Original Article

Accurate Prediction of Peanut Allergy in One-Third of Adults Using a Validated Ara h 2 Cutoff

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What is already known about this topic? The diagnostic value of peanut components is extensively studied in children, but less in adults. An Ara h 2 cutoff level (≥1.75 kU_A/L) with 100% positive predictive value has been reported in adults.

What does this article add to our knowledge? sIgE to Ara h 2 and 6 have equally high discriminative ability in adults. The validated Ara h 2 cutoff predicts peanut allergy in one-third of adults.

How does this study impact current management guidelines? sIgE to Ara h 2 should be used to reduce the need for double-blind placebo-controlled food challenges using the validated 100% positive predictive cutoff level of ≥1.75 kU_A/L in adults.

BACKGROUND: The diagnostic value of peanut components is extensively studied in children, but to a lesser extent in adults with suspected peanut allergy. The use of peanut components in daily practice may reduce the need for double-blind placebo-controlled food challenges (DBPCFCs); however, validation studies are currently lacking.

OBJECTIVE: To evaluate the diagnostic value of (combined) peanut components and validate a previously found Ara h 2 cutoff level with 100% positive predictive value (PPV) in adults with suspected peanut allergy.

METHODS: Adults who underwent a peanut DBPCFC were included: 84 patients from a previous study (2002-2012) and 70 new patients (2012-2019). Specific IgE (sIgE) to peanut extract, Ara h 1, 2, 3, 6, and 8 was measured using ImmunoCAP. Diagnostic value was assessed with an area under the curve (AUC) analysis.

RESULTS: In total, 95 (62%) patients were peanut allergic. sIgE to Ara h 2 and Ara h 6 were the best predictors with an AUC (95% confidence interval) of 0.85 (0.79-0.91) and 0.85 (0.79-0.92), respectively. The Ara h 2 cutoff level with 100% PPV (≥1.75 kU_A/L) was validated in the 70 new patients. Thirty percent of all included patients could be classified correctly as peanut allergic using this validated cutoff level.

CONCLUSION: sIgE to Ara h 2 and Ara h 6 have equally high discriminative ability. Peanut allergy can be predicted accurately in one-third of adults using a validated cutoff level of sIgE to Ara h 2. **(J Allergy Clin Immunol Pract 2021;9:1667-74)**

Key words: *Adults; Ara h 2; Ara h 6; Component-resolved diagnostics; Food challenge; Peanut allergy; Peanut components; Specific IgE*

Peanut allergy affects up to 0.60% of adults in Europe.[1-3] The reference standard to diagnose or exclude peanut allergy is a double-blind placebo-controlled food challenge (DBPCFC).[4] However, a DBPCFC is time consuming, labor intensive, costly, and not without risks because of the potential for severe allergic reactions including anaphylaxis.[5] Given these limitations, new diagnostic strategies have been evaluated that could reduce the number of DBPCFCs.[6]

Serology tests may be used to reduce the number of DBPCFCs when validated cutoff levels are used.[7-9] Sensitization to peanut component allergens (eg, component-resolved diagnostics) has emerged as a useful tool to diagnose peanut allergy with increased diagnostic accuracy compared with sensitization

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Abbreviations used
AUC- Area under the curve
CI- Confidence interval
DBPCFC- Double-blind placebo-controlled food challenge
LASSO- Logistic least absolute shrinkage and selection operator
NPV- Negative predictive value
PPV- Positive predictive value
ROC- Receiver-operating characteristic
sIgE- Specific IgE

to crude peanut extract or skin prick testing.[8,10-16] Currently, 17 peanut allergens have been described by the World Health Organization and International Union of Immunological Societies Allergen Nomenclature Sub-committee.[17] Ara h 2 and Ara h 6 are seed storage proteins and 2S albumins with highly similar protein structure,[18] and have been recognized as the most important allergens associated with peanut allergy.[8,10,15] Ara h 7 is a third 2S albumin and is gaining attention as a predictor for peanut allergy.[11] Ara h 1 and Ara h 3 are also seed storage proteins and, together with Ara h 2, have been designated as the major peanut allergens.[19,20] Ara h 8 is a Bet v 1 homologous pathogenesis-related 10 protein that cross-sensitizes with major-birch pollen allergen Bet v 1 in adults.[21] Isolated sensitization to Ara h 8 is associated with mild or no symptoms, although the role of Ara h 8 in multiple sensitized patients remains unclear.[22] Finally, Ara h 9 is a lipid transfer protein, and a major allergen in the Mediterranean area.[23]

Current evidence on cutoff levels for peanut components in adults is limited, as the majority of research has been conducted in children. Furthermore, validation studies of the diagnostic value of peanut components are currently lacking. Validation studies are warranted to implement research findings into daily practice. A previous study performed in our center included 84 adults and showed that peanut allergy can be predicted with 100% positive predictive value (PPV) using a specific IgE (sIgE) to Ara h 2 cutoff level of $\geq$1.75 kU_A/L.[16] Other recent studies have shown that the discriminative ability of sIgE to Ara h 6 is comparable with sIgE to Ara h 2.[8,15,24] Peanut component Ara h 6 has mainly been studied using a multiplex assay as opposed to a singleplex assay.[14,15,24,25] The results of a multiplex assay are semiquantitative, and therefore, these results cannot be used to determine cutoff levels.[26]

In the current study, we aimed to evaluate the diagnostic value of sIgE to peanut extract, Ara h 1, 2, 3, 6, and 8, and combinations, using a singleplex assay in adults with suspected peanut allergy. Furthermore, we aimed to validate the previously published cutoff level of sIgE to Ara h 2 with 100% PPV (ie, $\geq$1.75 kU_A/L).

