

RESEARCH LETTER

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Diagnostic accuracy of the ALEX² test in peanut-sensitized children

To the editor,

The fundamental concept when diagnosing allergies is that detection of immunoglobulin E (IgE) sensitization and the presence of allergy are not identical. Complementary to conventional testing that is based on allergen extracts, molecular allergy diagnosis reduces the need for oral food challenges (OFCs) in food allergy diagnosis. To detect IgE specific to allergen molecules, different singleplex or multiplex tests are available. Multiplex tests consume less serum and are less costly when a higher number of singleplex measurements are required, and they reveal IgE reactivity against a broad allergen panel in one test run.

Currently, there are two commercial allergen microarrays available for clinical use, that is, ISAC113 (Thermo Fisher, Uppsala, Sweden) and ALEX² (Macro Array Diagnostics, Vienna, Austria) which showed comparable sensitivity and specificity in previous studies.^{1,2} Here, we aimed to investigate the diagnostic accuracy of ALEX² in Turkish children with peanut sensitization compared to conventional serological and skin tests as a reference.

This retrospective study, reported in line with the current STARD guideline, included 123 children who had been referred to the Hacettepe University Paediatric Allergy Department due to suspected peanut allergy for diagnostic workup. Peanut sensitization had been confirmed either by skin prick test (SPT) and/or peanut extract-specific IgE (ImmunoCAP, Thermo Fisher). For all sera, data on sIgE to peanut components, detected by the ALEX² test, were also available (Figure 1). While 31 children (25%) were clinically reactive to peanut, 92 (75%) were tolerant. Clinical reactivity was assessed either by OFC ($n=8$) or by convincing clinical history ($n=23$); (see supplementary Appendix Table S1 in additional material, <https://zenodo.org/record/7836201#.ZD0d6exBz9E>). The details of study population, study design, measurements of SPT, sIgE, and ALEX² test are available in additional material. The study protocol was reviewed and approved by Hacettepe University Institutional Review Board (GO-21/1248).

There were significant differences between the peanut-reactive and the peanut-tolerant groups in SPT wheal size, peanut extract sIgE, total IgE and sIgE to Ara h 1, 2, 3 and 6 (see Table in additional material). Based on the recommended cut-off level of ≥ 0.3 kU/L for the ALEX² test, Ara h 2 and Ara h 6 were detected in 83.9% (26/31) and 77.4% (24/31) of the clinically reactive group, but only in 15.2% (14/92) and 10.9% (10/92) of the tolerant group, respectively ($p < .001$ for both). For Ara h 1 and Ara h 3, the corresponding values for the

clinically reactive group were 96.8% (30/31) and 90.3% (28/31), and for the tolerant group 70.7% (65/92) and 37.0% (34/92), respectively ($p = .002$ and $p < .001$). In the clinically reactive group, if we consider only sensitization to the 2S albumins (Ara h 2 and Ara h 6), the proportion of IgE-reactivity only to Ara h 2, only to Ara h 6, to both and to neither was 9.7% (3/31), 3.2% (1/31), 74.2% (23/31) and 12.9% (4/31), respectively. Those four patients (12.9%) in the clinically reactive group who were neither sensitized to Ara h 2 nor to Ara h 6, were sensitized to both Ara h 1 and 3. Ara h 8 was detected in 3 patients, all of whom were peanut tolerant. Ara h 9 was detected in 48.4% (15/31) of the clinically reactive group and 38.0% (35/92) of the tolerant group ($p = .332$). Hierarchical cluster analysis showed a pairwise association of Ara h 2 and Ara h 6, as well as of Ara h 1 and Ara h 3 (Figure 1B).

In the ROC curve analysis, SPT (AUC = 0.927), peanut extract sIgE (AUC = 0.854), IgE to Ara h 1 (AUC = 0.829), Ara h 2 (AUC = 0.882), Ara h 3 (AUC = 0.857) and Ara h 6 (AUC = 0.853) showed similar potential to diagnose peanut allergy ($p < .001$ for all) (Figure 1C). Comparison of AUCs revealed no significant difference between SPT, extract-sIgE and IgE to components (see supplementary Appendix Table S2 in additional material). Using recommended threshold levels (≥ 0.3 kU/L for ALEX², ≥ 3 mm wheal diameter for SPT and ≥ 0.35 kU/L for peanut extract sIgE by ImmunoCAP), highest accuracies were achieved for Ara h 2 and Ara h 6 (84.6% and 86.2%, respectively), compared with 59.4% and 30.3% for SPT and peanut extract sIgE, respectively (Table 1). The optimal cut-off values for SPT, peanut extract-sIgE, sIgE to Ara h 1, 2, 3 and 6, according to the Youden index, were 6.5 mm, 21.4 kU/L, 6.21, 0.84, 0.92 and 0.16 kU/L, respectively.

