

Cor a 14, Hazelnut-Specific IgE, and SPT as a Reliable Tool in Hazelnut Allergy Diagnosis in Eastern Mediterranean Children



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What is already known about this topic? Data regarding the value of diagnostic tests in determining clinical reactivity to hazelnut allergy are very limited.

What does this article add to our knowledge? Component testing improves the diagnostic accuracy, and Cor a 14 is a more useful marker than hazelnut sIgE, skin prick test wheal diameter, Cor a 1, and Cor a 9 in determining clinical reactivity to hazelnut in children.

How does this study impact current management guidelines? These data highlight the importance of component testing in discriminating between reactive and nonreactive children to hazelnut, and established probability curves will reduce the need for oral food challenges.

BACKGROUND: Improving the diagnostic efficacy of laboratory tests might reduce the need for oral food challenges and facilitate our daily practice.

OBJECTIVE: We aimed to determine cutoff values and probability curves, as well as to investigate the role of component-resolved diagnosis in predicting clinical reactivity in children with hazelnut allergy and to evaluate the association with pollen sensitivity.

METHODS: A total of 56 children with hazelnut allergy who underwent double-blind placebo-controlled food challenge and 8 children who experienced anaphylaxis after accidental hazelnut intake were included. Serum IgE levels to hazelnut extract, Cor a 1, Cor a 8, Cor a 9, Cor a 14, and Bet v 1 were measured with the ImmunoCAP system. Skin prick tests (SPT) with hazelnut, other implicated foods, and aeroallergens were performed.

RESULTS: The optimal cutoff levels for hazelnut sIgE and SPT wheal diameter that predicted clinical reactivity with the highest sensitivity and specificity were 3.15 kU/L and 7.5 mm, respectively. Among the components, only Cor a 14

discriminated between reactive and nonreactive children. The area under curve (AUC) at the optimal cutoff point of 0.63 kU/L for Cor a 14 (0.936) was higher than the AUC of hazelnut sIgE (0.818) and SPT wheal diameter (0.803). For the first time, a 95% probability for clinical reactivity was estimated for SPT wheal diameter, IgE to hazelnut extract, and to Cor a 14 at 12 mm, 10.2 kU/L, and 1.0 kU/L, respectively.

CONCLUSION: Cor a 14 was found to be a useful and reliable tool for predicting clinical reactivity in children with hazelnut allergy in the Eastern Mediterranean area. © 2016 American Academy of Allergy, Asthma & Immunology (J Allergy Clin Immunol Pract 2016;4:265-72)

Key words: Hazelnut allergy; Children; Component-resolved diagnosis; Cor a; Food challenge; Pollens; Probability

Tree nuts are common causes of food allergy that are often implicated in severe systemic reactions, resulting even in death.¹ In Europe, the prevalence of food-challenge-defined allergy to tree nuts has been reported to be 0.5%.² In the United States, the prevalence is reported to be 1.3% in adults and parent-reported prevalence is estimated to be 1.1% in children.³ Recently, a study assessing the prevalence of food sensitization in different regions of Europe found that the most common IgE sensitization in adults was to hazelnut.⁴ In our previous study, we demonstrated that hazelnut allergy ranks third among food allergies and is the most common nut allergy in Turkish children.⁵

Hazelnut allergy has a considerable negative impact on quality of life. Therefore, it is crucial to make an accurate diagnosis and prevent unnecessary elimination diets. In contrast to the general consideration that tree nut allergies are a lifelong condition, Fleischer et al⁶ demonstrated that 9% of patients outgrow their tree nut allergy. Although specific IgE (sIgE) and skin prick tests (SPT) are most commonly used, double-blind placebo-controlled food challenge (DBPCFC) tests are the gold standard tests not

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Abbreviations used

DBPCFC- Double-blind placebo-controlled food challenge

LTP- Lipid transfer protein

NPV- Negative predictive value

OFC- Oral food challenge

PPV- Positive predictive value

ROC- Receiver operating characteristics

sIgE- Specific immunoglobulin E

SPT- Skin prick test

only for diagnosis but also for deciding on the development of tolerance. However, DBPCFC tests are time consuming, labor intensive, and notably have a severe reaction risk. Moreover, there is uncertainty how, when, and in whom these tests can be performed. Determining cutoff values of sIgE and SPT measures would be helpful to physicians in the management of the disease. Although the cutoff values regarding sIgE and SPT diameter for some foods including cow's milk, egg, and peanut have been determined in previous studies, there is limited information available regarding hazelnut allergy.⁷⁻¹¹

Furthermore, contemporary research has opened up new horizons to our knowledge by the discovery of component-resolved diagnosis (CRD) in the last decade. In birch endemic areas, hazelnut allergy is often observed as a mild oral allergy syndrome (OAS) and is associated with cross-reactivity between Cor a 1 and Bet v 1, the major allergen from birch pollen.¹² Sensitization to Cor a 8, a lipid transfer protein (LTP), has been reported to cause more severe reactions to hazelnut in the Mediterranean region.¹³ Recently, Cor a 9, an 11S globulin, and Cor a 14, a 2S albumin, have been implicated in an association with severe reactions in hazelnut allergic children with no pollen sensitization.¹⁴ Nevertheless, the current limitations of data concerning the role of geographic region, population, and patterns of birch pollen sensitization in birch endemic and nonendemic areas must be addressed to improve hazelnut allergy management.

The purposes of this study were to determine the cutoff values and probability curves of hazelnut sIgE and SPT wheal size for predicting clinical reactivity in our geographical region, which is located in the Mediterranean area. We intended to elucidate the role of Cor a 1, Cor 8, Cor a 9, Cor a 14, and Bet v 1 in a pediatric population with hazelnut allergy. We also aimed to evaluate hazelnut extracts and component sIgE in light of our observation that birch pollen allergy is rare in our cohort of hazelnut allergic patients. Thus, the results of our exploratory study will provide data for further studies.

METHODS**Study population**

Children with IgE-mediated hazelnut allergy being followed up at the Division of Pediatric Allergy in Hacettepe University between January 2011 and January 2014 participated in the study. All parents were questioned diligently to determine reliable cause and effect relationships between the ingestion of hazelnut and the presentation of symptoms. Data collected included age, gender, baseline symptoms, sIgE levels, SPT wheal diameters, other food allergies, and associated atopic diseases.

The diagnosis of IgE-mediated hazelnut allergy was established when the children satisfied all of the following criteria:

1. A consistent and clear-cut history of an early phase reaction that was decisively related to the consumption of hazelnut along with obtaining benefit from an elimination diet.
2. Titers of hazelnut sIgE of ≥ 0.35 kU/L.
3. Positive SPT result for hazelnut, defined as a wheal diameter of ≥ 3 mm above the negative control.

Children with an ongoing allergy underwent DBPCFC tests in an attempt to determine the resolution of hazelnut allergy. Children who experienced anaphylaxis within the last year after accidental hazelnut intake did not undergo the food challenge test. The World Allergy Organization anaphylaxis guideline was followed for the diagnosis of anaphylaxis.¹⁵ The hazelnut sIgE levels of children with anaphylaxis were measured within a 1-month period after anaphylaxis.

Subjects more than 2 years of age underwent SPT with tree, grass, and weed pollens. The results of hazelnut extract and component sIgE were compared in children who were reactive or tolerant to hazelnut. Also, we compared the results of these children according to the sensitivity to pollens to evaluate the degree of cross-reactivity with homologous pollen allergens in a nonbirch endemic area. All the pollen-sensitized subjects had a wheal response greater than 3 mm with at least 2 allergens of tree, grass, or weed pollens.

