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IgE binding to peanut components by four different techniques: Ara h 2 is the most relevant in peanut allergic children and adults

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Summary

Background Several studies have analysed the diagnostic value of specific IgE (sIgE) for individual peanut allergens. However, little is known about the concordance between different techniques available in both children and adults.

Objective To evaluate the value of individual peanut allergens by different techniques, i.e. multi-plexed microarray, single-plexed IgE assay, skin prick test (SPT) and immunoblot in both peanut allergic adults and children.

Methods Sensitization patterns to peanut allergens Ara h 1, 2, 3, and 8 were evaluated using four different techniques: multi-plexed microarray immunoassay, single-plexed IgE assay, SPT and immunoblot. Twenty-two peanut allergic adults and 15 children scored on clinical severity according to double-blind, placebo-controlled food challenges and 27 atopic control patients were included.

Results Comparable sensitivity values were found between all four techniques in adults, with the highest sensitivity for Ara h 2 (76.2–95.5%, compared to 100% with all techniques in children). The multi-plexed assay to Ara h 1 (93.3%) demonstrated a higher sensitivity compared with the other three techniques ($P = 0.04$) in children, but absolute values were perfectly correlated. There were no differences between adults and children. The area under the receiver operating characteristic curve (AUC) of sIgE to Ara h 1 was higher with the multi-plexed assay compared with the single-plexed assay (0.91 vs. 0.75). In adults, sIgE to Ara h 1, 2, and 3 was correlated with clinical severity. No such correlation was found in children.

Conclusion and Clinical Relevance In conclusion, the single- and multi-plexed assay, SPT and immunoblot perform equally in both peanut allergic adults and children, with Ara h 2 being most often recognized with all techniques. Specific IgE to Ara h 1, 2, and 3 in adults was correlated with severity.

Keywords immunoblot, multi-plexed microarray immunoassay, peanut allergy, single-plexed IgE assay, skin prick test

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Introduction

Peanut allergy is a common health problem affecting more than 1% of the population in the western world [1–4]. Its prevalence may be on the rise, especially among children [2, 5]. As peanut allergy is rarely outgrown and accidental exposure can elicit severe or even fatal reactions, accurate diagnostics is important [6].

The current gold standard, the double-blind placebo-controlled food challenge (DBPCFC), is expensive, laborious and burdensome. Routinely used diagnostic tools, such as skin prick test (SPT) and serum-specific IgE (sIgE) tests often fail to discriminate between true allergy and asymptomatic sensitization [6].

Component-resolved diagnostics (CRD) for peanut allergy might be more discriminative by using

individual-specific allergens instead of whole peanut extracts, which contain both true peanut allergens and cross-reactive allergens (e.g. PR-10 family members) next to large amounts of non-allergenic proteins [7, 8]. Until now, 10 different specific peanut allergens have been characterized, e.g. Ara h 1 to 11, with Ara h 3 and 4 being the same protein. Among the three major peanut allergens (Ara h 1, 2, and 3/4), recent studies have demonstrated the superior value of Ara h 2 [9–17]. However, none of these studies compared different techniques to analyse these peanut components. In addition, all these studies were performed in children. The sensitization pattern of peanut allergens in an adult peanut allergic population remains largely unknown yet.

Therefore, our aim was to evaluate the value of individual peanut allergens by different diagnostic techniques, i.e. multi-plexed microarray, single-plexed IgE assay, SPT and immunoblot in both peanut allergic adults and children. Peanut allergy was confirmed by DBPCFC, and the sensitization profile of peanut allergic patients was compared with atopic control patients. Furthermore, we analysed the possible association between IgE binding to peanut components and severity in both adults and children.

Methods

Patients

In total 37 peanut allergic patients were included from the outpatient department of the University Medical Centre in Utrecht, the Netherlands.

Twenty-two adults were included with a history of allergic reactions to peanut, sensitization and a positive DBPCFC [18]. The median age was 24 years (interquartile range (IQR) 21–29 years).

Fifteen children were included based on sensitization to peanut and a positive DBPCFC [19]. Most of the children had a history of allergic reactions to peanut, but some had never consumed peanut due to elimination diets. Median age was 6 years (IQR 4–7 years). The clinical characteristics of all peanut allergic subjects are summarized in Table 1.