METHODS

Study population

All 84 adult patients who participated in the previous diagnostic study and underwent a DBPCFC for peanut at the University Medical Centre Utrecht between 2002 and June 2012 were eligible for inclusion.[16] Furthermore, we expanded the cohort with patients who underwent a DBPCFC for peanut between June 2012 and May 2019 (n = 120). The DBPCFC was performed because of a suspected peanut allergy based on clinical history or sensitization. Patients with an inconclusive DBPCFC result (n = 18) or without leftover serum (n = 32) were excluded from the analysis. Included and excluded patients were comparable in age, gender, presence of allergic rhinitis, severity of peanut allergy, and levels of sIgE to peanut extract and components (see Table E1 in this article's Online Repository at www.jaci-inpractice.org). The previous and current studies were approved by the ethical committee of the University Medical Center Utrecht (no. 18-428).

Oral food challenges

DBPCFCs were performed in a clinical setting equipped for resuscitation in accordance with the international consensus protocol as previously described.[27-29] The DBPCFC result was considered positive when objective symptoms occurred ("positive objective") or when subjective symptoms lasted for at least 45 minutes, or occurred to at least 3 subsequent doses ("positive subjective"). The severity of the DBPCFC was based on the adapted Mueller score: 0 = oral symptoms; 1 = cutaneous symptoms; 2 = gastrointestinal symptoms; 3 = respiratory symptoms; and 4 = cardiovascular symptoms.[16,30] Both the results and the severity of the DBPCFC were discussed among 2 food allergy experts (HMK, T-ML). Any discrepancies between the 2 experts were resolved by discussion and consensus, or by consulting a third expert (FCE), if necessary.

Sensitization (sIgE) measurements

sIgE values to peanut extract and peanut components Ara h 1, 2, 3, 6, and 8 were determined using the ImmunoCAP platform (provided by Thermo Fisher Scientific, Uppsala, Sweden). In the previous diagnostic study, sIgE to peanut extract, Ara h 1, 2, 3, and 8 was already measured in all patients (n = 84). In these patients, Ara h 6 was determined as part of the current study in those with available leftover serum (n = 48). In all newly included (n = 70) patients, we determined Ara h 1, 2, 3, 6, and 8.

In peanut-allergic patients without sensitization to peanut components or peanut extract as measured using the ImmunoCAP platform, sensitization to Ara h 1, 2, 3, 5, 6, 7, 8, 9, 10, 11, 14, and 15 was assessed using a line blot (EUROLINE; EUROIMMUN, Lübeck, Germany) as described elsewhere.[11]

Statistical analyses

The differences between patients with a positive and a negative DBPCFC result were analyzed using the χ^2 test and Mann-Whitney *U* test, when appropriate. The diagnostic value of sIgE to peanut extract and peanut components was assessed by the area under the curve (AUC) of the receiver-operating characteristic (ROC). The AUC values were compared using DeLong's test for correlated ROC curves.[31] To evaluate the diagnostic value of all serology tests combined (ie, sIgE to peanut extract, Ara h 1, 2, 3, 6, and 8), logistic least absolute shrinkage and selection operator (LASSO) regression analysis was performed. The LASSO regression method is based on the regular least-squares method but includes an additional parameter λ to prevent overfitting and automatically deletes unnecessary covariates.[32] We used cross-validation to select λ.

The previously published cutoff level of sIgE to Ara h 2 with 100% PPV was validated by calculating the PPV of this cutoff level ($\geq$1.75 kU_A/L) in the newly included patients (43 peanut-allergic and 27 peanut-tolerant patients). The sample size needed to have 95% confidence and 80% power to detect a difference of 5% from a presumed PPV of 98% was 31 cases.[33] In addition, the cutoff levels with optimal PPVs and negative predictive values (NPVs) with corresponding sensitivities and specificities were calculated for peanut extract and all peanut components. Statistical analyses were performed in SPPS for Windows (version 25.0; IMB Corp.,

Armonk, NY) and R (pROC package v 1.15.3, randomForest package v 4.6-14, OptimalCutpoints package v 1.1-4).

RESULTS

In total, 154 adults were included after a positive (n = 95) or a negative (n = 59) DBPCFC result.

A positive DBPCFC result was based on objective symptoms in 69 (73%) patients and on only subjective symptoms in 26 (27%) patients. Patients with a positive DBPCFC result were significantly younger compared with patients with a negative DBPCFC result (median age 25 and 34 years, respectively) and had higher levels of sIgE to peanut extract, Ara h 1, 2, 3, and 6 (Table I). The level of sIgE to Ara h 8 was comparable between patients with a positive and a negative DBPCFC result.