Diagnosis of atopic dermatitis, asthma, and allergic rhinitis was made by the investigator. This study was approved by the Ethics Committee of Hacettepe University. All parents provided written informed consent for the study procedures.

DBPCFC tests

DBPCFC tests were performed based on the recommendations of the recent guidelines with an aim to document the symptoms objectively or assess the tolerance status of children previously diagnosed with hazelnut allergy.^{16,17} See [Data E1](#) available in this article's Online Repository at www.jaci-inpractice.org for details of DBPCFCs.

Study procedures and measurements

Total serum IgE and sIgE to natural extract of hazelnut and other implicated foods, rCor a 1, rCor a 8, nCor a 9, rCor a 14, and rBet v 1 antigens were measured with the ImmunoCAP system in accordance with the manufacturer's specifications (Thermo Fisher Scientific, Uppsala, Sweden). sIgE concentrations to natural extract of foods higher than 0.35 kU/L and levels of IgE to rCor a 1, rCor a 8, nCor a 9, rCor a 14, and rBet v 1 higher than 0.1 kU/L were considered positive.

SPT with hazelnut and other implicated foods (Alyostal ST-IR, Stallergenes SA, Antony, France) were performed on patients' upper back of the trunk and measured after 15 minutes. Histamine (10 mg/mL of histamine phosphate) and sterile saline solutions served as positive and negative controls, respectively. In children less than 2 years, SPT with only house dust mites (*Dermatophagoides farinae* and *D. pteronyssinus*) were applied, whereas in children more than 2 years old, SPT with a standard panel of aeroallergens (Stallergenes) including grass, weed, tree pollens (*Phleum pratense*, *Poa pratensis*, *Dactylis glomerata*, *Lolium perenne*, *Festuca pratensis*, *Avena sativa*, *Cynodon dactylon*, *Parietaria judaica*, *Artemisia vulgaris*, *Plantago*, *Chenopodium* mixture, *Salsola kali*, *Salix caprea*, *Ulmus campestris*, *Quercus robur*, *Betula alba*, *Populus alba*, *Platanus vulgaris*, and *Olea europaea*), house dust mites (*D. farinae* and *D. pteronyssinus*), molds (*Alternaria alternata*, *Aspergillus fumigatus*, *Cladosporium herbarum*, and *Penicillium* mixture of *Penicillium digitatum*, *P. expansum*, and *P. notatum*), cockroach (*Blattella germanica*), dog, cat, and horse

dander were performed. A mean wheal diameter 3 mm greater than the negative control was considered positive.

Statistical analysis

Statistical analyses were performed with SPSS 21 for Windows (IBM SPSS Statistics Chicago, Ill). Continuous variables including age, age of onset of the symptoms, total IgE, Cor a 1, Cor a 8, Cor a 9, Cor a 14, and Bet v 1, and hazelnut sIgE levels and SPT wheal diameter were not normally distributed; thus, the data were given as median and interquartile ranges (IQR). The statistical comparisons were carried out using the Mann-Whitney *U* test for continuous variables and using the χ^2 and Fisher's exact test for discrete variables, as appropriate. A *P* value less than .05 was considered statistically significant for all analyses.

We used a receiver operating characteristics (ROC) curve analysis to determine the accuracy of hazelnut sIgE, hazelnut SPT wheal diameter, and hazelnut component sIgE to predict clinical reactivity. Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were calculated for each selected cutoff point that significantly differentiated between reactive and nonreactive patients. Two-by-two tables were created to calculate sensitivity, specificity, PPV, and NPV. The optimal cutoff point was determined according to the Youden index (sensitivity + specificity – 1). Predicted probability curves were created using the results derived from logistic regression models. Analyses concerning the ROC curve, cutoff levels, and probability curves were performed in children who underwent DBPCFC. The children who were not challenged on account of a recent history of anaphylaxis were not included because the pretest likelihood of a positive reaction of these children was very high. In fact, inclusion of patients with a close to 100% certain diagnosis does not contribute to the reliability of the predictive value of a test and falsely increases the apparent *P* value.

RESULTS

A total of 64 children (70.3% boys) with a median (IQR) age of 3.4 (2.1–7.2) years participated in the study (Table I). There was a male predominance in the children and most of them had atopic dermatitis in their clinical history. DBPCFC tests were performed in 56 children to determine the clinical reactivity to hazelnut. Eight children were included in the study without performing food challenge on account of experiencing anaphylaxis within the last 12 months after accidental hazelnut intake. In medical history, the most commonly affected organs after hazelnut consumption were skin (87.3%) and respiratory system (36.5%).

Challenge results

Of the 56 DBPCFC tests, 24 resulted in positive reaction and 10 of these reactions were anaphylaxis. The most common symptoms in the study group elicited by hazelnut were cutaneous (87.5%), respiratory (33.3%), and gastrointestinal (29.2%) systems. Among the children who experienced anaphylaxis, we observed cutaneous and respiratory symptoms in 3 children, cutaneous, respiratory, and gastrointestinal symptoms in 2 children, cutaneous and gastrointestinal symptoms in 3 children, respiratory and gastrointestinal symptoms in 1 child, and cutaneous, respiratory, gastrointestinal, and neurologic symptoms in 1 child. Eight children who had anaphylaxis after accidental exposure in the previous year did not undergo confirmatory DBPCFC tests. The detailed characteristics of the children with a

recent history of anaphylaxis along with the reactive and nonreactive children in the DBPCFC tests are shown in Table I. In the whole study group, 64 subjects were hazelnut sensitized and 32 (50%) were reactive to hazelnut. A total of 18 children (28%) had an anaphylactic reaction to hazelnut (see Table E1 available in this article's Online Repository at www.jaci-inpractice.org). The median hazelnut sIgE was 2.3 (0.9–6.6) kU/L.

Hazelnut extract sIgE, SPT, and Cor a 14 significantly discriminate between children reactive and nonreactive to hazelnut

The levels of sIgE to hazelnut extract sIgE (*n* = 64) and the components, including Cor a 1 (*n* = 55), Cor a 8 (*n* = 55), Cor a 9 (*n* = 51), Cor a 14 (*n* = 51), and Bet v 1 (*n* = 58), were determined in available sera from the patients. The median level of IgE to hazelnut extract and Cor a 14 was significantly higher in children who were reactive to hazelnut compared with nonreactive children (*P* < .001, *P* < .001, respectively) (Table II). The median levels of IgE to Cor a 1, Cor a 8, and Cor a 9 were similar between reactive and nonreactive children. In addition, the SPT wheal diameter of reactive children was significantly higher than that of the nonreactive children (*P* = .004).

Cutoff levels of hazelnut extract sIgE, SPT wheal diameter, and IgE to Cor a 14 for predicting clinical reactivity

The ROC curve with the area under curve (AUC) for hazelnut sIgE fulfilled a satisfying relationship between sensitivity and specificity. The highest AUC was observed with Cor a 14 for distinguishing between reactive and nonreactive children (Figure 1). The cutoff hazelnut sIgE level that predicted clinical reactivity with the sum of highest sensitivity and specificity (Youden index) was 3.15 kU/L (see Table E2 available in this article's Online Repository at www.jaci-inpractice.org). Sensitivity, specificity, PPV, and NPV were 70.8, 90.6, 85.0, and 80.6, respectively. Remarkably, all the children with hazelnut sIgE $\geq$ 5.93 kU/L showed clinical reactivity to hazelnut. The cutoff hazelnut SPT wheal diameter that predicted clinical reactivity at the highest sensitivity (58.8%) + specificity (90%) was 7.5 mm (see Table E2). PPV and NPV were 90.9% and 56.3%, respectively. The optimal cutoff point of IgE to Cor a 14 according to the Youden index was 0.63 kU/L, with a sensitivity and specificity of 81.8% and 100%, respectively (see Table E3 available in this article's Online Repository at www.jaci-inpractice.org).

