

The Utility of Peanut Components in the Diagnosis of IgE-Mediated Peanut Allergy Among Distinct Populations

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What is already known about this topic? Recent studies have suggested the IgE to peanut components may be a more specific marker of peanut allergy than IgE to whole peanut.

What does this article add to our knowledge? This study adds confirmatory evidence to suggest IgE to the peanut component Ara h 2 may be the most specific, available marker for peanut allergy when used in patients with peanut sensitization and suspected peanut allergy.

How does this study impact current management guidelines? IgE to Ara h 2 may be a key diagnostic test to better differentiate patients with sensitization to whole peanut, but questionable peanut allergy.

BACKGROUND: Increasing data suggest that analysis of IgE to peanut components can be clinically helpful and possibly more accurate than IgE to whole peanut. Not all studies examining this topic, however, have used prospective samples, multiple components, and peanut challenges.

OBJECTIVE: We sought to determine the utility of peanut component testing, using a standardized, commercially available

test done before oral peanut challenge in various populations of patients with suspected peanut allergy from 2 different countries.

METHODS: IgE to whole peanut and the recombinant allergen components Ara h 1, 2, 3, and 8 were analyzed from serum samples drawn before double-blind peanut challenge from 4 distinct cohorts of patients with suspected peanut allergy from 2 nations (United States and Sweden).

RESULTS: Patients (n = 167; median age, 11.7 years; interquartile range, 7.0-15.0 years) had serum analyzed for peanut components and completed an oral food challenge to peanut. Although IgE to peanut was the most sensitive test (0.93), Ara h 2 was the most specific (0.92) and provided the best positive predictive value (0.94) of all the tests. Ara h 2 was also the best overall diagnostic test by receiver operating characteristic analysis (area under the curve, 0.84; $P < .05$).

CONCLUSIONS: In patients with suspected peanut allergy, IgE to peanut is a sensitive test but is not specific. IgE to Ara h 2 is a more specific and more accurate diagnostic test in this sampling of patients with suspected peanut allergy. Given each tests attributes, a stepwise approach to testing may provide clinicians with a way to minimize the need for peanut challenges. © 2013 American Academy of Allergy, Asthma & Immunology (J Allergy Clin Immunol: In Practice 2013;1:75-82)

Key words: Peanut allergy; Peanut sensitization; Food allergy; Ara h 2; Ara h 1; Ara h 3; Ara h 8; Anaphylaxis

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The prevalence of IgE-mediated peanut allergy appears to be increasing in developed nations. In the United States, the prevalence of self-reported peanut allergy increased from 0.4% in 1997 to 1.4% in 2008.¹ This increasing prevalence has led to higher numbers of visits to emergency departments and outpatient physician offices because of food-allergic reactions.² High rates of peanut sensitization have been found in population-based and birth cohort studies from around the world.³⁻⁵ It is important that physicians are able to accurately distinguish true peanut allergy (IgE-mediated allergic reaction to the ingestion of

Abbreviations used

IQR- Interquartile range

OFC- Oral food challenge

ROC- Receiver operating characteristic

SPT- Skin prick test

peanuts) from peanut sensitization alone (presence of IgE antibody to peanut but no clinical reaction).

Early studies reported the utility of skin prick testing and levels of IgE to peanut for establishing predictive values that have helped guide physicians in determining the likelihood of true peanut allergy and thus helped in many cases to distinguish sensitization from true allergy.⁶⁻⁹ Although these tests are quite sensitive, they lack specificity. *Component-resolved diagnostics* refers to the detection of IgE antibody against particular component allergens in an allergen mixture and has been described for peanut allergy.¹⁰⁻¹⁸ IgE against peanut storage proteins (Ara h 1, Ara h 2, and Ara h 3) are associated with severe allergic reactions, whereas IgE to Ara h 8, a structural homolog to the major allergen in birch pollen, is associated with mild or no symptoms.^{16,19,20} However, many of these studies were retrospective, did not challenge all patients with peanut, and/or did not use standardized methods for detecting peanut components.

In this study, we sought to determine the utility of peanut component testing, using a standardized, commercially available test done before oral peanut challenge in various populations and clinical settings from two different countries.

METHODS

Patient selection

The study was approved by local institutional review board for the following 4 sites: Mount Sinai, Consortium of Food Allergy Research (CoFAR), Sachs Open, and Sachs Double-blind.

At the Mount Sinai site, patients referred for a double-blind oral food challenge (OFC) to peanut from a university-based outpatient pediatric allergy clinic (Mount Sinai, New York, NY) were invited to participate. They were eligible for entry if they had a clinical history for peanut allergy and evidence of peanut sensitization, either a positive skin prick test (SPT) to peanut extract (≥ 3 mm) and/or IgE to peanut ≥ 0.35 kU_A/L and <15 kU_A/L. Serum samples were drawn from all patients before the OFC and stored in freezer at -80°C until analysis.

