

Ana o 3—specific IgE is a predictive marker for cashew oral food challenge failure



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Clinical Implications

- Ana o 3—specific IgE is an important complement to cashew-specific IgE measurement as an aid in the diagnosis of cashew allergy due to its higher clinical specificity.

TO THE EDITOR:

Tree nut allergies are a public health problem and a challenge for the health care providers.¹ Although oral food challenge (OFC) is the criterion standard in food allergy diagnosis, diet restrictions for nuts are often given on the basis of proof of nut sensitization, even if there is no history of immediate reaction to nuts. This is mostly due to the fear of anaphylactic reactions during OFC.²

Cashew is a commonly eaten tree nut renowned for its ability to cause severe allergic reactions,^{3,4} and cashew allergy has increased.^{4,5} Ana o 3 is a major cashew allergen belonging to the 2S albumin protein family.⁵ Studies have shown that Ana o 3—specific IgE (sIgE) is diagnostic for cashew allergy.⁶⁻⁸ However, in these studies, not all patients were diagnosed using cashew OFC,^{6,7} or an experimental test based on purified native Ana o 3 was used.⁸ In the present study, we evaluated the clinical performance of a commercially available recombinant Ana o 3 test in a cohort of cashew-allergic and -tolerant Japanese children and adolescents, all diagnosed by OFC.

Ninety-five patients who were suspected to be cashew allergic on the basis of adverse reactions or just a sensitization to cashew (sIgE > 0.35 kU_A/L) were analyzed in the study (see Figure E1 in this article's Online Repository at www.jaci-inpractice.org). Twenty-one patients had a history of immediate reaction to cashew and 9 of them had experienced an anaphylactic reaction (Table 1). All patients underwent an OFC with up to 0.5 to 3 g of cashew powder following Japanese Food Allergy Guidelines as described in this article's Online Repository at www.jaci-inpractice.org. IgE antibodies to Ana o 3, cashew, walnut, hazelnut, Brazil nut, and almond were measured using ImmunoCAP (Thermo Fisher Scientific/Phadia AB, Uppsala, Sweden). Ethics approvals were obtained from the institutional review boards of the participating hospitals. Informed consent was obtained from all children and/or their parents.

Twenty-six patients failed the OFC (allergic) and 69 passed (tolerant) (Table 1). The median cumulative dose of cashew resulting in objective symptoms during OFC was 1.35 g (range, 0.1-10 g). The most commonly observed symptoms during OFC were from the oral mucosa (69%) and the gastrointestinal tract

TABLE 1. Demographical and clinical characteristics of cashew-allergic and -tolerant subjects diagnosed by OFC

Description	Allergic	Tolerant	P value
No. of subjects	26	69	
Sex (male/female)	11/15	47/22	.033
Age (y), median (min, max)	7 (2, 20)	6 (2, 26)	.146
Total IgE (kU/L), median (min, max)	755 (24, 14,000)*	1,390 (60, 16,300)	.024
History of allergic diseases and symptoms, n (%)			
Immediate reaction to cashew	14 (56)†	7 (10)‡	<.0001
Anaphylaxis to cashew	5 (20)†	4 (5.9)‡	.055
Other food allergies (all)	17 (68)†	52 (79)‡	.288
Other tree nut or peanut allergies	15 (60)†	35 (53)‡	.640
Allergic rhinitis	8 (30.5)	25 (36.2)	.809
Bronchial asthma	10 (38.5)	21 (30.4)	.471
Atopic dermatitis	12 (46.2)	52 (75.4)	.013
Oral food challenge			
Cumulative reacting dose of cashew			
0.1-1 g	10	NA	
>1-5 g	11	NA	
>5-10 g	5	NA	
Severity of reaction, no. of subjects (%)			
Grade 1	7 (27)	0 (0)	
Grade 2	15 (58)	0 (0)	
Grade 3	4 (15)	0 (0)	
Organ involvement, no. of subjects (%)			
Oral mucosa	18 (69)	0 (0)	
Skin	13 (50)	0 (0)	
Respiratory	10 (38)	0 (0)	
Gastrointestinal	19 (73)	0 (0)	
Cardiovascular	4 (15)	0 (0)	

NA, Not applicable.

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

*Missing values: 2.

†Missing values: 1.

‡Missing values: 3.

(73%). Four patients had severe reactions that were successfully treated.

