Diagnostic Value of Antigen-Specific Immunoglobulin E Immunoassays against Ara h 2 and Ara h 8 Peanut Components in Child Food Allergy

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Key Words

Peanut allergy · Pediatric allergy · Ara h 2 · Ara h 8 ·
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Abstract

Background: Peanut allergy is one of the most severe food allergies in children. The diagnostic gold standard is the oral food challenge (OFC). However, OFC has inherent risks and is time consuming. The measurement of specific immunoglobulin E (sIgE) to peanut components in blood detects peanut sensitization, but the decision point predicting allergy is still unclear. The aim of this study was to determine the diagnostic value of these tests for the evaluation of child peanut allergy. **Methods:** In this retrospective study, 81 children were referred for peanut allergy. The diagnosis of peanut allergy was based on the clinical context and a positive OFC. Levels of sIgE against whole peanuts or peanut components (Ara h 2 and Ara h 8) were determined by immunoassay. **Results:** The Ara h 2 sIgE assay has the best negative predictive value (0.93) and positive predictive value (1) at a cutoff of 0.1 kU/l. Ara h 2 sIgE titers can predict the risk of anaphylaxis (<0.44 kU/l, low risk; >14 kU/l, high risk). The

Ara h 8 sIgE assay is not able to discriminate peanut-allergic patients but can be used to evaluate possible cross-reactions to birch pollen with a low risk of anaphylaxis. The best diagnostic strategy is to first determine the Ara h 2 sIgE level and, if negative, evaluate Ara h 8 sIgE. **Conclusions:** We propose an algorithm for a better use of peanut component sIgE immunoassays that should improve their diagnostic value and avoid unnecessary OFC.

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Introduction

Peanut allergy is one of the most frequent food allergies capable of eliciting severe reactions in childhood, with an increased prevalence over the years [1]. Disease onset generally occurs within the first years of life and spontaneously resolves in only 15% of patients [2]. Allergic reaction to this allergen is associated with fatal anaphylaxis [3, 4]. Therefore, as peanut intake is common in Western countries, a profound change in dietary habits is required for allergic patients, with an impact on their quality of life [5–8]. Currently, oral food challenge (OFC)

Table 1. Characteristics of the population grouped into peanut-allergic and tolerant patients

	Total	Allergic	Tolerant
Patients	81	48	33
Mean age $\pm$ SD, years	7.7 $\pm$ 4.4	7.6 $\pm$ 4.3	7.7 $\pm$ 4.7
Male gender	60	31	29
Polyallergy	50	36	14
Asthma, n (%)	11 (13.6)	6 (12.5)	5 (15.1)
OFC clinical reactions (<50 mg)			
Urticaria		3	0
Angioedema		2	0
Rhinoconjunctivitis		2	0
Frequent coughing, wheezing		1	0
Important abdominal pain and repeated vomiting		1	0
Hypotension		0	0
OFC clinical reactions (>50 mg)			
Urticaria		30	0
Angioedema		29	0
Rhinoconjunctivitis		20	0
Frequent coughing, wheezing		8	0
Important abdominal pain and repeated vomiting		5	0
Hypotension		5	0
OFC clinical severity			
No clinical reaction	33	0	33
Mild clinical reaction	33	33	0
Severe clinical reaction	15	15	0

Values are presented as numbers unless otherwise stated.

is the gold standard for the diagnosis of peanut allergy. Furthermore, OFC allows evaluation of the food amount that triggers anaphylaxis [9, 10]. Laboratory tests measuring the serum levels of specific immunoglobulin E (sIgE) directed against whole-peanut protein extracts have been used to evaluate peanut sensitization. However, even when combined with skin tests, these assays are not able to accurately diagnose peanut allergy and evaluate the risk of anaphylaxis because of a lack of sensitivity and specificity [11, 12].

Component sIgE immunoassays have been proposed to improve the diagnosis of allergy. Notably, they are meant to better categorize patients into groups with different prognoses, i.e. those at a low or high risk for anaphylaxis. Regarding peanut allergy, Ara h 2, a storage protein also referred to as conglutin, has been identified as a major allergen [13–15]. Another peanut allergen, i.e. Ara h 8, a so-called pathogenesis-related protein (PR-10), is associated with mild peanut-induced reactions and cross-reacts with other PR-10 such as birch pollen protein Bet v 1 [16]. The diagnostic value of these component sIgE immunoassays, and notably their role in the

diagnostic strategy, requires more thorough investigation [17, 18].

