

Increasing the accuracy of peanut allergy diagnosis by using Ara h 2

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Background: Measurement of whole peanut-specific IgE (sIgE) is often used to confirm sensitization but does not reliably predict allergy. Ara h 2 is the dominant peanut allergen detected in 90% to 100% of patients with peanut allergy and could help improve diagnosis.

Objectives: We sought to determine whether Ara h 2 testing might improve the accuracy of diagnosing peanut allergy and therefore circumvent the need for an oral food challenge (OFC).

Methods: Infants from the population-based HealthNuts study underwent skin prick tests to determine peanut sensitization and subsequently underwent a peanut OFC to confirm allergy status. In a stratified random sample of 200 infants (100 with peanut allergy and 100 with peanut tolerance), whole peanut sIgE and Ara h 2 sIgE levels were quantified by using fluorescence enzyme immunoassay.

Results: By using the previously published 95% positive predictive value of 15 kU_A/L for whole peanut sIgE, a corresponding specificity of 98% (95% CI, 93% to 100%) was found in this study cohort. At the equivalent specificity of 98%, the sensitivity of Ara h 2 sIgE is 60% (95% CI, 50% to 70%), correctly identifying 60% of subjects with true peanut allergy compared with only 26% correctly identified by using whole

peanut sIgE. We report that when using a combined approach of plasma sIgE testing for whole peanut followed by Ara h 2 for the diagnosis of peanut allergy, the number of OFCs required is reduced by almost two thirds.

Conclusion: Ara h 2 plasma sIgE test levels provide higher diagnostic accuracy than whole peanut plasma sIgE levels and could be considered a new diagnostic tool to distinguish peanut allergy from peanut tolerance, which might reduce the need for an OFC. (J Allergy Clin Immunol 2012;■■■■:■■■-■■■.)

Key words: Peanut allergy, diagnosis, diagnostic testing, Ara h 1, Ara h 2, Ara h 3, skin prick tests, ImmunoCAP, HealthNuts, IgE, oral food challenge

Diagnosis of peanut allergy is relatively straightforward when there is an unequivocal history of clinical reaction to peanut ingestion.¹ However, diagnosis can be more complicated in cases in which the clinical history is not clearly defined or in children who have not yet been exposed to a food. Positive results on both skin prick tests (SPTs) and blood tests for peanut-specific IgE (sIgE; ImmunoCAP fluorescence enzyme immunoassay) have high sensitivity and low specificity for the diagnosis of peanut allergy. Thus the likelihood of having peanut allergy in the presence of peanut sIgE (ie, the positive predictive value [PPV] of these tests) is low. The use of 95% PPV threshold values can improve the usefulness of these tests in the prediction of true peanut allergy, and various studies have reported 95% PPV thresholds for both SPTs and ImmunoCAP tests to aid in the diagnosis of food allergy in these clinical settings.^{2,3} Nevertheless, for those patients who have positive SPT or ImmunoCAP results of less than these 95% PPVs, it remains uncertain whether there is clinical allergy, and an oral food challenge (OFC) is required to confirm or exclude a diagnosis of food allergy. Although definitive, the OFC is time-consuming, costly, and associated with a risk of anaphylaxis. Thus new approaches that allow the accurate diagnosis of peanut allergy while reducing the need for an OFC are needed.

Because of the rapid increase in rates of sensitization to foods, allergy services are overwhelmed, and food challenge tests might be difficult to access. Clinicians faced with the difficult task of having to assess the presence of food allergy based solely on a positive SPT or ImmunoCAP result must err on the side of caution and accept a diagnosis of “possible” food allergy in these situations. This approach can lead to overdiagnosis of peanut allergy in the community and a potentially unnecessary burden on

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Abbreviations used

OFC: Oral food challenge

PPV: Positive predictive value

ROC: Receiver operating characteristic

sIgE: Specific IgE

SPT: Skin prick test

the health care system. Moreover, an incorrect diagnosis of food allergy unnecessarily imposes allergen avoidance and impaired quality of life on the patient and family.

Recently, a number of studies have investigated the use of major peanut allergens to improve the diagnosis of peanut allergy. Eleven peanut allergens have been identified, with Ara h 2 recognized as the predominant allergen.⁴ Ara h 2 sIgE has been detected in 90% to 100% of patients with peanut allergy, and although these results have been based on small study cohorts, it has been suggested that the sensitivity and specificity of Ara h 2 sIgE testing is higher when compared with those of the current tests used to diagnose peanut allergy.⁵⁻⁷ In a study of 29 patients with peanut allergy, Nicolaou et al⁸ reported a 93% sensitivity and 100% specificity for Ara h 2 sIgE at a level of 0.55 kU_A/L. However, this small study was carried out in a patient population selected from the clinic, and negative control subjects were not used.

In this study we assessed whether Ara h 2 sIgE measurement could be used to accurately identify peanut allergy by using subjects recruited into the population-based HealthNuts study, in which an OFC was performed in all infants with positive peanut SPT results irrespective of wheal size or history of previous reaction. This design allows validation of the ability of Ara h 2 to predict peanut allergy in a population-based setting.