Specific IgE to Ara h 2 and Ara h 6 are the best predictors of peanut allergy

The levels of sIgE to peanut extract, Ara h 1, 2, 3, and 8 were available in all 154 patients and the level of sIgE to Ara h 6 in 118 patients. The diagnostic values of sIgE to peanut extract, Ara h 1, 2, 3, and 8 were comparable in the cohort of 118 and 154 patients (see Table E2 in this article's Online Repository at www.jaci-inpractice.org). The discriminative abilities of sIgE to Ara h 2 and sIgE to Ara h 6 for predicting peanut allergy were high and comparable, with an AUC (95% confidence interval [CI]) value of 0.85 (0.79-0.91) and 0.85 (0.79-0.92), respectively (Figure 1, *A*). The discriminative ability of the combination of sIgE to Ara h 2 and 6 was comparable with the individual components, with an AUC (95% CI) of 0.85 (0.78-0.92). The AUC values of sIgE to Ara h 2 and Ara h 6 were significantly higher than the AUC values of sIgE to peanut extract, Ara h 1, Ara h 3, and Ara h 8 (see Table E3 in this article's Online Repository at www.jaci-inpractice.org). When serology tests were used to predict peanut allergy with objective symptoms, the AUC values of sIgE to Ara h 2 and Ara h 6 increased slightly to respectively 0.90 (0.85-0.95) and 0.90 (0.84-0.96) (Figure 1, *B*). The concentrations of sIgE to Ara h 2 and Ara h 6 were strongly correlated (Spearman's rho correlation coefficient 0.93; $P < .01$). The concentration of sIgE to Ara h 2 was weakly correlated with the eliciting dose of objective symptoms (Spearman's rho correlation coefficient 0.30; $P = .04$), whereas the concentration of sIgE to Ara h 6 was not correlated with the eliciting dose.

The final covariates included in the LASSO model for predicting peanut allergy were sIgE to Ara h 1 and Ara h 6, with Ara h 6 as the most influential predictor. The AUC value of the LASSO model was 0.85 (0.78-0.92).

The Ara h 2 cutoff level with 100% PPV is validated

A previous study performed in our center, including 84 adults, showed that peanut allergy can be diagnosed in 28% of patients by using an sIgE to Ara h 2 cutoff level with 100% PPV of $\geq$1.75 kU_A/L.[16] In the expanded cohort, including 84 adults from the previous study and 70 adults who were challenged after the first study, this cutoff level has again 100% PPV (Table II). Thus, we were able to confirm the previously published cutoff level of Ara h 2 in a larger group of adult patients. Thirty-two percent of patients had an Ara h 2 value above the validated cutoff level and could be predicted correctly as peanut allergic. Eighty-eight percent of patients above the Ara h 2 cutoff level had a positive DBPCFC result with objective symptoms. The optimal Ara h 2 cutoff level with 100% PPV in patients who were not part of the previous diagnostic study (n = 70) was $\geq$0.43 kU_A/L. Six patients had an Ara h 2 level between 0.43 and 1.74 kU_A/L and were all peanut allergic (4 with objective symptoms and 2 with subjective symptoms).

Cutoff levels for Ara h 6 and other peanut components

The Ara h 6 cutoff level with 100% PPV was $\geq$1.80 kU_A/L, comparable with the Ara h 2 cutoff level (Table II). Twenty-eight percent of patients had an Ara h 6 value above this cutoff level and could be predicted correctly as peanut allergic. The highest NPVs were observed for Ara h 6 and Ara h 8 at a cutoff of 0.1 kU_A/L and were 81% and 88%, respectively. The NPVs for sIgE to peanut extract, Ara h 1, Ara h 2, and Ara h 3 (at a cutoff of 0.1 kU_A/L) were 78%, 58%, 79%, and 62%, respectively.

Higher titers in patients with objective symptoms

Figure 2 shows the percentage of patients with and without sensitization (cutoff 0.10 kU_A/L) to peanut components and peanut extract per DBPCFC result (ie, positive with objective symptoms, positive with subjective symptoms, and negative) and the titers of sensitization. Patients with a positive DBPCFC result with objective symptoms recognized Ara h 1, 2, 3, and 6 more often and had a higher titer compared with patients with subjective symptoms or a negative DBPCFC result. A large proportion of patients recognized Ara h 8 (75%) and peanut extract (89%), irrespective of the DBPCFC result.

Peanut-allergic patients without sensitization to Ara h 1, 2, 3, and 6

In 16 (17%) patients with a positive DBPCFC result, we did not detect sensitization to Ara h 1, 2, 3, and 6 (Figure 3). Five of 16 patients had objective symptoms Mueller grade 3 during the DBPCFC. Peanut-allergic patients with undetectable sensitization to Ara h 1, 2, 3, and 6 were sensitized to Ara h 8 and peanut extract (n = 8), to Ara h 8 only (n = 2), to peanut extract only (n = 2), or were not sensitized to peanut extract or any of the peanut components (n = 4).

