risk.

Materials and Methods

Ethics Approval and Consent to Participate

The protocol was approved by the Institutional Review Board (Ethikkommission der Fakultät für Medizin der Technischen Universität München). Informed consent was obtained from every patient participating in this study.

Study Population

A total of 6 individuals were tested, 2 patients with confirmed diagnosis of AGS based on positive OPT (patients 1 and 2), 2 patients as proven by medical histories of anaphylaxis after exposure to alpha-gal-containing food products (patients 3 and 4), and 2 healthy controls without any previous medical history for allergies. Serologically, none of the tested patients showed a sensitization toward gelatin (sIgE <0.1 kU/L). Furthermore, none of the tested individuals had been immunized before with V or V-Zoster (VZ) vaccine, but all had gone through V infection in childhood. However, information on immunization with MMR live vaccine is lacking. Patients' characteristics, medical history, and allergy workup are summarized in Table 1.

Reagents for BATs

BAT was performed using different concentrations (undiluted, 1:5, 1:25, 1:125, 1:625, and 1:3,250 diluted) of vaccines, which were prepared according to the manufacturer's specifications (reconstitution of vaccine with water for injection). Gelatin-containing, attenuated V vaccine (Varivax®; Sanofi Pasteur MSD, France) was tested in all patients and healthy controls. Gelatin-containing MMR live vaccine (M-M-RVax®; Chiron Behring, Germany, MMR vaccine) was tested in patients 1, 2, and 3 as well as healthy controls. Due to lack of availability, another MMR live vaccine (Priorix®; GlaxoSmithKline, Germany, non-gelatin MMR vaccine) not containing gelatin was tested in patient 4. For the same reason, BATs with gelatin-containing, attenuated VZ vaccine (Zostavax®; Sanofi Pasteur MSD, France) could only be performed in patients 1 and 2 and healthy controls.

In all patients, additional BATs were performed using alpha-gal (BAG2-GAL, alpha-gal-HSA allergen; BÜHLMANN Laboratories AG, Schönenbuch, Switzerland) at concentrations of 1,000, 200, 40, 8, 1.6, and 0.32 ng/mL (patients 1 and 2) or of 22.5, 4.5, 0.9, 0.18, 0.036, and 0.0072 ng/mL (patients 3 and 4). The different concentrations are due to the amount of allergen per vial charged by the company.

Basophil Activation Tests

The Flow CAST (BÜHLMANN Laboratories AG, Schönenbuch, Switzerland) was used for quantitative measurement of in vitro basophil activation, as previously described [11]. Venous blood was collected in 10-mL EDTA tubes from each subject. For each subject and allergen, polystyrene tubes were prepared with 50 µL of allergen at the defined concentration and diluted in 100 µL of stimulation buffer (containing heparin, Ca²⁺, and IL-3 [2 ng/mL]). An anti-FcεRI mAb and anti-N-formyl-methionyl-leucyl-phenylalanine (fMLP) were used as positive controls. Background values were assessed with 50 µL of stimulation buffer. We added and gently homogenized 50 µL of each subject's whole blood (with EDTA), added 20 µL of staining reagent (anti-CD63-fluorescein isothiocyanate and anti-CCR3-phycoerythrin mAbs), and mixed and incubated the solution at 37°C for 25 min in an incubator. Two

Table 1. Patients' characteristics, medical history, and allergy workup

Characteristic	Patient 1	Patient 2	Patient 3	Patient 4
Gender	F	F	M	F
Age, years	81	68	68	46
Atopy	No	No	No	No
History	One episode with rash, shortness of breath, and loss of consciousness 3 h after ingestion of pork kidney	One episode with rash, shortness of breath, and loss of consciousness 3 h after ingestion of pork kidney	Three delayed anaphylactic reactions (rash, generalized urticaria, and shortness of breath) following exposure to red meat	Since 2 years recurrent episodes with severe abdominal pain 2–3 h after ingestion of red meat, and tolerance of chicken and turkey
SPT (mm diameter wheal size)	Raw pork kidney suspension (3)	Raw pork kidney suspension (3)	Raw pork kidney suspension (6)	Raw pork kidney suspension (4)
IDT (mm diameter wheal size)	Gelafundin 4% diluted 1:1 (5)	Gelafundin 4% diluted 1:1 (6)	Gelafundin 4% diluted 1:1 (9)	Gelafundin 4% diluted 1:1 (8)
Total IgE	38.7 IU/mL	78.4 IU/mL	736 IU/mL	796 IU/mL
sIgE alpha-gal	15.2 kU/L	3.65 kU/L	68.5 kU/L	38 kU/L
sIgE gelatin	<0.1 kU/L	<0.1 kU/L	<0.1 kU/L	<0.1 kU/L
OPT	Anaphylactic reaction to 1.5 g cooked pork kidney	Anaphylactic reaction to 18 g cooked pork kidney	Refused	Pending

SPT, skin prick test; IDT, intradermal test; OPT, oral provocation test; Gelafundin, a gelatin-derived colloid rich in alpha-gal.

