

Letter to the Editor

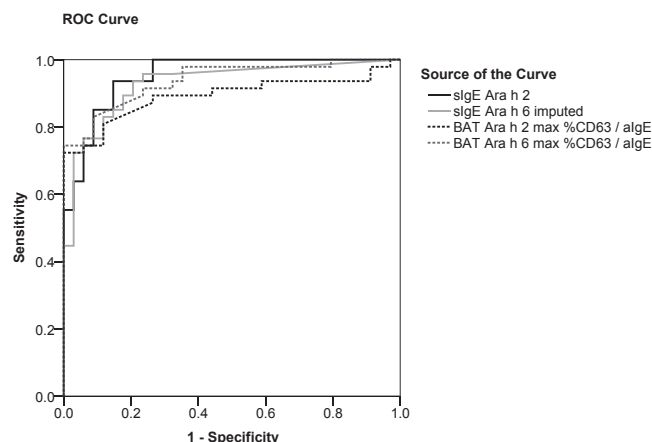
The IgE and basophil responses to Ara h 2 and Ara h 6 are good predictors of peanut allergy in children

To the Editor:

The diagnosis and severity of peanut allergy are ideally established with a double-blind placebo-controlled food challenge (DBPCFC).¹ The DBPCFC is however a burdensome and expensive procedure.^{2,3} Component-resolved diagnostics showed promising results in predicting peanut allergy.⁴ However, IgE tests can be affected by cross-reactivity with other allergens and do not take into account functional crosslinking between IgE molecules. The basophil activation test (BAT) is a functional assay that could be an alternative way to differentiate between sensitization and relevant allergy. This is the first prospective study that evaluates the most accurate diagnostic approach in children suspected of peanut allergy using specific IgE (sIgE) and the BAT using peanut components with focus on Ara h 2 and Ara h 6.

We included 81 children referred to the Wilhelmina Children's Hospital, a tertiary centre for food allergy, with suspected peanut allergy based on an sIgE level of more than 0.35 kU/L to peanut and/or a previous reaction (see Fig E1 in this article's Online Repository at www.jacionline.org). sIgE levels to peanut and recombinant components Ara h 1, 2, 3, 6, 8, and 9 were determined using the ImmunoCAP method (Thermo Fisher Scientific, Uppsala, Sweden). For the BAT, basophils in whole heparinized blood samples were stimulated with increasing concentrations of purified peanut proteins Ara h 2 and Ara h 6 within a median (interquartile range) time of 2.5 hours (1.6-23.9 hours) of blood collection. Results were expressed as the ratio between the maximal degranulation response (percentage CD63-positive cells) to allergen and anti-IgE. A 2-day DBPCFC was performed in all children and considered positive when objective or severe long-lasting subjective symptoms occurred. The area under the curve (AUC) of the receiver-operating characteristic (ROC) were used to evaluate the performance and discriminative capacity of the diagnostic tests. Statistical analyses were performed using SPSS for Windows (version 21; SPSS, Chicago, Ill). A *P* value of less than .05 was considered statistically significant, and a Bonferroni correction was used when performing multiple testing. Complete methodology of sensitization tests, DBPCFC, the BAT, and statistical procedures are described in this article's Methods section in the Online Repository at www.jacionline.org.

Baseline characteristics and diagnostic test results are presented in Tables E1 and E2 in this article's Online Repository at www.jacionline.org. sIgE levels to Ara h 2 and Ara h 6 were strong predictors of peanut allergy and showed high discriminatory capacity (AUC, 0.95; 95% CI, 0.91-0.99 and AUC, 0.93; 95% CI, 0.87-0.99). sIgE levels to Ara h 2 and Ara h 6 were highly correlated ($r^2 = 0.948$), and there were no allergic children who recognized Ara h 6 without Ara h 2. When using the best performing cutoff points (highest negative predictive value and positive predictive value) of Ara h 2 to exclude or confirm peanut allergy (0.1 and 5 kU_A/L), we could classify 50 of 81 (62%) children correctly. Ara h 2 and Ara h 6 were also highly correlated in the BAT ($r^2 = 0.842$), but differences were seen at the individual patient level. Five children (including 2 mild allergic and 1 severe allergic) were positive (maximal CD63% >5%) in the BAT to



Diagnostic test	AUC	95% CI
sIgE Ara h 2	.952	.910-.994
sIgE Ara h 6	.929	.873-.986
BAT Ara h 2*	.892	.818-.967
BAT Ara h 6*	.939	.890-.988

*BAT is expressed as the ratio between the maximal CD63% to allergen and alGE.

FIG 1. ROC curves and AUC values of Ara h 2 and Ara h 6 (n = 81).