The Ana o 3—sIgE levels were significantly higher in the allergic group than in the tolerant group (Figure 1, A). Significantly higher cashew sIgE levels were also found in the allergic group (Figure 1, B), but not with any of the other tree nut tests or almond. In fact, the cashew-tolerant patients had significantly higher sIgE levels than the cashew-allergic patients to both hazelnut and almond, probably a consequence of the patient

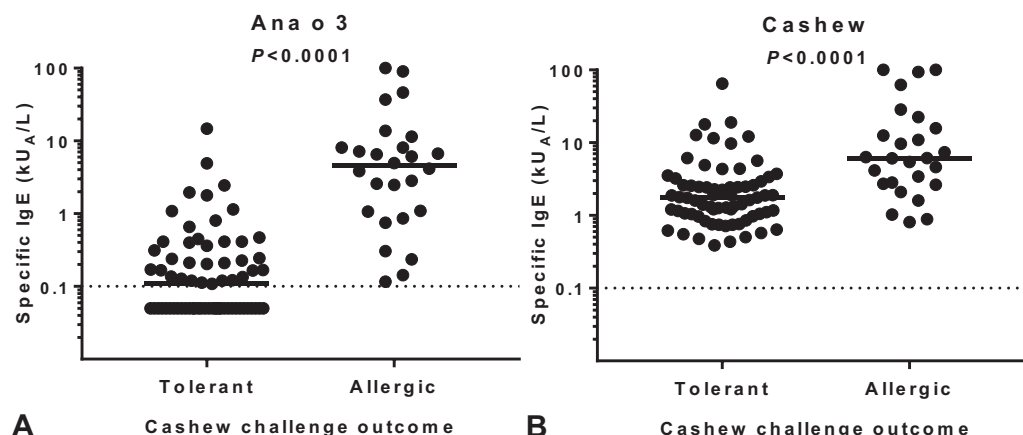


FIGURE 1. Specific IgE to (A) Ana o 3 and (B) cashew in cashew-allergic (n = 26) and -tolerant patients (n = 69), all diagnosed by OFC.

selection criteria (see Figure E2 in this article's Online Repository at www.jaci-inpractice.org). Severe symptoms at OFC was associated with higher specific IgE to Ana o 3 and cashew (see Figure E3 in this article's Online Repository at www.jaci-inpractice.org).

Receiver-operating characteristic analysis showed that the Ana o 3 test had a larger area under the curve (AUC, 0.920) compared with the cashew test (AUC, 0.791) (see Figure E4 in this article's Online Repository at www.jaci-inpractice.org). All cashew-allergic patients and about half the tolerant but cashew-sensitized patients (51%) had detectable Ana o 3-sIgE antibodies (>0.1 kU_A/L). By using 0.35 kU_A/L as cutoff, Ana o 3-sIgE had a sensitivity of 85% and a specificity of 77% (see Table E1 in this article's Online Repository at www.jaci-inpractice.org). The statistically optimal cutoff was 0.70 kU_A/L for Ana o 3 and 2.62 kU_A/L for cashew. It corresponded to a clinical sensitivity and specificity of 85% and 88% for Ana o 3 and 81% and 75% for cashew, respectively. Thus, 12 patients (13%) in the study would have been misclassified using the optimal cutoff for Ana o 3, and 22 (23%) using cashew. A positive decision point (PDP) based on at least 95% clinical specificity was calculated for the Ana o 3 test. The PDP was 2.2 kU_A/L and gave a clinical sensitivity of 69% and specificity of 96%. It means that only 1 of 20 tolerant patients would be wrongly misclassified as allergic by having Ana o 3-sIgE level greater than 2.2 kU_A/L, whereas about 7 of 10 cashew-allergic patients could be correctly diagnosed. It should be noted though that calculated PDPs often differ between studies, mainly due to choice of statistical methods and diagnostic measures (eg, based on clinical specificity, positive predictive value, or predictive probability from logistic regression) but also due to disease prevalence and demographic factors such as age of the patients.⁹ Accordingly, the present study with a wide patient age range should be repeated in more discrete age groups.