In addition, 27 adult atopic controls, median age of 33 years (IQR 22–40 years), were included. At time of inclusion, all control patients indicated to consume peanut regularly without allergic symptoms. Ten patients had a mixed allergy to grass and birch pollen, 11 patients had a mono-allergy to grass pollen and 1 to birch pollen. The other 5 patients had a food allergy (other than peanut) in addition to their grass and birch pollen allergy.

The study was approved by the Medical Ethical Committee of the University Medical Center Utrecht. All patients gave written informed consent.

Design

Clinical information regarding history and results of earlier performed SPT, immunoblot and DBPCFC were obtained [14, 18, 19]. The DBPCFC was performed according to the international consensus protocol [20]. The challenge consisted of nine doses ranging from 0.01 mg to 3 g peanut flour. The recipe has been described in detail earlier [18, 19]. The challenge was discontinued in case of objective symptoms or convincing subjective symptoms at three consecutive doses. The SPT was performed with purified Ara h 1, Ara h 2 and Ara h 3 in serial tenfold dilutions [14, 18]. In this study, we describe the SPT reactions on the highest dilution: 100 µg/mL, as this concentration gave the highest response. The preparation of the dilutions has been described in our previous studies of Flinterman *et al.* and Peeters *et al.* [18, 19]. Histamine dihydrochloride (10 mg/mL) and saline were used as positive and negative controls respectively. The SPT reactivity was measured after 15 min using computer scanning and expressed by the ratio of the weal reaction to peanut in millimetres squared, divided by the area of the weal reaction of the positive control [21]. A ratio ≥ 0.25 was regarded positive. For quantitative analysis of the immunoblots, a 5-point scale was used from 0 (negative) to 4 (strongly positive). A value ≥ 1 was considered positive. The severity of peanut allergy was expressed by a severity score based on results of the DBPCFC. Specific IgE binding to whole peanut extract and recombinant peanut allergens Ara h 1, Ara h 2, Ara h 3 and Ara h 8 was measured using single-plexed IgE assay and a multi-plexed microarray as well.

Single-plexed IgE assay

Specific IgE to whole peanut extracts and four recombinant peanut allergen components were assessed using ImmunoCAP® Specific IgE (Thermo Fisher Scientific, Uppsala, Sweden) according to manufacturer's instructions. A value of sIgE < 0.35 kU_A/L was considered negative.

Multi-plexed microarray immunoassay

Immuno Solid-phase Allergen Chip (ImmunoCAP ISAC®, VBC Genomics and Thermo Fisher Scientific) with 103 allergen components was used following manufacturer's instructions [22]. A value of < 0.3 ISU was considered negative.

Clinical severity

Severity of allergic reactions according to history and during DBPCFC was graded according to Muller [23].

Table 1. Clinical characteristics of the peanut allergic patients (22 adults and 15 children)