Ara h 6 but negative to Ara h 2. Those children did respond with sIgE to both Ara h 2 and Ara h 6 although levels were low (<2 kU_A/L). ROC curves and AUC values of the sIgE and basophil response to Ara h 2 and Ara h 6 are presented in Fig 1 (see also Fig E2 and Table E3 in this article's Online Repository at www.jacionline.org). The BAT was especially useful when used as a second step in the diagnostic process or when used with sIgE to Ara h 2 simultaneously (both strategies resulted in 65 of 81 [80%] correctly predicted patients). A diagnostic flow chart with different diagnostic approaches to predict and exclude peanut allergy is shown in Fig 2. The diagnostic performance and a diagnostic flow chart including sIgE and skin prick test to peanut extract are presented in the Results section and Fig E4 in this article's Online Repository at www.jacionline.org. sIgE to Ara h 2 and Ara h 6 were the best predictors for severe peanut allergy. ROC curves and AUC values for severe peanut allergy are shown in Fig E3 in this article's Online Repository at www.jacionline.org.

Ara h 2 and Ara h 6 performed almost similar in predicting the presence of peanut allergy, which can be explained by the homology and cross-reactivity between these 2 2S albumins.⁵ It should be kept in mind that rare cases of (severe) peanut allergy were described in the absence of reactivity to Ara h 2.⁶ Our study demonstrates that in contrast to sIgE the BAT was able to identify children with peanut allergy who were responsive to Ara h 6 and not to Ara h 2. This might reflect the more functional nature of the BAT, which takes into account the concentration and affinity of sIgE and other (blocking) antibodies. We feel it is unlikely that some discordance in performance of both tests was caused by the different source (purified vs recombinant) and manufacturer

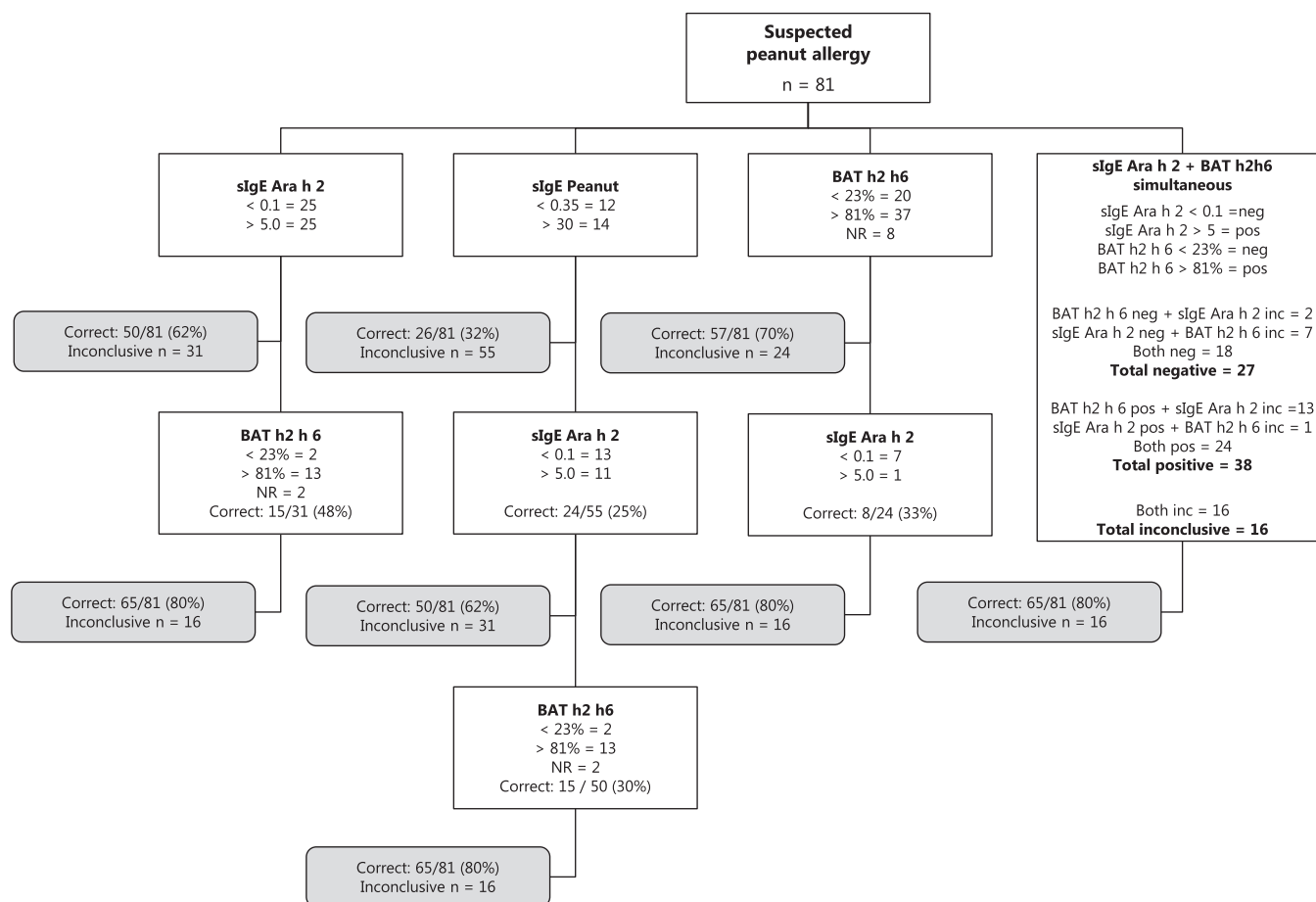


FIG 2. Diagnostic flow chart for the prediction of peanut allergy. The number of inconclusive tests is indicating the number of children who could not be classified by the diagnostic test and therefore will need a DBPCFC to diagnose peanut allergy. Inc, Inconclusive; neg, negative; pos, positive.