Additional diagnostic testing on a line blot (EUROLINE) was performed, measuring Ara h 1, 2, 3, 5, 6, 7, 8, 9, 10, 11, 14, and 15, in 14 of 16 patients with leftover serum available after the ImmunoCAP measurements. Again, no sensitization to Ara h 1, 2, 3, or 6 was measured on the line blot. Furthermore, all patients were tested negative to Ara h 5, 7, 10, 11, 14, and 15. Two patients were sensitized to Ara h 9 (patient ID 3 and ID 14 in Figure 3) on the line blot.

DISCUSSION

In the current study, we showed that Ara h 2 and Ara h 6 are the best predictors of peanut allergy in adults with suspected peanut allergy. Our validated Ara h 2 cutoff level with 100% PPV (cutoff $\geq$1.75 kU_A/L) can be used to accurately predict peanut allergy in one-third of patients. Furthermore, we were the first to investigate the diagnostic value of sIgE to Ara h 6 using a singleplex assay in adults and were able to identify a cutoff level of Ara h 6 with 100% PPV (cutoff $\geq$1.80 kU_A/L), again applicable to almost one-third of patients.

Our results are in line with previous research that identified Ara h 2 and Ara h 6 as the best predictors of peanut allergy.[8,14,15] The AUC value of sIgE to Ara h 2 was lower in our study in adults (AUC 0.85) compared with the AUC values reported in

TABLE I. Characteristics of participating patients and details of double-blind placebo-controlled food challenges

	Total	Positive DBPCFC result	Negative DBPCFC result	
	N = 154	n = 95 (62%)	n = 59 (38%)	*P* value
Age, median (IQR)	27 (22-38)	25 (21-31)	34 (24-43)	<.0005
Male gender	52 (34)	35 (37)	17 (29)	.306
Allergic rhinitis*	69 (45)	30 (32)	22 (37)	.236
Sensitization, median (IQR)				
sIgE peanut extract	1.65 (0.35-11.05)	6.20 (0.72-23.70)	0.44 (0.16-1.65)	<.0005
sIgE Ara h 1	0.02 (0.00-0.61)	0.10 (0.01-7.58)	0.00 (0.00-0.03)	<.0005
sIgE Ara h 2	0.16 (0.03-3.50)	2.20 (1.14-10.00)	0.03 (0.00-0.08)	<.0005
sIgE Ara h 3	0.03 (0.01-0.18)	0.07 (0.01-0.92)	0.01 (0.00-0.03)	<.0005
sIgE Ara h 6†	0.07 (0.00-3.14)	1.37 (0.08-9.50)	0.00 (0.00-0.03)	<.0005
sIgE Ara h 8	2.02 (0.11-8.57)	2.05 (0.24-8.70)	1.50 (0.03-8.53)	.198
DBPCFC period				.952
2003-2012	84 (55)	52 (55)	32 (54)	
2012-2019	70 (45)	43 (45)	27 (46)	
DBPCFC result				NA
Positive objective	69 (45)	69 (73)	NA	
Positive subjective	26 (17)	26 (27)	NA	
Negative	59 (38)	NA	59 (100)	
Severity positive DBPCFC result‡				NA
Mueller 0	15 (16)	15 (16)	NA	
Mueller 1	16 (17)	16 (17)	NA	
Mueller 2	32 (34)	32 (34)	NA	
Mueller 3	28 (29)	28 (29)	NA	
Mueller 4	2 (2)	2 (2)	NA	

Value are n (%) unless otherwise indicated.
DBPCFC, Double-blind placebo-controlled food challenge; *IQR*, interquartile range; *NA*, not applicable; *sIgE*, specific IgE.
*Missing data n = 85.
†Missing data n = 36.
‡Missing data n = 1. Severity of symptoms during the oral food challenge based on the Mueller score with 0 = oral symptoms; 1 = cutaneous symptoms; 2 = gastrointestinal symptoms; 3 = respiratory symptoms; and 4 = cardiovascular symptoms.[16,30]