By combining different diagnostic tests, criteria to diagnose peanut sensitization are more stringent which could improve diagnostic accuracy. When applying recommended threshold levels, the combination of Ara h 2 and SPT had 80.0% sensitivity, 92.2% specificity, 89.2% diagnostic accuracy and 10.3 likelihood ratio (LR+) and for the combination of Ara h 6 and SPT 76.7%, 94.4%, 90.0% and 13.7, respectively (see supplementary Appendix Table S3 in additional material). Overall, both the combinations of SPT + Ara h 2 + Ara h 6, and SPT + Ara h 3 + Ara h 6 had the highest diagnostic accuracies (90.9%) (Figure 1D). However, the former (SPT + Ara h 2 + Ara h 6) had a better LR+ than the latter (22.2 vs 3.6, respectively).

The observation in our cohort that AUC values for sIgE to Ara h 2 and 6 revealed no significant difference to the other peanut

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components may be explained by the fact that AUCs for Ara h 1 and Ara h 3 were found to be higher in this study, compared to previous studies.^{3,4} In the present study, although the best accuracies to predict clinical reactivity were achieved with sIgE to Ara h 2 and Ara h 6, we found higher rates of IgE-reactivity to Ara h 1 and Ara h 3 than to Ara h 2 and Ara h 6 in peanut-allergic patients of our cohort which was rarely reported previously.^{5,6} Variations in the reported prevalence of sIgE to Ara h 1 and Ara h 3 may be potentially linked to differences in geographical locations, particularities of the respective study populations, differences in the amount/ way how peanuts are consumed (roasted nut versus cooked forms) and the allergen of the assay.

Using the recommended cut-off of 0.3 kUA/L for Ara h 2, the sensitivity to diagnose peanut allergy was 83.9% and the specificity was 84.8%. When using the optimal cut-off for Ara h 2 (0.83 kUA/L), the high sensitivity was maintained, while specificity slightly increased to 89.1%. Similarly, in the ISAC IgE-microarray, the optimal cut-off value for Ara h 2 was 0.81 ISU with a sensitivity and specificity of 86% and 82%, respectively.⁷ Those results are in accordance with a recently published systematic review of paediatric studies where Ara h 2 sIgE with a cut-off of 0.35 kU/L (ImmunoCAP) was reported to have a sensitivity of 85.8% (95% CI 79.3–90.6) and a specificity of 83.6% (95% CI 76.0–89.2).⁸

We found that the combination of Ara h 2, Ara h 6 and SPT had the best accuracy (90.9%) with the highest LR+ (22.2). Recently,

Key Messages

- We evaluated diagnostic accuracy of ALEX² microarray for peanut allergy in a Turkish population.
- SPT, sIgE to whole extract, Ara h 1, Ara h 2, Ara h 3 and Ara h 6 had good diagnostic accuracy.
- Combining SPT with sIgE to Ara h 2 and Ara h 6 increased diagnostic accuracy >90%.

Hemmings reported that the combination of IgE to the 4 major peanut components Ara h 1, 2, 3 and 6 had the highest AUC (0.94) in ISAC.⁹ However, SPT was not included in that analysis. We, therefore, conclude that SPT, in combination with sIgE to Ara h 2 and Ara h 6 are a highly sensitive and specific approach to diagnose allergy to peanuts.

