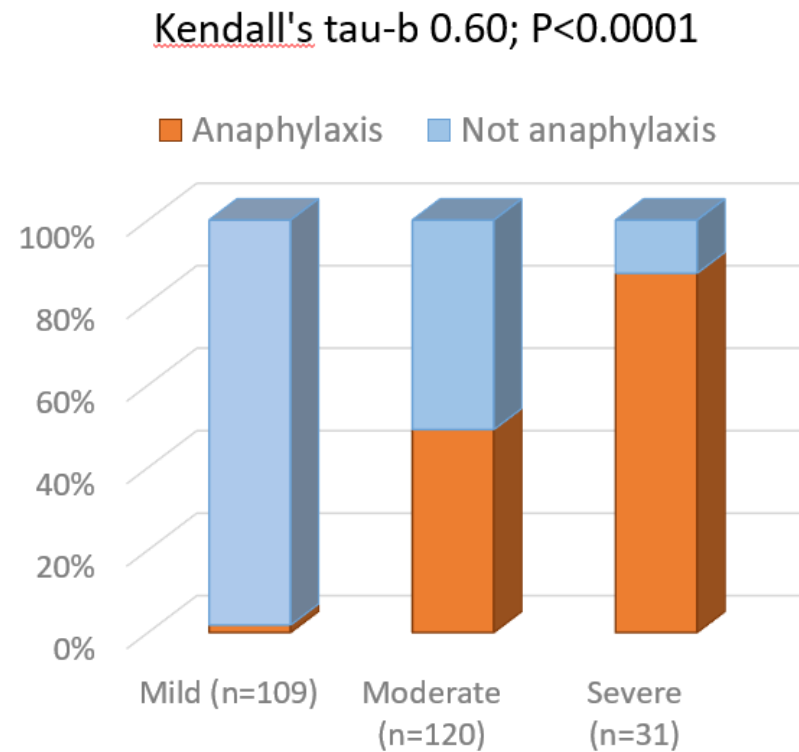


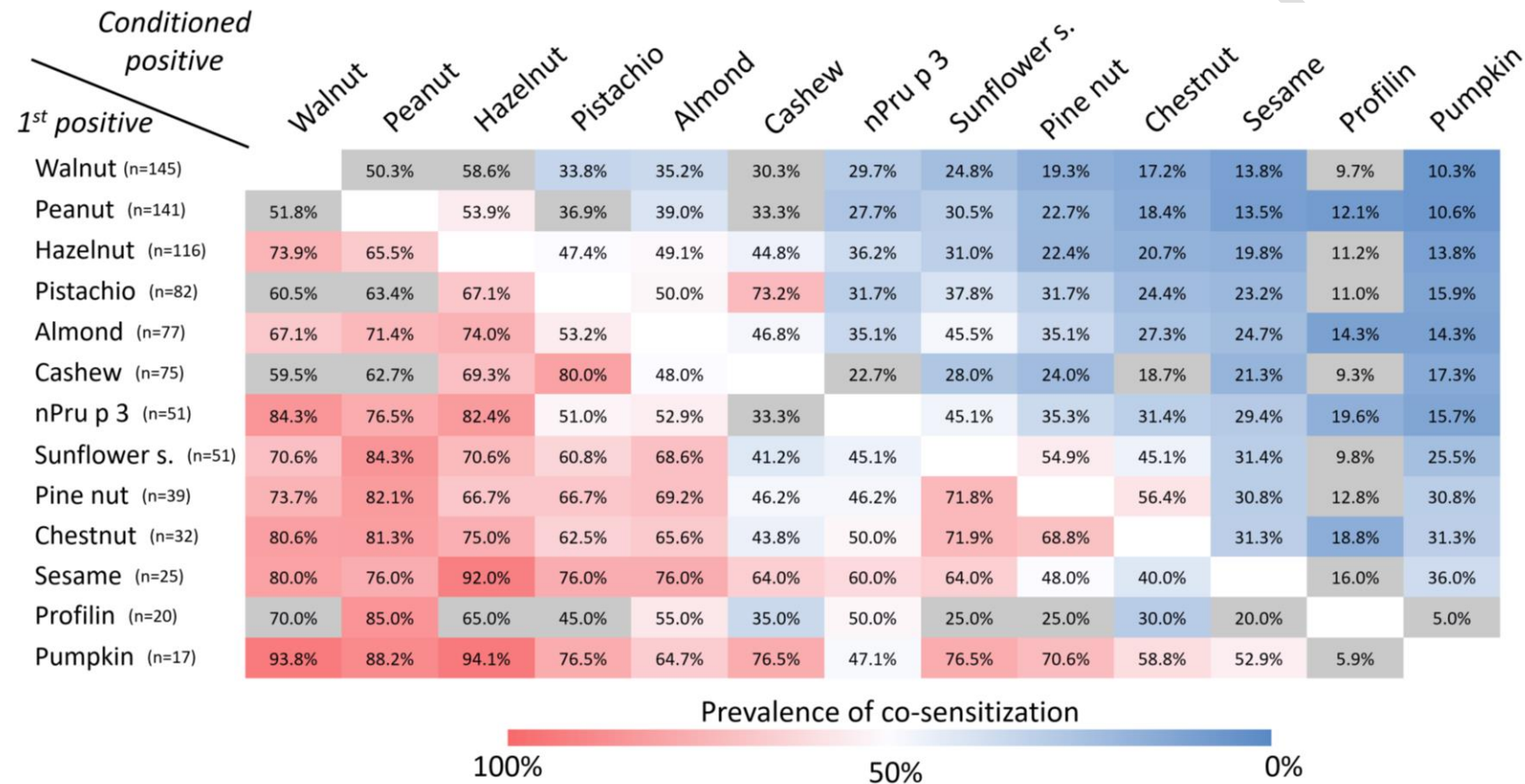
SUPPLEMENTARY MATERIAL

Supplementary Figure 1. Anaphylaxis in index reaction compared to doctors criteria for severity



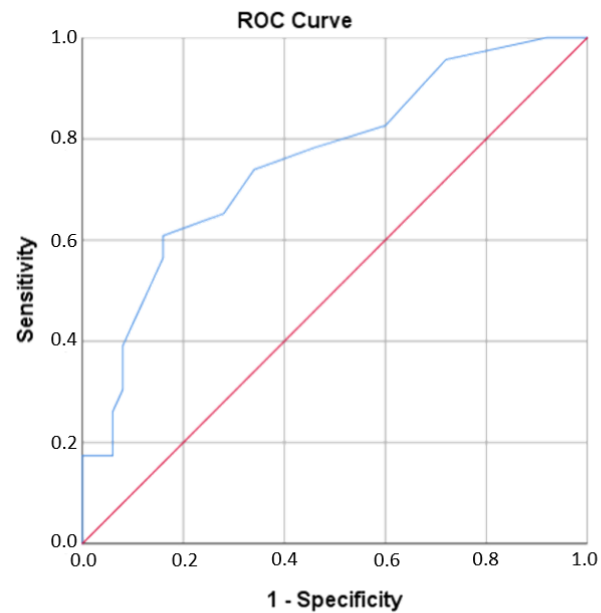
Legend Suppl. Figure 1: For the 260 index reactions occurring in allergic subjects, a high level of correlation was found for IR severity and anaphylaxis (n=91), as all but 1 anaphylactic reaction were considered moderate or severe by the attending physician. It is also worth noting that 17.6% of reactions considered severe by the doctor failed to meet the criteria for anaphylaxis.

Supplementary Figure 2. Heatmap of SPT nut cosensitization in nut/seed-allergic patients



Legend supplementary figure 2 displays a heat map for SPT co-sensitization for all the nuts and seeds tested. The percentage in each cell represents the number of subjects sensitized to each of the nuts shown on each line and positive for the allergens displayed in each column. As an example, among 145 subjects showing positive SPT for walnut, 50.3% had a positive peanut SPT and 58.6% were positive for hazelnut; gray cells are for non significant ($p > 0.05$) associations. Cells colored on a red-to-blue scale represent more to less significant cross-sensitization respectively.