At the CoFAR site, serum samples from patients with peanut allergy who are a part of an ongoing trial of peanut sublingual immunotherapy at 5 centers in the United States were analyzed. Inclusion criteria for subjects in this study included physician-diagnosed peanut allergy or a convincing clinical history of peanut allergy, evidence of peanut sensitization (positive SPT and/or IgE to peanut ≥ 0.35 kU_A/L), and a positive double-blind OFC to peanut.

At the Sachs Open site, patients referred to Sachs's Children's Hospital, Stockholm, Sweden, for evaluation of peanut allergy were eligible for entry if they had a suspicion for peanut allergy and evidence of peanut sensitization, either a positive SPT to peanut extract (≥ 3 mm) and/or IgE to peanut ≥ 0.35 kU_A/L. Patients in this group typically had lower suspicion of peanut allergy than patients in the Sachs Double-blind group below, or chose not to participate in a double-blind challenge. These subjects therefore had open food challenge performed in the

TABLE I. Symptom score to evaluate reactions during OFC

Symptom score	Symptoms
0	No symptoms
1	Abdominal pain that resolved without medical treatment, rhinoconjunctivitis, or urticaria <10 papules/hives, rash
2	One organ involved * Abdominal pain requiring treatment * Generalized urticaria * Nonlaryngeal angioedema * Mild asthma (cough, fall of peak expiratory flow $<20\%$)
3	Two organs involved (of symptoms mentioned under 2)
4	Three organs involved (of symptoms mentioned under 2) or asthma requiring treatment or laryngeal edema, or hypotension
5	Cardiac and respiratory symptoms requiring hospitalization in the intensive care unit

clinical setting. Serum samples were drawn from all patients before the OFC.

At the Sachs Double-blind site, patients referred to Sachs's Children's Hospital, Stockholm, Sweden, for evaluation of possible peanut allergy were eligible for entry if they had a suspicion for peanut allergy and evidence of peanut sensitization, either a positive SPT to peanut extract (≥ 3 mm) and/or IgE to peanut ≥ 0.35 kU_A/L. These patients had higher suspicion for peanut allergy on the basis of baseline peanut testing and clinician assessment and were considered higher risk than the Sachs Open group above. All subjects in this group agreed to participate in double-blind peanut challenge. Serum samples were drawn from all patients before the double-blind OFC and stored until analysis.

OFC

OFCs were performed per each respective facility's practices. Briefly, peanut was fed in increasing doses, every 15 to 30 minutes until a cumulative dose of 5 g of peanut protein or 10 g of peanut flour was reached. Peanut challenge and placebo challenge (if conducted) were done on sequential days or done on same day, separated by 4 hours. Symptoms for this analysis were scored according to previous published scoring system (Table I).²¹ A negative test was defined as no objective allergic symptoms during and for 2 hours after the OFC was completed.

Serum IgE analysis

IgE to whole peanut and the recombinant allergen components Ara h 1, 2, 3, and 8 were determined in serum with ImmunoCAP (Thermo Fisher Scientific, Uppsala, Sweden), according to the manufacturer's instructions. In some cases, serum was frozen at -80°C before analysis.

Statistical analysis

IgE levels for both peanut and peanut components were reported with lower limit detection values of either 0.35 kU_A/L or 0.1 kU_A/L, depending on the laboratory and software used for analysis. For groups that used a lower limit of detection of 0.35 kU_A/L, all values <0.35 kU_A/L were recorded as 0.175 kU_A/L for analysis. For groups that used a lower limit of detection of 0.10 kU_A/L, all values <0.10 kU_A/L were recorded as 0.05 kU_A/L for analysis.

TABLE II. Patient characteristics and peanut challenge results

	All patients (n = 167)	Mount Sinai (n = 45)	CoFAR (n = 35)	Sachs Open (n = 48)	Sachs Double-blind (n = 39)
Age, y, median (IQR)	11.7 (7.0-15.0)	7.3 (5.0-10.5)	15.0 (13.6-18.1)	9.0 (6.0-13.5)	11.8 (7.7-14.1)
Sex, male, no. (%)	108 (64.7)	31 (68.9)	23 (65.7)	32 (66.7)	22 (56.4)
Positive oral peanut challenge, no. (%)	106 (63.5)	24 (53.3)	35 (100)	21 (43.8)	26 (66.7)
Reaction severity, mean $\pm$ SD	2.4 $\pm$ 1.1	1.9 $\pm$ 0.6	2.5 $\pm$ 0.8	2.0 $\pm$ 1.5	2.8 $\pm$ 1.2