between the allergens. The trace levels and therefore the risk for contamination with other allergens in the purified allergens were less than 0.05%. Still, this cannot be completely excluded. Although the BAT was useful as a second step in the diagnostic process, it had no added value in predicting severe peanut allergy, which is in contrast to previous studies.^{7,8} The difference can be explained by the study design (children with proven peanut allergy) that was used in other studies, which is not the design suitable to evaluate its diagnostic value. It should be noted that several limitations of BAT need to be considered before implementation. The occurrence of nonreleasers (10% in our study) is a major drawback because in those subjects the BAT cannot be interpreted. Interestingly, the peanut allergics who were nonreleasers still demonstrated a positive skin prick test response, indicating that the nonreleaser phenotype was limited to basophils and not mast cells. Furthermore, the BAT needs to be performed on whole blood samples within 24 hours of blood collection and requires a specialized laboratory with expertise in cell activation. For efficiency reasons, in a clinical setting we would propose to use the BAT after sIgE to Ara h 2 (in case of an inconclusive sIgE to Ara 2 response) and perform the BAT with a mixture of Ara h 2 and Ara h 6. Because our primary aim was to compare the diagnostic value of the sIgE response and BAT response to Ara h 2

and Ara h 6, we did not perform the BAT with peanut extract. On the basis of the results of this study, we therefore did not compare the performance of peanut extract with Ara h 2 and Ara h 6 in the BAT. However, a previous study of Glaumann et al⁹ already showed that Ara h 2 leads to higher CD-sens values in the BAT compared with peanut extract. A limitation of this study is that we did not perform internal or external validation and therefore did not adjust for overfitting of the diagnostic models. Moreover, generalizability to other populations such as secondary care is not clear because the positive predictive value and the negative predictive value of a diagnostic test are dependent on the prevalence of peanut allergy in the population investigated. However, our study was prospective, peanut allergy was diagnosed accurately in all patients, and it comprises a realistic representation of children referred to tertiary care. Further research should focus on validation of diagnostic accuracy in other settings and efficiency including costs and quality of life in daily practice.

The results of our study show that in most children, peanut allergic status can be predicted using a stepwise approach including sIgE to Ara h 2 and subsequently the BAT to Ara h 2 and Ara h 6. For optimal insight into eliciting doses or severity, a food challenge remains the test of choice.

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METHODS

Ethical aspects

This study was reviewed and approved by the ethical committee of the University Medical Centre Utrecht. Written informed consent was obtained from parents and/or children before enrolment in the study.

Food challenges

The diagnosis of peanut allergy was made after DBPCFC and if no reaction occurred on both days, a consecutive open challenge. Challenges were performed in a clinical setting equipped for resuscitation with monitoring of vital signs and lung function throughout the whole challenge procedure. Intravenous access was established before the first portion. The challenge protocol consisted of 7 increasing portions of ginger bread containing 8 g defatted peanut flour (50% peanut protein) on the verum day but not on the placebo day.^{E1} All signs and symptoms during challenge were classified and recorded on a food challenge score sheet adapted from Nowak et al.^{E2} Challenges were stopped and treated according to protocol when objective (ie, rash, urticaria, angioedema, rhinitis, conjunctivitis, vomiting, diarrhea, dyspnea, wheezing, hypotension) or severe subjective symptoms (ie, oral allergy, abdominal pain) that lasted longer than 45 minutes indicating an allergic reaction occurred. Children were observed for 2 (no symptoms) or 4 (symptoms) hours after DBPCFC was stopped. If no or inconclusive symptoms occurred during either verum or placebo challenge, a confirmatory open challenge with 10 g of whole peanuts was performed. An expert panel of 3 independent experienced allergologists blinded for the sensitization tests and the BAT individually classified children as peanut allergic or tolerant and determined severity of symptoms (by Sampson grade 1-5) and eliciting dose on the basis of all available information of DBPCFC open challenge and subsequent introduction at home. In the absence of consensus in the first instance, a face-to-face panel discussion meeting was held until agreement among all experts was reached. *Severe peanut allergy* was defined as peanut-related symptoms of Sampson grade 4 to 5 and/or objective symptoms to peanut after ingestion of up to 300 mg peanut protein during challenge.