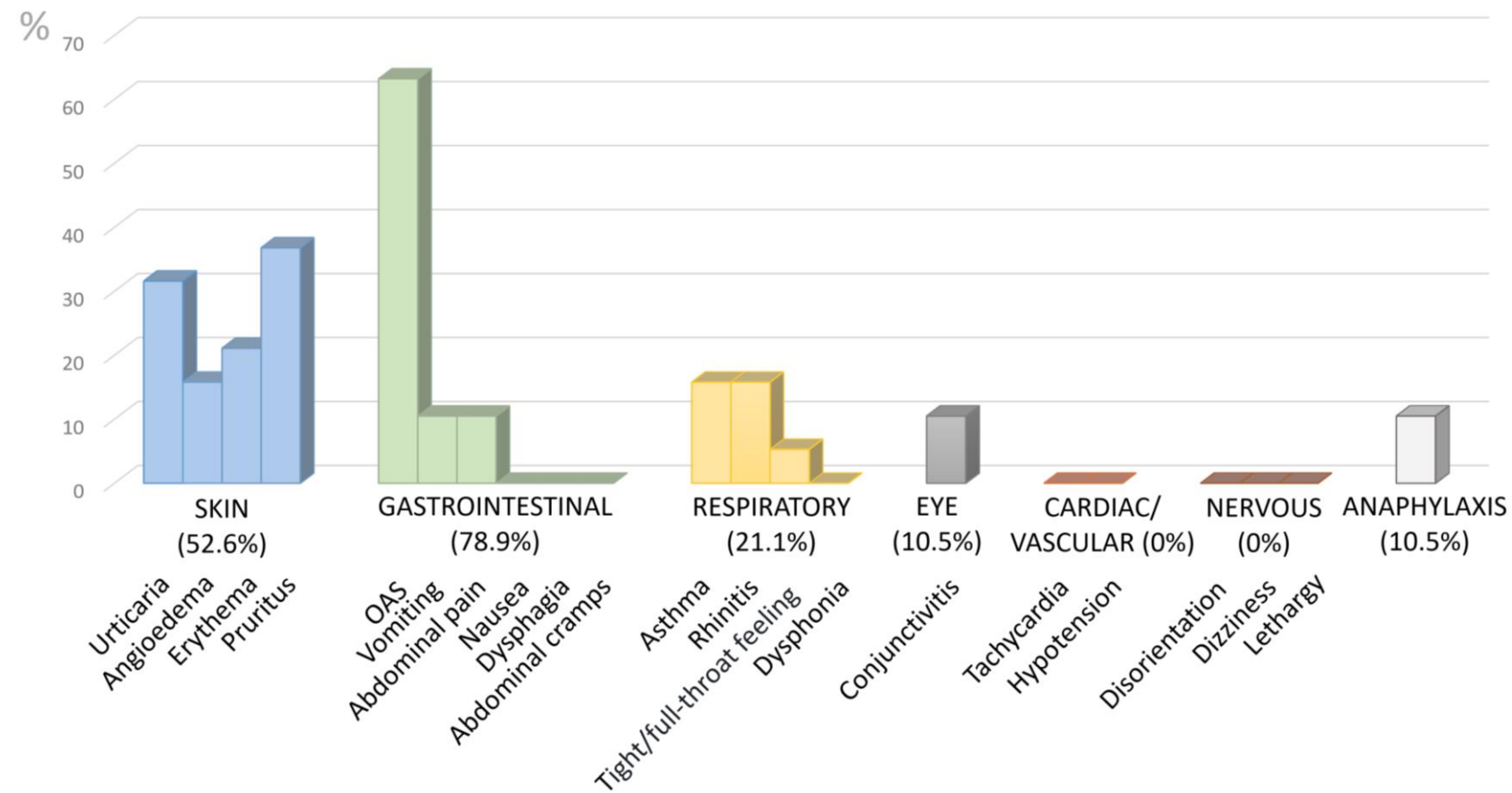
Supplementary figure 3 supplementary: Receiver operating characteristic (ROC) curve for the utility of SPT size to peanut to predict anaphylaxis in a peanut-triggered reaction