In this study, we show that testing for Ana o 3-sIgE improves the diagnostic workup in patients with a positive cashew test result by increasing the clinical specificity. This is aligned with other studies. Ana o 3-sIgE was strongly associated with cashew OFC failure in a recent Dutch study.⁸ However, that study used a native Ana o 3 preparation, and no sensitivity or specificity data were given. In a Greek pediatric study, sensitivity and specificity values as high as 94% were reported for Ana o 3-sIgE

(>0.35 kU_A/L).⁶ A German study including 61 infants and children with suspected cashew allergy showed that Ana o 3-sIgE had a sensitivity of 90% and specificity of 93% at 0.3 kU_A/L.⁷ The AUC for Ana o 3-sIgE in the present study was slightly lower than in the other 2 studies (0.944-0.977),^{6,7} which may be explained by differences in the inclusion criteria including the age of the patients and the diagnosis of cashew allergy. In our study, all patients were diagnosed by OFC, which reduced the risk of wrong allergy classification. Twenty-seven percent were found cashew allergic compared with 69% in the German study.⁷ In the Greek study, 63 cashew-allergic children were preselected and compared with 36 tolerant controls.⁶

We conclude that Ana o 3 is an important cashew allergen also in Japanese patients, and that Ana o 3-sIgE is a valuable marker for cashew allergy with a high clinical sensitivity and specificity.

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ONLINE REPOSITORY

METHODS

Inclusion criteria

We analyzed data from patients who received cashew OFC at participant facilities between November 2010 and December 2016. Inclusion criteria of this study were (1) history of immediate reaction to cashew, (2) positive cashew sIgE (>0.35 kU_A/L), and (3) sera available from patients within 365 days of cashew OFC. We excluded patients using oral steroid, anti-IgE antibody, and immunotherapy. We also excluded patients with insufficient serum sample volume for Ana o 3–sIgE analysis.

Oral food challenge

Open OFC was performed in the outpatient department or during hospitalization. The OFC was conducted following the routines of the included hospitals and according to the Japanese Food Allergy Guidelines.^{E1} Generally, increasing doses of roasted cashew, starting with 0.1 g (powder weight), were administered to patients every 15 to 60 minutes up to a dose of 0.5 to 3 g or until an objective allergic reaction was observed. The reproducibility of symptoms during OFC was investigated in doubtful cases where only slight or subjective symptoms were induced. It was done by repeated administration of cashew products at home. Alternatively, the patients were receiving up to 10 g of cashew until they developed an objective allergic reaction. For the OFC we used baked bread including roasted cashew powder or roasted cashew grains. The most common bread used was a pumpkin cake that besides cashew powder included a mixture of pumpkin, sorghum bicolor, sugar, baking soda, and water. 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 the European Academy of Allergy and Clinical Immunology.^{E2,E3} In case no objective allergic reactions were observed during the OFC, the patients were endorsed to regularly eat cashew at home. Tolerance to cashew was confirmed by the absence of any symptoms after ingestion of 3 to 10 g of roasted cashew.

Statistical analysis

The statistical evaluation was performed using GraphPad Prism version 7.03 (GraphPad Software, San Diego, Calif). The Mann-Whitney *U* test (2-tailed) was used for comparisons between the groups, and *P* values of less than .05 were considered significant.

Receiver-operating characteristic (ROC) analysis was performed for the Ana o 3 and cashew ImmunoCAP tests used for specific IgE measurement.^{E4} The optimal cutoff for a test was estimated as the level that minimizes the distance between corresponding point on the ROC curve and the point (1, 0), that is, “Distance to ROC.” From the ROC analysis also, sensitivity, specificity, and likelihood ratios were given for different cutoff levels of specific IgE.

Before statistical evaluation, all specific IgE values below the assay cutoff (0.1 kU_A/L) were assigned a value of 0.05 kU_A/L and all values above 100 kU_A/L (higher limit of quantitation) were assigned a value of 100 kU_A/L.

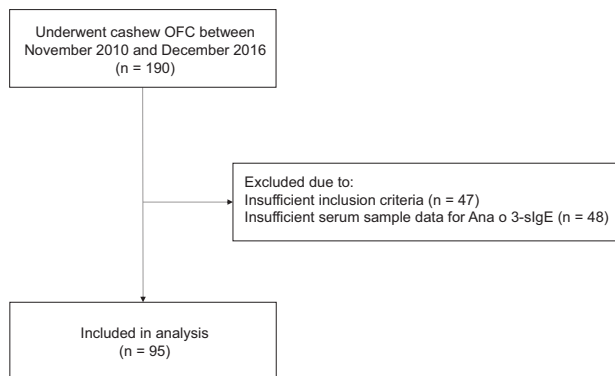


FIGURE E1. Flow diagram showing patient inclusion of this study.