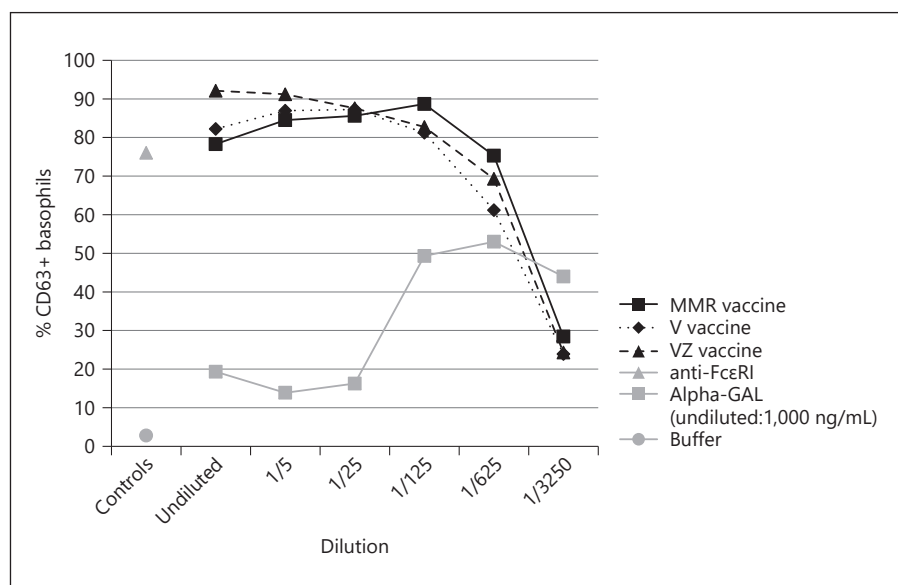


Fig. 1. Basophil activation for different dilutions of tested vaccines, alpha-gal, and positive/negative controls in patient 1. MMR, measles, mumps, and rubella; V, varicella; VZ, V-zoster.

milliliters of lysing buffer stopped the stimulation within 5 min of incubation in the dark at room temperature, followed by centrifugation of the solution for 5 min at 460 g. The supernatant was decanted, and stained cells were washed with 300 μ L of washing buffer. Cells were resuspended by means of gentle vortexing and analyzed by using FACSCalibur (Becton Dickinson, Heidelberg,

Germany) with CellQuestTM software. Basophils were gated as low side scatter CCR3⁺/side scatter^{low}. Anti-CCR3 was used as a selection marker to separate basophils from other leukocytes. Upregulation of the basophil activation marker CD63 was calculated based on the percentage of CD63⁺ cells compared with the total number of identified basophils. In each assay, a minimum of 300 events

Fig. 2. Basophil activation for different dilutions of tested vaccines, alpha-gal, and positive/negative controls in patient 2. MMR, measles, mumps, and rubella; V, varicella; VZ, V-zoster.