ID	Sex	Age	Muller history	IgE peanut	SPT peanut	Lowest ED	Dose score	Symptoms DBPCFC	Muller DBPCFC	Severity score
04	f	22	0	15.2	1.3	10	3	ap	2	$3 \times (2 + 1) = 9$
16	m	21	0	< 0.35	0.9	10	3	oas	0	$3 \times (0 + 1) = 3$
20	f	21	0	< 0.35	1.4	10	3	oas	0	$3 \times (0 + 1) = 3$
01	m	21	1	3.6	1.9	300	1	oas	0	$1 \times (0 + 1) = 1$
11	m	20	1	2.4	1.8	0.1	6	oas	0	$6 \times (0 + 1) = 6$
19	f	35	1	3.4	1.4	1	4	rc	1	$4 \times (1 + 1) = 8$
06	m	32	2	1.1	2.4	100	2	n, ap	2	$2 \times (2 + 1) = 6$
14	f	27	2	16.1	6.8	10	3	n	2	$3 \times (2 + 1) = 9$
18	f	29	2	0.9	2.9	10	3	ap, n, d	3	$3 \times (3 + 1) = 12$
03	f	19	3	1.6	1.9	100	2	oas	0	$2 \times (0 + 1) = 2$
05	f	37	3	8.7	3.8	10	3	dia	2	$3 \times (2 + 1) = 9$
13	f	24	3	4.7	0.6	10	3	n, ap	2	$3 \times (2 + 1) = 9$
15	f	23	3	91.4	5.9	0.1	6	d	3	$6 \times (3 + 1) = 24$
21	m	33	3	100	6.0	1	4	ap, d	3	$4 \times (3 + 1) = 16$
23	m	18	3	5.5	2.6	0.5	5	ap, n, v	2	$5 \times (2 + 1) = 15$
25	f	29	3	1.9	8.8	100	2	n, ap	2	$2 \times (2 + 1) = 6$
29	m	27	3	29.8	2.0	1	4	d, ap	3	$4 \times (3 + 1) = 16$
02	m	16	4	101	1.9	100	2	d	3	$2 \times (3 + 1) = 8$
09	m	25	4	28.1	6.4	0.1	6	n, v	2	$6 \times (2 + 1) = 18$
10	f	22	4	101	6.2	0.1	6	n	2	$6 \times (2 + 1) = 18$
22	f	24	4	9.2	4.2	10	3	d, h	3	$3 \times (3 + 1) = 12$
26	f	28	4	18	14.8	0.1	6	oas	0	$6 \times (0 + 1) = 6$
33	m	6	no	4	7	3000	2	rc, v	2	$2 \times (2 + 1) = 6$
37	f	6	no	101	10	1000	3	rc, v, b	3	$3 \times (3 + 1) = 12$
38	f	5	no	3.9	8	3000	2	oas, rc, ap, dia, d	3	$2 \times (3 + 1) = 8$
40	m	4	no	9.5	1	1000	3	oas, fs, rc, ap, d	3	$3 \times (3 + 1) = 12$
34	f	5	1	74.1	10	100	5	rc, d	3	$5 \times (3 + 1) = 20$
35	m	6	1	101	6	10	6	oas, v	2	$6 \times (2 + 1) = 18$
39	m	12	1	17.9	6	300	4	oas, rc, ap, v	2	$4 \times (2 + 1) = 12$
42	f	4	1	24.7	2	1000	3	oas, ap, v	2	$3 \times (2 + 1) = 9$
45	m	7	1	52.4	10	>3000	1	gu, rc, ap, v, dia	2	$1 \times (2 + 1) = 3$
46	m	3	1	2	13	300	4	oas, v, rc, d	3	$4 \times (3 + 1) = 16$
48	m	7	1	43	3	3000	2	oas, cu, fs	1	$2 \times (1 + 1) = 4$
47	m	12	2	2.3	3	1000	3	n, v	2	$3 \times (2 + 1) = 9$
41	m	4	3	58.7	10	1000	3	oas, gu, rc, b, t	4	$3 \times (4 + 1) = 15$
43	m	7	3	101	3	10	6	oas, ap	2	$6 \times (2 + 1) = 18$
44	m	12	3	2.4	9	100	5	oas, fs, urt, rc, ap	2	$5 \times (2 + 1) = 15$

The solid line in the middle separates the adult (above) and paediatric (below) population

Severity score = dose score $\times$ (Mueller according to DBPCFC + 1).

no, no symptoms; oas, oral allergy syndrome; n, nausea; ap, abdominal pain; urt, urticaria; cu, contact urticaria; gu, generalised urticaria; fs, facial swelling; rc, rhinoconjunctivitis; v, vomiting; dia, diarrhoea; d, dyspnoea; h, hoarseness; b, bronchoconstriction; t, tachycardia.

