

Detection of Salivary Tryptase Levels in Children following Oral Food Challenges

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Keywords

Allergy and immunology · Food hypersensitivity · Diagnosis · Saliva · Tryptase

Abstract

Background: Oral food challenge (OFC) is commonly used to diagnose food allergy. This test is time and resource intensive, and conclusions are not always unequivocal as this relies on the interpretation of symptoms. Therefore, an objective marker would improve the accuracy of the diagnostic workup of food allergy. **Objectives:** The aim of this study was to investigate whether tryptase can be detected in saliva of children following OFC. **Method:** Children from 3 to 18 years of age were eligible for inclusion if an OFC for peanut or tree nut had been recommended. Saliva samples were collected prior to the first dose and 5, 10, and 15 min following the last administered dose during OFC. Assay precision, spike-and-recovery, and assessment of lower limit of detection of the tryptase immunoassay were examined before analysis of tryptase in saliva was performed. **Results:** A total of 30 chil-

dren were included (median age 8 years, 63.3% male, 53.3% positive OFC outcome). Tryptase was detected in saliva samples. The mean of the change in baseline tryptase value to each saliva collecting time point was significantly different in patients with a positive OFC outcome compared to a negative outcome ($p < 0.01$). **Conclusions:** This study showed that tryptase can be detected in saliva of children following OFC. Increased levels of tryptase compared to baseline were found if the OFC outcome was positive, suggesting that measuring tryptase in saliva may be useful in the diagnosis of food allergy. Further research is needed to evaluate the potential association between tryptase levels and symptoms.

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Introduction

Food allergy has a substantial impact on the daily life of children and their families [1]. Therefore, it is important to accurately establish or exclude the diagnosis. Although sensitization to a specific food can be easily determined, it cannot adequately predict food allergy nor its severity [2, 3]. Today, the oral food challenge (OFC) is recommended to diagnose food allergies [4]. The OFC outcome is based on the interpretation of symptoms, which may be subject to variability [5]. Therefore, it would be valuable to have an objective parameter such as the release of specific allergic mediators [6].

Biomarkers may assist to diagnose food allergy [7–10]. Tryptase can be detected in saliva of adults, although reported results were conflicting [7, 8]. To our knowledge, no data have been published on salivary tryptase levels in food allergic children. To explore the utility of salivary tryptase as a non-invasive objective tool to support interpretation of OFCs in children, we first aimed to investigate whether tryptase can be detected accurately in saliva of children following an OFC.

Materials and Methods

This prospective study took place between July 2018 and October 2018 at the paediatric allergy centre of a large teaching hospital. All children from 3 to 18 years of age were eligible for inclusion if referred for suspected peanut or tree nut allergy for which an OFC had been recommended by the attending physician. The Medical Ethics Committee (MEC) of Martini Hospital, Groningen, The Netherlands, approved the study protocol (MEC 2018-066). Written informed consent was obtained from parents and children aged 12 years and older.

Open or double-blind placebo controlled food challenges (DBPCFCs) were performed according to the European Academy of Allergy and Clinical Immunology (EAACI) guidelines [4]. In short, all food challenge days consist of a maximum of 7 steps with increasing dose (starting dose 3 mg, total amount 4443 mg) and 30-min waiting time between each dose. All OFCs were conducted by trained paediatric nurses. The occurrence of symptoms was registered based on the classification as mentioned in the EAACI guidelines [4]. Sensitization to the suspected food allergen was defined as serum specific IgE level ≥ 0.35 kU/L (Phadia 250, Uppsala, Sweden).

Saliva samples were planned to be collected prior to the first dose (baseline or T0) and at 5, 10, and 15 min (T1, T2, and T3, respectively) following the last administered dose, based on previous research [7, 8]. Saliva was collected by chewing on a synthetic swab (Salivette, Sartedt, Germany) for approximately 1 min. In case of DBPCFCs, saliva samples collected on the verum day were analysed. If allergic symptoms occurred and/or medication had to be administered, saliva was collected when deemed feasible. After collection, the saliva samples were labelled, stored at 4°C, and sent to

the laboratory within 24 h. Hereafter, swabs were centrifuged according to the manufacturer's instructions, and saliva was stored at –20°C before analysis with ImmunoCAP Tryptase immunoassay on the UniCAP1000 (Phadia, Uppsala, Sweden). Details on the analysis of salivary tryptase can be found in online supplementary part A (see www.karger.com/doi/10.1159/000519374 for all online suppl. material).