Predicted probability curves of hazelnut extract sIgE, SPT wheal diameter, and IgE to Cor a 14

A logistic regression model provided the opportunity to depict the predicted probability curves for positive clinical reactivity at a given hazelnut sIgE, SPT wheal diameter, and IgE to Cor a 14. The curve of predicted probabilities showed that the hazelnut sIgE of 4.0 and 10.2 kU/L had a 50% and a 95% probability for clinical reactivity, respectively (Figure 2, A). Regarding the hazelnut SPT wheal diameter, the curve of predicted probability determined that 5.25 and 12.0 mm had a 50% and a 95% probability of clinical reactivity, respectively (Figure 2, B). Furthermore, the predicted probability curves showed that the level of IgE to Cor a 14 of 1.0 kU/L had a 50% and a 95% probability for clinical reactivity, respectively (Figure 2, C).

TABLE I. Characteristics of children with hazelnut allergy

	Whole study group (n = 64)	Children with a recent history of anaphylaxis (n = 8)	Children who were submitted to DBPCFC		P†
			Reactive (n = 24)	Nonreactive (n = 32)	
Current age*	3.4 (2.1-7.2)	4.3 (1.5-14.2)	2.9 (1.73-6.5)	3.7 (2.6-9.6)	NS
Gender (male/female), n (%)	45 (70%)/19 (30%)	6 (75%)/2 (25%)	16 (66.7%)/8 (33.3%)	23 (71.9%)/9 (28.1%)	NS
Family history of atopy, n (%)	31 (49.2%)	6 (75%)	10 (43.5%)	15 (46.9%)	NS
Age of onset (y)*	1.0 (0.8-2.0)	1.0 (0.9-3.5)	1.0 (0.9-1.9)	1.0 (0.8-2.7)	NS
Concomitant atopic dermatitis, n (%)	52 (81.3%)	5 (62.5%)	21 (87.5%)	26 (81.3%)	NS
Concomitant asthma, n (%)	33 (51.5%)	7 (87.5%)	11 (45.8%)	15 (46.9%)	NS
Concomitant allergic rhinitis, n (%)	24 (37.5%)	5 (62.5%)	10 (41.7%)	9 (28.1%)	NS
Total IgE (kU/L)*	215 (69-489)	294 (87-534.7)	221 (55-694)	194 (81.8-475)	NS
Hazelnut sIgE (kU/L)* at initial visit	6.8 (3.4-14.3)	8.9 (3.58-24.5)	12.3 (4.35-36.0)	4.76 (2.38-7.58)	.005
Concomitant other nut allergy, n (%)	32 (50%)	5 (62.5%)	17 (70.8%)	10 (31.3%)	.003
Peanut	16 (25%)	4 (50%)	6 (25%)	6 (18.8%)	NS
Walnut	26 (40.6%)	5 (62.5%)	14 (58.3%)	7 (21.9%)	.005
Pistachio	9 (14.1%)	1 (12.5%)	5 (20.8%)	3 (9.4%)	NS
Almond	2 (3.1%)	0	1 (4.2%)	1 (3.1%)	NS
Pollen sensitization‡, n (%)	28 (54.9%) (n = 51)	4 (57.1%) (n = 7)	12 (66.7%) (n = 18)	12 (46.2%) (n = 26)	NS
Tree	4 (7.8%)	1 (14.2%)	2 (11.1%)	1 (3.8%)	NS
Grass	24 (47%)	3 (42.9%)	10 (55.6%)	11 (42.3%)	NS
Weed	13 (25.4%)	2 (38.5%)	5 (27.8%)	6 (23%)	NS

DBPCFC, Double-blind placebo-controlled food challenge; sIgE, specific immunoglobulin E.

*Median (interquartile range).

†Skin prick tests with pollens were performed in 51 children who were more than 2 years of age, and the percentage of pollen sensitization gives the ratio in total 51 children.

‡P value is given for the comparison of reactive and nonreactive children who were submitted to DBPCFC.

Association of hazelnut allergy with pollen sensitization

SPT with pollens were performed in 51 children and 28 (54.9%) had sensitization to pollens. Grass, weed, and tree pollen sensitization frequencies were 47% (n = 24), 25.4% (n = 13), and 7.8% (n = 4), respectively. Contrary to the common birch pollen sensitization in central and northern parts of Europe and the USA, grass allergy is the most common pollinosis and tree allergy is relatively rare compared with grass and weed sensitization in Turkey, a Mediterranean area country. There was no difference regarding clinical reactivity between pollen-sensitized and nonsensitized patients (data not shown). Bet v 1 and Cor a 1 are homolog proteins and they were slightly higher in subjects with pollen sensitization than those without pollen sensitization ($P = .006$ and $P = .011$, respectively) (Table III). Bet v 1 is a birch pollen antigen, and in our pollen-sensitized group there were only 4 subjects with tree pollinosis. The low frequency of tree allergy and being located in a nonbirch endemic area may be the reasons for the comparable and low levels of Bet v 1 and Cor a 1 in the study group. Cor a 8, which is an LTP, was again slightly higher in the pollen-sensitized than the non-sensitized group ($P \leq .001$). Even though the median of hazelnut extract IgE, Cor a 14, and SPT wheal diameter showed a trend of higher levels in the pollen-sensitized group, the comparison was not significant.

When the subjects were analyzed separately according to the sensitivity to pollens and the reactivity to hazelnut, the highest median IgE levels of hazelnut extract, Cor a 1, Cor a 9, and Cor a 14 were obtained in the subgroup that was reactive to hazelnut and sensitized to pollens (see Table E4 available in this article's

TABLE II. Comparison of hazelnut extract sIgE, Cor a 1, Cor a 8, Cor a 9, Cor a 14, Bet v 1, and SPT wheal diameter in children reactive and nonreactive to hazelnut

	Nonreactive n = 32	Reactive n = 32	P*
Hazelnut sIgE (kU/L)	n = 32	n = 32	
Positive if ≥ 0.35	25 (78.1%)	29 (90.6%)	.168
Median (IQR)	1.07 (0.50-2.19)	6.43 (2.64-26.85)	<.001
rCor a 1 (kU/L)	n = 26	n = 29	
Positive if ≥ 0.1	0	4 (13.8)	.113
Median (IQR)	0.01 (0.0-0.22)	0.02 (0.01-0.05)	.065
Bet v 1 (kU/L)	n = 29	n = 29	
Positive if ≥ 0.1	1 (3.4%)	6 (20.7%)	.102
Median (IQR)	0.02 (0.01-0.04)	0.03 (0.01-0.08)	.183
rCor a 8 (kU/L)	n = 27	n = 28	
Positive if ≥ 0.1	11 (40.7%)	12 (42.9%)	.874
Median (IQR)	0.03 (0.01-0.20)	0.05 (0.02-2.59)	.168
nCor a 9 (kU/L)	n = 25	n = 26	
Positive if ≥ 0.1	18 (72%)	19 (73.1%)	.931
Median (IQR)	0.58 (0.06-1.91)	0.89 (0.07-4.81)	.213
rCor a 14 (kU/L)	n = 25	n = 26	
Positive if ≥ 0.1	9 (36%)	25 (96.2)	<.001
Median (IQR)	0.03 (0.02-0.18)	4.04 (0.82-13.9)	<.001
SPT wheal diameter (mm)	n = 10	n = 22	
Positive if ≥ 7.5	1 (10%)	13 (59.1%)	.009
Median (IQR)	4.5 (3.0-6.25)	8.0 (5.0-11.25)	.004

IQR, Interquartile range; sIgE, specific immunoglobulin E; SPT, skin prick test.