Muller grade 0 was defined as local symptoms of the oral cavity, grade 1 as symptoms of the skin and mucous membranes, grade 2 as gastrointestinal symptoms, grade 3 as respiratory symptoms and grade 4 as cardiovascular symptoms. Muller grades do not take eliciting dose (ED) into account, which is an important clinical factor. Therefore, we applied a severity score which combines the severity of the reaction and the lowest ED during challenge in a similar way as previously described [24, 25]. Severity score = (Muller according to DBPCFC + 1) $\times$ dose score (which is a 1–6 ranked score corresponding to

ED from 300 mg to 0.1 mg in adults and ED from > 3 g to 10 mg in children). Muller scores were increased by one to avoid multiplying with zero. Dose score was defined according to the range of ED in each of the two subpopulations. The range of ED in adults was from 300 mg to 0.1 mg; therefore, the dose score was 1 for an ED of 300 mg, 2 for 100 mg, 3 for 10 mg, 4 for 1 mg, 5 for 0.5 mg and 6 for 0.1 mg. In children, the range of ED was from > 3 g to 10 mg; therefore, the dose score was 1 for an ED of > 3 g, 2 for 3 g, 3 for 1 g, 4 for 300 mg, 5 for 100 mg and 6 for 10 mg.

Statistics

All analyses were performed using non-parametric tests as the data were not normally distributed. Correlations were analysed using Spearman's rank. Comparisons of two independent groups of continuous variables were tested by Mann–Whitney *U* test and comparisons of more independent groups were tested by Kruskal–Wallis test. Dichotomous variables were analysed using chi-square test. The area under the receiver operating characteristic (ROC) curve (AUC) was used to discriminate between peanut allergic individuals and controls with a value of 0.5 indicating 'no discrimination' and a value of 1 indicating 'perfect discrimination'.

All analyses were performed using SPSS (version 15; Chicago, IL, USA). *P*-values < 0.05 were considered statistically significant.

Results

Sensitivity values between four different techniques in both adults and children

Sensitivity values were assessed as the percentage of peanut allergic adults and children with a positive test result with any of the four different techniques to the peanut components Ara h 1, 2, 3 and 8 (Table 2). In the

Table 2. Sensitivity of four different techniques in both adults and children

	Sensitivity adults (%)	Sensitivity children (%)	Children vs. adults (<i>P</i> -value)
Ara h 1			
ImmunoCAP*	52.4	60.0	0.74
ImmunoCAP ISAC	68.2	93.3	0.11
SPT	59.1	50.0 (<i>n</i> = 8)	0.70
Immunoblot	68.2	46.7	0.31
<i>P</i> -value	0.66	0.04	
Ara h 2			
ImmunoCAP*	76.2	100	0.06
ImmunoCAP ISAC	86.4	100	0.26
SPT	95.5	100 (<i>n</i> = 7)	1.00
Immunoblot	90.9	100	0.51
<i>P</i> -value	0.27	NA [†]	
Ara h 3			
ImmunoCAP*	42.9	60.0	0.50
ImmunoCAP ISAC	54.5	60.0	1.00
SPT	59.1	55.6 (<i>n</i> = 9)	1.00
Immunoblot	68.2	53.3	0.49
<i>P</i> -value	0.41	0.98	
Ara h 8			
ImmunoCAP*	68.4	60.0	0.72
ImmunoCAP ISAC	59.1	53.3	0.75
<i>P</i> -value	0.75	1.00	

*ImmunoCAP was measured in 21 of 22 adults.

[†]Not able to calculate.

adult population, we found no significantly different sensitivity values between all four techniques for all peanut components. Ara h 2 was most often recognized by all tests with the highest sensitivity established by SPT (95.5%). In addition, significant correlation coefficients between all techniques were found for individual components (data not shown).

In children a significantly higher sensitivity value for Ara h 1 was seen with the multi-plexed assay (93.3%) compared with the single-plexed assay, SPT and immunoblot (60%, 50% and 46.7%, respectively, *P* = 0.04, Table 2). Still, the absolute value of the multi-plexed assay was perfectly correlated with the absolute value of the single-plexed assay ($\rho = 0.86$, *P* < 0.001), SPT ($\rho = 0.84$, *P* < 0.001) and immunoblot ($\rho = 0.59$, *P* = 0.004). No significant difference in sensitivity was found between the four techniques with respect to Ara h 2, 3 and 8. The correlation coefficients between all techniques were also significant for these individual peanut components in children (data not shown). Also in children, Ara h 2 was more often recognized, with a sensitivity of 100% for all four techniques.