Analysing individual IgE-reactivity patterns to peanut components, our data showed that both peanut allergens belonging to the 2S albumin family, that is, Ara h 2 and Ara h 6, had a high rate of co-sensitization (74.2%) in peanut-allergic patients, and, in line with previous reports, monosensitization to Ara h 6 was rarely observed (3.2%). It was previously shown that sensitization to Ara h 8 (Bet v 1 homologue) is frequent in peanut-allergic patients from regions, where birch allergy is common. Low rate of sensitization in our study is probably due to the low frequency of birch allergy in our region. Moreover,

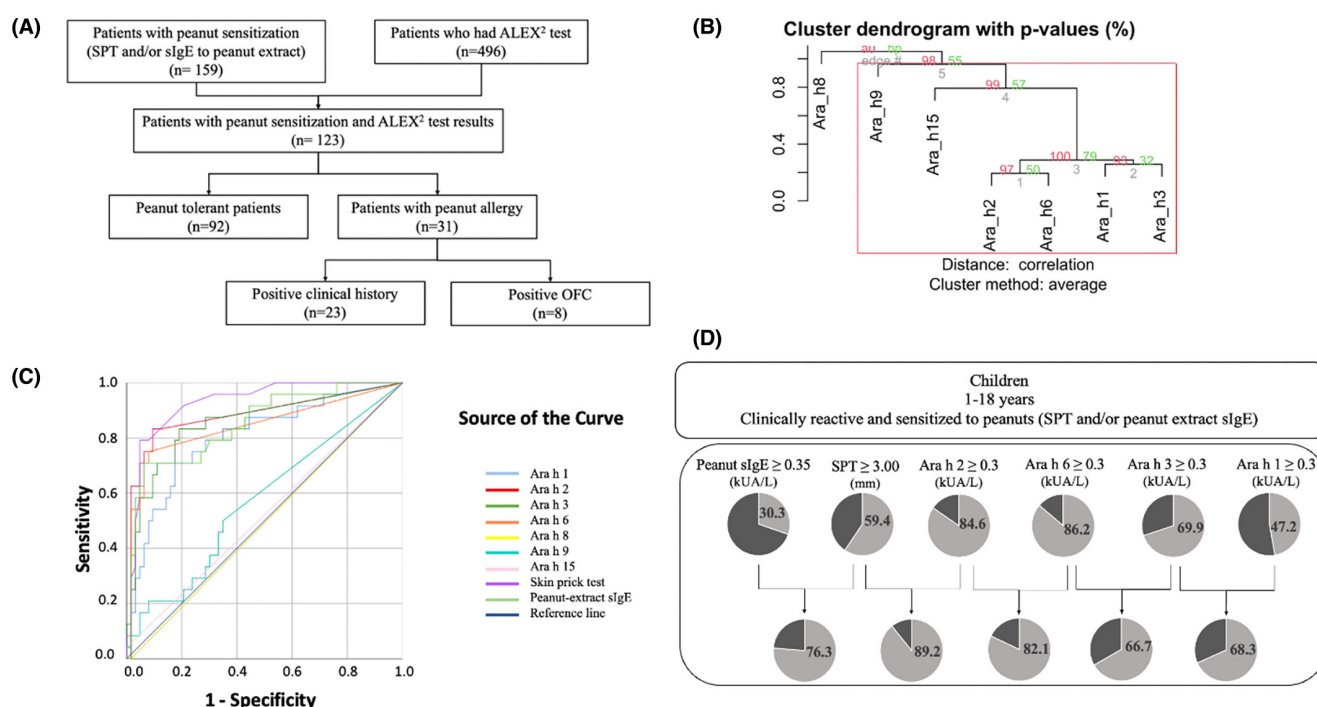


FIGURE 1 (A) Patient flow chart for the study cohort. (B) Hierarchical clustering of the peanut components. Each branch of the dendrogram represents a subcluster; components within subclusters are more similar than those in other subclusters. Clusters with *au*-values (*au* - approximately unbiased, numbers in red) higher than 95% are marked by red boxes and identify clades with high reliability. (*bp*—bootstrap probability, numbers in green; *edge*—numeric vector of edge numbers, numbers in grey). (C) ROC Curve analysis for sIgE to peanut extract, Ara h 1, Ara h 2, Ara h 3, Ara h 6, Ara h 8, Ara h 9 and Ara h 15, and of peanut extract SPT. (D) Flow chart showing diagnostic accuracy (light grey segments of pie charts, % diagnostic accuracy) of sIgE specific to peanut components, to peanut extract and of SPT, as well as of combinations of these parameters.

TABLE 1 Effects of different cut-off values on indices characterizing the diagnostic performance of the respective parameters.