All statistical analyses were performed using Analyse-it for Microsoft Excel Method Comparison Edition (v5.20; Analyse-it Software Ltd., Leeds, UK). Data were not normally distributed (Shapiro-Wilk's test, $p < 0.05$). Therefore, the Mann-Whitney U test was used to compare the salivary tryptase values in children with both positive and negative OFC outcome ($p < 0.05$ indicates statistical significance).

Results

In total, 30 patients were included with a median age of 8 years (IQR: 5.5–10.3) and 63.3% were male. Twenty-three (82.1%) patients were sensitized. A total of 30 OFCs were performed, of which 26 (86.7%) were open food challenges and 4 DBPCFCs. Fourteen (46.7%) out of 30 OFCs had a negative outcome and 16 (53.3%) positive. Most symptoms that occurred during the OFCs were categorized as ear, nose, and throat (e.g., itching of mouth, angioedema, or rhinitis) as well as cutaneous symptoms (e.g., erythema or urticaria). Detailed clinical information can be found in online supplementary part B.

Salivary tryptase levels for all time points for both negative and positive OFC outcomes are shown in Figure 1a and b. Given the measurement uncertainty in absolute terms regarding the salivary tryptase levels, it was difficult to identify exact peak concentrations. Therefore, the mean of the change in baseline value to each saliva collecting time point was calculated (delta T1, T2, and T3). This was significantly higher in patients with a positive OFC outcome compared to those with a negative outcome ($z = -2.678$, $p < 0.01$; Fig. 1c).

Discussion

This study demonstrates that tryptase can be detected in saliva from children following OFC. The increase in salivary tryptase levels was significantly higher in OFCs with a positive outcome compared to those with a negative outcome.

While our study is the first to demonstrate that salivary tryptase levels can be detected after positive OFC in children, several limitations need to be considered. First, the kinetics of tryptase following OFC are largely unknown.