With all different techniques to measure Ara h 1, 2, 3 and 8 comparable sensitivity values were seen between adults and children, although there was a trend that Ara h 2 was more often recognized by the single-plexed assay in children compared with adults (100% vs. 76.2%, *P* = 0.06, Table 2).

sIgE values comparable between the single- and multi-plexed assay in both adults and children

Potential differences between the techniques were in more detail analysed between the single- and multi-plexed assay with regard to absolute values because of the comparable outcome measure and their frequent use in routine clinical practice.

No significantly different median sIgE levels were found between the single- and multi-plexed assay in both adults and children to all peanut components (Fig. 1). Moreover, no significant difference was seen when the children and adult data were put together and analysed as one group (data not shown).

In addition, the absolute values of the SPT reactivity and immunoblot between adults and children were comparable. The exact median values for both adults and children of all four techniques to all peanut components can be found in Table 3.

sIgE to Ara h 2 is the most capable to discriminate between peanut allergy and peanut tolerance with both single-plexed and multi-plexed assay

We analysed the discriminative ability of peanut components as measured by both single- and multi-plexed assay

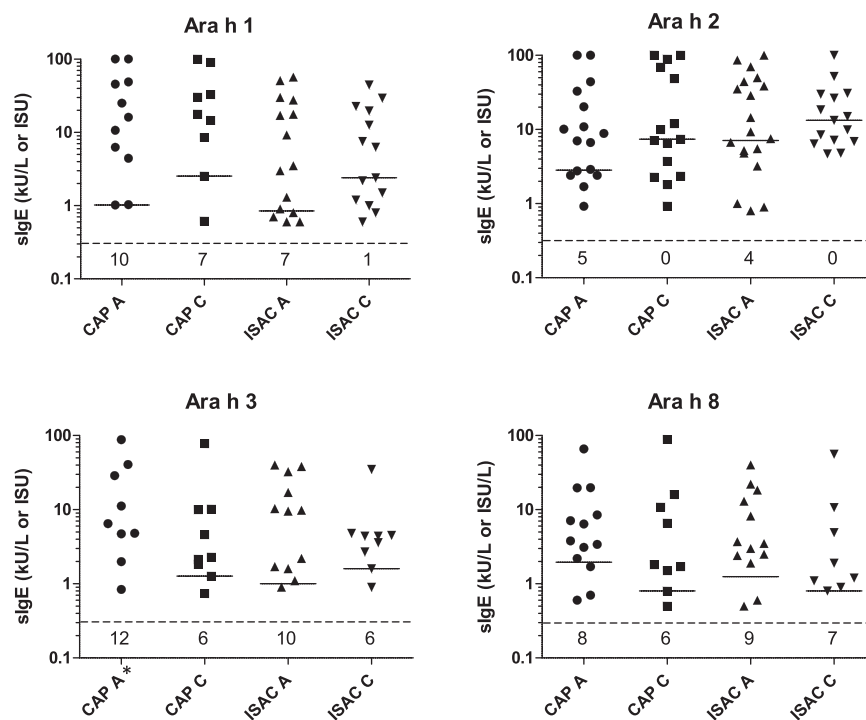


Fig. 1. Median-specific IgE (sIgE) values measured by both single-plexed IgE assay (in kU/L) and multi-plexed microarray immunoassay (in ISU) in adults and children. The dotted line indicates the cut-off value for the multi-plexed assay (0.3). The number below the dotted line indicates the number of patients having a value below this cut-off value. A, adults; C, children; CAP, single-plexed assay; ISAC, multi-plexed assay; *median value lies below the cut-off value.

Table 3. Median values in adults and children of all four test methods to Ara h 1, 2, 3 and 8