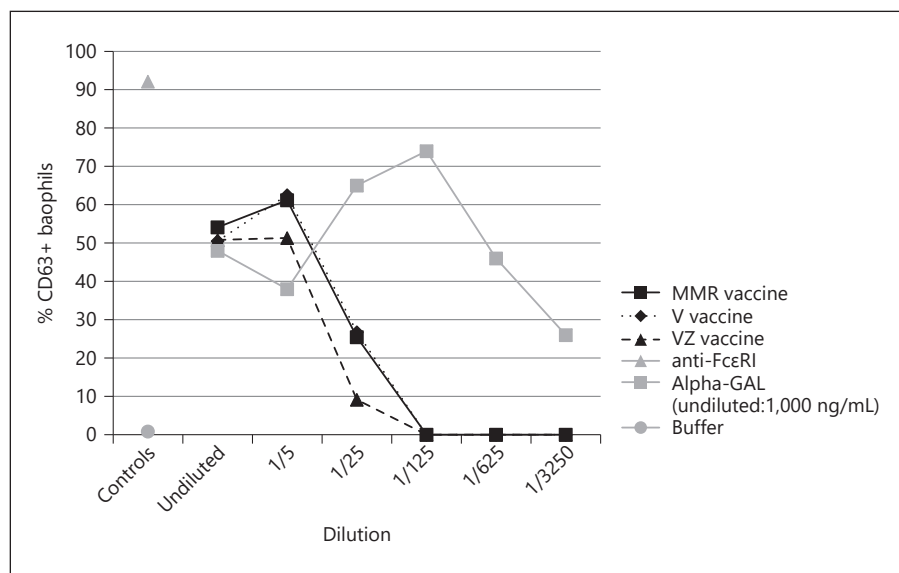
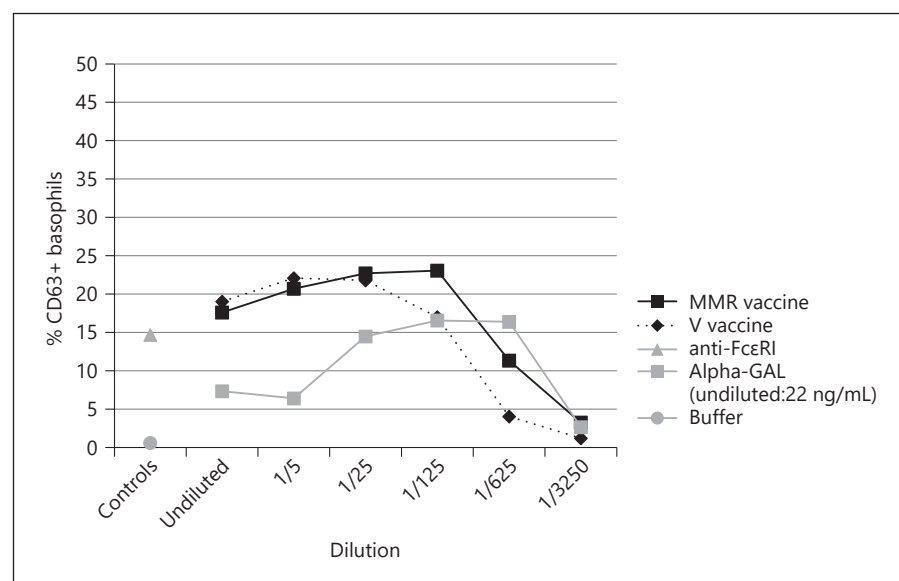


Fig. 3. Basophil activation for different dilutions of tested vaccines, alpha-gal, and positive/negative controls in patient 3. MMR, measles, mumps, and rubella; V, varicella; VZ, V-zoster.



(i.e., basophils) was recorded. A cut-off of 15% CD63⁺ cells was used as recommended for food allergens by the supplier and according to a previous study [7].

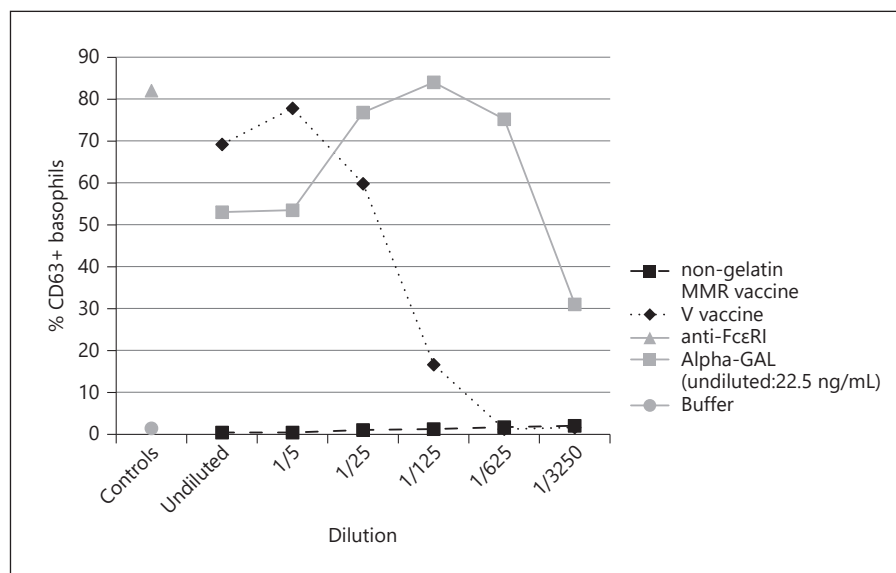
Results

Patient 1 showed positive basophil activation for all tested vaccines. The highest activation with a CD63 expression of 92.1% was found for VZ vaccine undiluted. For V vaccine at a dilution of 1:25, the CD63 expression was 87.3%, and for MMR vaccine at a dilution of 1:125,