* χ^2 test was applied for binary results of IgE; Mann-Whitney U test was applied for continuous results (median) of IgE.

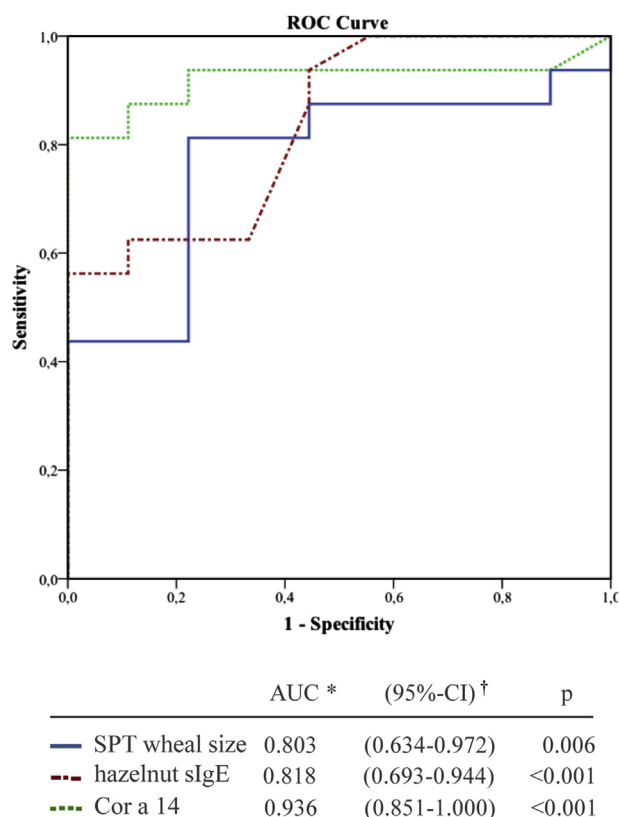


FIGURE 1. Receiver operating characteristic (ROC) curve predicting clinical reactivity for hazelnut (*AUC: area under curve, †95% CI: 95% confidence intervals).

Online Repository at www.jaci-inpractice.org. In both groups of subjects, with and without pollen sensitization, sIgE to hazelnut extract and Cor a 14 was significantly higher in the hazelnut-reactive children than the hazelnut-tolerant children (Figure 3, A and B). When compared with natural hazelnut extract, Cor a 14 could be significantly distinguished with a larger difference and a higher accuracy for the children reactive to hazelnut compared with nonreactive children in pollen-sensitized and non-pollen-sensitized subjects.

DISCUSSION

This study is a comprehensive evaluation of hazelnut allergy combining the previous methods consisting of SPT and sIgE to natural hazelnut extract and the new recombinant and natural hazelnut allergens including Cor a 1, Cor a 8, Cor a 9, and Cor a 14 in hazelnut-reactive versus hazelnut-tolerant and pollen-sensitive versus non-pollen-sensitive subjects. In this study, we determined optimal cutoff values and probability curves of natural hazelnut extract sIgE, SPT wheal diameter, and IgE to Cor a 14 for predicting clinical reactivity in Turkish children with hazelnut allergy. Among the components of Cor a 1, Cor a 8, Cor a 9, and Cor a 14, only Cor a 14 successfully discriminated between hazelnut reactive and nonreactive children, whether the subjects were sensitive or nonsensitive to pollens. For the first time, a 95% probability for clinical reactivity was estimated for hazelnut sIgE, SPT wheal diameter, and Cor a 14 at 10.2 kU/L, 12 mm, and 1.0 kU/L, respectively.

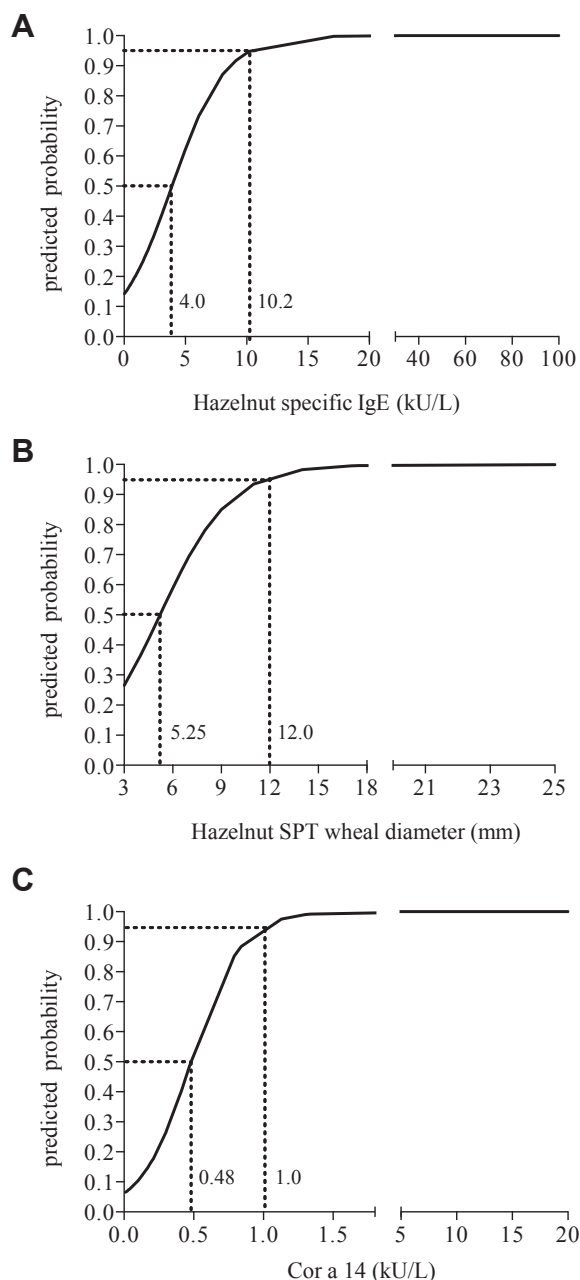


FIGURE 2. Probability of clinical reactivity to hazelnut at a given hazelnut specific IgE (A), hazelnut skin prick test (SPT) wheal diameter (B), and IgE to Cor a 14 (C).

It is widely agreed that oral food challenges (OFC) are troublesome, require an appropriate setting, and have potential severe reaction risks. Therefore, most clinicians are reluctant to perform these tests. With this in view, reliable and practical laboratory tests would reduce the need to perform OFC and facilitate our daily practice. Some investigators have previously studied the use of sIgE and SPT instead of OFC with food allergies, mainly for milk, egg, and peanut.⁷⁻⁹ In addition, there is an increasing interest in CRD for the purpose of improving diagnostic test accuracy. Although there are a number of studies of CRD in peanut, milk, egg, and shrimp, our knowledge about hazelnut related to CRD is limited.