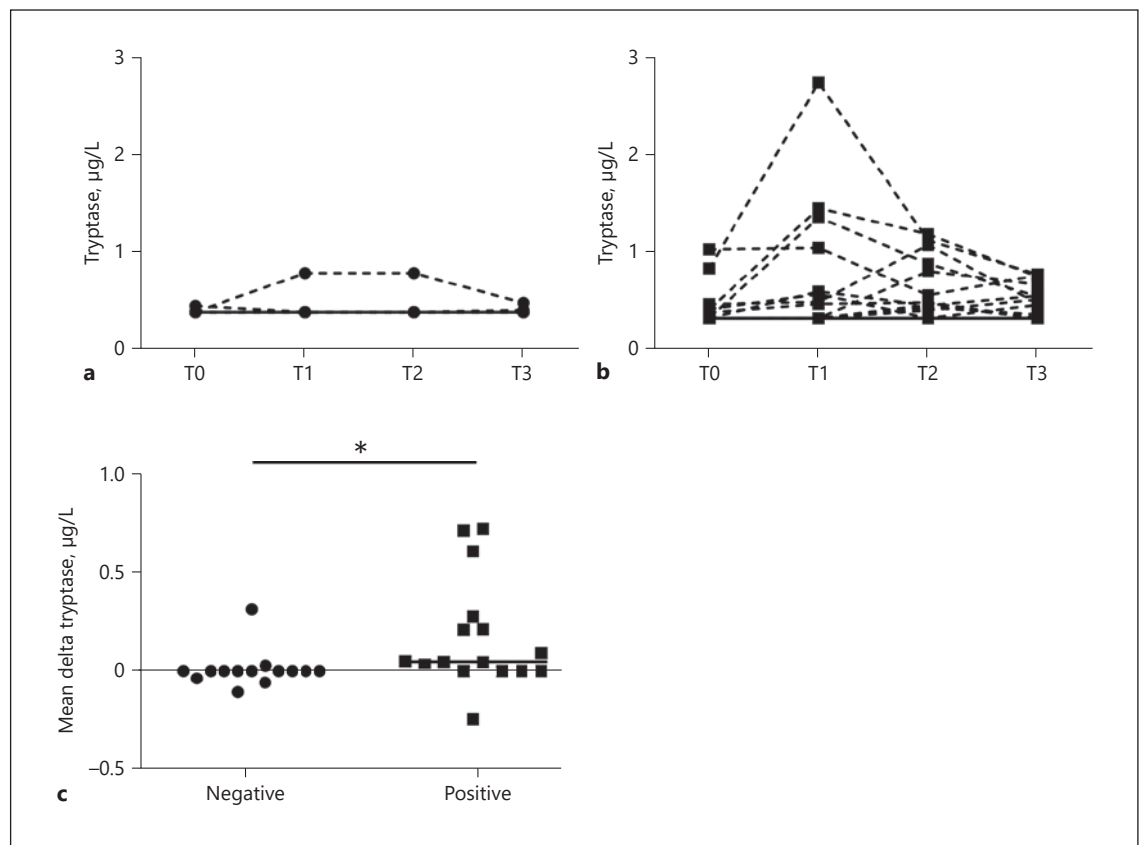


Fig. 1. Salivary tryptase levels. **a** Tryptase values from saliva samples collected prior to and following OFCs with negative outcome ($n = 14$). The continuous line represents the tryptase values of 12 out of 14 participants, all with values below $0.34 \mu\text{g/L}$. **b** Tryptase values from saliva samples collected prior to and following OFCs with positive outcome ($n = 16$). The continuous line represents the tryptase values of 4 out of 16 participants, all with values below $0.34 \mu\text{g/L}$. **c** Comparison of delta tryptase values calculated from saliva

samples collected prior to and following OFCs with negative and positive outcome, respectively ($n = 30$). Delta tryptase values were calculated as the mean of the change in baseline value to each saliva collecting time point. T0: saliva sample collected at baseline; T1–T3: time points of collected saliva samples when OFC was ended (range 0–30 min); OFC: oral food challenge. *Significant difference.

Tryptase is mainly secreted by activated mast cells [6]. The molecular weight of the active form of tryptase makes transport from serum to other bodily fluids impossible [8]. Therefore, the amount of tryptase in saliva is fully dependent on release by local mast cells. The fixed time points we chose to collect saliva were consistent with previously published reports, but in practice turned out to be variable (range 0–30 min) due to several practical reasons (e.g., difficulties while collecting saliva if symptoms occurred). This makes it challenging to conclude whether or not we detected peak tryptase levels, as these may have either been on the rising or declining part of the kinetic curve. To gain more insight into the kinetics of tryptase, multiple saliva samples prior to, during, and following OFC should be collected.

Second, the aforementioned limitations hampered further analysis regarding the possible association between symptoms and salivary tryptase. However, the aim of this study was to evaluate whether tryptase could be detected in saliva of children following OFC. Further research should evaluate the association between salivary tryptase levels and symptoms (both severity and type) and use a controlled study design using DBPCFCs. This is of special interest because it may be helpful to draw more firm conclusions about the OFC outcome since the broad spectrum of possible symptoms during OFC can be challenging to interpret. It might be interesting to determine whether salivary tryptase levels can differentiate between objective and subjective symptoms and/or local and systemic allergic reactions. Furthermore, additional

analyses in a larger population of healthy subjects may be of interest to better characterize sensitivity and specificity of salivary tryptase.

Third, a sandwich immunoassay intended for blood-derived matrices in the range of 1–200 µg/L was used to measure salivary tryptase levels, as previously described in 2 studies [7, 8]. Within our study, a significant number of samples were below the detection limit, which is similar to the result previously reported by Vila et al. [7]. Therefore, a partial method validation was performed to validate the measurement in saliva in the lower range of the assay (for details see online suppl. part A). Expanding the calibration curve to lower (salivary) tryptase levels in future studies will strengthen the results.

In conclusion, the results of our study demonstrate that tryptase can be detected in saliva samples of children following OFC. Further research is needed to define optimal sampling times and evaluate the potential association between tryptase levels and symptoms.

Statement of Ethics

The study was conducted in accordance with the World Medical Association Declaration of Helsinki. The study protocol was reviewed and approved by the Medical Ethics Committee (MEC) of Martini Hospital, Groningen, The Netherlands (Approval No. MEC 2018-066). Written informed consent was obtained from parents and children aged 12 years and older.

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Conflict of Interest Statement

The authors report no proprietary or commercial interest in any product mentioned, concept discussed, or personal relationships with other people or organizations that could influence their work and conclusions in this article.