		Adults	Children	P-value
Single-plexed assay (sIgE in kU/L)	Ara h 1	1.0 (0.0–21)	2.5 (0.0–30)	0.70
	Ara h 2	2.9 (0.5–16)	7.4 (2.3–69)	0.16
	Ara h 3	0.0 (0.0–5.6)	1.3 (0.0–4.6)	0.70
	Ara h 8	2.2 (0.0–7.1)	0.8 (0.0–6.6)	0.49
Multi-plexed assay (sIgE in ISU)	Ara h 1	0.9 (0.0–17)	2.4 (1.0–20)	0.23
	Ara h 2	7.1 (0.9–40)	13 (6.9–30)	0.33
	Ara h 3	1.0 (0.0–9.9)	1.6 (0.0–4.4)	0.94
	Ara h 8	1.2 (0.0–4.8)	0.8 (0.0–1.9)	0.51
SPT (ratio)	Ara h 1	0.3 (0.2–1.1)	0.3 (0.1–0.7)	0.70
	Ara h 2	1.6 (0.8–2.4)	1.3 (0.6–3.1)	0.90
	Ara h 3	0.4 (0.1–0.7)	0.3 (0.2–0.5)	0.54
Immunoblot (scale 0–4)	Ara h 1	1.0 (0.0–1.3)	0.0 (0.0–1.0)	0.32
	Ara h 2	1.0 (1.0–2.3)	2.0 (1.0–4.0)	0.28
	Ara h 3	1.0 (0.0–2.0)	1.0 (0.0–1.0)	0.30

Data are presented as median values with interquartile ranges.

to discriminate between peanut allergic patients ($n = 37$) and atopic controls ($n = 27$). With both methods, sIgE to Ara h 2 had the best discriminative ability with an AUC of 0.92 (single-plexed assay) and 0.96 (multi-plexed assay) respectively (Fig. 2). The AUC values for sIgE to Ara h 3 and Ara h 8 were comparable between both methods (0.74 vs. 0.78 and 0.67 vs. 0.66 respectively). A difference was seen for sIgE to Ara h 1 with an AUC of 0.75 when mea-

sured by single-plexed assay and 0.91 when measured by the multi-plexed assay.

sIgE to Ara h 1, 2, and 3 in adults correlated with clinical severity

Specific IgE titres measured by the multi-plexed assay to the four peanut components in challenged adults and children ($n = 37$) were plotted against the severity score, indicating the clinical severity observed during DBPCFC (Fig. 3). In adults, the sIgE level was positively correlated with clinical severity for Ara h 1 ($r = 0.74$, $P < 0.001$), Ara h 2 ($r = 0.63$, $P = 0.002$) and Ara h 3 ($r = 0.78$, $P < 0.001$). No significant correlation in children could be found between severity and sIgE to any of these components (P -values of 0.32, 0.36 and 0.25, respectively, Fig. 3). Ara h 8 showed no significant correlation in both adults and children ($P = 0.28$ and $P = 0.07$ respectively) and even showed a negative correlation trend. Similar results were obtained when sIgE levels were measured by the single-plexed assay.

Discussion

To our knowledge, this is the first study that thoroughly evaluated the use of the peanut components Ara h 1, 2, 3 and 8 in both peanut allergic adults and children with

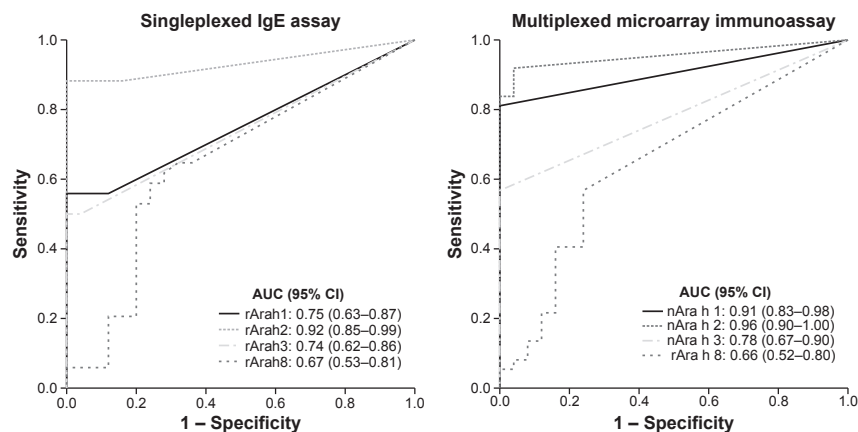


Fig. 2. Area under the receiver operating characteristic curve (AUC) values of specific IgE (sIgE) to the peanut components between single-plexed IgE assay and multi-plexed microarray immunoassay. AUC, area under the curve; CI, confidence interval.

