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Two Different Composite Markers Predict Severity and Threshold Dose in Peanut Allergy

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What is already known about this topic? Peanut allergy is one of the most frequent causes of anaphylaxis in children with food allergies. Oral food challenge remains the gold standard to evaluate the threshold dose and severity of peanut allergy.

What does this article add to our knowledge? This study reports the relevance of allergen-specific and non-allergen-specific basophil activation test parameters to determine the severity and threshold dose of a peanut-allergic reaction in children.

How does this study impact current management guidelines? Introduction of these multivariable models in routine practice could avoid an oral food challenge in some children with peanut allergy.

BACKGROUND: Safe and cost-effective biological surrogate markers to evaluate the severity and threshold dose of peanut allergy (PA) reactions during an oral food challenge (OFC) are lacking.

OBJECTIVE: To evaluate biological markers associated with the severity and threshold dose of an allergic reaction during an OFC in a population of children with PA.

METHODS: Demographic and biological parameters of children with peanut OFC and basophil activation test (BAT) results were collected. Patients were stratified into 2 severity groups (mild-to-moderate and severe) and 2 cumulative threshold dose groups: low (LCTG) ≤ 100 mg crushed peanut and high >100 mg.

RESULTS: Among the 68 children included, there was a 96% concordance between the OFC and BAT result for the diagnosis of PA. Of the 56 children with a positive OFC and BAT to peanut (median age: 8.8 years), the severity of an allergic reaction and the cumulative threshold dose were not correlated ($P = .24$). Higher Ara h 2-specific IgE and FcεRI-positive control values were both associated with severe reactions to peanut. Combining these 2 markers led to a 92% sensitivity (84%-97%) and an 82% specificity (71%-89%) for severe

reactions in all subjects. For children in the LCTG, a 4-variable composite marker, including age, normalized basophil sensitivity (EC₅₀), and FcεRI- and fMLP-positive control values, resulted in a 97% sensitivity (89%-99%) and 61% specificity (49%-71%).

CONCLUSION: Distinct composite markers including BAT allergen-specific and non-allergen-specific parameters appear to be associated with severity and cumulative threshold dose in children with PA. © 2020 American Academy of Allergy, Asthma & Immunology (J Allergy Clin Immunol Pract 2020; ■■■)

Key words: Peanut allergy; Basophil activation test; FcεRI-positive control; Oral food challenge

Peanut allergy (PA) is one of the most frequent food allergies in children accounting for 25% of food allergies overall.^{1,2} PA is diagnosed before the age of 6 in approximately 80% of children with PA.³ The prevalence of PA is increasing throughout the world, and self-reported allergy studies record a prevalence of 2.1% in the United States and from 0.3% to 0.75% in France.^{4,5} In 2019, the incidence of food-induced anaphylaxis in Europe was higher compared with other regions in the world.

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Abbreviations used

Ara h 2-sIgE- Ara h 2-specific IgE
 Ara h 2-sIgG4- Ara h 2-specific IgG4
 AUC- Area under the curve
 BAT- Basophil activation test
 HCTG- High cumulative threshold dose group
 LCTG- Low cumulative threshold dose group
 OFC- Oral food challenge
 PA- Peanut allergy
 pp- Peanut protein
 Ps-IgE- Peanut specific IgE
 ROC- Receiver operating characteristic
 Se- Sensitivity
 Sp- Specificity
 VIF- Variance inflation factor

(America, Oceania, and Asia)⁶—and more prevalent among children and adults younger than 24 years—resulting in an increase in hospitalizations for anaphylaxis.^{6,7} Along with the increased prevalence, the severity of allergic reactions to peanut has increased with PA being the most common cause of food anaphylaxis since 2007.^{7,8} PA is rarely outgrown and tolerance develops in only 20% of cases.^{2,9} Current PA management is avoidance of culprit foods, which is risky, exposes subjects to accidental allergic reactions, and impairs quality of life.^{10,11}

Oral food challenge (OFC) is the gold standard for the diagnosis of food allergy and to define severity and a threshold dose for a clinical reaction.¹² However, OFC is resource-intensive, time-consuming, and carries a risk of anaphylaxis,¹³ justifying investigations for biological surrogates for diagnosis and to evaluate threshold dose and severity. Many studies have already demonstrated the usefulness of clinical and biological markers to diagnose PA to avoid an OFC. Among these markers, Ara h 2-specific IgE (Ara h 2-sIgE) levels and the basophil activation test (BAT) have been extensively studied.¹⁴⁻¹⁸ Although some studies have suggested that the association of these biomarkers could predict threshold dose during an OFC, fewer studies have focused on their usefulness in predicting the severity of an allergic reaction during an OFC.¹⁹⁻²² Some tools, mainly incorporating biological parameters, have been developed to stratify patients according to the severity of an allergic reaction during an OFC.²³⁻²⁶

In this context, the aim of our study was to assess the reliability of a model including BAT and various composite markers to predict the threshold dose and the severity of an allergic reaction during an OFC in children with PA.