Sensitization tests

Levels of sIgE to peanut and recombinant components Ara h 1, 2, 3, 6, 8, and 9 were determined using the ImmunoCAP method (Thermo Fisher Scientific, Uppsala, Sweden) and expressed as continuous variables ranging from 0.1 to 100 kUA/L or higher after sample dilution as required. Skin prick test (SPT) was performed with peanut extract and a single-headed lancet (ALK-Abello, Nieuwegein, The Netherlands). SPT was expressed as the peanut wheal index, which was calculated by dividing the peanut wheal size by the wheal size of the positive control (histamine).

Basophil activation test

Whole heparinized blood samples were collected at the start of DBPCFC and stored at room temperature for a maximum of 24 hours. Basophils were stimulated for 30 minutes at 37°C with increasing concentrations (0.1, 1, 3, 10, 100, and 1000 µg/mL) of purified peanut proteins Ara h 2 and Ara h 6 (Indoor Biotechnologies, Warminster, United Kingdom) diluted in RPMI (Gibco, Life technologies, NY) supplemented with 1 ng/mL IL-3. Polyclonal goat antihuman-IgE (Anti-IgE AI-3040, 1 µg/mL, Vector Laboratories, Burlingame, Calif) and formyl-methionyl-leucyl-phenylalanine (1 µM, Sigma F3506-10MG) were used as positive controls and RPMI medium and supplemented with IL-3 as negative controls. Leukocytes were stained with a cocktail of CD63-PE (Monosan, MON9000R; Uden, The Netherlands), CD203C-APC (Biolegend, 324610), CD123-FITC (Biolegend, 306014; San Diego, Calif), CD45-PO (Invitrogen, MHCD4530; Carlsbad, Calif), CD41-PECY7 (Beckman Coulter, 6607115; Woerden, The Netherlands), and HLA-DRPB (Biolegend, 307624) and analyzed by flow cytometry (FACS Canto II) with FACSDiva software (version 8.0.1, BD Biosciences, San Jose, Calif). Basophils were defined as CD45⁺ CD203⁺ CD123⁺ and HLA-DR⁺ and CD41⁺ in the lymphocyte scatter region. Degranulating basophils were determined as CD63-bright cells and calculated as percentage positive cells. Results of the BAT were expressed as the percentage CD63-positive cells after stimulation with 0.1 to 1000 µg/L allergen. For this study, different outcomes of the BAT were evaluated: (1) the maximal CD63 response after allergen

stimulation, (2) the ratio between the maximal response to allergen and the response to anti-IgE, (3) the mean CD63 response after 10 and 100 µg allergen, (4) the ratio between the mean CD63 response after 10 and 100 µg allergen and the response to anti-IgE, (5) the inverse of the concentration allergen with 50% of maximum CD63 upregulation (CD-sens), and (6) the area under the allergen-CD63 response curve. Children with basophils that after anti-IgE stimulation responded with less than 5% CD63-positive basophils were regarded as nonreleasers. The BAT was performed and interpreted by an investigator who was blinded for patient characteristics and the reference test.

Statistical methods

Continuous variables were expressed as mean $\pm$ SD or as the median and interquartile range in case of a skewed distribution. Comparison of differences in diagnostic test parameters between allergic and tolerant or mild and severe allergic children for continuous variables was done by the *t* test or the nonparametric Mann-Whitney *U* test, as appropriate. The significance of difference in proportions was tested with use of the chi-square statistic. To avoid bias that might result from an incomplete case analysis, missing sensitization data (SPT and sIgE to peanut components) were imputed by using multiple imputations in SPSS with the automatic method setting and *n* = 10 imputation sets.^{E3} We used age, sex, history of peanut allergic reaction, sIgE to peanut components Ara h 2, h 6, and h 8, and peanut allergic status as predictor variables to impute for the missing values. Nonreleasers in the BAT were excluded for the determination of optimal cutoffs but included when evaluating the diagnostic capacity. The ability of each diagnostic test (or combination of tests) to discriminate between peanut allergic and tolerant patients (or nonseverely and severely reacting patients) was estimated using the AUC. Optimal cutoff points and corresponding sensitivity, specificity, negative predictive value, and positive predictive value were determined for diagnostic tests (or combination of tests) with the highest AUC. The added value of the BAT was also evaluated as a second or third diagnostic step in the diagnostic procedure in the population that could not be predicted using sensitization tests. Based on cutoffs with the highest negative predictive value and positive predictive value, a flow chart was created, showing how many children could be predicted correctly using different diagnostic approaches. All statistical analyses were performed using SPSS for Windows (version 21) and a *P* value of less than .05 was considered statistically significant. A Bonferroni correction was used when performing multiple testing.