SPT (mm)	Sensitivity	Specificity	PPV	PNV	+LR	P value
≥ 8.5	0.739	0.66	50	84.6	2.2	<0.0001

Legend Supplementary Figure 3: Area under the curve: 0.758 (95% CI 0.638-0.879, $p<0.0001$). Table displays optimal cut-off points of peanut SPT size to predict risk of anaphylaxis, SPT: skin prick test, PPV: positive predictive value, PNV: negative predictive value, +LR: positive likelihood ratio.

Supplementary Figure 4. Clinical features of positive oral food challenges (n=19)



Legend Supplementary Figure 4: Symptoms occurring during controlled food challenge sorted by organ and frequency

Supplementary table 1. **Demographic and baseline characteristics of the population (N=271)**

Personal history of allergy	Allergic subjects, n, (%)	Non-allergic subjects, n, (%)	Total, n/total subjects for the variable (%)
Rhinitis (any trigger)	118 (46.3%)	7 (63.6%)	125/266 (47%)
Mite rhinitis	70 (27.5%)	1 (9.1%)	71/266 (26.7%)
Pollen rhinitis	59 (23.1%)	5 (45.5%)	64/266 (24.1%)
Mold rhinitis	13 (5.1%)	1 (9.1%)	14/266 (5.3%)
Epithelia rhinitis	21 (8.2%)	0	21/266 (7.9%)
Asthma (any trigger)	110 (43%)	3 (30%)	113/266 (42.5%)
Mite asthma	59 (23%)	1 (10%)	60/266 (22.6%)
Pollen asthma	39 (15.2%)	3 (30%)	42/266 (15.8%)
Mold asthma	12 (4.7%)	1 (10%)	13/266 (4.9%)
Epithelia asthma	19 (7.4%)	1 (1%)	20/266 (7.5%)
Positive family history of allergy	195 (81.9%)	9 (90%)	204/248 (75.3%)
Total allergic events by nut (IR+Secondary nuts)			
	Events among allergic subjects, (302, %)	Events in nonallergic subjects (35, %)	Total number of events (337, %)
Walnut	106 (35.1%)	5 (14.3%)	111/337 (32.9%)
Peanut	81 (26.7%)	4 (11.5%)	85/337 (25.2%)
Cashew	29 (9.6%)	0	29/337 (8.6%)
Hazelnut	31 (10.3%)	5 (14.3%)	36/337 (10.7%)
Pistachio	19 (6.3%)	8 (22.8%)	27/337 (8.0%)
Almond	16 (5.3%)	8 (22.8%)	24/337 (7.1%)
Sunflower seed	6 (2%)	1 (2.9%)	7/337 (2.1%)
Pine nut	4 (1.3%)	2 (5.7%)	6/337 (1.8%)
Pumpkin seed	1 (0.3%)	0 (0%)	1/337 (0.3%)
Sesame	2 (0.7%)	2 (5.7%)	4/337 (1.2%)
Chestnut	1 (0.3%)	0	1/337 (0.3%)
Other	6 (2%)	0	6/337 (1.8%)

Legend Supplementary Table 1. There were no significant differences between allergic and non-allergic subjects. Of note: total number of allergic and non-allergic subjects is 260 and 11; however, for some variables there was missing information, resulting in a slight change in the total number of patients as indicated for each variable.

IR: Index reaction; y.o.: years old.