METHODS

Selection of subjects for Ara h 2 testing

The methods used in the HealthNuts study have been detailed previously.⁹ In brief, 11- to 15-month-old infants were recruited from 131 council-run immunization sessions across Melbourne, Australia, between September 2007 and August 2011. All infants underwent SPTs to peanut and 3 other foods. Those with any detectable SPT wheal reactions (wheal size ≥ 1 mm after subtracting the negative control, $n = 463$) were invited to the HealthNuts research follow-up clinic at Melbourne's Royal Children's Hospital within the next 4 to 8 weeks for a formal OFC result (Fig 1). Infants with a negative SPT result to peanut, egg, sesame, and cow's milk in the context of a positive histamine control response were considered highly unlikely to have IgE-mediated allergy to these foods. A subset of these infants were invited to attend an OFC as a negative healthy control group.

For an estimated sensitivity or specificity of 95% from the Ara h 2 sIgE data in the study by Nicolaou et al,⁵ a sample size of 100 allergic and 100 nonallergic participants randomly selected from the HealthNuts study is sufficient to provide a lower limit of 92% for the corresponding 95% CI.

Study sample

Two hundred subjects (median age, 12 and 14 months [range, 11-15 and 11.5-20 months] at the time of SPTs and peanut OFCs, respectively) were randomly selected based on the patients' peanut allergy status and food challenge outcomes from 5276 one-year-old infants enrolled in the HealthNuts population-based cohort study.⁹ One hundred of the 200 subjects had peanut allergy, as confirmed by a peanut food challenge. The remaining 100 infants with peanut tolerance confirmed by a peanut food challenge consisted of 58

peanut-sensitized and 42 non-peanut-sensitized infants who were randomly selected as negative controls (Fig 1).

Predefined criteria for a positive OFC result

Peanut challenges were performed as previously described.⁹ In brief, at least 1 of the following signs was present during the OFC to satisfy the criteria for a positive reaction: 3 or more concurrent noncontact hives (urticarial lesion) lasting for more than 5 minutes, perioral or periorbital angioedema, vomiting (excluding immediate postingestion gag/vomits), or evidence of anaphylaxis as defined by the Australian Society of Clinical Allergy and Immunology (evidence of circulatory or respiratory compromise) within 2 hours of the last dose of the OFC.¹⁰

Definitions

- Infants with peanut allergy were defined as having peanut SPT results of 1 mm or greater and a positive allergic reaction confirmed by an OFC ($n = 100$).
- Peanut-tolerant infants had a negative challenge outcome confirmed by a peanut OFC ($n = 100$). These subjects were further characterized into the following groups:
 - peanut-sensitized tolerant infants with a peanut SPT result of 1 mm greater and negative peanut challenge outcome ($n = 58$) or
 - non-peanut-sensitized infants with a 0-mm SPT result to peanut and a negative peanut challenge outcome ($n = 42$).

Cell separation, plasma collection, and allergen sIgE analysis

Blood was collected into a sodium heparin tube (Sarstedt, Inc, Newton, NC) 1 to 2 hours after the last dose of the OFC. The blood was centrifuged at 700 g for 10 minutes within 2 hours after the blood was taken, and the plasma was collected and frozen at -20°C until use.

Allergen sIgE was measured with the ImmunoCAP System FEIA (Phadia AB, Uppsala, Sweden). Plasma samples were analyzed for IgE to whole peanut and Ara h 2. Samples with Ara h 2 IgE levels of less than 0.35 kU_A/L were tested for the presence of allergen sIgE to the other major peanut allergens Ara h 1, Ara h 3, Ara h 8, and Ara h 9.

Statistical analysis

Data were analyzed by generating the receiver operating characteristic (ROC) curve, and analyses were performed with GraphPad Prism 5.03 software (GraphPad Software, Inc, La Jolla, Calif). Sensitivities and specificities were generated for a range of cutoffs for the ROC curve. *P* values were reported for the curve, testing the null hypothesis that the area under the curve was equal to .50. We also quote estimated positive and negative likelihood ratios because their interpretation is not dependent on the underlying disease prevalence or the pretest probability of the subject, which potentially permits the reader to then transfer results to their own patients. A full discussion of the role of the likelihood ratio and interpretation of thresholds is given by Roberts and Lack.¹¹ PPVs were calculated for a series of Ara h 2 and peanut IgE thresholds based on the estimated positive likelihood ratio (ie, Sensitivity/[1 - Specificity]) by using previously published estimates of the population prevalence of peanut allergy from the same (HealthNuts) study. CIs for the estimated PPVs were generated by applying the Fieller theorem to the positive likelihood ratio. The SPT, peanut sIgE, and Ara h 2 sIgE results had a skewed distribution and are reported as medians and ranges; they were also reported as means $\pm$ SDs. The proportions comparing the population were tested by using the 2-proportion *z* test to determine significance between the 2 groups. Significance was indicated by a *P* value of less than .05.