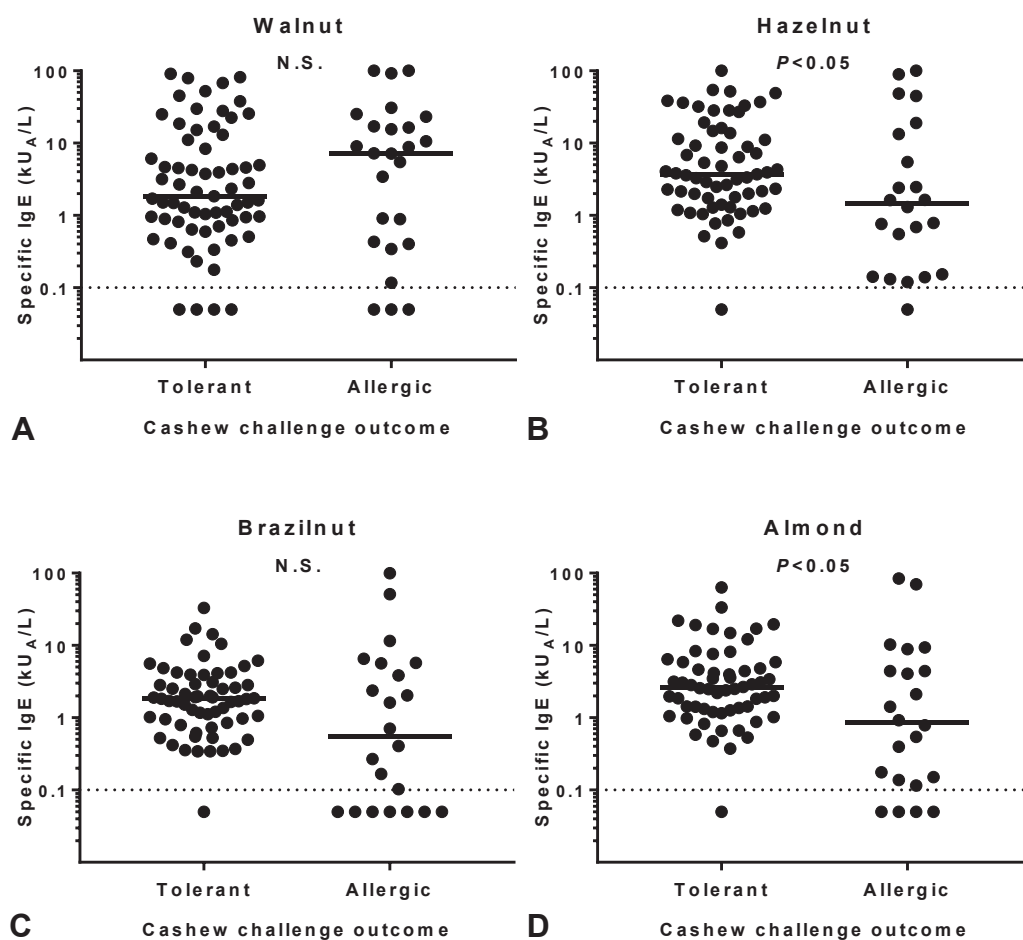


FIGURE E2. Specific IgE to (A) walnut, (B) hazelnut, (C) Brazil nut, and (D) almond in cashew-allergic (n = 26) and -tolerant patients (n = 69), all diagnosed by OFC. N.S., Not significant. Median levels are indicated.