TABLE III. Total peanut and peanut component serum IgE levels

IgE	All patients (n = 167)	Mount Sinai (n = 45)	CoFAR (n = 35)	Sachs Open (n = 48)	Sachs Double-blind (n = 39)
Peanut, kU _A /L, median (IQR)	3.14 (0.97-31.29)	1.43 (0.70-2.66)	26.10 (3.10-63.00)	1.90 (0.55-5.70)	36.46 (1.30-136.00)
Positive peanut, %*	89.7	86.4	100	85.1	89.7
rAra h 1, kU _A /L, median (IQR)	0.18 (0.05-9.20)	0.05 (0.05-0.11)	8.16 (0.50-38.80)	0.18 (0.18-0.18)	11.47 (0.05-32.41)
Positive Ara h 1, %*	40.5	8.9	77.1	20.5	66.7
rAra h 2, kU _A /L, median (IQR)	0.55 (0.18-13.95)	0.25 (0.05-0.53)	19.82 (1.96-55.25)	0.18 (0.18-0.74)	12.70 (0.05-67.01)
Positive Ara h 2, %*	54.2	37.8	91.4	29.8	69.2
rAra h 3, kU _A /L, median (IQR)	0.18 (0.05-1.79)	0.05 (0.05-0.15)	0.56 (0.05-8.88)	0.18 (0.18-0.18)	1.94 (0.12-12.84)
Positive Ara h 3, %*	34.2	11.1	54.3	14.3	64.1
rAra h 8 IgE, kU _A /L, median (IQR)	0.18 (0.05-6.13)	0.17 (0.05-3.01)	0.05 (0.05-0.28)	1.02 (0.18-15.5)	0.30 (0.05-10.58)
Positive Ara h 8, %*	43.1	40.0	20.0	58.3	48.7

*Positive IgE considered value at ≥ 0.35 kU_A/L.

Comparison of medians was done with the Mann-Whitney test and comparison between categorical data used the Fisher exact test. For reaction severity according to Astier, one-way ANOVA was used to measure variance among the four groups.

RESULTS

Baseline characteristics

In total, 167 patients had serum analyzed for peanut components and completed an OFC to peanut. The median age of all patients was 11.7 years [interquartile range (IQR), 7.0-15.0 years]. The patients in the CoFAR group were older than the other patients ($P < .005$) and those in the Mount Sinai cohort were younger than the other patients ($P < .005$). Most were male ($n = 108$; 64.7%), and this breakdown was similar in all groups (Table II).

OFC results

Breakdown of results of peanut challenges are presented in Table II. Including all patients, 106 (63.5%) of the patients reacted on peanut challenge. By definition, all patients in the CoFAR cohort reacted on peanut challenge, whereas 66.7% reacted in the Sachs Double-blind group, 53.3% reacted in the Mount Sinai group, and 43.8% reacted in the Sachs Open group.

The severity of reaction was different among the groups ($P = .01$). The Sachs Double-blind group had the most severe reactions overall (mean score, 2.8), whereas the Mount Sinai cohort had the milder reactions overall (mean score, 1.9).

Serum peanut and peanut component IgE levels

The median peanut IgE level was 3.14 kU_A/L (IQR, 0.97-31.29 kU_A/L) in the whole group. The levels were the highest in the Sachs Double-blind group (median, 36.46 kU_A/L; IQR, 1.30-136.00 kU_A/L) and lowest in the Mount Sinai group (median, 1.43 kU_A/L; IQR, 0.70-2.66 kU_A/L; Table III).

When examining the peanut components, IgE to Ara h 2 was most often found to be positive (≥ 0.35 kU_A/L) in each group other than the Sachs Open cohort, in which IgE to Ara h 8 was most often present. (The cutoff of 0.35 kU_A/L is used because software for some laboratories involved in this study set this as the limit and did not report values below this level.) In addition, the median value of IgE to Ara h 2 was higher than other component values for each group other than the Sachs Open cohort.

IgE to Ara 1 was present in 40.5% of all samples and was highest in the Sachs Double-blind group with a median of 11.47 kU_A/L. IgE to Ara 3 was present in only 34.2% of all samples and was the also highest in the Sachs Double-blind group, but with a median of only 1.94 kU_A/L.