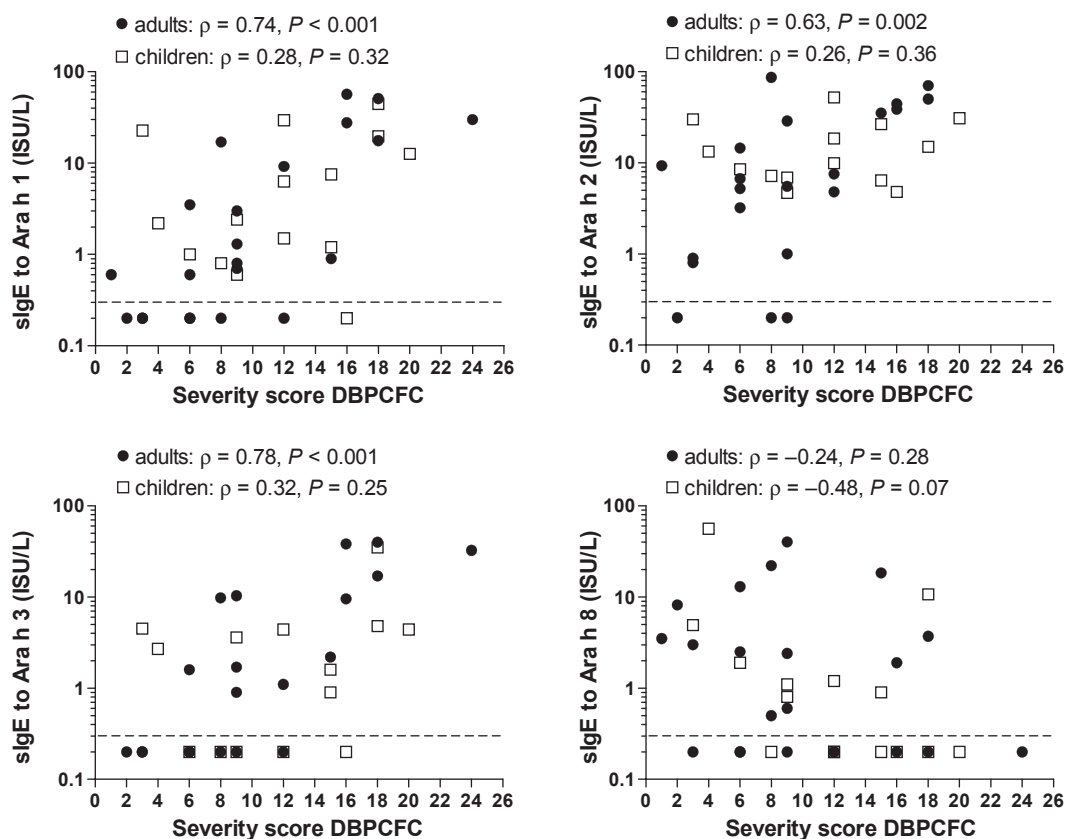


Fig. 3. Peanut components measured by multi-plexed microarray immunoassay related to clinical severity in both adults and children. Spearman's rho and its corresponding P -value (2-tailed) are given for the two subgroups. The dotted line indicates the cut-off value of 0.3 ISU/L were given the value 0.2 in this figure. One circle or square may represent several patients with the same severity score and sIgE titre.

four different techniques: multi-plexed microarray, single-plexed IgE assay, SPT and immunoblot.

The sensitivity and median absolute values of all four techniques to all peanut components were not significantly different between children and adults. However, a trend

of a higher sensitivity in children was found for Ara h 2 with the single-plexed assay (100% vs. 76.2%). We believe this can be explained by the fact that in adults more peanut allergic patients were also pollen allergic and therefore more often sensitized to the Bet v 1-related

Ara h 8 instead of Ara h 2. This hypothesis was supported by higher sensitivity and median values to Ara h 8 in the adult population compared with the paediatric population. In our study population two peanut allergic adults but no children were mono-sensitized to Ara h 8. In addition, Ara h 8 was least often recognized of all peanut components by both single-plexed and multi-plexed assay in children, whereas in the peanut allergic adults this was Ara h 3.