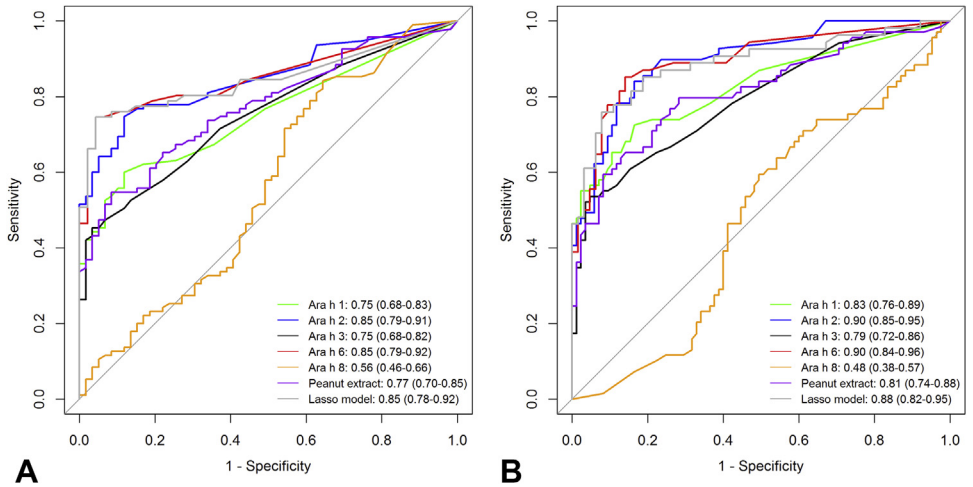

FIGURE 1. ROC curves of serology tests to predict a positive DBPCFC result (**A**) or a positive DBPCFC result with objective symptoms (**B**). The LASSO model included sIgE to Ara h 1 and Ara h 6 to predict peanut allergy (**A**) and sIgE to peanut extract, Ara h 1, Ara h 6, and Ara h 8 to predict peanut allergy with objective symptoms (**B**). *DBPCFC*, Double-blind placebo-controlled food challenge; *LASSO*, logistic least absolute shrinkage and selection operator; *ROC*, receiver-operating characteristic; *sIgE*, specific IgE.

studies in children (AUC 0.90-0.96).[14,34] In addition, we observed a lower cutoff level of Ara h 2 with 100% PPV (cutoff ≥1.75 kU_A/L) compared with children (cutoff 4.0-5.0 kU_A/L).[8,35] The Ara h 2 cutoff with 100% PPV was even lower in the subset of patients who were not part of the previous diagnostic study and underwent a DBPCFC between 2012 and 2019

TABLE II. Validation of sIgE to Ara h 2 cutoff level and cutoff levels with optimal positive and negative predictive values

	n	Cutoff (kUA/L)	PPV, % (N/D)*	NPV, % (N/D)†	Sens, % (N/D)‡	Spec, % (N/D)§
Validation						
sIgE to Ara h 2	70	1.75	100 (25/25)	60 (27/45)	58 (25/43)	100 (27/27)
Optimal PPV cutoff levels						
sIgE to Ara h 2	154	1.75	100 (49/49)	56 (59/105)	52 (49/95)	100 (59/59)
sIgE to Ara h 6	118	1.80	100 (33/33)	55 (47/85)	46 (33/71)	100 (47/47)
sIgE to Ara h 1	154	2.47	100 (34/34)	49 (59/120)	36 (34/95)	100 (59/59)
sIgE to Ara h 3	154	0.88	100 (25/25)	46 (59/129)	2625 (95)	100 (59/59)
sIgE to Ara h 8	154	100	100 (1/1)	39 (59/153)	1 (1/95)	100 (59/59)
sIgE to peanut extract	154	15.10	100 (32/32)	48 (59/122)	34 (32/95)	100 (59/59)
Optimal NPV cutoff levels						
sIgE to Ara h 2	154	0.02	71 (89/126)	79 (22/28)	94 (89/95)	37 (22/59)
sIgE to Ara h 6	118	0.04	86 (56/65)	72 (38/53)	79 (56/71)	81 (38/47)
sIgE to Ara h 1	154	0.05	89 (57/64)	58 (52/90)	60 (57/95)	88 (52/59)
sIgE to Ara h 3	154	0.01	67 (84/125)	62 (18/29)	88 (84/95)	31 (18/59)
sIgE to Ara h 8	154	0.01	64 (94/146)	88 (7/8)	99 (94/95)	12 (7/59)
sIgE to peanut extract	154	0.11	68 (94/139)	78 (14/18)	96 (94/98)	24 (14/59)

D, Denominator; *FN*, false-negative results; *FP*, false-positive results; *n*, number of patients included; *N*, numerator; *NPV*, negative predictive value; *PPV*, positive predictive value; *Sens*, sensitivity; *sIgE*, specific IgE; *Spec*, specificity; *TN*, true-negative results; *TP*, true-positive results.
*Numerator: TP, denominator: TP + FP.
†Numerator: TN, denominator: TN + FN.
‡Numerator: TP, denominator: TP + FN.
§Numerator: TN, denominator: TN + FP.