Ethics

Ethics approval was obtained for the HealthNuts study from the Victorian State Government Office for Children (reference no. CDF/07/492), the

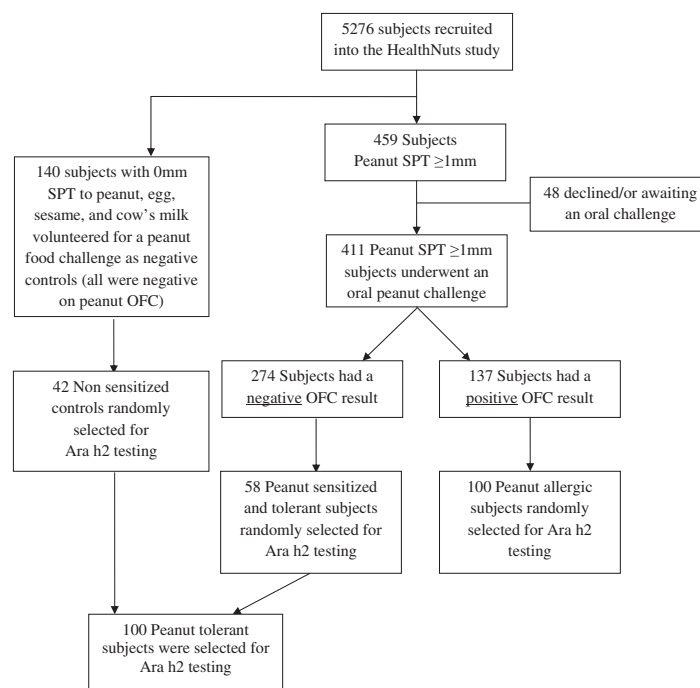


FIG 1. Selection of subjects for Ara h 2 testing.

TABLE I. Clinical features of the study group

Allergy sensitization	Patients with peanut allergy (n = 100)	Peanut-tolerant subjects (n = 100)		
		All peanut-tolerant subjects (n = 100)	Peanut-sensitized subjects (n = 58)	Non-peanut-sensitized subjects (n = 42)
Male subjects (%)	65	50	49	51
Coexistent sensitization to peanut and 1 other food* (%)	61	22	38	0
Coexistent sensitization to peanut and ≥2 other foods* (%)	17	3	6	0
Coexistent food allergy to peanut and 1 other food* (%)	48	18	31	0
Coexistent food allergy to peanut and ≥2 other foods* (%)	9	0	0	0
Eczema (%)	52	36	38	33 [†]
Family history of eczema (%)	42	36	33	41
Family history of food allergy (%)	9	20	10	33 [‡]

*Other foods might include egg, sesame, shellfish, or cow's milk.

[†]*P* < .05 and [‡]*P* < .001 compared with the group with peanut allergy.

Victorian State Government Department of Human Services (reference no. 10/07), and the Royal Children's Hospital Human Research Ethics Committee (reference no. 27047).

RESULTS

Clinical features of the study sample

Infants with peanut allergy were more likely to have current eczema (52%) compared with non-peanut-sensitized infants (33%, *P* < .05). Conversely, the proportion of infants with a family history of food allergy was higher (*P* < .001) in the non-peanut-sensitized group (33%) compared with that seen in the group with peanut allergy (9%, Table I). Otherwise, there were no differences in clinical features between the groups.

Accuracy of diagnosing peanut allergy with peanut sIgE measurements and peanut SPTs

Using previously defined thresholds for diagnosing peanut allergy (SPT wheal ≥8 mm or peanut sIgE level ≥15 kU_A/L), we

assessed the utility of peanut SPTs and ImmunoCAP peanut sIgE measurements to diagnose peanut allergy in our cohort. Of 100 infants with peanut allergy, 57% had SPT results of 8 mm or greater and could be given a diagnosis of peanut allergy; however, 35% with SPT results of 3 to 7 mm would require an OFC to confirm the presence of allergy (Table II). For peanut sIgE measurement, 26 of 100 infants with peanut allergy had levels of 15 kU_A/L or greater and could be given a diagnosis of peanut allergy; however, 65 of 100 with levels between 0.35 and 15 kU_A/L would require an OFC to confirm the presence of allergy (Table II). Conversely, 3% or less of the peanut-sensitized tolerant subjects would receive an incorrect diagnosis of peanut allergy by using the 95% PPV thresholds for SPT results (≥8 mm) and peanut sIgE levels (≥15 kU_A/L).

Ara h 2 ImmunoCAP testing

To describe the accuracy of Ara h 2 sIgE testing, we report a number of Ara h 2 sIgE and whole peanut sIgE cutoff levels along

TABLE II. Accuracy of diagnosing peanut allergy with peanut sIgE measurements and peanut SPTs

Allergy sensitization	Patients with peanut allergy (n = 100)	Peanut-tolerant subjects (n = 100)	
		Peanut-sensitized subjects (n = 58)	Non-peanut-sensitized subjects (n = 42)
Positive peanut SPT result <8 mm, no. (%)	43 (43)	57 (98)	42 (100)
Mean ($\pm$ SD) peanut SPT result (mm)	8.67 $\pm$ 3.97	3.15 $\pm$ 2.22	0
Peanut sIgE <15 kU _A /L, no. (%)	74 (74)	56 (96)	42 (100)
Mean ($\pm$ SD) peanut sIgE (kU _A /L)	11.48 $\pm$ 15.27	2.98 $\pm$ 6.59	0.17 $\pm$ 0.87
Median peanut sIgE (kU _A /L)	4.97 (0.02-77)	0.61 (0-46.8)	0.02 (0-3.6)
Ara h 2 sIgE <0.35 kU _A /L, no. (%)	18 (18)	51 (88)	42 (100)
Mean ($\pm$ SD) Ara h 2 sIgE (kU _A /L)	7.11 $\pm$ 11.49	0.25 $\pm$ 0.53	0.02 $\pm$ 0.01
Median Ara h 2 sIgE (kU _A /L)	1.88 (0.01-56.5)	0.02 (0-0.34)	0.01 (0-0.11)