Serum IgE levels and challenge outcomes

Peanut and peanut component IgE levels were compared between those patients that failed OFCs and those patients that passed OFC. To examine the utility of each test, comparisons were made within each cohort (excluding CoFAR, because there were no negative OFC in this cohort). Median IgE levels and frequency of positive tests compared between patients with positive and negative OFCs are displayed in Table IV. IgE to Ara h 2 was the only test that was different between those patients that failed and those patients that passed OFC in each cohort, and this was true when comparing the medians, as well as when comparing percentage of positive tests between the two challenge outcome groups. For the Sachs Double-blind cohort, IgE to peanut, Ara h 1, and Ara h 3 was also statistically different between challenge outcome groups.

When combining all patient data, the median values of IgE to peanut, Ara h 1, Ara h 2, and Ara h 3 were each significantly higher in those patients that failed the OFC, while Ara h 8 was significantly higher in those patients that passed the OFC. When

TABLE IV. IgE results by challenge outcome

IgE	Mount Sinai (n = 45)			Sachs Open (n = 48)			Sachs Double-blind (n = 39)			All patients (n = 167)		
	Positive OFC (n = 24)	Negative OFC (n = 21)	P value	Positive OFC (n = 21)	Negative OFC (n = 27)	P value	Positive OFC (n = 26)	Negative OFC (n = 13)	P value	Positive OFC (n = 106)	Negative OFC (n = 61)	P value
Peanut IgE, kU _A /L, median (IQR)	1.32 (0.59-2.40)	1.58 (0.73-3.37)*	.42	2.80 (0.61-14.0)	1.80 (0.43-3.60)*	.18	102.2 (27.76-214.4)	1.01 (0.25-6.78)	<.001 [†]	8.29 (1.42-64.95)	1.50 (0.61-3.80)	<.001 [†]
Positive peanut IgE, % [‡]	83.3	90.0	.67	85.7	84.6	.999	100	30.8	.009 [†]	93.4	83.1	.06
rAra h 1 IgE, kU _A /L, median (IQR)	0.05 (0.05-0.05)	0.05 (0.05-0.13)	.58	0.18 (0.18-7.54)	0.18 (0.18-0.18) [§]	.34	20.76 (10.15-48.38)	0.05 (0.05-0.14)	<.001 [†]	1.86 (0.05-19.37)	0.18 (0.05-0.18)	<.001 [†]
Positive Ara h 1, % [‡]	4.2	9.5	.999	27.8	15.4	.45	92.3	15.4	<.001 [†]	56.3	13.3	<.001 [†]
rAra h 2 IgE, kU _A /L, median (IQR)	0.44 (0.27-0.78)	0.05 (0.05-0.16)	<.001 [†]	0.64 (0.18-6.85)	0.18 (0.18-0.18)*	.005 [†]	52.44 (12.33-93.02)	0.05 (0.05-0.18)	<.001 [†]	4.30 (0.44-46.07)	0.18 (0.05-0.18)	<.001 [†]
Positive Ara h 2, % [‡]	66.7	4.8	<.001 [†]	55.0	8.0	<.001 [†]	96.2	15.4	<.001 [†]	80.0	8.2	<.001 [†]
rAra h 3 IgE, kU _A /L, median (IQR)	0.05 (0.05-0.10)	0.05 (0.05-0.15)	.72	0.18 (0.18-0.83)	0.18 (0.18-0.18) [§]	.13	7.24 (0.76-32.13)	0.05 (0.05-1.39)	<.001 [†]	0.32 (0.05-7.20)	0.18 (0.05-0.18)	<.001 [†]
Positive Ara h 3, % [‡]	16.7	4.8	.35	29.4	4.0	.03 [†]	80.8	30.8	.004 [†]	48.0	10.2	<.001 [†]
rAra h 8 IgE, kU _A /L, median (IQR)	0.20 (0.05-2.40)	0.14 (0.05-6.57)	.76	0.93 (0.18-16.00)	1.10 (0.18-8.90)	.93	0.20 (0.05-6.97)	6.13 (0.05-27.54)	.16	0.18 (0.05-3.72)	1.20 (0.16-10.88)	.005 [†]
Positive Ara h 8, % [‡]	37.5	42.9	.77	47.6	63.0	.38	38.5	69.2	.10	34.9	57.4	.006 [†]

*One patient with missing serum IgE data.

[†]Statistically significant.[‡]Positive IgE considered value ≥ 0.35 kU_A/L.[§]Three patients with missing serum IgE data.