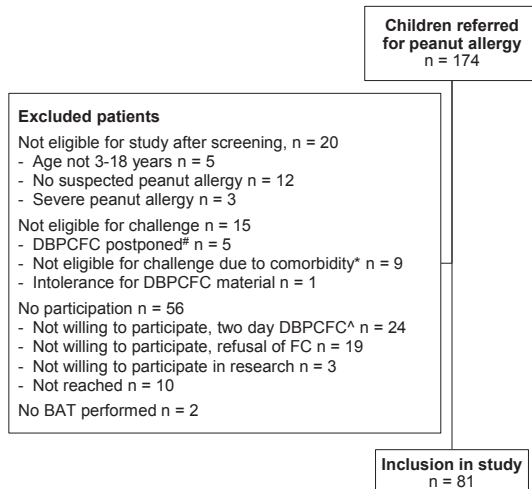
RESULTS

Diagnostic value of sensitization tests

When using the best performing cutoff points of sIgE to Ara h 2 to exclude or confirm peanut allergy (0.1 and 5 kUA/L), we could classify 50 of 81 (62%) children correctly (including 25 of 34 [71%] tolerant and 25 of 47 [53%] allergic children). With the best cutoff points of sIgE to Ara h 6 (0.1 and 6.67 kUA/L), we could predict 44 of 81 (54%) patients correctly (including 23 of 34 [74%] tolerant and 21 of 47 [45%] allergic children). However, 2 false-negative tests were also found. The SPT and sIgE to peanut extract had a lower discriminant capacity compared with sIgE to Ara h 2 and Ara h 6. sIgE to peanut extract had no added value when used before sIgE to Ara h 2 and the BAT. The SPT had very limited added value when used before Ara h 2 and the BAT (1 extra patient could be predicted correctly). Diagnostic test results of the sensitization tests are presented in Table E3 and Figs E2 and E4.

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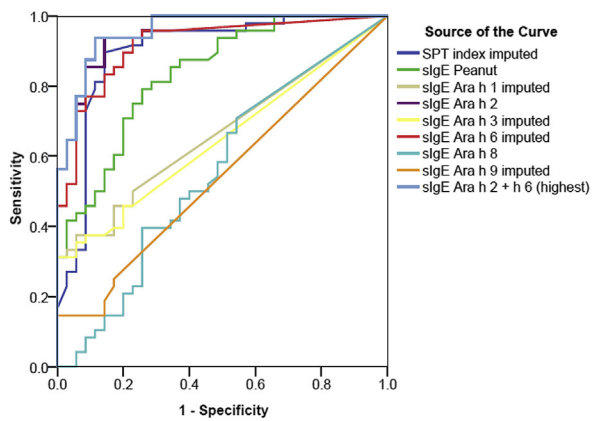


[#] Postponed due to: Young age (n = 4) Severe nut allergy (n = 1)

* Comorbidity: Asthma (n = 5), Hay fever (n = 1), MRSA (n = 1), eating disorder (n = 2)

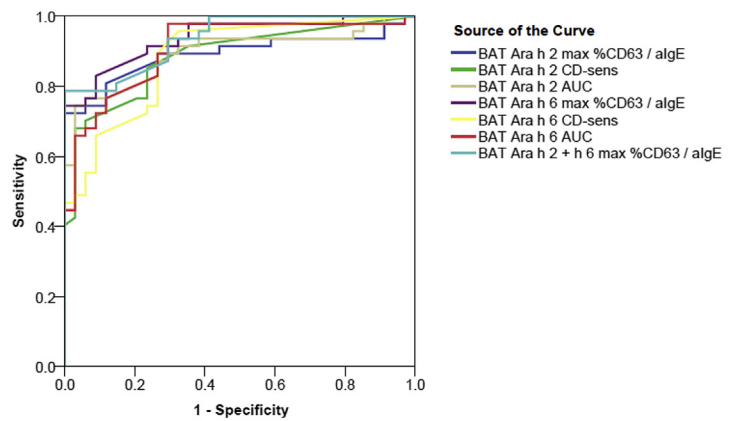
[^] At the time of study a one day food challenge was the routine procedure

FIG E1. Flowchart of in- and exclusion of patients.

A Sensitization tests, n = 81

A. Sensitization test, n = 81	AUC	95%CI	
SPT index imputed	.896	.819	.974
slgE peanut	.842	.757	.927
slgE Ara h 1 imputed	.660	.544	.777
slgE Ara h 2	.952	.910	.994
slgE Ara h 3 imputed	.637	.518	.756
slgE Ara h 6 imputed	.929	.873	.986
slgE Ara h 8	.569	.440	.698
slgE Ara h 9 imputed	.550	.424	.676
slgE Ara h 2 + Ara h 6 (highest value)	.953	.911	.996