Supplementary table 2. Complementary information to Patients and index-reaction characteristics stratified for nuts with at least 10 cases (n=245 allergic subjects)

	Walnut (n=95)	Peanut (n=74)	Hazelnut (n=22)	Cashew (n=27)	Pistachio (n=14)	Almond (n=13)	Total (n=245)	p value
Personal history of allergy								
Atopic dermatitis (%)	48.4	52.8	40.9	55.6	42.9	30.8	48.5	0.648
Allergy to any plant food (%)	20.0	12.3	28.6	11.1	7.1	30.8	17.2	0.218
Mite respiratory allergy (R/A) (%)	44.1*	23.3	31.8	18.5	28.6	23.1	31.8	0.045*
Mold respiratory allergy (R/A) (%)	8.8	2.8	4.5	3.7	7.1	0.0	5.5	0.609
Pollen respiratory allergy (R/A) (%)	22.0	27.8	9.1	22.2	35.7	38.5	24.3	0.28
Epithelia respiratory allergy (R/A) (%)	11.0	5.6	13.6	7.4	28.6	7.7	10.1	0.193
Family history (FH) of allergy								
Positive FH of allergy (%)	82.6	83.3	95.2	76.9	92.3	58.3	82.6	0.117
FH of atopic dermatitis (%)	9.8*	25.8	15.0	26.9	41.7*	27.3	19.8	0.036*
FH of food allergy (%)	19.0	26.9	30.0	26.9	16.7	16.7	23.1	0.769
Severity of the index reaction according to allergist judgement and anaphylaxis								
Mild (%)	40.0	50.0	36.4	29.6	57.1	46.2	42.9	0.542
Moderate (%)	50.5	39.2	59.1	51.9	28.6	46.2	50.5	
Severe (%)	9.5	10.8	4.5	18.5	14.3	7.7	9.5	
Treatment of index reaction								
Visited emergency room (%)	54.3	52.1	68.2	69.2	38.5	46.2	55.3	0.335
Hospitalization (%)	1.1	4.1	4.5	0.0	7.7	0.0	2.5	0.503
No treatment at all (%)	33.0	35.7	22.7	19.2	23.1	41.7	31.2	0.518
Antihistamines (%)	54.9	51.4	63.6	69.2	69.2	53.8	57.0	0.575
Corticosteroids (%)	44.0	41.4	36.4	65.4	30.8	30.8	43.4	0.196
Adrenaline (%)	11.0	8.6	9.1	15.4	7.7	0.0	9.8	0.750
Beta-agonists (%)	8.8	8.6	9.1	7.7	15.4	0.0	8.5	0.868
Fluids (%)	1.1	2.9	0.0	0.0	7.7	0.0	1.7	0.414
Oxygen (%)	1.1	4.3	4.5	0.0	7.7	0.0	2.6	0.497

Sensitization patterns for each pollen source in SPT and/or any ISAC allergen								
Pollen-sensitized (any pollen) (%)	81.3	68.1*	85.7	62.5	85.7	100.0*	76.7	0.0325*
<i>Phleum</i> sensitization (%)	50.6	49.3	61.9	45.8	78.6	76.9	53.9	0.137
<i>Olea</i> sensitization (%)	49.4	36.1	52.4	30.4	38.5	69.2	44.0	0.124
Plane tree sensitization (%)	49.4	39.1*	64.7	43.5	53.8	90.9*	49.1	0.026*
<i>Cupressus</i> sensitization (%)	35.7	25.4	31.6	37.5	38.5	36.4	32.4	0.769
<i>Betula</i> sensitization (%)	18.1	13.1	31.3	18.2	0.0	42.9	17.6	0.143
Ragweed sensitization (%)	18.4	8.8	12.5	9.1	8.3	30.0	13.7	0.324
Parietaria sensitization (%)	13.2	6.3	14.3	0.0	9.1	28.6	9.7	0.175
Pollen polysensitization (%)	78.5	75.5	83.3	80.0	58.3	83.3	77.2	0.669

Legend Supplementary Table 4: detailed atopic background, index reaction characteristics, and pollen sensitization profile for the most frequent onset-triggering nuts in the population. When a significant difference ($p < 0.05$) between the value for a given nut with respect to the total was identified, it was marked with an asterisk (*).

A: asthma; R: rhinitis; Min: minutes; FH: family history; Rhin: rhinitis; SPT: skin prick test; y.o.: years old.