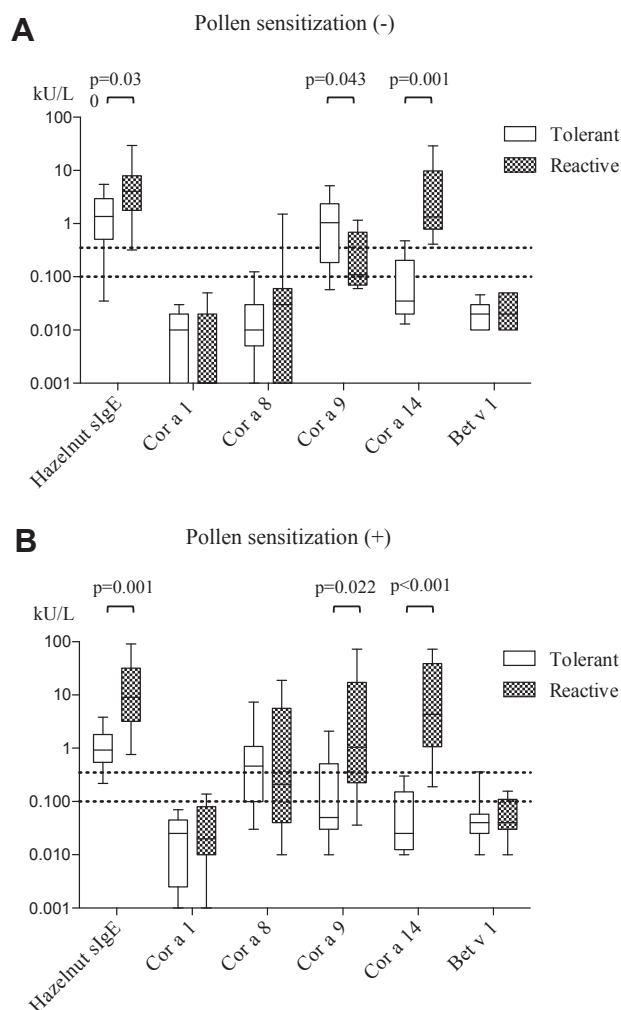
TABLE III. Comparison of hazelnut extract sIgE, Cor a 1, Cor a 8, Cor a 9, Cor a 14, Bet v 1, and SPT wheal diameter in children with and without sensitization to tree, grass, or weed pollens

	Pollen sensitization (-) n = 23	Pollen sensitization (+) n = 28	P*
Hazelnut sIgE (kU/L)	n = 23	n = 28	
Positive if ≥ 0.35	20 (87.0%)	25 (89.3%)	1.000
Positive if ≥ 3.15	8 (34.8%)	14 (50.0%)	.275
Median (IQR)	1.92 (0.58-4.69)	3.15 (0.91-10.17)	.320
rCor a 1 (kU/L)	n = 20	n = 23	
Positive if ≥ 0.1	0	3 (13.0%)	.236
Median (IQR)	0.01 (0.0-0.02)	0.02 (0.01-0.05)	.011
Bet v 1 (kU/L)	n = 20	n = 25	
Positive if ≥ 0.1	0	5 (20.0%)	.056
Median (IQR)	0.02 (0.01-0.03)	0.04 (0.03-0.09)	.006
rCor a 8 (kU/L)	n = 20	n = 24	
Positive if ≥ 0.1	3 (15.0%)	16 (66.7%)	.001
Median (IQR)	0.01 (0.00-0.04)	0.33 (0.04-2.80)	<.001
nCor a 9 (kU/L)	n = 19	n = 21	
Positive if ≥ 0.1	15 (78.9%)	13 (61.9%)	.240
Median (IQR)	0.69 (0.11-1.38)	0.54 (0.05-2.63)	.755
rCor a 14 (kU/L)	n = 19	n = 21	
Positive if ≥ 0.1	11 (57.9%)	16 (76.2%)	.217
Median (IQR)	0.21 (0.03-1.13)	0.84 (0.06-12.88)	.238
SPT wheal diameter (mm)	n = 12	n = 17	
Positive if ≥ 7.5	4 (33.3%)	9 (52.9%)	.296
Median (IQR)	5.75 (4.62-10.50)	8.0 (5.0-10.0)	.399

IQR, Interquartile range; sIgE, specific immunoglobulin E; SPT, skin prick test.

* χ^2 test was applied for binary results of IgE; Mann-Whitney U test was applied for continuous results of IgE.

Attempts to investigate the performance of diagnostic tests, similar to the purpose of our research, have been carried out in a few studies regarding tree nut or hazelnut allergy. In an observational study of 1000 subjects in the United Kingdom, Clark and Ewan¹⁸ observed that only 5% of patients with SPT ≥ 8 mm and sIgE ≥ 15 kU/L could tolerate nuts, and SPT and sIgE could be used in practice for confirming nut allergy. In Australia, Ho et al¹⁹ found that an SPT wheal diameter ≥ 8 mm predicted a clinical reaction to tree nuts with more than 95% accuracy. The cutoff level for hazelnut at ≥ 8 mm showed a poor sensitivity (8%) but 100% specificity. Masthoff et al.²⁰ demonstrated that SPT was a better tool than the level of sIgE in predicting allergy to hazelnut in Dutch children. When compared with the previous study, the level of SPT ≥ 8 mm gave a PPV of 74%, but an SPT size ≥ 17 mm had a 100% PPV. Collectively, the results of the studies vary in a wide range presumably owing to differences in age groups, inclusion criteria, and geographic locations. We found that the optimal cutoff points according to the Youden index for hazelnut sIgE was 3.15 kU/L with a sensitivity of 70.8% and a specificity of 90.6%, and for the SPT wheal diameter it was 7.5 mm with a sensitivity of 58.8% and specificity of 90.0%, respectively, for predicting clinical reactivity in hazelnut allergic children (Table IV and Table E2, available in this article's Online Repository at www.jaci-inpractice.org). Unlike the above-mentioned studies, SPT did not show superiority over hazelnut sIgE in our findings. As explained in the Methods section, we determined cutoff levels in children with an

**FIGURE 3.** Comparison of hazelnut extract sIgE, Cor a 1, Cor a 8, Cor a 9, Cor a 14, and Bet v 1 in children reactive and tolerant to hazelnut and with and without sensitization to tree, grass, or weed pollens.**TABLE IV.** Different cutoff levels of hazelnut extract sIgE, SPT wheal diameter, and sIgE to Cor a 14 in predicting clinical reactivity

	Cutoff	Sensitivity	Specificity	PPV (%)	NPV (%)
Hazelnut extract sIgE (kU/L)	3.15	70.8	90.6	85.0	80.6
	5.0	50.0	96.9	92.3	72.1
	5.93	50.0	100	100	72.7
SPT wheal diameter (mm)	7.5	58.8	90.0	90.9	56.3
	10.0	35.3	100	100	47.6
rCor a 14 sIgE	0.48	81.8	96.0	94.7	85.7
	0.63	81.8	100	100	86.2

NPV, Negative predictive value; PPV, positive predictive value; sIgE, specific immunoglobulin E; SPT, skin prick test.

appreciable uncertainty in their pretest clinical diagnosis. The children with a recent history of anaphylaxis after accidentally intake of hazelnut were not included because mixing groups of

subjects with very different pretest likelihoods is not statistically helpful.²¹ As for hazelnut sIgE, although a 95% PPV could not be determined in previous studies so far, a level of 5.0 kU/L gave a PPV of 92.3% and a level of 5.93 kU/L gave a PPV of 100% in this study. For the SPT wheal diameter, the level of 10 mm yielded a PPV of 100% (Tables IV and E2). Generally, physicians might opt for using PPVs to have information on the likelihood of a positive challenge outcome. Nevertheless, it should be kept in mind that the prevalence of the disease in the population influences the PPV results.⁹

Moreover, we established 50% and 95% probability curves for clinical reactivity, which may be helpful to clinicians in daily practice, as suggested by Sampson.⁷ To our knowledge, a 95% probability for clinical reactivity has not been calculated for sIgE and SPT size for hazelnut allergy to date. The sIgE and SPT wheal diameter values indicating a 95% probability for clinical reactivity were 10.2 kU/L and 12 mm, respectively. By using probability curves, practitioners may be able to estimate the possibility of a clinical reaction to hazelnut and consequently decide whether or not to perform OFC.