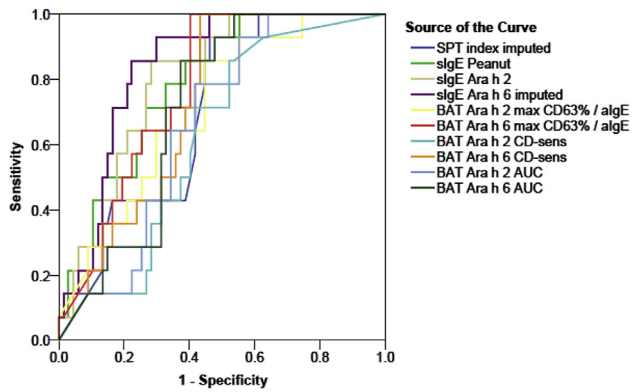
AUC, Area under the curve; ROC, receiver operating characteristic

B Basophil activation test, n = 81

B. Basophil Activation Test, n = 81	AUC	95%CI	
Ara h 2			
Maximal CD63%	.898	.828	.968
Maximal CD63% / algE*	.892	.818	.967
Mean CD63% 10–100µg	.901	.833	.968
Mean CD63% 10–100µg / algE	.907	.838	.976
CD-sens*	.886	.814	.958
AUC*	.894	.823	.965
Ara h 6			
Maximal CD63%	.925	.866	.984
Maximal CD63% / algE*	.939	.890	.988
Mean CD63% 10–100µg	.920	.861	.979
Mean CD63% 10–100µg / algE	.913	.852	.975
CD-sens*	.885	.813	.956
AUC*	.909	.845	.973
Ara h 2 + Ara h 6 (highest value)			
Maximal CD63% / algE*	.938	.892	.985

* BAT-outcomes displayed in ROC figure

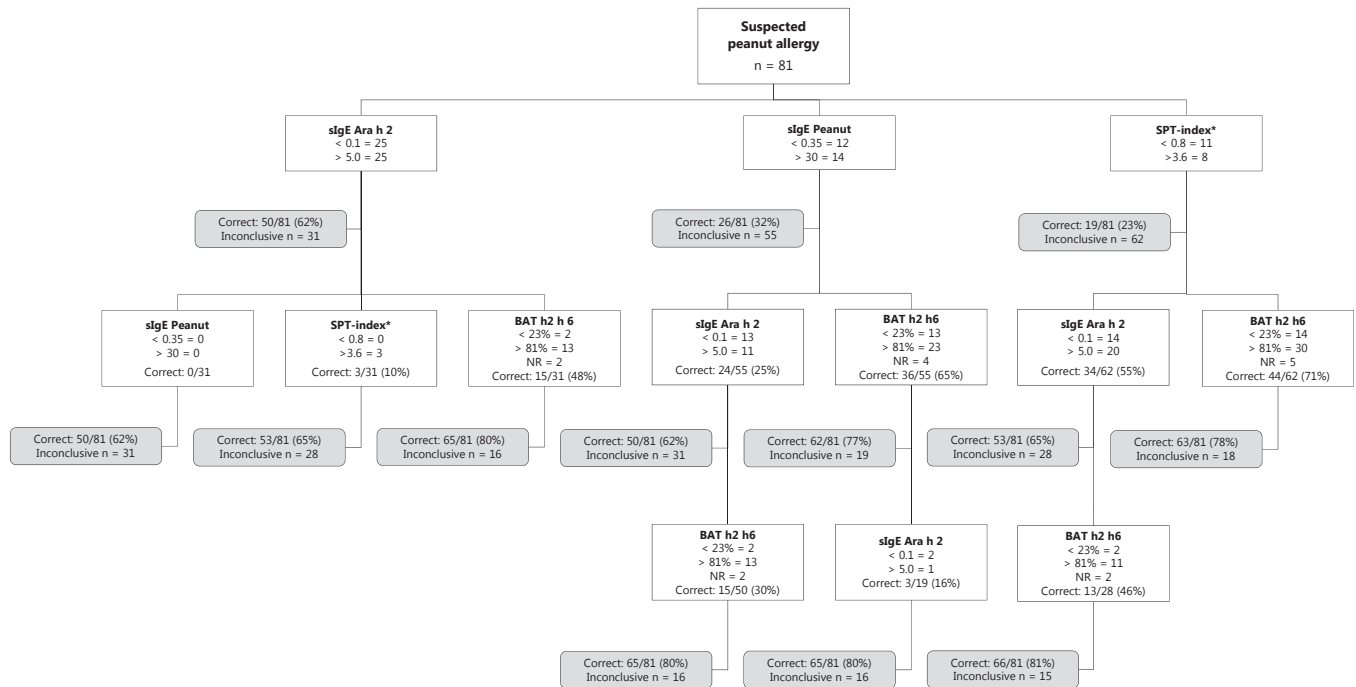
FIG E2. ROC-curves and AUC values of all the diagnostic tests under study.