TABLE III. Sensitivity and specificity of various cutoffs for Ara h 2 and whole peanut

Ara h 2 sIgE							Peanut sIgE						
Cutoff (kU _A /L)	Sensitivity (%)	95% CI	Specificity (%)	95% CI	PLR*	NLR†	Cutoff (kU _A /L)	Sensitivity (%)	95% CI	Specificity (%)	95% CI	PLR*	NLR†
Patients with peanut allergy (n = 100) vs all peanut-tolerant subjects (sensitized and nonsensitized, n = 100)													
>0.01	100	96-100	15	7-21	1.2	—	>0.09	99	95-100	54	40-60	1.4	0.02
>0.10	95	89-98	86	78-92	6.6	0.06	>0.23	95	89-98	66	56-75	2.8	0.08
>0.15	91	84-96	89	81-94	8.3	0.10	>0.35	91	83-96	68	58-77	2.8	0.13
>0.35	81	72-88	93	86-97	11.6	0.20	>0.40	90	83-95	69	59-78	2.9	0.15
>0.46	73	66-84	95	87-98	12.7	0.28	>2.12	68	58-77	90	82-95	6.8	0.36
>1.00	60	50-70	97	92-99	20.0	0.41	>6.20	44	34-54	95	89-98	8.8	0.56
>1.19	60	50-70	98	93-100	30.0	0.41	>14.9	26	18-36	98	93-100	13.0	0.76
>3.51	43	33-53	100	96-100	—	0.43	>54.2	3	1-9	100	96-100	—	0.97
Patients with peanut allergy (n = 100) vs peanut-sensitized tolerant subjects (n = 58)													
>0.01	100	96-100	8.6	3-19	1.1	—	>0.02	100	96-100	14	6-25	1.2	—
>0.05	95	89-98	72	59-83	3.4	0.07	>0.23	95	89-98	49	35-62	1.8	0.10
>0.35	81	72-88	88	77-95	6.7	0.22	>7.45	41	31-51	95	86-99	7.9	0.62
>0.88	62	52-72	95	86-99	12.0	0.40	>14.9	26	18-36	97	88-100	7.5	0.67
>3.51	43	33-53	100	94-100	—	0.57	>54.2	3	1-9	100	94-100	—	0.97
Patients with peanut allergy (n = 100) vs non-peanut-sensitized tolerant subjects (n = 42)													
>0.01	100	96-100	23	12-40	1.3	—	>0.02	100	96-100	52	36-68	2.1	—
>0.03	96	90-99	85	51-88	4.1	0.05	>0.23	95	89-98	90	77-97	10.0	0.06
>0.08	95	89-98	97	87-100	39.9	0.05	>0.50	87	79-93	95	84-99	18.3	0.14
>0.13	94	87-98	100	92-100	—	0.06	>3.34	58	48-68	97	87-100	24.4	0.43
							>5.64	46	36-56	100	92-100	—	0.54

*PLR is the positive likelihood ratio calculated as (Sensitivity/[1 – Specificity]) and indicates the likelihood of having peanut allergy.

†NLR is the negative likelihood ratio calculated as ([1 – Sensitivity]/Specificity) and indicates the likelihood of not having peanut allergy.

with the sensitivities and specificities (Table III). An Ara h 2 sIgE level of 0.46 kU_A/L provides 95% specificity and 73% (95% CI, 66% to 84%) sensitivity, whereas a whole peanut sIgE level of 6.2 kU_A/L provides 95% specificity with a significantly lower sensitivity of 44% (95% CI, 34% to 54%; $P < .001$; Table III). If the previously reported threshold of 15 kU_A/L is adopted, which provides the 95% PPV and 98% specificity, the sensitivity of the whole peanut sIgE test reduces to 26% (95% CI, 18% to 36%). At a comparative specificity of 98%, provided by an Ara h 2 sIgE level of 1.19 kU_A/L, Ara h 2 sIgE testing detects 60% (95% CI, 50% to 70%; $P < .001$).

Compared with both SPTs and whole peanut sIgE measurements, measurement of Ara h 2 sIgE correctly identified more patients with true peanut allergy when 95% specificity or a 95% PPV were applied. The mean Ara h 2 sIgE level for the 100 patients with peanut allergy was 7.11 $\pm$ 11.49 kU_A/L compared with 0.25 $\pm$ 0.53 kU_A/L in the peanut-sensitized subjects who did not have peanut allergy. The area under the curve for the

peanut sIgE ROC curve is 0.89 (95% CI, 0.85-0.94) compared with an area under the curve of 0.95 (95% CI, 0.92-0.98) for Ara h 2, indicating that Ara h 2 performs significantly better ($P < .027$, Fig 2). The ROC curve also shows the current conventional cutoffs for sensitization measured by using SPTs and whole peanut sIgE measurements (3 mm and 0.35 kU_A/L, respectively), along with the current conventional 95% PPV cutoffs for peanut allergy of 8 mm and 15 kU_A/L, respectively. Compared with these cutoffs, an Ara h 2 sIgE cutoff of 0.1 kU_A/L discriminated peanut sensitization more accurately (ie, with higher specificity compared with whole peanut sIgE measurement and SPTs; Fig 2). Conversely, an Ara h 2 sIgE cutoff of 1.19 kU_A/L, which represents the 98% specificity threshold for Ara h 2 sIgE, detected more patients with true allergy compared with whole peanut sIgE measurements and SPTs.