TABLE V. Test characteristics

IgE	Mount Sinai (n = 45)				Sachs Open (n = 48)				Sachs Double-blind (n = 39)				All patients (n = 167)			
	Sensitivity	Specificity	PPV	NPV	Sensitivity	Specificity	PPV	NPV	Sensitivity	Specificity	PPV	NPV	Sensitivity	Specificity	PPV	NPV
Peanut	0.83	0.10	0.53	0.33	0.86	0.15	0.45	0.57	1.00	0.31	0.74	1.00	0.93	0.17	0.67	0.59
rAra h 1	0.08	0.90	0.50	0.46	0.28	0.85	0.56	0.63	0.92	0.85	0.92	0.85	0.56	0.87	0.88	0.54
rAra h 2	0.67	0.95	0.94	0.71	0.55	0.92	0.85	0.72	0.96	0.85	0.93	0.92	0.80	0.92	0.94	0.73
rAra h 3	0.17	0.95	0.80	0.50	0.29	0.96	0.83	0.67	0.81	0.69	0.84	0.64	0.48	0.90	0.89	0.50
rAra h 8	0.38	0.57	0.50	0.44	0.48	0.37	0.37	0.48	0.38	0.31	0.53	0.20	0.35	0.43	0.51	0.27

PPV, Positive predictive value; NPV, negative predictive value.

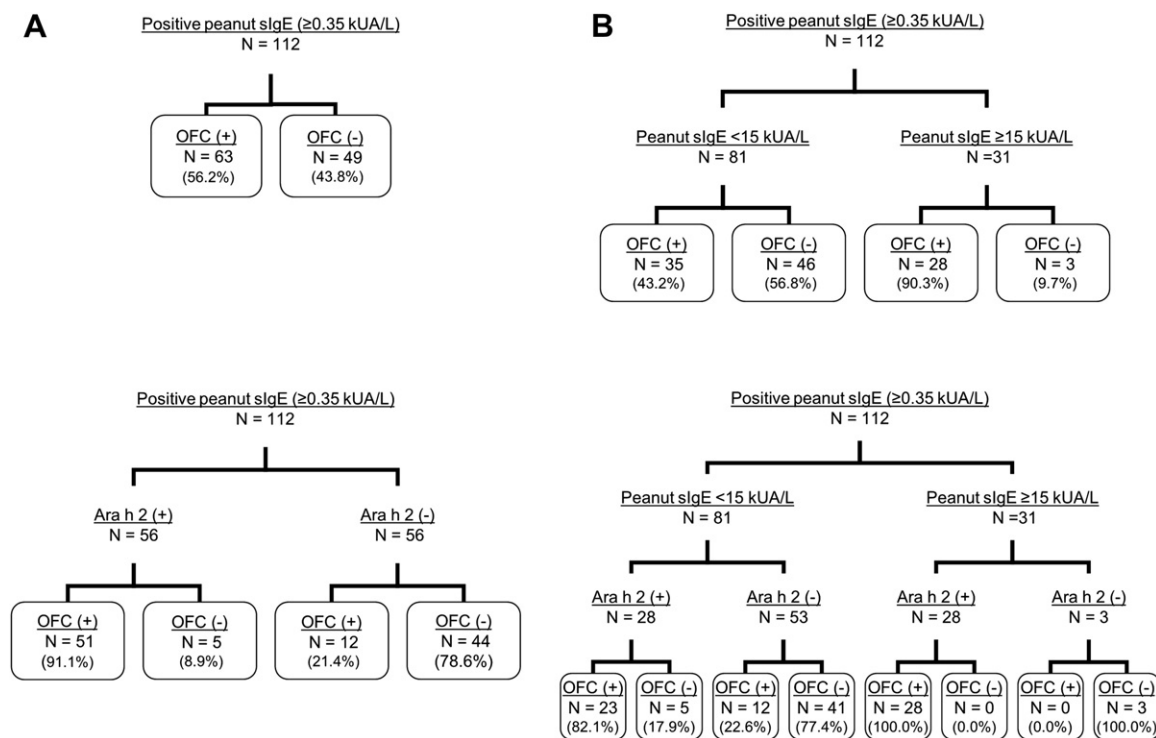


FIGURE 1. Stepwise diagnostic approach that used IgE to peanut and then Ara h 2 with cutoff values of <0.35 kUA/L. Results are broken down (A) by taking all subjects with a positive IgE to peanut in the study and then (B) by separating out those subjects with a high likelihood of reaction on the basis of IgE to peanut ≥ 15 kUA/L.

comparing the percent positive tests for all patients combined, IgE values to Ara h 1, Ara h 2, and Ara h 3 were more often positive in those patients that failed the OFC, whereas IgE values to Ara h 8 were more often positive in those patients that passed the OFC.