In Northern and Central Europe, where birch pollinosis is common, hazelnut allergy associated with cross-reactivity between Bet v 1 and Cor a 1 in adults usually results in reactions mostly localized to the oral cavity, known as OAS.²² In this study, IgE levels to Cor a 1 and Bet v 1 were very low and not different between reactive and nonreactive children, but slightly higher in pollen-sensitized subjects. This result stems particularly from low sensitization to birch pollen in our country, which was reported to be 1.7% in a previous study.²³ Notably, there was no patient with a history of OAS in our study group. Nevertheless, sensitization to Cor a 1 can be detected in patients who had been exposed to Birch pollen although not prevalent in Turkey. Therefore, in patients with OAS who had lived for a while in Central or Northern Europe (the region where birch pollen is common) or traveled to this region in the past, measuring Cor a 1 may be appropriate. Likewise, the history concerning travel could be questioned in patients with high levels of IgE to Cor a 1. Besides, in our country, OAS is seen very rarely in pediatric patients, but more frequently reported in adults. A travel history to the countries where Bet v 1 allergen is common may be an explanation for the birch pollen sensitization in these patients.

In the Mediterranean area, sensitization to hazelnut LTP (Cor a 8) has been shown to cause severe reactions to hazelnut in individuals without pollen sensitization, but this relation does not hold true for other regions.^{13,24} Contrary to expectations, our results revealed no difference between reactive and nonreactive children regarding sensitization to Cor a 8. When we analyzed Bet v1, Cor a 1, and Cor a 8 in a further step according to the sensitization status for pollen, we again did not observe a significant difference between subjects reactive and nonreactive to hazelnut (Table E4, available in this article's Online Repository at www.jaci-inpractice.org, and Figure 3). Similar to Cor a 8, Cor a 9 and Cor 14 were also reported to cause severe reactions in hazelnut allergic children. Masthoff et al¹⁴ demonstrated that Cor a 9 and Cor a 14 are associated with objective symptoms to hazelnut in children without pollen allergy among Dutch children. IgE levels to Cor a 9 ≥ 1 kU/L or Cor a 14 ≥ 5 kU/L reached a sensitivity of 83% and a specificity of 93% when used in combination. Furthermore, Kattan et al²⁵ demonstrated that Cor a 9 ≥ 2.0 kU/L or Cor a 14 ≥ 1.0 kU/L had a sensitivity of 92% and a specificity of 93% for clinical reactivity

among children in the USA. In contrast to these studies from birch endemic areas, the median levels of Cor a 9 were comparable between reactive and nonreactive children in this study, accentuating the regional differences in sensitization patterns. Recently, Beyer et al²⁶ investigated the role of component testing in the diagnosis of peanut and hazelnut allergy in German children. Cor a 14 discriminated tolerant and allergic children better than Cor a 1, Cor a 8, and Cor a 9, and also hazelnut sIgE. A cutoff level 0.35 kU/L for Cor a 14 revealed a sensitivity of 85% and specificity of 81%, while giving a PPV and NPV of 64% and 91%, respectively. Although a 95% probability could not be determined, a 90% probability for a positive reaction was estimated for IgE to Cor a 14 at 47.8 kU/L. In Beyer's study, the sensitization patterns to pollens including birch tree were not mentioned, but in line with the current data, we observed that Cor a 14 was associated with objective symptoms to hazelnut in children with and without pollen sensitization. In addition, in this study, the highest AUC was achieved with Cor a 14 (0.93) for clinical reactivity when compared with hazelnut sIgE (0.81) and SPT wheal diameter (0.80). For Cor a 14, the optimal cutoff point of 0.63 kU/L according to the Youden index showed 100% specificity and 100% PPV, which are higher than those in previous studies (Table IV and Table E3, available in this article's Online Repository at www.jaci-inpractice.org). Furthermore, a 50% and a 95% probability for clinical reactivity were determined at a level of 0.48 and 1.0 kU/L, respectively. Of note is that the 95% probability level of 1.0 kU/L is far below the 90% probability level of the previous study, which was 48.5 kU/L. The underlying reason for this result is unknown, but the difference between the study designs, populations, and sensitization to pollens might partly explain this discrepancy. Our findings indicate that Cor a 14 and hazelnut extract sIgE have a tendency to be higher in subjects who are allergic to pollens and reactive to hazelnut, and this trend does not seem to be related to birch pollinosis in the current study. Cor a 14 is a seed storage protein, and, to our knowledge, a cross-reactivity between seed storage protein and grass pollen allergens, which is the predominant pollen in the current studied group, has not yet been described. Even though the comparison of Cor a 14 and hazelnut extract IgE in hazelnut-reactive children with and without pollen sensitization was not significant (see Table E4 in this article's Online Repository at www.jaci-inpractice.org), our results suggest that positive results of Cor a 14 (>0.1 kU/L) and hazelnut extract (>0.35 kU/L) with a very low median level are strongly meaningful for reactivity in children without pollen sensitization.

Taken together, we suggest that utilizing Cor a 14 sensitization in clinical practice might improve the diagnosis of hazelnut allergy, especially in children with objective symptoms, and decrease the need for OFC and severe reaction risk. However, OFC is still required for the results under the cutoff value. In addition, clinical presentation and sensitization patterns of hazelnut allergy differ between children and adults and between geographic regions. Therefore, our results may not be valid for adults and for other countries. Finally, while interpreting Cor a 14 and hazelnut extract sIgE and SPT to predict the reactivity, pollen sensitization should be taken into consideration.

The small sample size of this study may be a limitation, especially for subgroup analysis depending on the sensitization to pollens. However, our inclusion criteria were strict by means of having a consistent history together with positivity both in sIgE and SPT measurements, and except for the 8 children who

experienced anaphylaxis in the last 12 months, all children were submitted to a DBPCFC test. Moreover, our study group consisted of children who were referred to a tertiary care facility and therefore may not be representative of the general population.

As being a descriptive report of an extensive exploratory study, our data will be of much help for subsequent studies specifically aimed at establishing predictive protocols in several restricted and well-defined study groups.

In conclusion, Cor a 14 is a more reliable diagnostic test than Cor a 1, Cor a 8, Cor a 9, hazelnut extract sIgE, and SPT wheal diameter for predicting clinical reactivity in children with hazelnut allergy in Turkey, which is located in the Mediterranean region. We suggest that established probability curves will be helpful to clinicians when making decisions on appropriate timing for performing food challenges. Because there is diversity in clinical presentation of hazelnut allergy according to age, geographic region, and pollen sensitization, further studies from different countries and populations are required for optimizing the use of these tests in hazelnut allergy.