We aimed to assess the possibility of predicting severe peanut allergy, as well as the diagnostic accuracy values of whole-peanut and peanut component sIgE immunoassays, by studying a cohort of children referred to our center for peanut OFC, and to propose a diagnostic strategy to guide the evaluation and interpretation of whole-peanut and peanut component sIgE.

Patients and Methods

Study Design

Eighty-one children referred to the Pediatric Department of Rouen University Hospital for investigation of peanut allergy were included in this retrospective study. This study was approved by the Research and Ethics Committee of Rouen University Hospital under the reference E2014-15.

The inclusion criteria were: (i) referral to the tertiary allergy center for peanut OFC and (ii) serum measurement of sIgE against whole peanut and Ara h 2 and Ara h 8 components. The diagnosis of peanut allergy was based on: (i) a documented self-reported immediate reaction to peanut and a positive OFC, (ii) a recent severe

anaphylaxis reaction after peanut feeding associated with a positive peanut prick test, and obviously no OFC. Clinical data were recorded and the patients were diagnosed as proven peanut allergic or tolerant.

Oral Food Challenge

The OFC was performed as an open challenge test with peanut oil and homogenized fresh peanuts in a hospital environment. The use of antihistamines had been suspended 7 days prior to the challenge. Amounts of peanut oil equal to 5 ml and peanut protein equal to 50, 100, 250, and 500 mg and 1, 2, and 4 g were given orally every 20 min [19]. The test was monitored and clinical reactions were evaluated. The OFC test was scored as positive and stopped if objective clinical reactions such as urticaria, angioedema, rhinoconjunctivitis, frequent coughing, wheezing, important abdominal pain with repeated vomiting, and hypotension were observed [20]. The patients remained under clinical supervision for at least 2 h after the end of the challenge.

As proposed by the European Academy of Allergy and Clinical Immunology (EAACI) Task Force on Anaphylaxis in Children, patients with a clinical reaction of at least 2 organs, regardless of the amount of peanut ingested, or patients with clinical reactions after ingestion of less than 50 mg of peanut after the OFC, were classified as having a severe peanut allergy [21]. Patients with a clinical reaction of only 1 organ after ingestion of more than 50 mg of peanuts were classified as having a mild peanut allergy.

sIgE Immunoassays

All samples were analyzed using ImmunoCAP (Phadia, Sweden) for IgE reactivity to whole peanut, Ara h 2, and Ara h 8. Twenty samples were analyzed for IgE reactivity to Bet v 1.

Statistics

The nonparametric Mann-Whitney U test was used for comparisons between peanut sIgE titer means. The Youden index (sensitivity + specificity – 1, target >0.8) was used to evaluate the accuracy of biological tests compared to clinical diagnosis and OFC. Sensitivity, specificity, negative predictive values (NPV), and positive predictive values (PPV) were evaluated for each test and compared with clinical diagnosis and OFC. They were determined at 6 different cutoff levels, i.e. 0.10 kU/l, corresponding to the detection threshold of the method; 0.35 kU/l, which is the currently admitted threshold for clinical relevance [22], and 0.5, 1, 5, and 15 kU/l. A receiver operating characteristic (ROC) curve was used to evaluate the positive threshold. Data were analyzed using Prism software (version 6.01, GraphPad, USA).

Results

Patient Characteristics

Eighty-one (74% boys) children and adolescents were included in this study (table 1). Most of them (n = 50; 62%) had a diagnosis of polyallergy (at least 1 confirmed allergy to other allergens) and 13.6% had developed asthma. The OFC confirmed a peanut allergy in

Fig. 1. Summary flowchart for patient selection.

Fig. 2. ROC curves for peanut sIgE immunoassays. Whole-peanut, Ara h 2, and Ara h 8 sIgE titers were compared between tolerant and allergic patients to evaluate sensitivity and specificity at different thresholds. The ROC curve (sensitivity vs. 100 – specificity) was calculated from these results.

48 patients (peanut-allergic group), whereas the remaining 33 children were peanut tolerant (peanut-tolerant group; fig. 1). Five patients were diagnosed as peanut allergic without performance of an OFC. Within the peanut-allergic group, 69% were classified as se-

Fig. 3. Algorithm for the evaluation and interpretation of whole-peanut, Ara h 2, and Ara h 8 sIgE immunoassays.

verely peanut allergic and 31% were classified as mildly peanut allergic.