We also examined the utility of each test as a diagnostic marker of food challenge outcome (Table V). IgE to peanut is the most sensitive marker (of those examined) for challenge-proven peanut allergy in each cohort, with sensitivities of 0.83, 0.86, and 1.00 in the Mount Sinai, Sachs Open, and Sachs Double-blind cohorts, respectively. The specificity of peanut IgE was lacking for all cohorts however, with a maximum specificity of 0.31. The individual peanut components were each more specific for peanut allergy than peanut IgE, but they lacked sensitivity. Ara h 2 IgE provided the best overall specificity and positive predictive values when looking at all groups, reaching specificity as high as 0.95, with a positive predictive value of 0.94 in the Mount Sinai cohort.

Stepwise testing approach

Because the analyzed serum tests have differing attributes for sensitivity and specificity, we sought to determine how a stepwise approach with the use of the tests in combination would predict challenge outcomes. A simple algorithm that used only 2 tests, IgE to peanut and Ara h 2, is displayed in Figure 1. Examining all 112 patients with a positive IgE to peanut (≥ 0.35 kUA/L), one can see that 63 (56.3%) of the OFCs resulted in a reaction. If one, however, examines the Ara h 2 IgE value in those 112 patients, only 56/112 (50.0%) had a positive IgE value to Ara h 2 (≥ 0.35 kUA/L). Of those 56 patients, 51 (91.1%) had reactions on challenge. However, of the 56 patients who had IgE to peanut, but negative IgE to Ara h 2, only 12 (21.4%) had reactions on challenge (Figure 1A).

We also analyzed the data by separating those patients with a peanut IgE level ≥ 15 kUA/L, a value that has been reported to be 95% to 100% predictive of reaction,^{9,22} because these patients

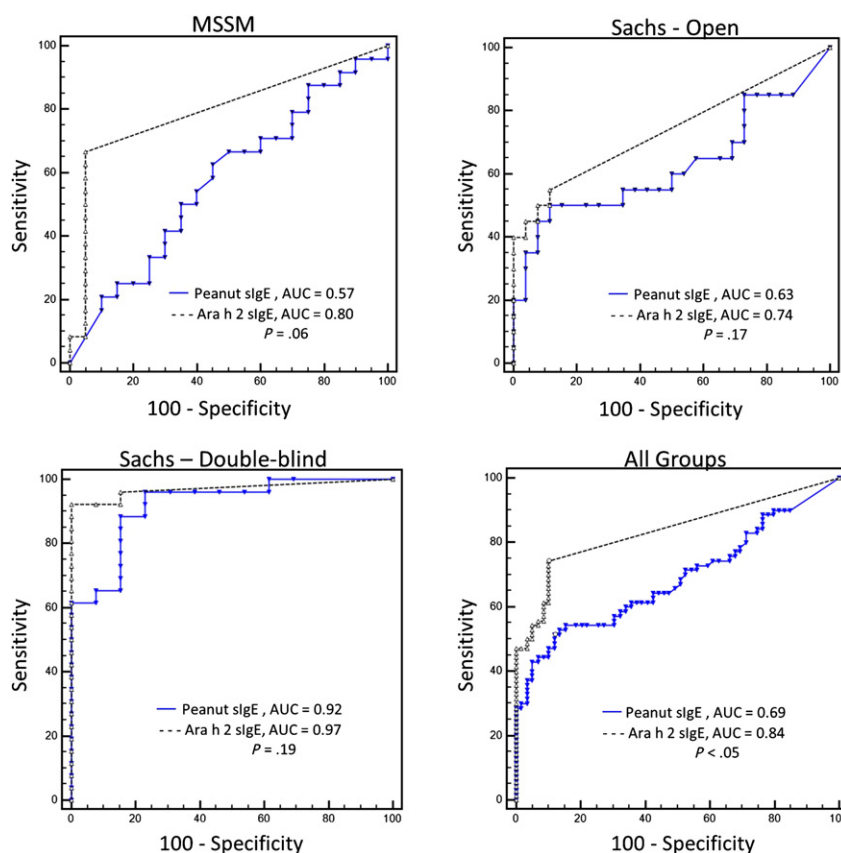


FIGURE 2. ROCs for each cohort that had both positive and negative peanut challenges and for all cohorts combined. *AUC*, Area under the curve, *MSSM*, Mount Sinai School of Medicine; *sIgE*, serum IgE.

would not likely be offered challenge in typical clinical settings on the basis of the IgE to peanut alone (Figure 1B). There were a resultant 81 patients with IgE to peanut <15 kU_A/L, of which 35 (43.2%) reacted on challenge. Of those 81 patients, 28 had a positive value of IgE to Ara h 2 (≥ 0.35 kU_A/L), of which 23/28 (82.1%) reacted. Interestingly, 53 of the 81 patients with positive peanut IgE <15 kU_A/L had negative IgE Ara h 2 test, and only 12 of those 53 patients (22.6%) reacted on challenge. By contrast, of the 31 patients who had peanut IgE ≥ 15 kU_A/L, all 28 who had concomitant positive IgE to Ara h 2 reacted, but the 3 who had no detectable IgE to Ara h 2 passed their peanut OFC without a reaction, despite high peanut serum IgE levels (21.00, 36.59, and 46.89 kU_A/L).