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Author Contributions

W.W.W., V.M.B., E.M.L., L.L., J.H.G., C.E.M.H., G.N.M., and A.W.A.K. contributed to conceptualization; W.W.W. and E.M.L. contributed to data acquisition; V.M.B. contributed to formal analysis; W.W.W. and V.M.B. contributed to writing – original draft preparation; E.M.L., L.L., J.H.G., A.B.S., G.H.K., and A.W.A.K. contributed to writing – review and editing; A.W.A.K. and J.H.G. contributed to supervision.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Part A. Analysis of saliva samples

Tryptase in saliva was analysed using a sandwich immunoassay on the UniCAP1000 platform (Phadia, Uppsala, Sweden) in one single run. This commercial assay is intended for blood-derived matrices but was previously used for saliva.[1-2] Due to the design of our study, the tryptase concentrations are hypothesized to be in the lower range of the assay. Therefore, a partial method validation was performed focussing on linearity of dilution, spike-and-recovery, repeatability, lower limit of detection (LoD) and carry-over.[3-6]

In addition to pooled saliva samples from patients who underwent an oral food challenge (OFC), the following samples were used: Quality control material (QC, Phadia, Uppsala, Sweden), pooled serum of patients with increased tryptase-levels (serum high) and pooled saliva samples from subjects whom did not undergo an OFC (control). The additional samples were obtained from the routine workflow of the department of Clinical chemistry, Martini Hospital, Groningen, the Netherlands.

First, linearity of dilution and spike-and-recovery was assessed by spiking control saliva samples with the commercial available antigen in the form of QC material and pooled saliva from patients with a high concentration (patient pool high). The dilution was diluted over seven increments (factor of 1; 0.8; 0.6; 0.4; 0.2; 0.1 and 0). These concentrations were measured in triplo. Second, repeatability was evaluated using 10 replicates of four different samples: 1) QC-material diluted in saliva samples from control subjects in a high concentration (QC-high); 2) QC-material diluted in saliva samples from control subjects in a low concentration (QC-low); 3) Pooled patient saliva samples in a low concentration (patient pool low); and 4) blank water sample (blank). Finally, the carry-over was determined with

the following sequence: B1-B2-B3-A1-A2-B4-A3-A4-B5-B6-B7-B8-A5-A6-B9-A7-A8-B10 (A: serum high, B: control).

Statistical analysis

Linearity of dilution was evaluated by fitting linear and polynomial (second and third order) curves between measured values and expected values (mean value of factor 1 multiplied by increments) of the QC material and the patient pool with a high concentration (patient pool high). The percentual difference between the mean of these expected and observed values was seen as recovery. Assay precision was assessed with coefficients of variation (CV%) in the repeatability study. The LoD was calculated as described in Armbuster and Pry (2008).[3] The percentage of carry-over was calculated with the following formula:
$$\frac{(\text{Mean}_{\text{OD control samples after control}} - \text{Mean}_{\text{OD control samples after pooled serum sample}})}{\text{Mean}_{\text{OD control samples after control}}} * 100\%$$
. This value is a percentual representation of the possible increase of the control sample when measured directly after a sample with a high concentration.

Assay characteristics

For linearity of dilution, the measured optical densities signals (OD) over the seven increments were plotted against the calculated expected values (figure A1). No 2nd/3rd order polynomial function fitted statistically better than a linear fit at 5% significance level, for both the QC material as the patient pool high. With a recovery of 99% (standard deviation (SD)=1,7%) of the QC material in control saliva, the assay is not affected by this matrix.

The commercial available calibration curve uses a linear function which includes the highest concentrations of the previously described curves. Therefore, an approach to estimate the corresponding concentrations to the measured OD in saliva samples is to fit a linear function through the in duplo measured OD signals of (commercial) calibrators with concentrations ranging from 1.00 to 12.50 µg/L and extrapolating the optical density along this line to the lower concentration range.

The repeatability of the OD and concentrations that were calculated by extrapolation, was assessed for QC-high, QC-low, patient pool low and blank sample (table A1). All CV% were below 20%, a term often used in the determination of a lower limit of quantification. The lower limit of the assay is therefore calculated to a LoD ($\text{LoD} = \text{mean}_{\text{blank}} + 1.645(\text{SD}_{\text{blank}}) + 1.645(\text{SD}_{\text{patient pool low}})$). [1] The calculated LoD is 0.34 µg/L. For the statistical analysis, concentrations below 0.34 µg/L were set to 0.33 µg/L to minimise overestimation of the difference between baseline and T1, T2 and T3.