METHODS**Study design**

This was a retrospective cross-sectional study performed in patients in whom PA had been confirmed by an open OFC. Patients were recruited from February 2016 to April 2020 at the allergy department of Trousseau Hospital in Paris, France, a pediatric, university-based outpatient practice. We included all children (aged <18 years) who had been addressed for a peanut OFC for the following reasons: (1) suspected PA with doubtful allergic history and evidence of peanut specific IgE (ps-IgE) sensitization; or (2) evidence of ps-IgE sensitization with avoidance of peanut

in their diet, and having undergone the BAT for the same indications.

Parameter assessment

Standardized open peanut OFC consisted of ingesting increasing doses of crushed peanut (Benenuts)—from 2 to 3000 mg of peanut (0.56-840 mg of peanut protein [pp])—every 20 minutes for a total cumulative dose of 7100 mg of peanut (1988 mg of pp). An OFC was considered positive on observation of objective symptoms (as defined by the European Academy of Allergy and Clinical Immunology or the modified 2010 World Allergy Organization grading system) and was considered negative when all doses were well tolerated.²⁷ The allergic symptoms during an OFC were graded in severity using the French guidelines (see Table E1 in this article's Online Repository at www.jaci-inpractice.org).²⁷⁻²⁹

The patients were stratified into 2 score severity groups for analysis²⁹: mild-to-moderate (scores I and II) allergic reaction and severe (scores III and IV when epinephrine was needed) allergic reaction during an OFC.

Patients were also stratified into 2 cumulative threshold dose groups defined in accordance with literature^{3,30,31}: a low cumulative threshold dose group (LCTG) and a high cumulative threshold dose group (HCTG) of allergic reactions, ≤ 100 mg of crushed peanut (28 mg of pp) and > 100 mg, respectively.

Biological parameters

The biological markers collected, on the same day of the OFC, were eosinophil count, total IgE, ps-IgE and peanut specific IgG4, Ara h 2-sIgE, and Ara h 2-specific IgG4 (Ara h 2-sIgG4) levels (ImmunoCAP; Thermo Fisher Scientific, Uppsala, Sweden). The BAT was performed only once: either on the same day of the OFC or, if that was not possible for logistic reasons (availability of a flow machine and technicians), within a year only if ps-IgE and Ara h 2-sIgE levels were stable indicating no change in the allergy status.

BATs were carried out using the FlowCAST assay kit (Bühlmann Laboratories, Schönenbuch, Switzerland), according to the FK-CCR supplier procedure, and are briefly summarized below.

After gentle stirring, 50 μ L of whole blood was incubated with 50 μ L of peanut extract solution at 100, 10, or 1 ng/mL for 15 minutes at 37°C. Control conditions included 50 μ L of unstimulated negative activation buffer and 50 μ L of anti-Fc ϵ RI antibody solution or fMLP solution (positive controls with nonspecific stimulation). Basophils were gated based on CCR3+/SSC-low window on the negative control, and activated basophils on CCR3+/CD63+ window on the Fc ϵ RI-positive control. The percentage of basophil activation was measured for each condition. A criterion of acceptability of a given sample was at least 1 of the 2 positive controls $> 15\%$. A sample was considered positive if 1 of the 3 tubes containing the peanut extract displayed a percentage of basophil activation $> 15\%$.

The following BAT parameters were considered for analysis:

- The absolute percentage of basophil activation without stimulation (negative control values).
- The absolute percentage of basophil activation with nonspecific stimulation (Fc ϵ RI- and fMLP-positive control values).
- The absolute percentage of basophil activation at different concentrations of peanut extract.
- The absolute allergen concentration able to activate 50% of basophils (EC₅₀).

TABLE I. Descriptive analysis of parameters associated with the severity of an allergic reaction during a peanut oral food challenge

