

Jug r 1 sensitization is important in walnut-allergic children and youth



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Clinical Implications

- Measurement of specific IgE to Jug r 1 could be an important complement in the diagnosis of walnut allergy in children and youth due to its improved clinical specificity compared with IgE to walnut extract.

TO THE EDITOR:

Tree nut allergies have become a public health problem over the past 2 decades and managing nut allergies is a remaining clinical challenge.¹ Walnut (*Juglans regia*) is the most prevalent eaten tree nut in Japan and is the most common tree nut causing anaphylaxis.² If an allergy to tree nut is suspected, guidance for food removal is often given without any oral food challenge (OFC) being performed due to the high risk of anaphylaxis. IgE measurement to nut allergen components has been shown to increase the clinical specificity compared with measuring IgE to whole nut extract.³⁻⁶ However, the only walnut component study in children presented so far was a retrospective study based on doctor's diagnosis without obligatory OFC.⁶ The aim of the present study was to investigate the predictive value of measuring IgE to walnut and the walnut components Jug r 1 (2S albumin, storage protein) and Jug r 3 (nonspecific lipid transfer protein [nsLTP]) in Japanese children and youth suspected of walnut allergy in comparison with the results of OFC.

One-hundred and eight patients with suspected walnut allergy, who had a history of immediate reaction to walnut or sensitized to walnut, were enrolled in the study. All patients were defined as allergic or tolerant to walnut based on an OFC outcome (Table 1). A majority of the patients had atopic dermatitis (59%) and suffered from other food allergies (70%), and 33% had an asthma diagnosis (Table 1). Forty-two children (39%) had a history of reported allergic reaction to walnut. OFC was conducted according to the Japanese Food Allergy Guidelines and is described in this article's Online Repository at www.jaci-inpractice.org. Ethics approvals were obtained from the institutional review boards of the participating hospitals. Informed consent was obtained from all children and/or their parents. Detailed statistic method descriptions are presented in this article's Online Repository.

The cumulative dose of walnut resulting in objective allergic symptoms during OFC varied from 0.1 to 10 g (median dose, 3 g). Skin reactions were the most commonly observed symptoms during OFC (71%) and 6 children had moderate oral allergy syndrome (OAS) as the only symptom. More than half of

subjects had respiratory symptoms due to the challenge. Two patients had anaphylactic shock and were successfully treated.

The clinical efficacy of measuring IgE antibodies to walnut, Jug r 1, and Jug r 3 was evaluated using the ImmunoCAP technology (Thermo Fisher Scientific/Phadia AB, Uppsala, Sweden). The sIgE levels to walnut were significantly higher in the allergic group when compared with the tolerant group (Table 1). This was also true for Jug r 1, but not for Jug r 3. There was a nonsignificant trend that children with anaphylactic shock or lower respiratory symptoms during challenge had higher sIgE levels to Jug r 1 ($n = 31$; 6.76 kU_A/L, range: <0.1-100 kU_A/L) than allergic children with moderate OAS, skin, digestive, or upper respiratory symptoms ($n = 29$; 2.15 kU_A/L, range: <0.1-37.6 kU_A/L, $P < .10$).

Receiver operating characteristic (ROC) analysis showed that the Jug r 1 ImmunoCAP test had the largest area under the curve (0.843; 95% confidence interval [CI], 0.574-0.854) compared with the walnut test (0.792; 95% CI, 0.418-0.711) (Figure 1). Using the statistically optimal cutoff for sIgE to Jug r 1 (0.42 kU_A/L), determined from the ROC analysis, a clinical sensitivity and specificity of 85% and 79% was obtained, respectively (Table E1, available in this article's Online Repository at www.jaci-inpractice.org). Thus, 19 children (18%) would have been misclassified using the cutoff of 0.42 kU_A/L. The walnut ImmunoCAP test had both a lower clinical sensitivity (73%) and specificity (70%) at the optimal cutoff (2.6 kU_A/L), and 30 of the 106 children tested (28%) would then have been misclassified. By using the traditional 0.35 kU_A/L cutoff, a sensitivity of 98% was obtained for the walnut test compared with 88% for Jug r 1. The corresponding specificities in this study population was 77% for Jug r 1 and only 17% for walnut.