REFERENCES

- Bock SA, Munoz-Furlong A, Sampson HA. Fatalities due to anaphylactic reactions to foods. *J Allergy Clin Immunol* 2001;107:191-3.
- Nwaru BI, Hickstein L, Panesar SS, Roberts G, Muraro A, Sheikh A, et al. Prevalence of common food allergies in Europe: a systematic review and meta-analysis. *Allergy* 2014;69:992-1007.
- Sicherer SH, Munoz-Furlong A, Godbold JH, Sampson HA. US prevalence of self-reported peanut, tree nut, and sesame allergy: 11-year follow-up. *J Allergy Clin Immunol* 2010;125:1322-6.
- Burney PG, Potts J, Kummeling I, Mills EN, Clausen M, Dubakiene R, et al. The prevalence and distribution of food sensitization in European adults. *Allergy* 2014;69:365-71.
- Yavuz ST, Sahiner UM, Buyuktiryaki B, Soyer OU, Tuncer A, Sekerel BE, et al. Phenotypes of IgE-mediated food allergy in Turkish children. *Allergy Asthma Proc* 2011;32:47-55.
- Fleischer DM, Conover-Walker MK, Matsui EC, Wood RA. The natural history of tree nut allergy. *J Allergy Clin Immunol* 2005;116:1087-93.
- Sampson HA. Utility of food-specific IgE concentrations in predicting symptomatic food allergy. *J Allergy Clin Immunol* 2001;107:891-6.
- Perry TT, Matsui EC, Kay Conover-Walker M, Wood RA. The relationship of allergen-specific IgE levels and oral food challenge outcome. *J Allergy Clin Immunol* 2004;114:144-9.
- Sampson HA, Ho DG. Relationship between food-specific IgE concentrations and the risk of positive food challenges in children and adolescents. *J Allergy Clin Immunol* 1997;100:444-51.
- Maloney JM, Rudengren M, Ahlstedt S, Bock SA, Sampson HA. The use of serum-specific IgE measurements for the diagnosis of peanut, tree nut, and seed allergy. *J Allergy Clin Immunol* 2008;122:145-51.
- Ortolani C, Ballmer-Weber BK, Hansen KS, Ispano M, Wuthrich B, Bindslev-Jensen C, et al. Hazelnut allergy: a double-blind, placebo-controlled food challenge multicenter study. *J Allergy Clin Immunol* 2000;105:577-81.
- Hofmann C, Scheurer S, Rost K, Graulich E, Jamin A, Foetisch K, et al. Cor a 1-reactive T cells and IgE are predominantly cross-reactive to Bet v 1 in patients with birch pollen-associated food allergy to hazelnut. *J Allergy Clin Immunol* 2013;131:1384-1392.e6.
- Schocker F, Luttkopf D, Scheurer S, Petersen A, Cistero-Bahima A, Enrique E, et al. Recombinant lipid transfer protein Cor a 8 from hazelnut: a new tool for in vitro diagnosis of potentially severe hazelnut allergy. *J Allergy Clin Immunol* 2004;113:141-7.
- Masthoff LJ, Mattsson L, Zuidmeer-Jonghean L, Lidholm J, Andersson K, Akkedaas JH, et al. Sensitization to Cor a 9 and Cor a 14 is highly specific for a hazelnut allergy with objective symptoms in Dutch children and adults. *J Allergy Clin Immunol* 2013;132:393-9.
- Simons FE, Arduoso LR, Bilo MB, El-Gamal YM, Ledford DK, Ring J, et al. World allergy organization guidelines for the assessment and management of anaphylaxis. *World Allergy Organ J* 2011;4:13-37.
- Sampson HA, Gerth van Wijk R, Bindslev-Jensen C, Sicherer S, Teuber SS, Burks AW, et al. Standardizing double-blind, placebo-controlled oral food challenges: American Academy of Allergy, Asthma & Immunology-European Academy of Allergy and Clinical Immunology PRACTALL consensus report. *J Allergy Clin Immunol* 2012;130:1260-74.
- Nowak-Węgrzyn A, Assa'ad AH, Bahna SL, Bock SA, Sicherer SH, Teuber SS, et al. Work Group report: oral food challenge testing. *J Allergy Clin Immunol* 2009;123:S365-83.
- Clark AT, Ewan PW. Interpretation of tests for nut allergy in one thousand patients, in relation to allergy or tolerance. *Clin Exp Allergy* 2003;33:1041-5.
- Ho MH, Heine RG, Wong W, Hill DJ. Diagnostic accuracy of skin prick testing in children with tree nut allergy. *J Allergy Clin Immunol* 2006;117:1506-8.
- Masthoff LJ, Pasmans SG, van Hoffen E, Knol MJ, Bruijnzeel-Koomen CA, Flinterman AE, et al. Diagnostic value of hazelnut allergy tests including rCor a 1 spiking in double-blind challenged children. *Allergy* 2012;67:521-7.
- Aalberse JA, Aalberse RC. A lesson from component-resolved testing: we need better extracts. *J Allergy Clin Immunol Pract* 2014;2:635-6.
- De Knop KJ, Verweij MM, Grimmelikhuijsen M, Philipse E, Hagendorens MM, Bridts CH, et al. Age-related sensitization profiles for hazelnut (*Corylus avellana*) in a birch-endemic region. *Pediatr Allergy Immunol* 2011;22:e139-49.
- Sahiner UM, Civelek E, Yavuz ST, Buyuktiryaki AB, Tuncer A, Sekerel BE. Skin prick testing to aeroallergen extracts: what is the optimal panel in children and adolescents in Turkey? *Int Arch Allergy Immunol* 2012;157:391-8.
- Hansen KS, Ballmer-Weber BK, Sastre J, Lidholm J, Andersson K, Oberhofer H, et al. Component-resolved in vitro diagnosis of hazelnut allergy in Europe. *J Allergy Clin Immunol* 2009;123:1134-41. 1141.e1-3.
- Kattan JD, Sicherer SH, Sampson HA. Clinical reactivity to hazelnut may be better identified by component testing than traditional testing methods. *J Allergy Clin Immunol Pract* 2014;2:633-634.e1.
- Beyer K, Grabenhenrich L, Hartl M, Beder A, Kalb B, Ziegert M, et al. Predictive values of component-specific IgE for the outcome of peanut and hazelnut food challenges in children. *Allergy* 2015;70:90-8.

DATA E1

Double-blind placebo-controlled food challenge tests

The challenge materials were always prepared and randomized by the same well-trained staff. Chocolate pudding was used as a test meal, whereas in milk allergic children mashed potato was the preferred alternative. For the purpose of masking odor, texture, and taste, crashed cookies (milk free), vanilla extract, and boiled (3 minutes) milled rice grains were also added. The active and placebo challenge materials before the study were tested by 20 volunteers. The physicians were blinded to the challenge materials.

Before the challenge tests, a detailed physical examination of the undressed child was made, the vital signs (heart rate, respiratory rate, blood pressure, and oxygen saturation) were recorded, and lung function test, in children who were able to perform the test, was measured. The measurements of hazelnut sIgE levels and the SPT wheal diameters used in the analyses were also

repeated on the day of the challenge. Emergency equipment was kept available in a rapidly accessible place and for children with a history of anaphylaxis an intravenous line was inserted. Powdered raw hazelnut was given orally under the supervision of a physician, and the dose was increased stepwise (12, 25, 100, 500, 860, 2500, 4000 mg [~ 4 small hazelnuts]) every 15 minutes, if no reaction was observed with the previous dose. In the event of any objective symptom, the challenge was ceased and appropriate medical treatment was initiated.

The child remained under observation at least 2 hours after the last dose given. If there was no reaction, the child was discharged and continued to receive 4000 mg of hazelnut every day during the following week. The parents were informed to observe their child for the late reactions and in case any new symptoms developed to get in contact with the physician who conducted the challenge test.