Supplementary table 3. Secondary nut evaluation for 66 events among 39 subjects

Nut for secondary evaluation	Allergic subjects (n=42, 63.6%)	Non-allergic subjects (n=24, 36.4%)	Total % (n=66, 100%)
Walnut	11 (26.1%)	2 (8.3%)	13 (19.7%)
Peanut	7 (16.7%)	2 (8.3%)	9 (13.6%)
Cashew	2 (4.8%)	0	2 (3%)
Hazelnut	9 (21.4%)	3 (12.5%)	12 (18.3%)
Pistachio	5 (11.9%)	7 (29.3%)	12 (18.3%)
Almond	3 (7.1%)	6 (25%)	9 (13.6%)
Sunflower seed	2 (4.8%)	0	2 (3%)
Pine nut	0	2 (8.3%)	2 (3%)
Pumpkin seed	0	0	0
Sesame	1 (2.4%)	2 (8.3%)	3 (4.5%)
Chestnut	1 (2.4%)	0	1 (1.5%)
Other	1 (2.4%)	0	1 (1.5%)

Legend Supplementary Table 3. This table represents diagnoses of other nut allergies in addition to the index-nut allergy. Such secondary allergy occurred in patients who reported additional reactions to other nuts after the index reaction or in the context of the clinical workup after identifying sensitizations of unknown clinical relevance.

Supplementary table 4. Description of relevant features in patients with exposure through cutaneous and respiratory route

Study ID	Gender	Age (years)	Nut	Route	Number of reactions	Distribution	Symptoms	Treatment
279	Female	6.92	Almond	Cutaneous	1	Systemic	Angioedema, rhinitis	Antihistamines, oral corticosteroids
178	Female	0.67	Cashew	Cutaneous	1	Systemic	Angioedema, erythema, Bronchospasm	Antihistamines, adrenaline, Beta 2 agonists
42	Female	4.92	Cashew	Cutaneous	2	Systemic	Oral pruritus, urticaria, angioedema, vomiting	Antihistamines, oral corticosteroids
128	Male	12.33	Cashew	Cutaneous	1	Systemic	Urticaria, erythema	Unknown
95	Female	5.17	Hazelnut	Cutaneous	4	Systemic	Nausea, vomiting, conjunctivitis	Antihistamines
60	Female	3.17	Peanut	Cutaneous	1	Systemic	Oral pruritus, vomiting, abdominal pain	Antihistamines, oral corticosteroids, adrenaline
70	Female	5.42	Peanut	Cutaneous	6	Systemic	Oral pruritus, Urticaria, Angioedema, erythema, pruritus, Bronchospasm, rhinitis, dysphonia,	Antihistamines, oral corticosteroids, adrenaline
77	Female	2.67	Peanut	Cutaneous	2	Systemic	Angioedema, erythema, pruritus	None
101	Female	6.58	Peanut	Cutaneous	2	Systemic	urticaria, Angioedema	Antihistamines
116	Female	5.17	Peanut	Cutaneous	1	Systemic	Oral pruritus, urticaria, nausea, vomiting, abdominal pain, rhinitis	None
317	Female	10.83	Peanut	Cutaneous	3	Systemic	Urticaria, erythema	None
277	Female	8.83	Walnut	Cutaneous	2	Systemic	Urticaria, angioedema	Antihistamines, oral corticosteroids
141	Male	1.17	Cashew	Cutaneous	1	Local	Oral pruritus,	Antihistamines, oral corticosteroids
14	Female	3.42	Peanut	Cutaneous	1	Local	Angioedema	Antihistamines, oral corticosteroids
204	Male	2.42	Peanut	Cutaneous	1	Local	Urticaria	None
230	Female	2.50	Peanut	Cutaneous	1	Local	Angioedema	Antihistamines, oral corticosteroids
260	Male	6.33	Peanut	Cutaneous	2	Local	Angioedema	oral corticosteroids
261	Male	6.33	Peanut	Cutaneous	2	Local	Angioedema	None
325	Male	9.25	Peanut	Cutaneous	1	Local	Oral pruritus, angioedema	Antihistamines
150	Male	3.92	Walnut	Cutaneous	1	Local	Angioedema, conjunctivitis	Adrenaline