In a subset of our analysis, we also report the sensitivity and specificity of Ara h 2 sIgE after stratifying the nonallergic group into (1) nonsensitized peanut-tolerant infants (n = 42) and (2)

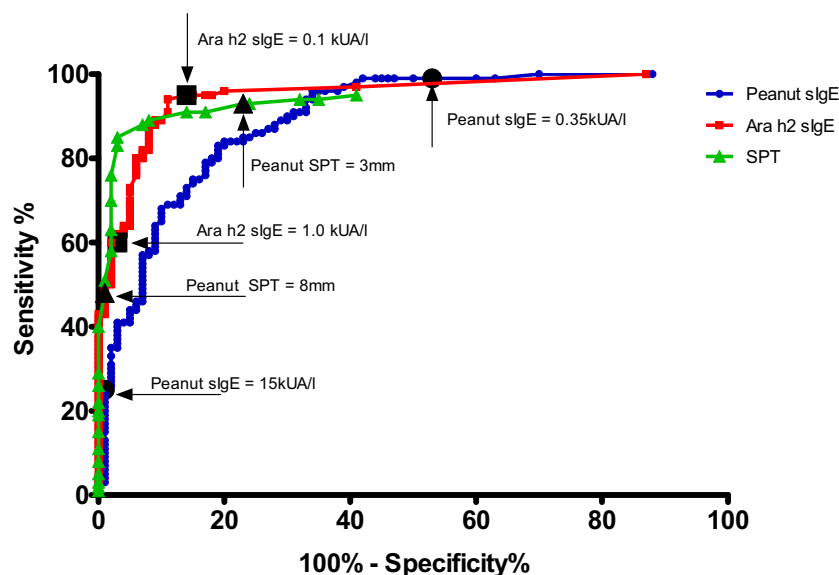


FIG 2. ROC curves showing true-positive rates (sensitivity) plotted against the false-positive rate (specificity) for different cutoff points of the quantified components of Ara h 2 (red squares) and whole peanut extract (blue circles). The points highlighted are the current conventional SPT (3 and 8 mm) and whole peanut sIgE (0.35 and 15 kU_A/L) thresholds. The points highlighted for Ara h 2 indicate putative levels for determining sensitization (0.1 kU_A/L) and peanut allergy (1.0 kU_A/L). The area under the curve is 0.95, 0.94, and 0.89 for Ara h 2 sIgE, peanut SPT, and peanut sIgE, respectively.

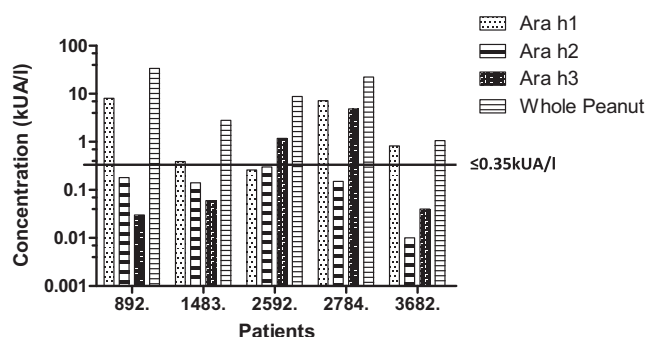


FIG 3. Ara h 1 or Ara h 3 concentrations of 0.35 kU_A/L or greater in patients with peanut allergy with levels of less than 0.35 kU_A/L for Ara h 2 (n = 19). The whole peanut concentrations are shown at the end of the 4 columns for each patient.

peanut-sensitized tolerant infants (n = 58, Table III). When only the nonsensitized peanut-tolerant subjects were included as the nonallergic group, Ara h 2 sIgE measurement achieved higher specificity (97%; 95% CI, 87% to 100%) by using a cutoff of 0.075 kU_A/L that provides 95% sensitivity (95% CI, 89% to 98%) than whole peanut sIgE, which achieved 90% specificity (95% CI, 77% to 97%), using an equivalent cutoff that provides 95% sensitivity (95% CI, 89% to 98%) of 0.225 kU_A/L. Although restriction of the nonallergic group to the peanut-sensitized tolerant subjects reduced the specificity of both tests, Ara h 2 sIgE measurement still showed higher specificities compared with peanut sIgE measurement. Ara h 2 sIgE measurement had 95% sensitivity (95% CI, 89% to 98) and 72% specificity (95% CI, 59% to 83%) for a cutoff of 0.05 kU_A/L. A cutoff of 1.92 kU_A/L generates a PPV of 96% (95% CI, 65% to 100%), with corresponding 50% sensitivity (95% CI, 40% to 60%) and 99% specificity (95% CI, 90% to 100%), assuming the prevalence of peanut allergy among

those with peanut sensitization is 33.3% (95% CI, 28.8% to 38.1%). Peanut sIgE had 95% (95% CI, 89% to 98%) sensitivity and 49% (95% CI, 35% to 63%) specificity at a cutoff of 0.225 kU_A/L.