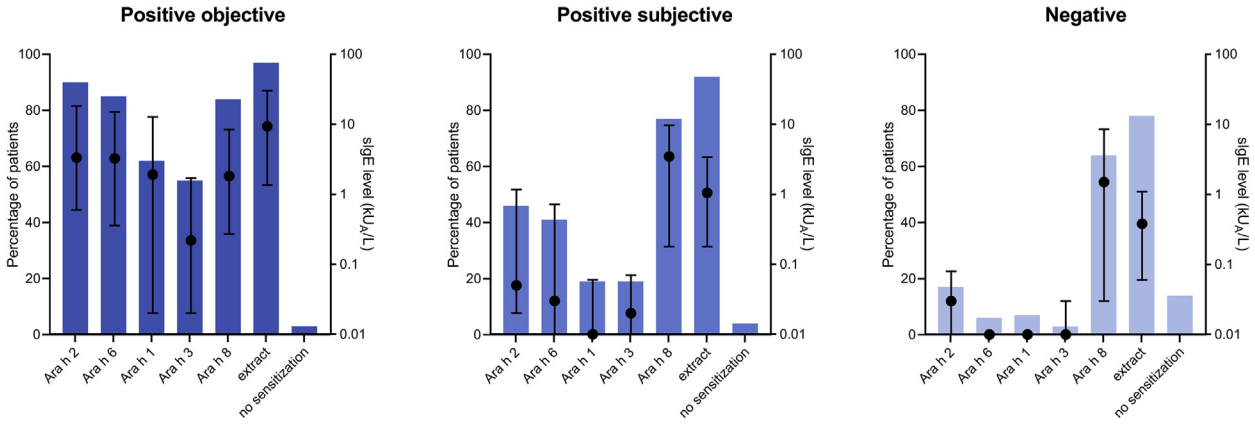

FIGURE 2. Percentage of patients with sensitization to peanut extract or peanut components and titers of sensitization, per DBPCFC result. The percentage of patients is shown is blue bars and the median (interquartile range) titers of sIgE are displayed in the center of the bars in black color. Sensitization is defined as a value of $\geq$0.10 kU_A/L. Specific IgE titers are displayed on logarithmic scales (base 10). *DBPCFC*, Double-blind placebo-controlled food challenge; *sIgE*, specific IgE.

(cutoff $\geq$0.43 kU_A/L). This disparity could not be explained by differences in age, sex, sensitization levels or DBPCFC outcome (ie, allergy vs tolerance) between both cohorts or by differences in the ratio of specific to total IgE (data not shown). The AUC value of sIgE to Ara h 6 using a singleplex assay in our study (AUC 0.85) was comparable with the AUC value of sIgE to Ara h 6 using a multiplex assay in adults (AUC 0.82), but lower than observed in children using a multiplex assay (AUC 0.98).[14,15] The lower diagnostic value of peanut components in adults compared with children could be explained by the higher proportion of birch pollen–related peanut allergy in adults. In patients with birch pollen allergy, cross-sensitization of Ara h 8 with major birch pollen allergen Bet v 1 occurs and these patients have mainly mild or subjective symptoms after peanut ingestion, making it more difficult to discriminate between the presence and absence of allergy.[12,14,16] As shown in the current study, adult peanut-allergic patients with subjective symptoms were less often sensitized to Ara h 2 and Ara h 6 and had lower titers, resulting in a lower diagnostic value. Further insight into the levels of IgE sensitization over time, from childhood to adulthood, could contribute to a better understanding of the differences between children and adults.

The concentrations of sIgE to Ara h 2 and Ara h 6 were highly correlated in our and previous studies.[36-38] In addition, the combination of Ara h 2 and 6 did not improve the diagnostic accuracy in the current study. The extensive overlap in sIgE