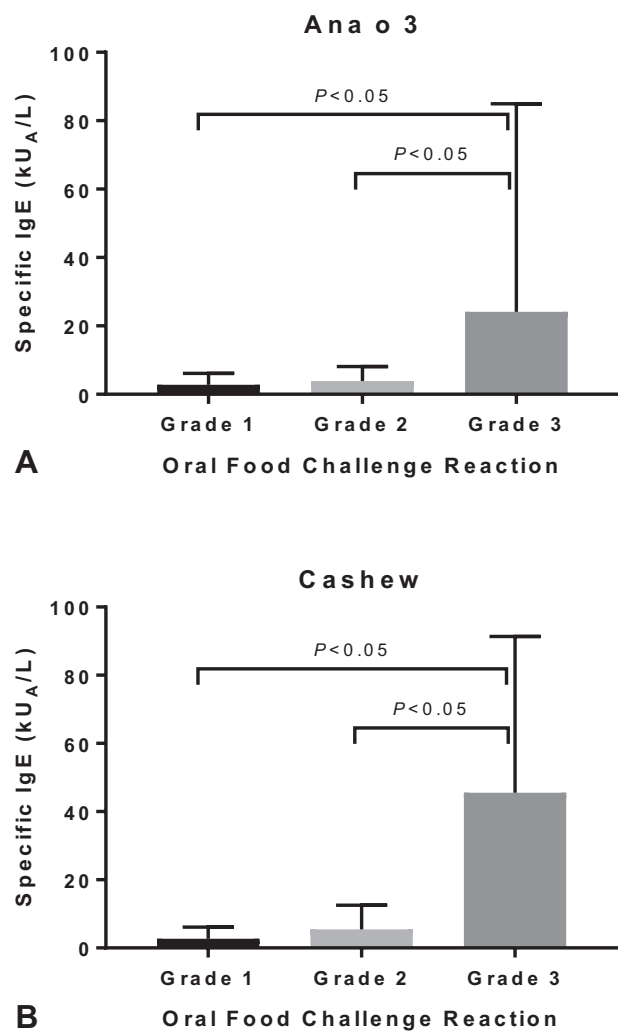


FIGURE E3. Specific IgE to (A) Ana o 3 and (B) cashew in cashew-allergic patients with mild (grade 1; $n = 7$), moderate (grade 2; $n = 15$), and severe (grade 3; $n = 4$) symptoms during OFC with cashew. Data are shown as median with interquartile range.

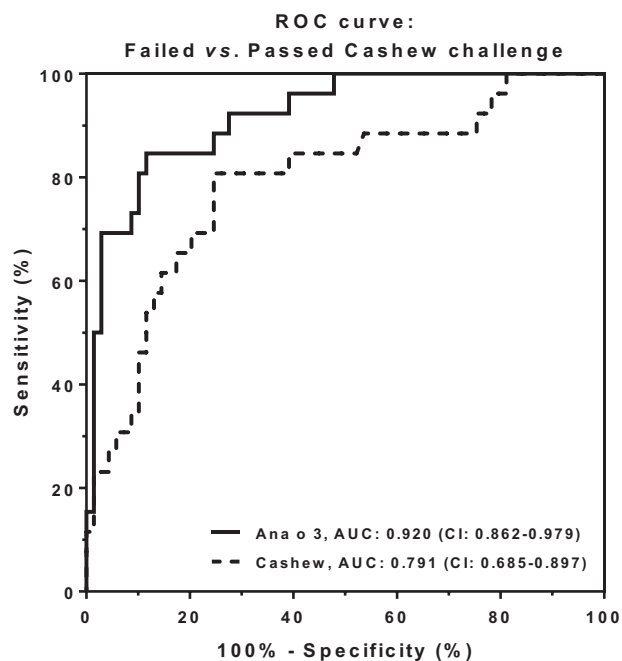


FIGURE E4. ROC curves for specific IgE to Ana o 3 (solid line) and cashew (dotted line) showing the ability to predict cashew OFC results in children and adolescents with suspected cashew allergy.

TABLE E1. Diagnostic performance of Ana o 3- and cashew-specific IgE at various cutoff levels (95% confidence intervals within parenthesis)

Test	Cut-off	Sensitivity, % (95% CI)	Specificity, % (95% CI)	Likelihood ratio
Ana o 3	>0.10	100.0 (86.8–100.0)	49.3 (37.0–61.6)	1.97
	>0.35	84.6 (65.1–95.6)	76.8 (65.1–86.1)	3.65
	>0.70*	84.6 (65.1–95.6)	88.4 (78.4–94.9)	7.30
	>2.20†	69.3 (48.2–85.7)	95.6 (87.8–99.1)	15.9
Cashew	>0.10	All positive	All positive	n/a
	>0.35	All positive	All positive	n/a
	>2.62*	80.8 (60.6–93.4)	75.4 (63.5–85.0)	3.28
	>14.20†	26.9 (11.6–47.8)	95.6 (87.8–99.1)	6.19

n/a, not applicable.

*Optimal cut-off (estimated as shortest distance to ROC).

†Positive decision point based on at least 95% clinical specificity.

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