the CD63 expression was 88.7%. Positive control 1 (anti-FcεRI) showed a CD63 expression of 76.0% and positive control 2 (fMLP) a CD63 expression of 58.0%. The highest CD63 expression with alpha-gal was 53.0% at a concentration of 1.6 ng/mL (Fig. 1). CD63 expressions with alpha-gal were 19.6, 13.9, 16.3, 49.3, and 44.0% at concentrations of 1,000, 200, 40, 8, and 0.32 ng/mL, respectively.

Patient 2 also showed positive basophil activation for all tested vaccines. The highest activation for VZ vaccine was found at a dilution of 1:5 with a CD63 expression of 51.3%, for V vaccine at a dilution of 1:5 with a CD63 expression of 62.5%, and for MMR vaccine at a dilution of

Fig. 4. Basophil activation for different dilutions of tested vaccines, alpha-gal, and positive/negative controls in patient 4. MMR, measles, mumps, and rubella; V, varicella; VZ, V-zoster.



1:5 with a CD63 expression of 61.2%. Positive control 1 (anti-FcεRI) showed a CD63 expression of 92.1%, and positive control 2 (fMLP), a CD63 expression of 5.0%. The highest CD63 expression with alpha-gal was 74.0% at a concentration of 8 ng/mL (Fig. 2). CD63 expressions with alpha-gal were 48.6, 38.9, 65.3, 46.3, and 26.0% at concentrations of 1,000, 200, 40, 1.6, and 0.32 ng/mL, respectively.

Due to lack of availability, only MMR vaccine and V vaccine were tested in patient 3, both vaccines showing positive basophil activation. The highest activation for MMR vaccine was found at a dilution of 1:125 with a CD63 expression of 23.1% and for V vaccine at a dilution of 1:5 with a CD63 expression of 22.1%. Positive control 1 (anti-FcεRI) showed a CD63 expression of 14.7% and positive control 2 (fMLP), a CD63 expression of 35.6%. The highest CD63 expression with alpha-gal was 16.6% at a concentration of 0.18 ng/mL (Fig. 3). CD63 expressions with alpha-gal were 7.4, 6.4, 14.5, 16.4, and 2.7% at concentrations of 22.5, 4.5, 0.9, 0.036, and 0.0072 ng/mL, respectively.

Patient 4 was tested with the same V vaccine as the other subjects but with another MMR vaccine not containing gelatin. The highest basophil activation was found for V vaccine at a dilution of 1:5 with a CD63 expression of 77.8%, and for the non-gelatin MMR vaccine, no activation was observed. Positive control 1 (anti-FcεRI) showed a CD63 expression of 83.4% and positive control 2 (fMLP), a CD63 expression of 47.7%. The highest CD63 with alpha-gal was 84.0% at a concentration of 0.18 ng/

mL (Fig. 4). CD63 expressions with alpha-gal were 53.0, 53.5, 76.8, 75.2, and 31.0% at concentrations of 22.5, 4.5, 0.9, 0.036, and 0.0072 ng/mL, respectively. The 2 healthy individuals did not show any basophil activation for any of the tested vaccines but showed positive results for the positive controls (84.8% CD63 expression and 93.7% for anti-FcεRI, respectively).

Discussion

The fact that patients with AGS might develop severe anaphylaxis after receiving VZ, zoster, or MMR has been described before [5, 6]. It has been postulated that the alpha-gal epitope in gelatin of bovine or porcine origin or in other sources of mammalian origin, which is acquired during the production process of such medical products, might be the trigger for anaphylaxis in those patients [3, 5, 6].

In this study, we could observe strong basophil activation when BAT was performed with gelatin-containing vaccines in patients with AGS. Maximum basophil activation by the vaccines exceeded the maximum alpha-gal and the FcεRI-induced basophil activation in 2 out of the 4 patients.