Accuracy of the Whole-Peanut, Ara h 2, and Ara h 8 sIgE Evaluation

The whole-peanut sIgE immunoassay sensitivity was 100% for an sIgE cutoff level of 0.1 kU/l, associated with an NPV of 1.00, and it then decreased for higher cutoff values (table 2). Accordingly, the specificity increased from 56 to 100%, and the PPV from 0.7 to 1.00, when the cutoff levels were raised. The Youden index reached its maximum value, i.e. 0.7 for a cutoff of 1.00 kU/l. Consistently, the best diagnostic value of the test, as determined based on the ROC curve and the Youden index, was found for the cutoff of 1 kU/l (fig. 2).

The best result for Ara h 2 sIgE was obtained for a cutoff value of 0.35 kU/l, yielding a Youden index of 0.94 and a specificity, sensitivity, PPV, and NPV of 100 and 94.4% and 1.00 and 0.93, respectively (fig. 2).

The Ara h 8 sIgE sensitivity decreased from 36.11 to 5.56%, and the specificity increased from 84 to 100%, when the cutoff values increased, with PPV ranging from 0.97 to 1 and NPV from 0.48 to 0.42 (table 2). The

Table 2. Performance evaluation of sIgE immunoassays against native and component peanut

sIgE	Threshold, kU/l	Sensitivity, %	Specificity, %	PPV	NPV	Youden's index
Whole peanut	0.10	100.00	56.00	0.77	1.00	0.56
	0.35	97.22	72.00	0.83	0.95	0.69
	0.50	94.44	76.00	0.85	0.90	0.70
	1.00	86.11	84.00	0.89	0.81	0.70
	5.00	63.89	100.00	1.00	0.66	0.64
	15.00	36.11	100.00	1.00	0.52	0.36
Ara h 2	0.10	94.44	96.00	0.97	0.92	0.90
	0.35	94.44	100.00	1.00	0.93	0.94
	0.50	75.00	100.00	1.00	0.74	0.75
	1.00	63.89	100.00	1.00	0.66	0.64
	5.00	38.89	100.00	1.00	0.53	0.39
	15.00	25.00	100.00	1.00	0.48	0.25
Ara h 8	0.10	36.11	84.00	0.76	0.48	0.20
	0.35	30.56	84.00	0.73	0.46	0.15
	0.50	27.78	84.00	0.71	0.45	0.12
	1.00	22.22	92.00	0.80	0.45	0.14
	5.00	13.89	96.00	0.83	0.44	0.10
	15.00	5.56	100.00	1.00	0.42	0.06
Combination of sIgE assays (fig. 3)	–	100.00	96.00	0.97	1.00	0.96

Fig. 4. Relationship between whole-peanut and component sIgE immunoassay titers and clinical reactions. Comparison of whole-peanut (**a**), Ara h 2 (**b**), and Ara h 8 (**c**) sIgE titers between mild ($n = 33$) and severe ($n = 15$) clinical reactions of peanut-allergic

children (mean $\pm$ SEM). **d** Ara h 2 sIgE titers were compared between mildly and severely allergic patients to evaluate the sensitivity and specificity at different thresholds. The ROC curve was calculated from these results.

Youden index ranged between 0.06 and 0.20. While this immunoassay was clearly of a lower diagnostic value than the other two tests, its best result was obtained at the cutoff of 0.1 kU/l, yielding a Youden index of 0.20 (fig. 2).

Ara h 8 sIgE and Cross-Sensitization

IgE reactivity against the PR-10 Ara h 8 protein is associated with cross-sensitization with the birch pollen protein Bet v 1 [16]. Twenty children were tested for Bet v 1 sIgE because of suspicion of an associated birch allergy. Twelve of them were found to be positive for Bet v 1 sIgE and they were also positive for Ara h 8 sIgE. After the OFC, 8 of these 12 Bet v 1 sIgE-positive patients were clinically diagnosed as peanut allergic and were therefore considered to be cross-allergic. Reciprocally, the remaining 4 patients who were positive for

Ara h 8 sIgE but not peanut allergic according to the OFC were considered to be only cross-sensitized but not cross-allergic. It should be mentioned that OFC-defined nut allergy was significantly more frequent (60%) in Ara h 8 sIgE-positive patients than in Ara h 8 sIgE-negative (22%) ones.