Peanut allergen sIgE profile in patients with peanut allergy with Ara h 2 levels ≤ 0.35 kU_A/L

Nineteen percent of the infants with peanut allergy in this study had Ara h 2 IgE levels of less than 0.35 kU_A/L (the conventional cutoff for a positive result). We examined the presence of sIgE to the other common major peanut allergens. Of these 19 infants, only 5 had levels of 0.35 kU_A/L or greater to 1 or more of the other major peanut allergens (Fig 3). Four infants had levels of 0.35 kU_A/L or greater of Ara h 1 sIgE (5 with Ara h 1 sIgE levels ≥ 0.1 kU_A/L), 2 had levels of 0.35 kU_A/L or greater of Ara h 3 sIgE, and the remaining 14 did not have detectable levels of Ara h 1 or Ara h 3 sIgE. None of these 19 patients with peanut allergy had Ara h 8 or Ara h 9 sIgE levels of 0.1 kU_A/L or greater (results not shown).

Diagnosing peanut allergy by using a combination of peanut sIgE and Ara h 2 sIgE measurements

Fig 4 shows the number of OFCs that would be required if the current thresholds for whole peanut sIgE measurements, SPTs, and Ara h 2 sIgE measurements were used to diagnose peanut allergy in the absence of any other tests. Of the 200 infants included in this study, 95, 50, and 44 would require OFCs with whole peanut sIgE measurements, SPTs, and Ara h 2 sIgE measurements, respectively. Fig 5 shows 2 strategies that incorporate the use of peanut sIgE measurements or SPTs as first-line tests, followed by Ara h 2 sIgE measurement as a second line of testing to help improve the accuracy of distinguishing patients with peanut allergy from those with peanut tolerance. In the first model Ara h

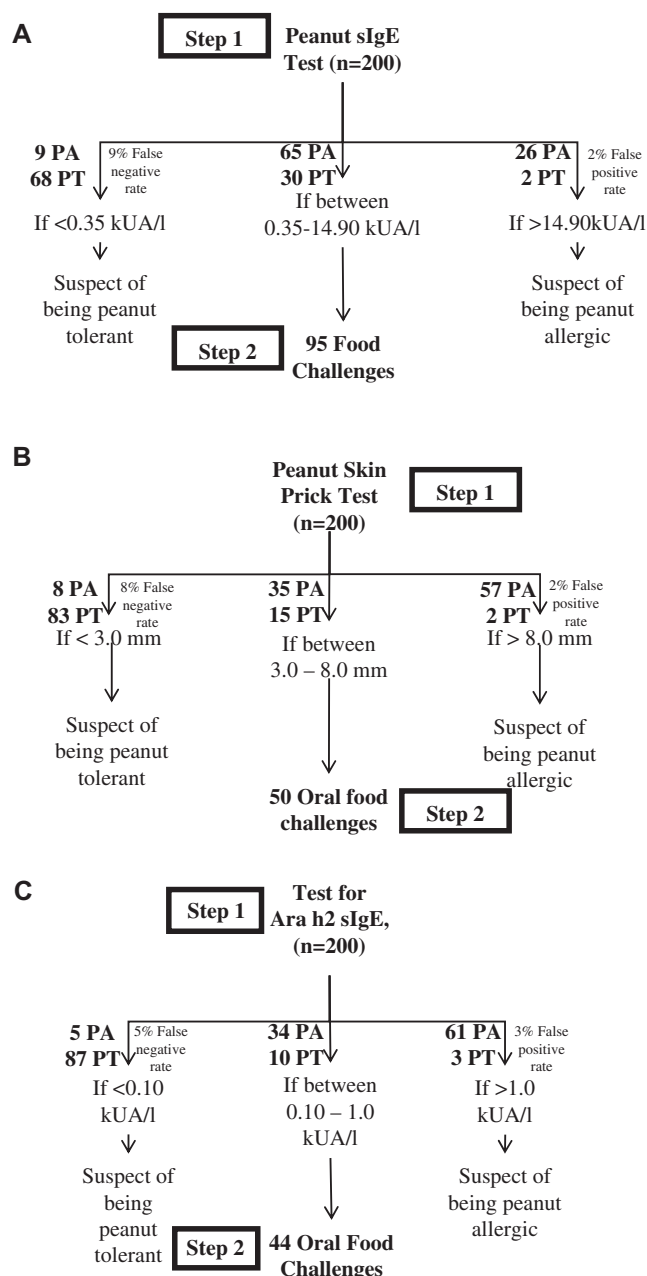


FIG 4. Various alternatives of diagnosing peanut allergy with peanut sIgE measurements (A), peanut SPTs (B), or Ara h 2 sIgE measurements (C) followed by an OFC. Patients from this study were examined by using the current conventional cutoffs for peanut sIgE measurements and SPTs to determine the stringency of each test. PA, Patients with peanut allergy; PT, peanut-tolerant subjects.