The saliva samples of the patients who underwent an OFC, were in the lower range of the assay. Therefore, the goal of the carry-over study was to determine to what extent a sample with a high concentration could affect the result of a sample with a low concentration. For this study, a pooled control saliva sample (mean OD=94, SD=4) was alternated with a serum sample (serum high: mean OD=7678, SD=243). The carry-over was 2,9%. This indicates that there is no significant carry-over, as this percentage is within the SD of the low concentration sample.

Table A1. Repeatability.

QC: quality control material; CV%: coefficients of variation; OD: optical density.
 QC-high: QC-material diluted in saliva samples from control subjects in a high concentration.
 QC-low: QC-material diluted in saliva samples from control subjects in a low concentration.
 Patient pool low: pooled patient saliva samples in a low concentration.
 Blank: blank water sample.

	Unit	QC High		QC Low		Patient pool low		Blank	
		Mean (Range)	CV%	Mean (Range)	CV%	Mean (Range)	CV%	Mean (Range)	CV%
Repeatability (n=10)	OD	296 (279-319)	3,8	186 (177-198)	3,0	167 (153-180)	4,8	95 (85-108)	7,1
	µg/L	1,09 (1,01-1,21)	5,1	0,67 (0,63-0,73)	4,1	0,46 (0,39-0,52)	8,6	0,22 (0,17-0,28)	15,6

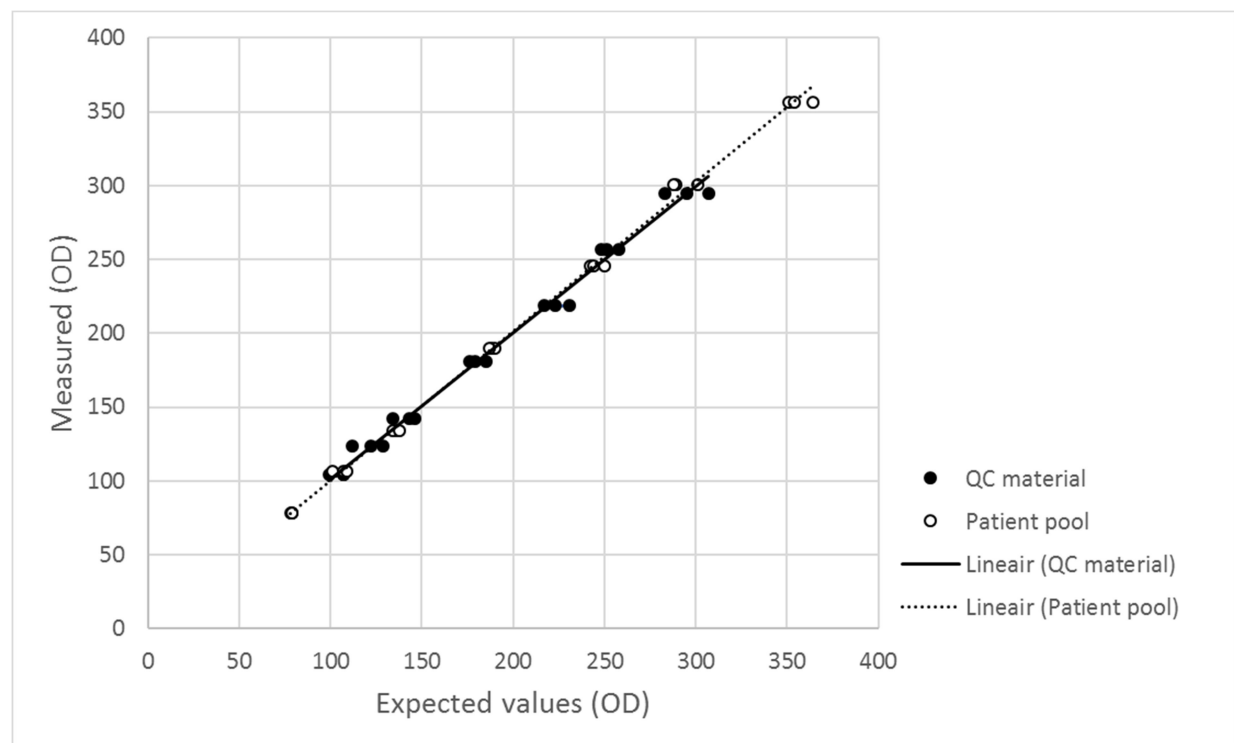


Figure A1. Linearity of dilution.