We show in this study that measuring sIgE to Jug r 1 increases the specificity of the diagnosis of walnut allergy compared with using complete walnut extract. This is aligned with other nut studies examining the clinical utility of sIgE testing to storage protein, for example, hazelnut,³ walnut,^{6,7} cashew and pistachio,⁴ and peanut.⁵ However, in a Dutch study by Blankstijn et al,⁸ IgE testing to Jug r 1 had no additional value compared with sIgE to walnut in diagnosing walnut allergy in adults. Masthoff et al³ observed that adult hazelnut-allergic patients had less sensitization to 2S albumin from hazelnut than children, indicating an age-related association. An explanation to their and our findings could be that tree pollen-related sensitization becomes a more frequent cause of nut allergy through adolescence and early adulthood. Only 24% of our subjects had allergic rhinitis compared with 86% in the Dutch walnut study, which supports this hypothesis. In addition, Jug r 1 is obviously not the only relevant walnut component. Other storage proteins besides Jug r 1 might also be of importance. The negative association between sIgE to Jug r 3 and walnut allergy observed in our study might be attributed to a primary sensitization to other highly cross-reactive nsLTPs in pollen or fruits, obviously with limited impact on tree nut allergy in the present population.

Despite the low prevalence, walnut allergy is one of the leading causes of food anaphylaxis. A high prevalence of respiratory symptoms in walnut- and pecan-allergic subjects was also observed by Maloney et al.⁹ We also observed that more than half of subjects who failed OFC reacted with respiratory symptoms

TABLE 1. Demographical and clinical characteristics of walnut-allergic and -tolerant subjects

Description	Allergic	Tolerant	P value
Number of subjects	60	48	
Sex, male/female	43/17	32/16	.68
Age (y), median [min, max]	6 [2, 20]	7 [2, 17]	.55
Total IgE (kU/L), median [min, max]	612 [36, 14,000]*	1260 [21, 7630]†	.013
History of allergic disease			
No. of subjects (%)			
Immediate reaction to walnut	32 (53%)	10 (21%)	.0007
Anaphylaxis to walnut	5 (8%)	0 (0%)	.06
Rhinitis	19 (32%)	5 (10%)	.01
Bronchial asthma	20 (33%)	16 (33%)	1
Atopic dermatitis	33 (55%)	31 (65%)	.33
Other food allergies	35 (58%)	41 (85%)	.003
Oral food challenge			
Total amount of reacting dose of walnut			
0.1-1 g	30	n/a	
>1-5 g	21	n/a	
>5-10 g	9	n/a	
No. of subjects (%) with reactions			
OAS symptoms	40 (67%)	0 (0%)	
Skin	43 (72%)	0 (0%)	
Respiratory	31 (52%)	0 (0%)	
Gastrointestinal	19 (32%)	0 (0%)	
Anaphylaxis shock	2 (3%)	0 (0%)	
Specific IgE (kU _A /L), median [min, max]			
Walnut	5.6 [0.34, >100]‡	1.1 [<0.1, 39.0]‡	<.001
Jug r 1	4.0 [<0.1, >100]	0.12 [<0.1, 52.8]	<.001
Jug r 3	<0.1 [<0.1, 13.3]‡	0.21 [<0.1, 8.5]§	.017

n/a, Not applicable; OAS, oral allergy syndrome.

Mann-Whitney *U*-test and Fisher's exact test (both 2-tailed) were used to compare the continuous and categorical data between groups, respectively.

*Missing values: 5.

†Missing values: 3.

‡Missing values: 1.

§Missing values: 2.

during the walnut provocation. This further implicates that improved diagnostics for walnut allergy is needed because challenge seems to be associated with severe symptoms in children.

We conclude that sIgE to Jug r 1 has additional value compared with extract-based testing in diagnosing walnut allergy in Japanese children and youth.

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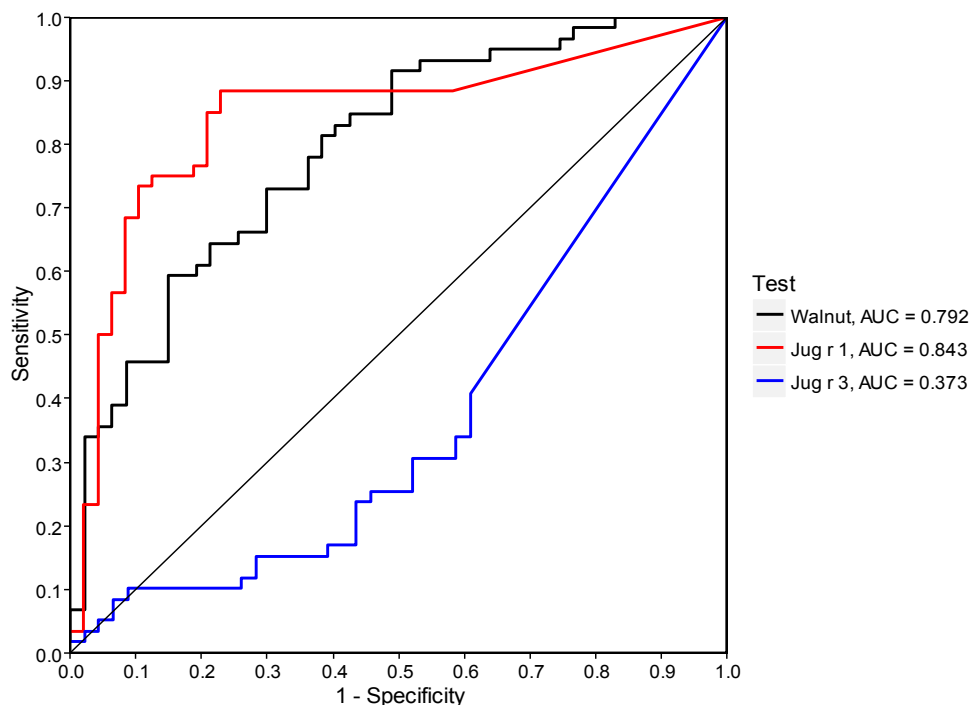