Test	AUC	95%CI	
SPT index imputed	.703	.576	.829
slgE peanut	.791	.682	.900
slgE Ara h 2	.802	.699	.905
slgE Ara h 6 imputed	.840	.751	.930
BAT Ara h 2 max CD63% / algE	.713	.581	.846
BAT Ara h 6 max CD63% / algE	.773	.668	.878
BAT Ara h 2 CD-sens	.623	.487	.758
BAT Ara h 6 CD-sens	.724	.610	.838
BAT Ara h 2 AUC	.663	.539	.788
BAT Ara h 6 AUC	.711	.597	.826

AUC, Area under the curve; ROC, receiver operating characteristic

FIG E3. ROC-curves and AUC values for severe peanut allergy, n = 81.



SPT, Skin prick test

* SPT values were missing in n = 15, missing values were imputed

FIG E4. Flowchart of different diagnostic approaches including sensitization tests.

TABLE E1. Baseline characteristics for tolerant and mild-moderate and severe allergic children (n = 81)

Characteristic	Total (81)	Tolerant (34)	Allergic (47)	Mild-Moderate (33)	Severe (14)	<i>P</i> value allergic vs tolerant	<i>P</i> value mild-moderate vs severe
Age (y), median (interquartile range)	7.83 (5.52-10.98)	6.91 (5.52-10.56)	7.88 (5.51-11.69)	8.45 (6.18-11.15)	6.99 (4.7-11.69)	.356	.146
Sex: male	56 (69.1)	25 (73.5)	31 (66.0)	24 (72.7)	7 (50.0)	.467	.133
Atopy							
Eczema	45 (55.6)	19 (55.9)	26 (55.3)	16 (48.5)	10 (71.4)	.960	.148
Food allergy	59 (72.8)	24 (70.6)	35 (74.5)	27 (81.8)	8 (57.1)	.698	.076
Asthma	39 (48.1)	13 (38.2)	26 (55.3)	18 (54.5)	8 (57.1)	.129	.870
Hay fever	46 (56.8)	16 (47.1)	30 (63.8)	20 (60.6)	10 (71.4)	.133	.480
Suspected peanut allergy							
Sensitization	23 (28.4)	12 (35.3)	11 (23.4)	5 (15.2)	6 (42.9)	.242	.040
Previous reaction	58 (71.6)	22 (64.7)	36 (76.6)	28 (84.8)	8 (57.1)		
Previous reaction*							
Never ingested peanut	23 (28.4)	12 (35.3)	11 (23.4)	5 (15.2)	6 (42.9)	.285	.290
Grade 1	9 (11.1)	5 (14.7)	4 (8.5)	3 (9.1)	1 (7.1)		
Grade 2	12 (14.8)	2 (5.9)	10 (21.3)	6 (18.2)	4 (28.6)		
Grade 3	5 (6.2)	2 (5.9)	3 (6.4)	2 (6.1)	1 (7.1)		
Grade 4	23 (28.4)	11 (32.4)	12 (25.5)	11 (33.3)	1 (7.1)		
Grade 5	2 (2.5)	1 (2.9)	1 (2.1)	1 (3.0)	—		
Contact†	7 (8.6)	1 (2.9)	6 (12.8)	5 (15.2)	1 (7.1)		

Values are n (%) unless otherwise indicated. *P* values in boldface indicate a significant difference between the outcome groups (allergic vs tolerant or mild/moderate vs severe).

*Most severe reaction according to the Sampson classification of anaphylaxis.

†No previous ingestion but a local reaction (redness or urtica) after skin contact with peanut.

TABLE E2. Diagnostic tests under study in tolerant, mild-moderate, or severe peanut allergic children