QC: quality control material; OD: optical density.
 Both the QC material (solid line) as the patient saliva pool with a high trypsin concentration (open, dotted line) were diluted with control saliva over seven increments (factor of 1; 0.8; 0.6; 0.4; 0.2; 0.1 and 0) in which factor 1 had the highest OD and 0 the lowest OD. Every increment was measured in triplo. The expected values were calculated by multiplying the factor with the mean of factor 1.
 Correlation coefficients for QC material: $R^2=0,9903$; for Patient pool: $R^2=0,9977$.

Table B1. Patient characteristics. Data stratified for OFC outcome.

OFC: open food challenge; sIgE: serum specific IgE.

Results presented as mean $\pm$ SD (normally distributed) or as median and 25th-75th percentiles (not normally distributed). Other parameters are presented as frequencies and percentages.

Differences between the groups were tested with Chi-square test (a) and Mann-Whitney U test (b).

An $\alpha < 0.05$ was defined as statistically significant and is presented with a bold p-value.

[†] sIgE was not determined in 2 out of 30 cases, yet medical history was suggestive for an allergic reaction (related OFCs were positive indeed)

[‡] an OFC was performed for these patients despite the lack of sensitisation, because medical history was suggestive for an allergic reaction

	Total	Negative	Positive	P-value
N	30	14 (46.7%)	16 (53.3%)	
Age (years)	8.0 (5.5-10.3)	7.5 (4.0-12.0)	8.5 (6.0-9.8)	0.736 ^b
Gender				
Female	11 (36.7%)	4 (28.6%)	7 (43.8%)	0.389 ^a
Male	19 (63.3%)	10 (71.4%)	9 (56.2%)	
Atopic comorbidities				
Asthma	13 (48.1%)	7 (53.8%)	6 (42.9%)	0.568 ^a
Eczema	25 (92.6%)	12 (92.3%)	13 (92.9%)	0.957 ^a
Allergic rhinitis	9 (33.3%)	5 (38.5%)	4 (28.6%)	0.586 ^a
Atopic disease (family)				
None	3 (10.7%)	2 (15.4%)	1 (6.7%)	0.457 ^a
≥ 1 family member	25 (89.3%)	11 (84.6%)	14 (93.3%)	
sIgE for specific food[†]				
<0.35 kU/L [‡]	5 (17.9%)	2 (14.3%)	3 (21.4%)	0.622 ^a
≥ 0.35 kU/L	23 (82.1%)	12 (85.7%)	11 (78.6%)	

Table B2. Details of performed food challenges.

DBPCFC: double-blind placebo controlled food challenge; OFC: oral food challenge

	DBPCFC		Open		Total		Total
Food	<i>negative</i>	<i>positive</i>	<i>negative</i>	<i>positive</i>	<i>negative</i>	<i>positive</i>	<i>number of OFCs</i>
peanut	0	0	2	2	2	2	4
cashew nut	0	0	5	3	5	3	8
hazelnut	3	0	1	4	4	4	8
macadamia	0	0	1	1	1	1	2
pistachio	0	1	0	2	0	3	3
walnut	0	0	2	3	2	3	5
Total	3	1	11	15	14	16	30

Table B3. Details of occurred symptoms during performed food challenges. **(A)** Symptoms per category (mild, moderate, severe). **(B)** Symptoms per tract. Multiple symptoms may occur during an OFC in an individual patient.

OFC: oral food challenge; DBPCFC: double-blind placebo controlled food challenge

[†] OFC outcome was considered negative if mild symptoms resolved quickly and did not reoccur during subsequent doses

B3a

	Open		DBPCFC		Total
Symptom (per category)	<i>negative[†]</i>	<i>positive</i>	<i>negative</i>	<i>positive</i>	
mild	8	63	0	4	75
moderate	0	22	0	1	23
severe	0	5	0	0	5

B3b

	Open		DBPCFC		Total
Symptom (per tract)	<i>negative[†]</i>	<i>positive</i>	<i>negative</i>	<i>positive</i>	
ear, nose and throat	2	31	0	2	35
respiratory	0	13	0	0	13
gastrointestinal	0	14	0	0	14
cutaneous	6	21	0	1	28
general	0	11	0	2	13

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