To our knowledge, this is the first report of BATs performed with such vaccines in these patients. Our findings are of particular interest as subcutaneous or intramuscular application of alpha-gal-containing vaccines leads to direct exposure to alpha-gal epitopes

without any delay. For this reason, severe immediate type-I reactions might be caused in AGS patients. Furthermore, the usefulness of BAT for identifying drugs of potential risk in patients with AGS could be proven. Also, BAT turned out to be an alternative screening method in cases where skin testing with a series of vaccines is unethical for patients not having reactions to these drugs in their history.

In contrast to the reports of Stone et al., there are cases in which patients with AGS tolerated vaccination with gelatin-containing vaccines without suffering from anaphylaxis. This might be due to the fact that higher doses of alpha-gal usually are required to elicit anaphylactic reactions [12]. It is known that cofactors (e.g., physical exercise, alcohol, and nonsteroidal analgesics) seem to play a role in anaphylactic reactions to alpha-gal when the allergen is ingested orally [4]. However, this may not play such a role when alpha-gal is administered parenterally as no gastrointestinal absorption occurs.

Beginning with reports on anaphylactic reactions toward the EGFR antibody cetuximab [2], other drugs have lately been identified in which alpha-gal is a possible trigger for anaphylaxis. Among these are antivenoms [9], porcine-derived drugs such as Creon and Enzynorm f [10], or vaginal capsules [13]. As SPT and IDT are more invasive, less sensitive, less standardized [4], and even carry a risk for systemic reactions and the possibility of sensitization when testing for alpha-gal and alpha-gal-containing drugs, BAT might be a good noninvasive alternative as it has shown its usefulness in the abovementioned drugs [8–10]. Furthermore, a recent study of Mehlich et al. could prove the utility of BAT in patients with AGS in distinguishing between patients with AGS and subjects with asymptomatic alpha-gal sensitization [7].

In the present study, we are limited with regard to a statement on clinical relevance in our observed patients, as none of them had shown anaphylactic reactions upon receipt of vaccination and none of them had been vaccinated after being diagnosed with AGS or after first symptoms hinting toward AGS had occurred. Interestingly, patient 4 did show positive basophil activation for a gelatin-containing vaccine but not for the non-gelatin MMR vaccine, providing an alternative vaccine for patients, if necessary.

To date, information on the exact source and concentration of gelatin in vaccines – as mentioned in the report from Pinson et al. [12] – are only available upon personal request and communication with the companies. Furthermore, source and concentration of gelatin – and

therefore quantities of alpha-gal – might vary between countries as different drugs from different companies are available on the market [14]. However, these information could be very useful to identify potentially dangerous vaccines for patients with AGS and therefore be life-saving. Taking into account that vaccines such as the VZ vaccine are especially used in older patients who are the population that is mainly affected by AGS [15] makes this point even more important.

Conclusions

All patients with confirmed AGS showed strongly positive basophil activation for all tested gelatin-containing vaccines, whereas healthy controls and the non-gelatin vaccine remained negative. Although this test does not prove clinical relevance in real-life situations, these vaccines should be administered with caution in patients with AGS. It has been postulated that alpha-gal epitopes in gelatin or other nonprimate mammal-derived products are the triggers. Information on the exact amount of gelatin in vaccines might be useful for better risk stratification. Also, BAT is a useful additional tool screening for potential high-risk and alternative drugs.

Acknowledgements

The authors thank Franziska Martin for her excellent technical support and also everyone who participated in this study.

Statement of Ethics

The study protocol was approved by the Institutional Review Board (Ethikkommission der Fakultät für Medizin der Technischen Universität München). Informed consent was obtained from every patient participating in this study.

Conflict of Interest Statement

BE reports nonfinancial support from Bühlmann Laboratories, outside the submitted work. The rest of the authors declare that they have no relevant conflicts of interest.

Funding Sources

There was no funding for this study.

Author Contributions

P.S. and B.E. had full access to all of the data in this study and are the guarantors of this study. They take responsibility for the content of the manuscript, including the data and analysis. P.S., T.B., and B.E. designed the study and wrote the manuscript. K.B. and U.D. contributed to the allergological workup of the patients, data interpretation, and revision of the manuscript.

Notation of Prior Abstract Publication/Presentation

Data of this study have been presented as an abstract at the Annual Congress of the EAACI, June 4, 2019, in Lisbon, Portugal.

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