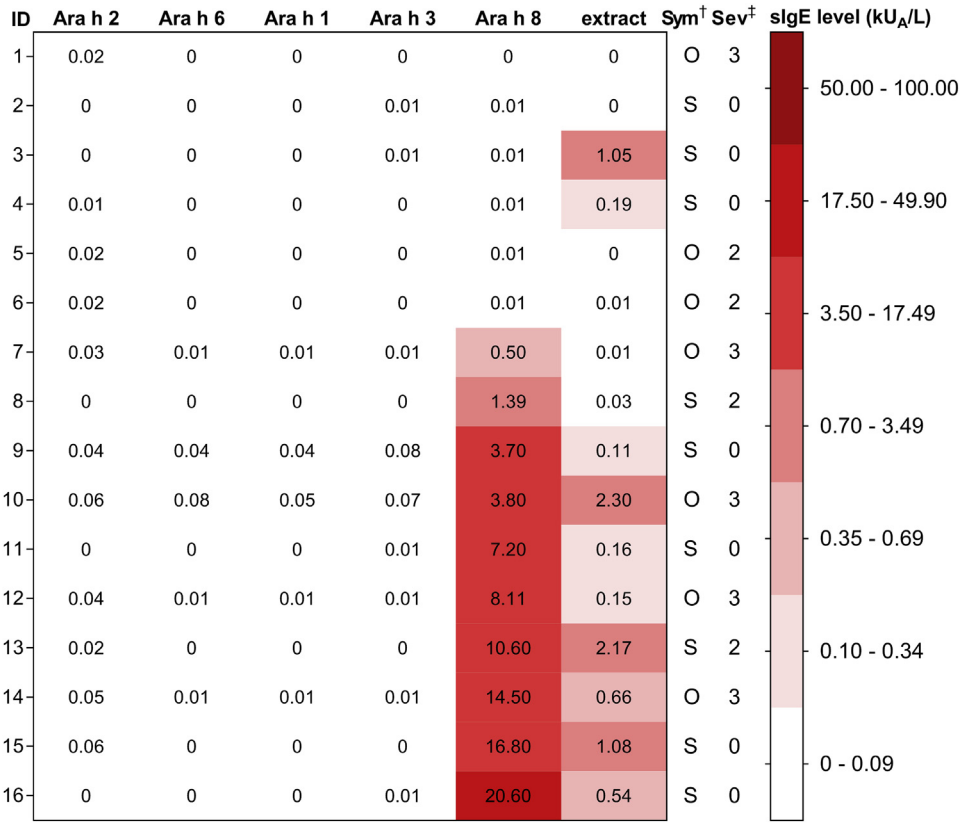

FIGURE 3. Sensitization pattern in 16 peanut-allergic patients without sensitization to Ara h 2 and Ara h 6. †Symptoms during the oral food challenge. ‡Severity of symptoms during the oral food challenge based on the Mueller score with 0 = oral symptoms; 2 = gastrointestinal symptoms; and 3 = respiratory symptoms.[16,30] *ID*, Patient number; *O*, objective; *S*, subjective; *Sev*, severity; *Sym*, symptoms.

reactivity and comparable diagnostic accuracy indicate that ImmunoCAP results for either Ara h 2 or Ara h 6 are sufficient in the diagnostic workup of a suspected peanut allergy in adults. Multivariable analyses revealed that a combination of Ara h 1 and Ara h 6 had the highest diagnostic accuracy, with an AUC comparable with those of Ara h 2 and Ara h 6 alone, despite regularization (ie, shrinkage of the coefficients) (AUC 0.85). At present, however, we recommend using sIgE reactivity to the peanut component Ara h 2 in daily practice, as the cutoff level is validated in the current study, implementation in daily practice is straightforward, and interpretation of sIgE levels to 1 peanut component is more intuitive than of a clinical prediction rule based on the LASSO model. Moreover, a recent study in children showed that Ara h 2 is likely the dominant allergen despite similarities with Ara h 6.[38]

Thirty-two percent of patients could be classified correctly as peanut allergic when using the validated cutoff level of sIgE to Ara h 2 with 100% PPV (cutoff $\geq$1.75 kU_A/L). This cutoff level of Ara h 2 could be used in daily clinical practice to select patients for oral food challenges. In patients with an Ara h 2 level above the cutoff level, peanut allergy is diagnosed with (almost) 100% accuracy without an oral food challenge. In these patients, a 1-day oral food challenge may still be performed, in accordance with the patient and physician preference, to assess the severity of the allergic reaction and the eliciting dose. In patients with an Ara h 2 level below the cutoff level, peanut allergic status cannot be predicted. In these patients, a 2-day DBPCFC is needed to confirm or exclude peanut allergy (see Figure E1 in this article's Online Repository at www.jaci-inpractice.org).

It must be noted that sIgE to Ara h 2 and Ara h 6 cannot be used to rule out peanut allergy in adults. In 16 peanut-allergic patients (17%) in our cohort, we did not detect sensitization to Ara h 2 and Ara h 6, nor for Ara h 1 or Ara h 3. In these patients, sensitization to peanut allergens might not have been detected by the ImmunoCAP platform or peanut allergy might have been caused by sensitization to other peanut components. Therefore, a different diagnostic testing modality was used to test a large number of peanut components (Ara h 1, 2, 3, 5, 6, 7, 8, 9, 10, 11, 14, and 15), resulting in 2 of 16 patients recognizing Ara h 9. Ten of 16 (63%) patients were sensitized to the birch pollen–related allergen Ara h 8, and 3 of these 10 patients who only recognized Ara h 8 experienced objective respiratory symptoms during the DBPCFC. However, the sensitization rate to Ara h 8 in peanut-tolerant patients was comparable (64%); thus sIgE to Ara h 8 was not predictive of clinical reactivity to peanut. A comparable frequency (18%) of allergic patients with negative serology tests, including Ara h 1, 2, 3, and 6, was reported in peanut-allergic patients in the EuroPrevall study.[36] The authors hypothesized that peanut oleosins may have caused allergic reactions in these patients. Interestingly, all peanut-

allergic patients with negative Ara h 1, 2, 3, and 6 results in our study were tested negative to peanut oleosins (ie, Ara h 10, 11, 14, and 15). Thus, peanut allergy in nonsensitized patients as detected in this and previous studies might be caused by sensitization to thus far unidentified peanut components.

A limitation of our study was that we only included patients with a conclusive DBPCFC result, and with leftover serum available from a tertiary center. However, we believe that our sample was a representative sample as we did not observe significant differences between included and excluded patients. Furthermore, the percentage of positive DBPCFCs in our study (62%) was comparable with other studies (43%-66%).[16,39,40] The strengths of our study were that we included a large cohort of adult patients who all underwent a standardized DBPCFC for peanut allergy, we measured a large number of peanut components, we performed a validation of our previous results, and we were able to confirm these findings.[16]

In conclusion, we showed that sIgE to Ara h 2 and Ara h 6 are the best predictors of peanut allergy in adults. Furthermore, we validated our previously published Ara h 2 cutoff level with 100% PPV. The validated Ara h 2 cutoff level should be implemented in daily practice to reduce the need for DBPCFCs, as all adults with an Ara h 2 $\geq$1.75 kU_A/L were peanut allergic.