	AUC	95%CI(LL-UL)	p	Cut-off	Sensitivity %	Specificity %	Positive LR	Negative LR	PPV %	NPV %	Accuracy	Probability curve	
												50% PPV	95% PPV
SPT (mm)	0.927	0.874–0.979	<.001	6.5 3.0	82.8 96.6	93.5 66.2	12.7 2.85	0.18 0.05	82.8 51.9	93.5 98.1	90.6 59.4	8.9	16.7
Extract sIgE (kUA/L)	0.854	0.766–0.943	<.001	21.35 0.35	72.0 100	91.9 68.0	8.8 1.07	0.30 0	75.0 26.6	90.7 100	86.9 30.3	85.3	240.3
Ara h 1 (kUA/L)	0.829	0.743–0.915	<.001	6.205 0.3	77.4 96.8	83.7 30.4	4.75 1.39	0.27 0.10	61.5 31.9	91.7 96.6	82.1 47.2	23.3	59.2
Ara h 2 (kUA/L)	0.882	0.797–0.966	<.001	0.835 0.3	83.9 83.9	89.1 84.8	7.70 5.51	0.18 0.19	72.2 65.0	94.3 94.0	87.8 84.6	17.2	42.4
Ara h 3 (kUA/L)	0.857	0.776–0.938	<.001	0.92 0.3	87.1 90.3	79.3 63.0	4.20 2.44	0.16 0.15	58.7 45.2	94.8 95.1	81.3 69.9	10.9	29.3
Ara h 6 (kUA/L)	0.853	0.760–0.946	<.001	0.16 0.3	77.4 77.4	89.1 89.1	7.10 7.10	0.25 0.25	70.6 70.6	92.1 92.1	86.2 86.2	11.9	31.8
Ara h 8 (kUA/L)	0.484	0.366–0.601	.787	- -	- -	- -	- -	- -	- -	- -	- -	-	-
Ara h 9 (kUA/L)	0.580	0.459–0.702	.183	4.28 0.3	19.4 48.4	95.6 61.5	4.40 1.26	0.84 0.84	60.0 30	77.7 77.8	76.2 58.2	-	-
Ara h 15 (kUA/L)	0.522	0.400–0.644	.714	0.35 0.3	6.7 6.7	97.8 97.8	3.04 3.05	0.95 0.95	50.0 50	76.1 76.1	75.2 75.2	-	-

Abbreviations: AUC, Area under the ROC curve; LR, likelihood ratio; NPV, negative predictive value; PPV, positive predictive value; sIgE, specific immunoglobulin E; SPT, skin prick test.

in our cohort, Ara h 9 (nsLTP) was not suitable to distinguish peanut allergies from tolerant children due to similar rates of IgE-reactivities (48.4% vs. 38.0%). Overall, Ara h 8 and 9 do not contribute to the diagnosis of peanut allergy in the young Turkish population.

Limitations of our study are the relatively small number of peanut-allergic patients and that OFC was only done in few participants, which was mainly attributable to COVID-19 restrictions and the consecutive decline in hospital admissions. However, there was no obvious difference in clinical, demographic and laboratory findings between patients where peanut allergy was confirmed by OFC or by clinical history. A final potential limitation is that due to the retrospective nature of the study, physicians who categorized patients as clinically reactive versus tolerant were not blinded and had access to the serological data.

In summary, we provide confirmatory evidence that IgE-antibodies specific to Ara h 1, 2, 3 and 6, measured by IgE-microarray, are predictors of clinical reactivity to peanuts in children. In particular, the combination of SPT with sIgE to Ara h 2 and Ara h 6 provided better diagnostic accuracy, compared to each individual parameter alone. In this real-life study of a young population from the Eastern Mediterranean region, we could show for the first time that the ALEX² test is complementary to SPT for the diagnosis of peanut allergy.

AUTHOR CONTRIBUTIONS

ESA contributed to the design, acquisition of data, interpretation of findings and original draft writing. OS contributed to the design, acquisition of data and interpretation of data. UMS contributed to the statistical plan and performed data analysis. CL and SW contributed to the design, interpretation of findings and language editing. BES contributed to the design, concept, acquisition and interpretation of data and revising critically for important intellectual content. All authors contributed to revising the manuscript and approved the final version.

KEYWORDS

allergy, diagnosis, microarray, molecular allergy diagnosis, peanut, skin prick test, specific IgE

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CONFLICT OF INTEREST STATEMENT

Elif Soyak Aytekin, Ozge Soyer, Umit Murat Sahiner and Bulent Enis Sekerel have no conflict of interest. Sandra Wieser and Christian Lupinek are employees of MADx (Macro Array Diagnostics).

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.