Test, median (IQR)	n	Tolerant (34)	Mild-moderate (33)	Severe (14)	P value allergic vs tolerant*	P value mild-moderate vs severe*
Sensitization tests						
SPT (mean wheal)†	66	4.5 (0-7.5)	9.75 (8-12.5)	9 (7-12.5)	<.001	.73
SPT (index)†	66	1.33 (0-1.71)	2.65 (2.25-3.25)	2.33 (2.18-3.5)	<.001	.78
SPT (index) imputed	81	1.33 (0-1.93)	2.67 (2.33-3.25)	2.36 (2.25-3.43)	<.001	.84
sIgE peanut (kU _A /L)	81	0.74 (0.11-2.16)	7.74 (2.06-22.79)	19.41 (6.94-79.2)	<.001	.08
sIgE Ara h 1‡ (kU _A /L)	77	0.1 (0.1-0.1)	0.1 (0.1-8.07)	0.17 (0.1-35.38)	.02	.18
sIgE Ara h 1 imputed (kU _A /L)	81	0.1 (0.1-0.1)	0.1 (0.1-7.72)	1.4 (0.1-35.38)	.005	.18
sIgE Ara h 2 (kU _A /L)	81	0.1 (0.1-0.39)	4.8 (1.06-20.18)	10.18 (3.54-38.97)	<.001	.19
sIgE Ara h 3‡ (kU _A /L)	77	0.1 (0.1-0.14)	0.1 (0.1-0.4)	0.26 (0.14-4.87)	.02	.04
sIgE Ara h 3 imputed (kU _A /L)	81	0.1 (0.1-0.14)	0.1 (0.1-0.4)	0.23 (0.1-4.87)	.02	.09
sIgE Ara h 6‡ (kU _A /L)	77	0.1 (0.1-0.21)	2.47 (1.12-23.57)	16.11 (5.29-24.09)	<.001	.06
sIgE Ara h 6 imputed (kU _A /L)	81	0.1 (0.1-0.21)	2.47 (1.12-18.06)	12.63 (5.29-24.09)	<.001	.04
sIgE Ara h 8 (kU _A /L)	81	0.47 (0.1-4.19)	2.1 (0.23-7.14)	0.33 (0.1-7.05)	.28	.34
sIgE Ara h 9‡ (kU _A /L)	77	0.1 (0.1-0.1)	0.1 (0.1-0.1)	0.1 (0.1-0.13)	.42	.03
sIgE Ara h 9 imputed (kU _A /L)	81	0.1 (0.1-0.1)	0.1 (0.1-0.1)	0.1 (0.1-0.17)	.27	.05
BAT§						
Ara h 2						
Maximal CD63%	73	2.3 (1-4.6)	50.05 (21.9-64.9)	41.1 (17.85-63.65)	<.001	.75
Maximal CD63%/aIgE	73	7.79 (4-22)	102.48 (62.64-139.97)	109.19 (66.47-167.98)	<.001	.73
Mean CD63% 10-100 µg	73	1.1 (0.6-1.95)	31.73 (14.43-52.08)	32.2 (10.68-47.33)	<.001	.89
Mean CD63%/aIgE	73	4 (1.73-10.39)	79.82 (33.45-105.77)	78.63 (43.73-110.89)	<.001	.67
CD-sens	73	0.01 (0.01-0.01)	23.31 (14.51-60.21)	19.81 (2.52-31.02)	<.001	.19
AUC	73	5.42 (2.3-7.77)	100.3 (36.42-160.8)	84.69 (32.89-123.8)	<.001	.47
Ara h 6						
Maximal CD63%	73	1.9 (1.2-3.5)	48.65 (25.55-65.6)	54 (41.7-58.55)	<.001	.67
Maximal CD63%/aIgE	73	6.49 (3.23-21.84)	104.07 (60.18-130.82)	120.56 (90.07-199.07)	<.001	.17
Mean CD63% 10-100 µg	73	1.15 (0.5-2.25)	36.15 (11.6-58.95)	37.45 (30.93-44.1)	<.001	.83
Mean CD63%/aIgE	73	4.18 (1.45-17.24)	85.12 (27.62-109.82)	93.01 (64.8-143.02)	<.001	.32
CD-sens	72	0.01 (0.01-0.01)	16.82 (1.98-21.9)	11.71 (3.89-26.15)	<.001	.47
AUC	73	4.52 (2.31-7.07)	91 (30.17-150.95)	97.66 (76.07-107.25)	<.001	.81

P values in boldface indicate a significant difference between the outcome groups (allergic vs tolerant or mild/moderate vs severe).

aIgE, Anti-IgE; AUC, area under the dose response curve; sIgE, specific IgE.

*A Bonferroni correction was used to account for multiple testing and therefore a P value of less than .002 was considered to be significant.

†SPT was not performed in n = 15 (19%) because of eczema, daily use of antihistamines, or logistic reasons.

‡sIgE to Ara h 1, 3, 6, 9 was not performed in n = 4 (5%) because of failed blood collection.

§BAT was performed in n = 81; the 8 nonreleasers are excluded from this table.

||Missing n = 1 concentration in the BAT because of small blood sample.

TABLE E3. Cutoff points of the diagnostic test under study to predict peanut allergy (n = 81)

Test	Correct classification, n (%) [*]	Cutoff	Sens	Spec	PPV	NPV
Sensitization tests (n = 81)						
SPT index imputed	19 (23)	0.84	100	31	67	100
		3.59	17	100	100	47
sIgE peanut	26 (32)	0.36	100	34	59	100
		30.33	31	100	100	51
sIgE Ara h 2	50 (62)	0.10	100	71	69	100
		5.00	54	100	100	61
sIgE Ara h 6 imputed	44 (54) [†]	0.10	92	66	67	92
		6.67	48	100	100	59
BAT (n = 81)						
Maximal CD63% to Ara h 2/aIgE	35 (43)	0.86	100	3	8	100
		65.19	72	100	100	72
Maximal CD63% to Ara h 6/aIgE	42 (52)	2.76	100	21	31	100
		80.61	74	100	100	69
Maximal CD63% to Ara h 2 + h 6/aIgE	57 (70)	23.22	100	59	42	100
		81.21	79	100	100	77

Sens, Sensitivity; *Spec*, specificity; *PPV*, positive predictive value; *NPV*, negative predictive value.

^{*}The total number of patients who were correctly classified as tolerant or allergic using the cutoff points with the highest PPV and NPV.

[†]Including n = 2 false-negative test results.