FIGURE 1. Receiver-operating characteristic curves for specific IgE to whole walnut extract and individual allergen components with regard to their ability to discriminate between allergy and tolerance to walnut. *AUC*, Area under the curve.

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METHODS

Oral food challenge

Oral food challenge (OFC) was performed in an open manner in the outpatient department or during hospitalization. The OFC was conducted according to the routines of the included hospitals and following the Japanese Food Allergy Guidelines.^{E1} Generally, increasing doses of roasted walnut, starting with 0.1 g (powder weight), were administered to patients every 15 to 60 minutes up to a cumulative dose of 0.5 to 3 g or until an objective allergic reaction was observed. In doubtful cases where slight or subjective symptoms were induced, the reproducibility of symptoms was investigated by repeated administration of walnut products at home, or patients were receiving up to 10 g of walnut until they developed an objective allergic reaction. Baked bread including roasted walnut powder or roasted walnut grains was used for the OFC. The most common bread used was a pumpkin cake (mixture of pumpkin, sorghum bicolor, sugar, baking soda, and water) including roasted walnut powder. Any immediate symptoms from skin, gastrointestinal tract, respiratory tract, and oral mucosa were observed and documented. Rescue medications (antihistamine, glucocorticoid, nebulized β_2 -agonist, and/or adrenaline) were immediately administered in the event of an allergic reaction based on the food allergy and anaphylaxis guidelines of European Academy of Allergy and Clinical Immunology (EAACI).^{E2,E3} If objective allergic reactions were not observed, patients were endorsed to regularly consume walnut products at home. Tolerance to walnut was confirmed by the absence of any symptoms after ingestion of walnut products including 3 to 10 g roasted walnut.

Statistical analysis

The statistical evaluation was performed in R version 3.3.1 (R Foundation for Statistical Computing, <https://www.R-project.org/>). Confidence intervals for receiver operating characteristic (ROC) curves were calculated with the method of DeLong et al^{E4} using the R package pROC.^{E5} The optimal cutoff for a test was estimated as the level that minimizes the distance between the corresponding point on the ROC curve and the point (1, 0). Confidence intervals for the binomial proportions, sensitivity, specificity, positive, and negative predictive values, were calculated with the Clopper-Pearson “exact” method.^{E6}

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TABLE E1. Assay performance for walnut and Jug r 1 ImmunoCAP at various cutoff levels

Test	Cutoff	Sensitivity		Specificity		PPV		NPV	
Walnut	>0.10	100.0	(93.9-100.0)	10.6	(3.5-23.1)	58.4	(48.2-68.1)	100.0	(47.8-100.0)
	>0.35	98.3	(90.9-100.0)	17.0	(7.6-30.8)	59.8	(49.3-69.6)	88.9	(51.8-99.7)
	>2.58*	72.9	(59.7-83.6)	70.2	(55.1-82.7)	75.4	(62.2-85.9)	67.3	(52.5-80.1)
Jug r 1	>0.1	88.3	(77.4-95.2)	41.7	(27.6-56.8)	65.4	(54.0-75.7)	74.1	(53.7-88.9)
	>0.35	88.3	(77.4-95.2)	77.1	(62.7-88.0)	82.8	(71.3-91.1)	84.1	(69.9-93.4)
	>0.423*	85.0	(73.4-92.9)	79.2	(65.0-89.5)	83.6	(71.9-91.8)	80.9	(66.7-90.9)

NPV, Negative predictive value; PPV, positive predictive value.

95% confidence intervals within parentheses.

*Optimal cutoff (estimated from receiver operating characteristic).

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