2 testing of the 95 infants with peanut sIgE levels between 0.35 and 14.9 kUA/L successfully identified an additional 22 infants as peanut tolerant and 35 infants as having peanut allergy (Fig 5, A). Hence incorporating Ara h 2 sIgE testing in combination with peanut sIgE testing would reduce the number of OFCs by almost two thirds from 95 (47.5%) to 32 (16%, $P < .001$). In the second model Ara h 2 testing of the 50 infants with SPT results of between 3 and 8 mm identified an additional 6 infants as peanut tolerant and 21 as having peanut allergy (Fig 5, B), reducing the number of OFCs from 50 (25%) to 21 (10.5%, $P = .007$).

Variability of Ara h 2 sIgE levels in infants with anaphylaxis

We attempted to assess whether Ara h 2 sIgE levels would predict the severity of the allergic reaction; however, our data suggest that Ara h 2 sIgE levels did not identify the 4 patients with peanut allergy who had anaphylaxis during the OFC. Their Ara h 2 sIgE levels ranged from 0.45 to 56.5 kUA/L. Infants with peanut allergy without anaphylaxis also had a wide range of Ara h 2 sIgE concentrations, predominantly skewing toward the lower end (range, 0.01-49.0 kUA/L).

It has been previously reported that results of SPTs to detect allergen sIgE have been reported to decrease after an anaphylactic reaction, hence we performed a sensitivity analysis by removing the 4 infants with an anaphylactic reaction after an oral peanut challenge. The sensitivity and specificity of Ara h 2 and whole peanut sIgE remained unchanged (data not shown).

DISCUSSION

This is the first study to report the utility of Ara h 2 sIgE levels to correctly predict peanut allergy in a population setting. We found that Ara h 2 sIgE testing is more accurate in determining peanut allergy compared with either peanut SPTs or whole peanut sIgE measurements alone by using the gold standard OFC to confirm the presence of true food allergy. We determined that by using the previously published cutoff levels that provide 14.9 kUA/L for peanut sIgE and 1.19 kUA/L for Ara h 2 sIgE and that provide 98% specificity for diagnosis of peanut allergy, the sensitivity of Ara h 2 sIgE testing is 60% compared with 26% for whole peanut sIgE. These findings might have profound clinical implications because they allow for increased peanut allergy diagnosis and reduce the number of patients requiring referral to specialist services for confirmation of food allergy by using OFCs.

The strengths of this study include the large cohort of clearly phenotyped children with peanut allergy and tolerant children through an SPT and an OFC. True population-negative control subjects provided better evaluation of the performance of these tests as a screening tool for peanut allergy. This is also the first study to present data on peanut SPTs in combination with Ara h 2 sIgE and whole peanut sIgE measurements on all subjects.

One limitation of this study is that all participants were 1 year old, and therefore our results might not generalize to other ages. Our data are population based and might not reflect specialized clinical practice. However, our results are likely to be useful in a community setting because most children with food allergy express it early in life and testing for peanut allergy by age 1 year is common in clinical practice.¹² In Australia 80% of infants have not introduced peanut into the diet by age 1 year and hence will not have a history of either tolerance or reaction to peanut.¹³ Ara h 2 sIgE testing will provide a method of screening for peanut allergy that reduces the need for OFCs. Ara h 2 sIgE measurement could also replace whole peanut sIgE measurement as a diagnostic test to confirm the diagnosis of peanut allergy in a patient with a history of a previous reaction to peanut.

Currently, both peanut SPTs and peanut sIgE measurements can confirm peanut allergy in patients with a recent history of a reaction. In cases in which there is no clear history of a reaction, it has previously been reported that application of 95% PPV thresholds for peanut allergy (8 mm for SPT results and 15 kUA/L for peanut sIgE levels) and, more recently, 0.35 kUA/L for