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ONLINE REPOSITORY

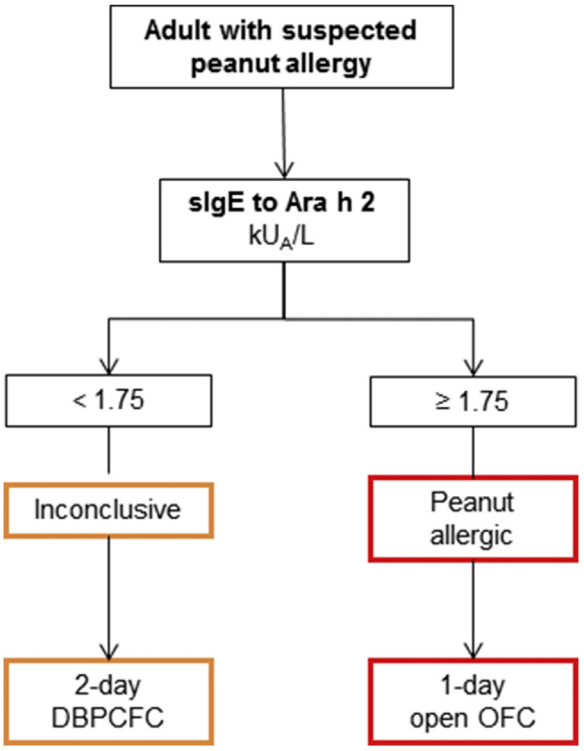

FIGURE E1. Diagnostic flowchart for adults with suspected peanut allergy. *DBPCFC*, Double-blind placebo-controlled food challenge; *OFC*, oral food challenge; *sIgE*, specific IgE.

TABLE E1. Baseline characteristics of included and excluded patients

	Included	Excluded		
	n = 154 (75%)	n = 50 (25%)	n available	*P* value
Age, mean (SD)	27 (22-38)	24.5 (20.3-31)	32	.055
Male gender	52 (34)	19 (38)	50	.585
Allergic rhinitis*	52 (75)	11 (73)	15	.869
Sensitization, median (IQR)				
sIgE peanut	1.65 (0.35-11.05)	2.35 (0.71-22.40)	23	.377
sIgE Ara h 1	0.02 (0.00-0.61)	0.00 (0.00-0.01)	4	–
sIgE Ara h 2	0.16 (0.03-3.50)	0.38 (0.00-1.37)	19	.424
sIgE Ara h 3	0.03 (0.01-0.18)	0.00 (0.00-0.01)	4	–
sIgE Ara h 6	0.07 (0.00-3.14)	0.02 (0.00-0.53)	6	–
sIgE Ara h 8	2.02 (0.11-8.57)	1.23 (0.01-8.75)	18	.428
DBPCFC result†			50	<.0005
Positive	95 (62)	20 (40)		
Inconclusive	0	18 (36)		
Negative	59 (38)	12 (24)		.213
Severity positive DBPCFC result‡			11	
Mueller 0	15 (16)	2 (18)		
Mueller 1	16 (17)	2 (18)		
Mueller 2	32 (34)	7 (64)		
Mueller 3	28 (30)	0		
Mueller 4	2 (2)	0		

Values are n (%) unless otherwise indicated.
DBPCFC, Double-blind placebo-controlled food challenge; *IQR*, interquartile range; *SD*, standard deviation; *sIgE*, specific IgE.
*Missing data included patients n = 85.
†One of the reasons for exclusion was an inconclusive DBCPFC result. The proportion of positive and negative DBPCFC results were comparable between groups ($P = .931$).
‡Missing data included patients n = 2. Severity of symptoms during the oral food challenge based on the Mueller score with 0 = oral symptoms; 1 = cutaneous symptoms; 2 = gastrointestinal symptoms; 3 = respiratory symptoms; and 4 = cardiovascular symptoms.[E1,E2]

TABLE E2. Diagnostic value of peanut components in the subset of cohort with available sIgE to Ara h 6 (n = 118)

	AUC (95% CI)
sIgE extract	0.76 (0.68-0.85)
sIgE Ara h 1	0.76 (0.68-0.84)
sIgE Ara h 2	0.85 (0.78-0.92)
sIgE Ara h 3	0.75 (0.67-0.84)
sIgE Ara h 8	0.57 (0.46-0.68)

AUC, Area under the curve; *CI*, confidence interval; *sIgE*, specific IgE.

TABLE E3. DeLong's test for comparing 2 ROC curves

	sIgE Ara h 6	sIgE extract	sIgE Ara h 1	sIgE Ara h 3	sIgE Ara h 8
sIgE Ara h 2	.87	**.02**	**<.01**	**<.01**	**<.01**
sIgE Ara h 6		**.02**	**<.01**	**<.01**	**<.01**
sIgE extract			.61	.56	**<.01**
sIgE Ara h 1				.93	**<.01**
sIgE Ara h 3					**<.01**
sIgE Ara h 8					

Data are *P* values corrected for multiple testing using the Benjamini-Hochberg procedure.
Significant *P* values are in bold.
ROC, Receiver-operating characteristic, *sIgE*, specific IgE.

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