With all techniques, Ara h 2 was most recognized in both peanut allergic adults and children and also had the highest median sIgE value. The significance of Ara h 2 was already suggested in an earlier study from 1998 on serological analysis and was in 2004 further underlined by the analysis of IgE binding to purified Ara h 2 on immunoblot, SPT and basophil activation tests [9, 26]. Recently, in a Swedish paediatric population, 83% of the children sensitized to Ara h 2 reported allergic symptoms to peanut [12]. Three other studies, again in paediatric populations, showed that Ara h 2 was a very important predictor of clinical allergy [13, 15, 16]. A recently published Japanese study showed an AUC for sIgE to Ara h 2 of 0.91 with a sensitivity of 88% by using the ImmunoCAP in a paediatric population [17]. A good agreement was found with their immunoblot results. Our results further extend the evidence of Ara h 2 being the most important peanut allergen for diagnosis, by demonstrating the recognition of Ara h 2 in both a paediatric- and adult DBPCFC-confirmed peanut allergic population with 4 different techniques. Recently, the importance of Ara h 2 was also found with an additional technique, the basophil threshold sensitivity, by Glaumann et al. [27]. They showed in peanut allergic children a CD-sens of Ara h 2 similar to peanut extract (92%). Unfortunately, because our results were retrospectively collected, we were not able to compare them with functional assays, such as the basophil activation test.

When comparing the discriminative ability of the single-plexed and multi-plexed assay to discriminate between peanut allergy and peanut tolerance, we found a higher AUC of sIgE to Ara h 1 with the multi-plexed assay (0.91 vs. 0.75). Although the median sIgE value measured with the multi-plexed assay was slightly higher than when measured with the single-plexed assay in the whole peanut allergic population, this difference was not significant. More important probably was the fact that three of the 27 atopic controls recognized Ara h 1 with the single-plexed assay compared with none of the controls with the multi-plexed assay. We cannot explain this but, because two different preparations of Ara h 1 are used: a recombinant in the single-plexed vs. a purified natural one in the multi-plexed assay, this might be a technical issue.

We would like to point out that the purpose of our study was not to assess a diagnostic value, which would need another design, but to compare the discriminative ability between different diagnostic techniques. As a result, the AUC values presented here should not be used for diagnostic purposes. In addition, we could not show specificity values as the control group we used was not suspected of peanut allergy, and peanut allergy was not ruled out by a food challenge but by convincing history. A diagnostic study design should include subjects suspected of peanut allergy, which results in both positive and negative outcomes after food challenge.

Another possible limitation of our study was that the SPT and immunoblot data of the atopic control group were lacking. Therefore, we could not compare all four techniques with regard to their discriminative ability. In addition, all atopic control patients were adults because no paediatric controls could be included. When dividing the peanut allergic group into adults and children, higher AUC values were found for the paediatric population. As the high diagnostic AUC values for Ara h 2 in literature were all established in paediatric populations [13, 15–17], this finding suggests that a diagnostic study in adults is essential to analyse whether these high AUC values can be reproduced in adults.

We have shown a significant positive correlation between clinical severity and the level of sIgE to Ara h 1, Ara h 2 and Ara h 3 in adults. In children, no such correlation could be found. Three earlier studies that mainly analysed peanut allergic children also found no relation between the clinical severity and sIgE level or size of SPT reactions [10, 16, 26]. A possible explanation could be that none of the children experienced a Muller 0 reaction during food challenge, whereas in adults 6 subjects did. Interestingly, the same analysis in our study performed with the severity score adapted from Muller, assessed by both history or by food challenge, resulted in the same findings in adults and children [23]. This was also found when the ED was used to assess clinical severity or when the scoring system of Clark and Ewan was used [28]. Nevertheless, interpretation of this correlation for patients has to be approached cautiously as correlations at population levels do not necessarily apply to individual patients.

In conclusion, the single-plexed IgE assay, the multi-plexed microarray immunoassay, SPT and immunoblot have comparable sensitivity values compared with each other and between adults and children, with Ara h 2 being most often recognized and with the highest outcome measure among all techniques. However, the exact diagnostic value of all techniques should be compared in future prospective studies in larger populations in both adults and children.

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Conflicts of interest

EF Knol and AC Knulst received speaking fee from Thermo Fisher Scientific. All other authors declared no conflict of interest.

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