227	Male	11.83	Walnut	Cutaneous	1	Local	Angioedema	None
268	Male	7.58	Walnut	Cutaneous	1	Local	Urticaria, erythema	None
271	Female	3.42	Walnut	Cutaneous	2	Local	Urticaria, erythema, pruritus, conjunctivitis	None
57	Male	4.25	Walnut	Cutaneous	3	Local	Erythema	None
155	Male	5.17	Walnut	Cutaneous	2	Local	No, erythema, pruritus	None
26	Male	3.92	Cashew	Inhalation	1	Systemic	Urticaria, Angioedema, pruritus, Bronchospasm, rhinitis	Antihistamines, Oral corticosteroids

*Systemic: involves more than single site in the patient, not collected if due to a massive direct contact or for extension of symptoms beyond direct contact area. In some patients, the cutaneous route was after lip exposure

METHODS FOR ONLINE SUPPLEMENTARY MATERIALS

Collection of patients' data

The database was available online, having been designed to minimize errors in data entry by using close-ended questions. All information was anonymized, making it impossible for analysts to identify the information for a given individual. The database was housed in a secure online hosting environment using Secure Sockets Layer (SSL) technology.

Data were gathered in a uniformed and structured manner following a preconceived questionnaire. For all subjects, an inclusion and exclusion criteria checklist had to be performed and detailed information on age, gender, and the patient's personal atopic background was gathered, including the following: presence of atopic dermatitis and other food allergies, presence of allergic rhinitis or asthma, age of onset, and triggers. Family atopic background was also collected. All patients were tested for a battery of pollen and nuts in prick tests. Patients in whom serum specific IgE to whole extract was performed at the participating center, the information could be included in the study. Detailed information on the IR included trigger nut, approximate date of index reaction, number of reactions, previous tolerance to the nut, route of exposure, chronology, and detailed symptoms according to the MedDRA dictionary (<https://www.meddra.org>). For those patients receiving treatment for the IR, drugs used and the setting were also specified. Severity of the reaction was established according to the personal impression of the investigator and anaphylaxis was a post-hoc variable created after independent evaluation by two of the researchers (MDI and PRR) of each reaction according to the criteria of Muraro 2014 (1); discrepancies were resolved upon discussion or with the help of a third researcher (CE). For those patients undergoing oral food challenge, a template with the same items used to report IR was also provided. Finally, all patients were clearly identified as allergic or non-allergic to a specific nut by the researcher.

SPT

All SPTs were performed using standardized techniques according to international guidelines (2). Skin prick test (SPT) mean diameter was recorded (mm), and a wheal that was 3 mm larger than the negative saline control was considered positive. For positive and negative controls, histamine phosphate at 10 mg/ml and saline solution were used.

Ethics

The study was approved by the Spanish Agency for Drug and Health Products as an observational study (AEMPS) and by the Ethics Committee of University Hospital Niño Jesús (Internal code: R-066/12) and was endorsed by the Ethics Committee of each of the participating centers. Written informed consent was signed by the parents or guardians and children over 12 years of age.

Statistical analysis

For the description of quantitative variables, the mean and SD were calculated. Descriptive statistics of the quantitative variables were mean, standard deviation, maximum, minimum, median, and standard deviation of the mean; and for qualitative variables, frequencies and percentages of the categories were used. Fisher's exact test or the Chi-square test were used to contrast the independence or influence between two qualitative variables. Pearson's correlation coefficient was used to analyze bivariate relationships between continuous variables. The predictive utility of SPTs size, nut-sIgE, and molecular sIgE to each nut was evaluated to predict

anaphylaxis using receiver operating characteristic (ROC) curve analysis. The correlation between severity and anaphylaxis was assessed by using Kendall correlation coefficient Tau- β .

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- 2.- Position Paper. Allergen standardization and skin tests. The European Academy of Allergy and Clinical Immunology. *Allergy* 1993;48(14 Supp):48-82