Diagnostic Strategy

Used alone, the Ara h 2 sIgE immunoassay had the best diagnostic value as determined by the Youden index (0.94). However, we hypothesized that the use of this test sequentially, in case of negativity, with Ara h 8 sIgE and whole-peanut sIgE immunoassays, might improve their diagnostic value. Indeed, use of the decision tree shown in figure 3 improved the diagnostic performance (Youden index = 0.96). This strategy augments the sensitivity of the immunoassay to 100% and provides the best specificity (96%).

sIgE Concentration Level and Clinical Reaction during OFC

In an attempt to predict the risk of anaphylaxis, we analyzed the relationship between the clinical reaction during an OFC and the titers of Ara h 2, Ara h 8, and whole-peanut sIgE. While Ara h 8 failed to categorize patients according to this risk, the titers of Ara h 2 and whole-peanut sIgE allowed accurate discrimination of high-risk children (fig. 4). Of note, following the proposed diagnostic strategy (fig. 3), Ara h 2 sIgE-negative patients who were positive for Ara h 8 sIgE were found to only develop oral syndrome reactions.

Finally, we evaluated within our series of peanut-allergic children the Ara h 2 sIgE cutoff value that was able to discriminate patients with mild or severe clinical reactions. The ROC curve analysis revealed that allergic children with Ara h 2 sIgE titers <0.44 kU/l had $>95\%$ probability of developing a mild clinical reaction, whereas those with titers >14 kU/l had $>95\%$ probability of a severe clinical reaction (fig. 4).

Discussion

This retrospective study evaluated the cutoff value of whole-peanut, Ara h 2, and Ara h 8 sIgE immunoassays in children who were referred for peanut allergy to a tertiary pediatric allergy center. Biologic assays were compared to OFC conducted as recommended [20]. In addition to accuracy values, test performance was also assessed using the Youden index and an ROC curve. Even at the best cutoff value of 0.5 kU/l, whole-peanut sIgE used alone appeared to be of a low diagnostic value, yielding a maximal Youden index below 0.80, with poor specificity and PPV. We conclude that it cannot reasonably be retained as an accurate tool to discriminate peanut allergy from tolerance, consistent with previous reports [12, 23–25]. At a cutoff of 0.35 kU/l, the Ara h 2 sIgE immunoassay was acceptable, with a Youden index of 0.94 and a PPV of 100%, in line with recent results [17, 26–28]. Thus, Ara h 2 sIgE immunoassay should be the first biological test performed for the diagnosis of peanut allergy.

The involvement of PR-10 such as Ara h 8 in severe peanut reactions is still a topic of debate [16, 17]. However, oral syndrome has commonly been reported with PR-10 and cross birch or hazelnut allergy [29]. The present study underlined that Ara h 8 sIgE was not useful for the diagnosis of peanut allergy when used alone but could be valuable when measured secondarily in case of

Ara h 2 sIgE negativity. In this series, all Ara h 8 sIgE-positive patients were also positive for Bet v 1 sIgE, suggesting that the assessment of only one PR-10 may be sufficient. Therefore, it is reasonable to restrict the use of Ara h 8 or Bet v 1 sIgE immunoassays to oral syndrome or suspicion of cross-allergy in patients negative for Ara h 2 sIgE.

Few studies have evaluated the role of sIgE titers in peanut allergy [18]. Therefore, we next investigated whether the titers of whole-peanut and component sIgE could inform as to the risk of anaphylaxis and determine the cutoff values to predict this risk. The results revealed that peanut-allergic children with Ara h 2 sIgE titers <0.44 kU/l under OFC have a mild peanut allergy and are at a low risk for anaphylaxis, while those with titers >14 kU/l develop, under OFC, a severe peanut allergy and are exposed to a higher risk. These results show a possible relationship between Ara h 2 sIgE titers and clinical reactions in patients. Similarly, Klemans et al. [30] demonstrated a relation between Ara h 2 sIgE titers and the dose response to the peanut allergen during an OFC.

Together, these observations support the proposal of the decision tree presented in figure 3 for our population: the best Youden index (0.96) was obtained for this strategy, including an excellent PPV (0.96). Further, we demonstrated that Ara h 2 sIgE titers could predict the clinical reaction after an OFC and, therefore, the risk of anaphylaxis. Following this flowchart, children with negative Ara h 2 and positive Ara h 8 sIgE immunoassays had a high probability of only developing a weak clinical reaction in contact with peanut. However, various other peanut components not evaluated herein, such as Ara h 9 in Mediterranean countries, could be clinically relevant allergens [31, 32]. That allergen was not tested here since our study focused on a pediatric cohort located in the north of France, and therefore Ara h 9-positive patients were unlikely to be encountered. These regional variations suggest that diagnosis algorithms may require optimization for distinct geographical regions [17, 33].

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