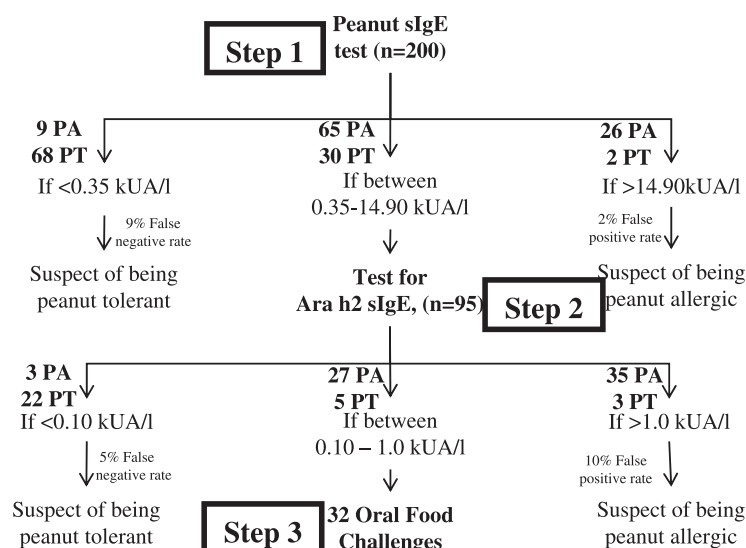
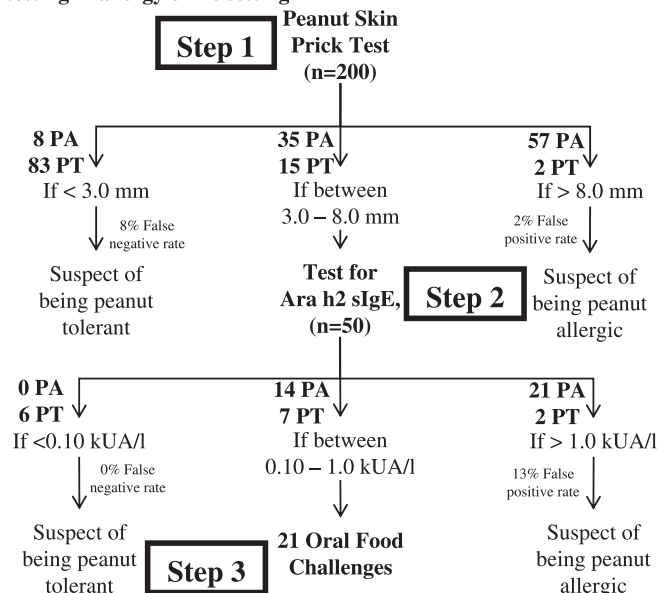
A Model for testing in community setting**B Model for testing in allergy clinic setting**

FIG 5. Various clinical scenarios for diagnosing peanut allergy by using a 3-step model in a community setting (A) or an allergy clinic setting (B). Peanut sIgE measurements or peanut SPTs were assessed as the first-line test followed by Ara h 2 sIgE measurements to help improve the diagnosis of peanut allergy when either peanut sIgE or peanut SPT test results fall into the respective cutoffs of either 0.35 to 15 kUA/L or 3 to 8 mm, respectively. PA, Patients with peanut allergy who fall into the designated ranges; PT, peanut-tolerant subjects who fall into the designated ranges.

Ara h 2 can aid in the diagnosis of peanut allergy.^{2,3,5} Although the specificity of Ara h 2 at 0.35 kUA/L in our study was not 100%, as previously reported in a clinically biased cohort, we established that Ara h 2 measurements are more accurate than SPTs and whole peanut sIgE measurements in correctly identifying infants with peanut allergy when used at a cutoff of 1.19 kUA/L.

Our study has demonstrated that the performance of both SPT and Ara h 2 sIgE testing to correctly identify infants with peanut allergy and peanut-tolerant infants was similar. SPTs are usually performed in a specialist setting. However, patient waiting times

are at present significant, exceeding 18 months in many centers in Australia.¹⁴ By comparison, blood testing for Ara h 2 and whole peanut sIgE can easily be accessed in the community by primary and secondary health care professionals with access to diagnostic laboratories. On the basis of our cohort, testing with peanut sIgE followed by Ara h 2 sIgE could substantially reduce the number of OFCs required to diagnose peanut allergy by almost two thirds. Given that the cutoff of 0.1 kUA/L or less for Ara h 2 can identify 87% of peanut-tolerant infants while only having a 5% false-negative rate, this would support the gradual introduction of

peanuts into the diet if the child has not already eaten the food. Conversely, a cutoff of 1.19 kU_A/L for Ara h 2 detects 60% of patients with peanut allergy with a false-positive rate of 2% and can be used to diagnose the presence of peanut allergy. The remainder of subjects that fall between these thresholds would require an OFC to confirm peanut allergy status. Although the use of Ara h 2 in the diagnosis of peanut allergy in the community has significant advantages, in an allergy clinic setting peanut SPTs still provide a rapid and accurate method for determining peanut allergy, and Ara h 2 sIgE measurement could be used as a subsequent test to reduce the number of patients requiring an OFC.

Ara h 2 has been identified as the predominant peanut allergen in a number of countries from Europe, North America, and Southeast Asia, as well as Australia, and might be considered in the diagnosis of peanut allergy.^{6,8,15-18} However, the use of Ara h 2 sIgE testing might not be applicable to all populations; for example, Ara h 9 is reported to be the dominant allergen in Spain.¹⁵ In the Australian population other recombinant allergens tested might not improve detection of peanut allergy among subjects with Ara h 2 sIgE levels of less than 0.35 kU_A/L. In our study only 9 of the 100 patients with peanut allergy had Ara h 2 sIgE concentrations of less than 0.35 kU_A/L, and only 5 of these 9 subjects had detectable Ara h 1, Ara h 3, Ara h 8, or Ara h 9 sIgE levels. Further additional testing of the other peanut allergens on all subjects would be required to identify whether there are other allergens that are dominant in our region.

In conclusion, Ara h 2 sIgE testing should be considered the preferred diagnostic tool for determining peanut allergy because we have shown greater diagnostic accuracy than that of whole peanut sIgE and SPT. This will reduce the need for an OFC and ultimately might reduce the strain and demand on clinical allergy services.

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Clinical implications: Our data suggest that Ara h 2 sIgE levels can distinguish peanut allergy from peanut tolerance more accurately than whole peanut and could change the current clinical approaches to the diagnosis of peanut allergy.

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