	All PA subjects with positive BAT (n = 56)	Severity groups		P value
		Mild-to-moderate* (n = 43)	Severe* (n = 13)	
Demographic characteristics				
Age (y)	9.5 (6.90-14.02)	9.7 (7.2-13.8)	9.2 (6.6-14.0)	.62
Male/female (n)	36/20	28/15	8/5	>.99
Astier score (I/II/III/IV)	17/26/11/2	—	—	
Cumulative threshold dose (mg of pp)	80 (20-317)	77 (17-258.5)	200 (37-317)	.72
Biological parameters				
Eosinophilia (10 ⁶ cells/mm ³)	0.42 (0.31-0.63)	0.43 (0.31-0.58)	0.39 (0.31-0.80)	.61
Total IgE log ₁₀ (kU _A /L)	618 (317-1139)	439 (261-823)	1256 (647-2177)	.02
Peanut-specific IgE log ₁₀ (kU _A /L)	87 (28.6-238.5)	76.1 (27.9-154)	259 (93.1-332)	.04
Ara h 2-specific IgE log ₁₀ (kU _A /L)	43.0 (11-105.5)	37.3 (51-69.5)	136 (43.9-168)	.02
Peanut-specific IgG4 (mg/L)	0.53 (0.19-1.18)	0.53 (0.19-1.03)	0.56 (0.19-2.59)	.66
Ara h 2-specific IgG4 (mg/L)	0.12 (0.06-0.5)	0.11 (0.06-0.35)	0.21 (0.05-1.49)	.33
Absolute peanut BAT parameters				
Negative control	0.4 (0.01-1)	0.3 (0.01-1)	0.4 (0.01-0.7)	.19
FcεRI-positive control	83.4 (76.1-90.8)	81 (70.8-87.8)	92.4 (89.3-93.8)	.01
fMLP-positive control	34.0 (21.9-45.1)	31.1 (21.1-42.5)	41 (26.1-56.9)	.04
Absolute percentage of basophil activation at 100 ng/mL of peanut extract	69.0 (47.0-86.5)	65.9 (46.4-83.3)	84.4 (60.3-90.2)	.50
Absolute percentage of basophil activation at 10 ng/mL of peanut extract	56.6 (11.2-85.1)	55.6 (11.0-83.8)	72 (29.6-88.7)	.56
Absolute percentage of basophil activation at 1 ng/mL of peanut extract	5.2 (0.5-40.6)	4.3 (0.5-35.5)	12.1 (0.4-53.3)	.52
Absolute basophil EC ₅₀ [†] (ng/mL)	97.1 (37.5-144.7)	95.8 (36.6-136.0)	120.3 (70.0-168.2)	.48
Absolute basophil activation AUC	8.9 (1.8->100)	10 (2.2->100)	6.3 (0.1-33.4)	.37
Normalized peanut BAT parameters				
Normalized percentage of basophil activation at 100 ng/mL of peanut extract	89.4 (74.8-99.3)	89.1 (74.7-98.6)	98.0 (85.4-99.4)	.43
Normalized percentage of basophil activation at 10 ng/mL of peanut extract	76.8 (13.5-102.8)	73.7 (13.3-104.8)	87.0 (31.4-98.8)	.94
Normalized percentage of basophil activation at 1 ng/mL of peanut extract	5.9 (0.7-50.0)	5.6 (0.7-41.0)	14.6 (0.4-55.2)	.66
Normalized basophil EC ₅₀ [†] (ng/mL)	131.0 (51.8-177.1)	126.4 (51.6-174.8)	148.1 (74.4-177.7)	.92
Normalized basophil activation AUC	6.7 (0.1-60.0)	6.8 (0.9-61.1)	4.4 (0.1-29.1)	.90

Values are expressed as count or medians (interquartile ranges). *P* values <.05 are boldface for 2 group comparisons of qualitative variables by using Fisher's exact test and for 2 group comparisons of quantitative variables by using permutation 1-way analysis of variance tests.

AUC, Area under the curve; BAT, basophil activation test; OFC, oral food challenge; PA, peanut allergy; pp, peanut protein.

*Mild-to-moderate allergic reactions during a peanut OFC (Astier scores I and II). Severe allergic reactions during a peanut OFC (Astier scores III and IV).

[†]EC₅₀ = allergen concentration able to activate 50% of basophils.

- The absolute area under the curve (AUC) of basophil activation at different concentrations of peanut extract, calculated as follows: $AUC = (\% \text{ activation for } 1 \text{ ng/mL}) + (\% \text{ activation for } 10 \text{ ng/mL}) + (\% \text{ activation for } 100 \text{ ng/mL} - \% \text{ activation for } 1 \text{ ng/mL})/2$.
- The relative percentage of basophil activation, EC₅₀, and AUC normalized against the FcεRI-positive control value to take into account allergen-specific activation relative to the potential maximum activation of a given patient.

Statistical analysis

Statistical analyses and plots were performed with R (Version 3.3.2; R Foundation for Statistical Computing, Vienna, Austria). The MASS, DescTools, beeswarm, pROC, coin, and qwraps2 packages were used for analysis and plotting.

Fisher's exact test was used for 2 group comparisons of binomial variables. Permutation 1-way analysis of variance tests were used for 2 group comparisons of quantitative variables. Multivariable logistic regressions were used to define composite markers, based on the OFC-positive BAT-positive subgroup. The multivariable step-forward selection strategy was adopted including the variables displaying a *P* value of <.05 by likelihood ratio tests and a significant drop in the residual variance, as compared with the *k* - 1 variables model, using the χ^2 test with *P* < .05. Variance inflation factors (VIFs) were computed to estimate multicollinearity between variables.