ROC analyses

Receiver operating characteristic (ROC) analyses for both IgE to peanut and Ara h 2 are displayed in Figure 2 for each group that had both positive and negative OFCs. The area under the curve was greater for Ara h 2 in all groups; however, this was only significant when combining all 3 groups ($P < .005$) because of the small sample sizes in each single group.

DISCUSSION

We present data from peanut component analysis drawn before oral peanut challenge in patients with suspected peanut allergy from various cohorts in 2 different nations. This work is similar to some prior studies on peanut component testing.

However, it is the first to examine peanut component analysis with the use of standardized methods from multiple cohorts of patients with suspected peanut allergy, all of whom underwent oral peanut challenges, which was not the case in many previous studies.

The data suggest that IgE to Ara h 2 is a more specific test for challenge-proven peanut allergy than IgE to peanut, with specificities ranging from 85% to 95%, depending on the cohort examined. This finding is in line with prior reports that used birth cohorts.⁴ Dang et al¹⁷ examined a birth cohort in Australia by examining IgE levels to peanut and Ara h 2 in patients with evidence of positive SPT to peanut (≥ 1 mm) who later had OFC to peanut. The investigators found that IgE to Ara h 2 provided a specificity of 93% at a cutoff value of 0.35 kU_A/L.¹⁷ Codreanu et al¹³ examined the utility of peanut components in French cohorts and reported a specificity of 93% for IgE to Ara h 2 at a cutoff value of 0.23 kU_A/L. These similar values across cohorts are mirrored in our data and suggest that the test should be applicable across most populations. One must understand, however, that sensitization patterns differ according to geographic locations and populations, as evidenced by the higher levels of IgE to Ara h 8 in the Sachs Open cohort and that these differences may be clinically relevant, as with Ara h 9 sensitization being clinically relevant in Mediterranean populations.¹⁴

With the use of ROC analysis for each group that had both positive and negative OFCs, there was a trend suggesting that IgE to Ara h 2 is a superior diagnostic test compared with IgE to

peanut. This trend became significant when combining the 3 patient populations. With the use of similar analyses, past studies have suggested values, above which most patients react, providing clinically valuable cutoffs. One must realize though that these predictive values are prevalence dependent; therefore, they change, depending on the prevalence of the disease in a given population, and must be interpreted with caution.²³ If we use the true prevalence of disease for each of our specific cohorts, we can create predictive values for each group individually. For the Mount Sinai cohort, an IgE to Ara h 2 of ≥ 0.35 kU_A/L provides a 94% predictive value. For the Sachs Open cohort a value of ≥ 0.75 kU_A/L provides 90% predictive value, and for the Sachs Double-blind cohort a value of ≥ 0.35 kU_A/L provides 93% predictive value. As one can see, although these values vary slightly, they suggest that in cohorts in which peanut allergy is suspected (at a prevalence of approximately 50%), any positive IgE to Ara h 2 (≥ 0.35 kU_A/L) is suggestive of a reaction. Clinicians must understand their own patient population, however, to know the best application of these diagnostic values.

We demonstrate, for the first time in a prospective, challenge-proven peanut allergy study, that, compared with IgE to Ara h 1, 3, and 8, Ara h 2 was the most sensitive and specific and offered the highest positive and negative predictive values (Table V, all patients).

With the increased specificity of IgE to Ara h 2 over IgE to peanut comes a decrease in sensitivity. This is to be expected, however, because examining only one component of the total peanut extract could miss sensitivity to other relevant proteins. Because of this, we suggest a stepwise approach with the use of IgE to peanut and then Ara h 2 to increase the diagnostic accuracy for challenge-proven peanut allergy. With the use of this stepwise approach applied to our cohorts (excluding the CoFAR group that had no negative challenges), IgE to peanut alone would lead to 81 OFCs to find 46 peanut-tolerant persons. This is excluding all patients with IgE to peanut ≥ 15 kU_A/L. By simply adding IgE levels to Ara h 2 as a second diagnostic step to all patients with a positive IgE to peanut and only challenging patients who have undetectable levels of IgE to Ara h 2 (< 0.35 kU_A/L) no matter their level of IgE to peanut, we can decrease the number of challenges to 56 and still find 44 tolerant patients. Although this approach would find 3 tolerant patients who had IgE to peanut ≥ 15 kU_A/L who would not otherwise have been challenged, it would incorrectly identify 5 of 46 (11%) peanut-tolerant patients with IgE to peanut < 15 kU_A/L who would not be challenged because of a positive IgE to Ara h 2. A similar approach with similar results was proposed by Dang et al.¹⁷ However, the study by Dang et al¹⁷ was directed at birth cohorts and therefore applicable to a general pediatric population, whereas our approach is directed toward patients referred to allergists for suspected peanut allergy and positive serum IgE to peanut.