TABLE E1. Reactions of the patients who experienced anaphylaxis during DBPCFC tests (n = 10) or after an accidental intake (n = 8)

Children who experienced anaphylaxis during DBPCFC (n = 10)	
Patient 1	Generalized urticaria, itching, cough, dyspnea
Patient 2	Swollen lips and uvula, cough, wheezing
Patient 3	Generalized urticaria, cough, dyspnea
Patient 4	Generalized urticaria, itching, nausea, vomiting, wheezing, rhinorrhea, sneezing
Patient 5	Generalized urticaria, flushing, crampy abdominal pain, vomiting, throat itching, stridor
Patient 6	Generalized urticaria, itching, abdominal pain, nausea, vomiting
Patient 7	Generalized urticaria, swollen lips, periorbital itching and edema, nausea, vomiting
Patient 8	Generalized urticaria, crampy abdominal pain, nausea, vomiting
Patient 9	Itching of tongue, palate, generalized urticaria, cough, wheezing, vomiting, dizziness
Patient 10	Nausea, vomiting, increased respiratory rate, dyspnea
Children with a history of recent anaphylaxis (n = 8)	
Patient 11	Generalized urticaria, swollen lips, periorbital itching, conjunctival erythema, cough, dyspnea
Patient 12	Swollen lips and uvula, itching of palate, wheezing, hypoxemia
Patient 13	Flushing, urticaria, stridor
Patient 14	Cough, dyspnea, crampy abdominal pain, nausea, vomiting
Patient 15	Generalized urticaria, wheezing, cough, sneezing
Patient 16	Itching, swollen lips, crampy abdominal pain, nausea, vomiting
Patient 17	Generalized urticaria, crampy abdominal pain, vomiting, cough
Patient 18	Cough, dyspnea, nausea, vomiting, nasal congestion, rhinorrhea

DBPCFC, Double-blind placebo-controlled food challenge.

TABLE E2. Different cutoff levels of hazelnut sIgE and skin prick test (SPT) wheal diameter in predicting clinical reactivity

	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
Hazelnut extract				
sIgE (kU/L)				
2.8	70.8	81.3	73.9	78.8
2.9	70.8	84.4	77.3	79.4
3.1	70.8	84.4	77.3	79.4
3.15	70.8	90.6	85.0	80.6
3.2	66.7	90.6	84.2	78.4
3.3	66.7	90.6	84.2	78.4
3.5	66.7	90.6	84.2	78.4
4.5	50.0	93.8	85.7	71.4
5.0	50.0	96.9	92.3	72.1
5.93	50.0	100	100	72.7
SPT wheal				
diameter (mm)				
4.0	100	40.0	73.9	100
5.0	88.2	50.0	75.0	71.4
5.5	64.7	60.0	73.3	50.0
6.0	64.7	70.0	78.6	53.8
6.5	64.7	80.0	84.6	57.1
7.0	58.8	80.0	83.3	53.3
7.5	58.8	90.0	90.9	56.3
8.0	58.8	90.0	90.9	56.3
9.0	47.1	90.0	88.9	50.0
10	35.3	100	100	47.6

NPV, Negative predictive value; PPV, positive predictive value; sIgE, specific immunoglobulin E; SPT, skin prick test.

Bold indicates the cut-off values (3.15 kU/L and 7.5 mm).

TABLE E3. Different cutoff levels of sIgE to Cor a 14 in predicting clinical reactivity

rCor a 14 sIgE (kU/L)	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
0.1	95.5	64.0	70.0	94.1
0.35	86.4	88.0	86.4	88.0
0.4	86.4	88.0	86.4	88.0
0.47	81.8	92.0	90.0	85.2
0.48	81.8	96.0	94.7	85.7
0.5	81.8	100	100	86.2
0.6	81.8	100	100	86.2
0.63	81.8	100	100	86.2
0.7	81.8	100	100	86.2
0.8	77.3	100	100	83.3
0.9	72.7	100	100	80.6
1.0	72.7	100	100	80.6
2.0	59.1	100	100	73.5
5.0	45.5	100	100	67.6

NPV, Negative predictive value; PPV, positive predictive value; sIgE, specific immunoglobulin E.

TABLE E4. Comparison of hazelnut extract sIgE, Cor a 1, Cor a 8, Cor a 9, Cor a 14, Bet v 1, and skin prick test wheal diameter in children reactive and tolerant to hazelnut, and with and without sensitization to tree, grass, or weed pollens

	Pollen (–)			Pollen (+) (grass and/or tree and/or weed)			<i>P</i> ^{1*}	<i>P</i> ^{2†}
	Reactive to hazelnut	Tolerant to hazelnut	<i>P</i>	Reactive to hazelnut	Tolerant to hazelnut	<i>P</i>		
	n = 9	n = 14		n = 16	n = 12			
Hazelnut sIgE (kU/L)								
Positive if ≥0.35	8 (88.9%)	12 (85.7%)	1.000	15 (93.8%)	10 (83.3%)	.560	1.000	1.000
Median (IQR)	4.06 (1.78-7.95)	1.36 (0.50-2.94)	.030	9.07 (3.19-31.70)	0.92 (0.54-1.81)	.001	.193	.625
rCor a 1 (kU/L)	n = 7	n = 13		n = 15	n = 8			
Positive if ≥0.1	0	0	—	3 (20%)	0	.526	.523	—
Median (IQR)	0.00 (0.00-0.02)	0.01 (0.00-0.02)	.834	0.02 (0.01-0.08)	0.02 (0.00-0.04)	.579	.077	.136
rCor a 8 (kU/L)	n = 7	n = 13		n = 15	n = 9			
Positive if ≥0.1	1 (14.3%)	2 (15.4%)	1.000	9 (60%)	7 (77.8%)	.657	.074	.007
Median (IQR)	0.03 (0.00-0.06)	0.01 (0.00-0.03)	.747	0.21 (0.04-5.65)	0.46 (0.10-1.08)	.788	.022	<.001
Bet v 1 (kU/L)	n = 7	n = 13		n = 15	n = 10			
Positive if ≥0.1	0	0	—	4 (26.7%)	1 (10%)	.615	.263	.435
Median (IQR)	0.02 (0.01-0.05)	0.02 (0.01-0.03)	.869	0.04 (0.03-0.11)	0.04 (0.02-0.05)	.779	.107	.037
nCor a 9 (kU/L)	n = 7	n = 12		n = 13	n = 8			
Positive if ≥0.1	4 (57.1%)	11 (91.7%)	.117	10 (76.9%)	3 (37.5%)	.164	.618	.018
Median (IQR)	0.11 (0.07-0.69)	1.04 (0.18-2.38)	.043	1.04 (0.22-17.25)	0.05 (0.03-0.51)	.022	.122	.031
rCor a 14 (kU/L)	n = 7	n = 12		n = 13	n = 8			
Positive if ≥0.1	7 (100%)	4 (33.3%)	.013	13 (100%)	3 (37.5%)	.003	—	1.000
Median (IQR)	1.34 (0.79-9.81)	0.03 (0.02-0.20)	.001	4.33 (1.07-38.90)	0.02 (0.01-0.15)	<.001	.405	.391

IQR, Interquartile range; sIgE, specific immunoglobulin E.

**P*¹: Comparison between reactive to hazelnut and without pollen sensitization and reactive to hazelnut and with pollen sensitization.

†*P*²: Comparison between tolerant to hazelnut and without pollen sensitization and tolerant to hazelnut and with pollen sensitization.

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