Perhaps even more interesting about using this algorithm is the utility Ara h 2 IgE determinations can add to patients with high IgE levels to peanut. Currently, most patients with IgE levels of peanut of ≥ 15 kU_A/L are considered allergic to peanuts without any further consideration. However, no test is 100% accurate, and clearly there are patients with high levels of IgE to peanut that tolerate peanut.^{20,24} In this analysis, of the 31 patients with IgE levels to peanut ≥ 15 kU_A/L, all 3 patients who tolerated peanut on challenge had an Ara h 2 IgE of < 0.35 kU_A/L, whereas all 28 with an Ara h 2 IgE of ≥ 0.35 kU_A/L failed the challenge. Thus, 9.7% of the patients with peanut IgE ≥ 15 kU_A/L tolerated

peanut in these cohorts and all of them could be identified by simply adding the Ara h 2 IgE testing. Thus, although it is clear that most patients with high IgE levels to peanut will react on challenge, the benefits of offering challenges to the selected group of these patients with no detectable IgE to Ara h 2 testing appear to outweigh the risks.

One must remember that no test is 100% accurate. There were 12 patients who had a positive IgE to peanut and negative IgE to Ara h 2 (< 0.35 kU_A/L) who reacted on challenge. Of note, all 12 of these patients had peanut IgE < 15 kU_A/L. Interestingly, 7 (58.3%) of these patients did have detectable IgE to Ara h 2 with the use of a cutoff of ≥ 0.10 kU_A/L. This suggests that even low levels of IgE to Ara h 2 may be clinically relevant.²⁵ However, these low levels point to a weakness of this study, which is that various laboratories report values below 0.35 kU_A/L and others do not. For that reason, we used 0.35 kU_A/L as the threshold for a positive test in this report. In addition, some of these patients' reactions (58.3%) can be explained by binding to other components; 1 had IgE to Ara h 3 and 6 had IgE to Ara h 8 (of which 4 had only oral pruritus on challenge). On the basis of these data, when there is clinical suspicion for peanut allergy, one should not rely solely on any single test, and, even in the setting of a negative serum IgE level to peanut and/or Ara h 2, a clinician must rely on the history to decide when and how safe it is to introduce peanut into a patient's diet.

By contrast, there were 5 patients who had a positive IgE to both peanut (range, 0.45-9.15 kU_A/L) and Ara h 2 (range, 0.75-2.32 kU_A/L) that still passed their OFC. These patients are quite interesting, and there is little data present to suggest why these patients do not react on challenge; however, it should be noted that they have low levels of IgE to Ara h 2. One theory is that these patients' IgE recognizes specific epitopes on Ara h 2 that are lost on heating and gastric digestion. Although we know most epitopes are still intact after these processes, we cannot say that all epitopes are available. It may also be that these patients need a higher dose of peanut to react.

Finally, for reaction severity, the Sachs Double-blind and CoFAR groups tended to have slightly more severe reactions as measured by a higher median reaction scores. This is not surprising because these 2 groups had higher clinical suspicion of peanut allergy at baseline. It could be argued that the higher baseline values of IgE to peanut and Ara h 2 could predict more severe reactions; however, values of IgE to peanut and Ara h 2 did not correlate well with reaction severity in these cohorts (data not shown). In fact, to our knowledge, there is only one study to suggest a positive correlation between the level of IgE to peanut and severity of reaction.²⁶ Therefore, we cannot suggest, based on these data, that higher levels of IgE to either peanut or Ara h 2 would predict more severe reactions; however, this cannot be ruled out. The data do however support that any positive value of IgE to peanut or Ara h 2 can lead to severe reactions as evidenced by patients having anaphylaxis with values of IgE to peanut and Ara h 2 of < 1.0 kU_A/L.

In conclusion, with these data, we have added confirmatory evidence that IgE to Ara h 2 is a more specific marker for challenge-proven peanut allergy than whole peanut IgE. This appears to hold true in patient cohorts referred to allergy departments for suspected peanut allergy, not just in larger population cohorts of patients with peanut sensitivity. In addition, we have shown that by using a combination of IgE to peanut and Ara h 2, one can more accurately predict reaction

outcomes and reduce the number of challenges than by using either value alone.

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