Model predictions for each individual in the whole cohort (including OFC/BAT discordant subjects) were computed using the weight coefficients found by the least-squares approach to the observed values of each variable. These predictions were compared with the actual classification of the subject, allowing analysis by

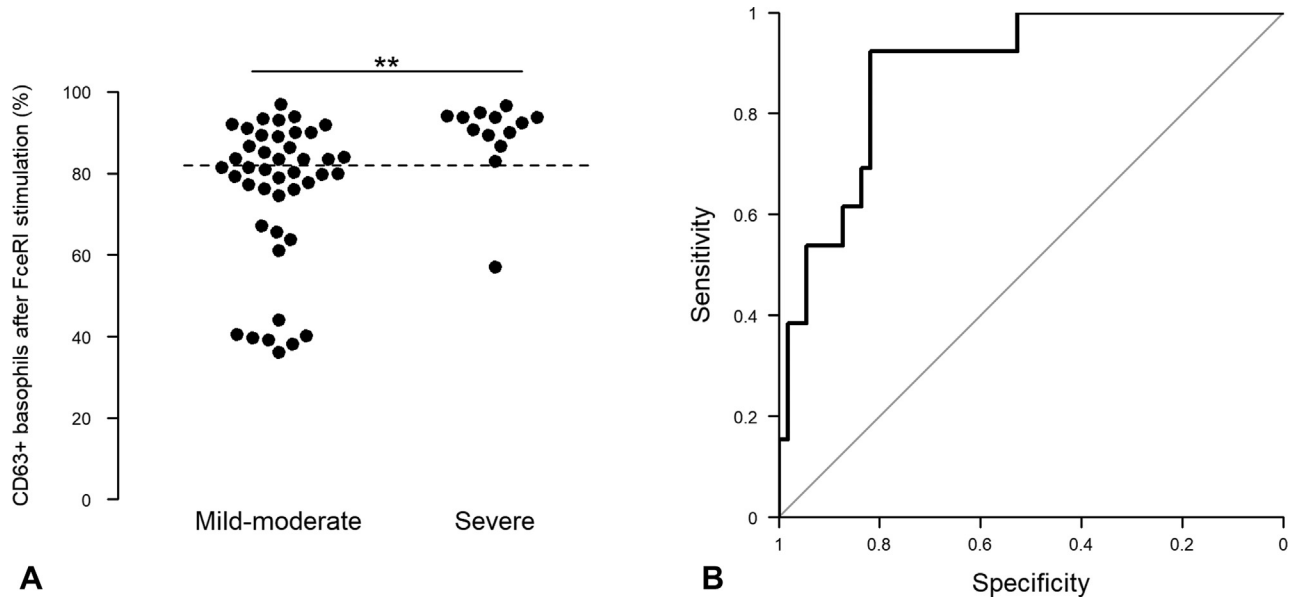


FIGURE 1. A, Basophile activation test FcεRI-positive control value in peanut-allergic patients presenting mild-to-moderate allergic reactions and severe allergic reactions during a peanut oral food challenge. $**P < .01$. **B**, Receiver operating characteristic curve of the multivariable logistic regression model presented in Table II (upper part) comparing the performances of this model in discriminating severe and nonsevere peanut-allergic children.

receiver operating characteristic (ROC) curves. Sensitivity (Se) and specificity (Sp) at best accuracy cutoffs for the candidate models of severity or cumulative threshold dose during an OFC were then computed.

All the tests were 2-sided, with significant P values below type I error risk $\alpha = 0.05$. EC_{50} were log-transformed.

Ethics

Because all the procedures reflected routine patient care at the study center, the protocol was endorsed by the direct procedure of the Institutional Review Board of the Medical Ethics Committee on Research of AP-HP (<http://recherche.aphp.fr/eds>).³²

RESULTS

Population characteristics

During the study period, 75 children with peanut OFC and available BAT results were considered for inclusion. Of these, 2 patients with subjective reactions during an OFC, negative peanut BAT, and negative Ara h 2-sIgE were excluded from analysis. Another 5 were excluded: 3 because of insufficient clinical information, and 2 because of uninterpretable peanut BAT for the other.

In the remaining 68 children with an OFC and BAT, 65 (96%) presented concordant OFC and BAT results: 56 with a positive OFC and BAT, and 9 sensitized nonallergic children with a negative OFC and BAT. One child reacted during the OFC with a negative BAT, and 2 children presented a positive BAT without reacting during the OFC. This result confirms the BAT as a valuable surrogate marker for children with PA as compared with the OFC.

Among the 57 patients with a positive OFC, there was no apparent correlation between the severity of the allergic reaction and the cumulative threshold dose during an OFC ($P = .27$).

The demographic and biological characteristics of these patients are presented in Table I.

Parameters associated with the severity of an allergic reaction during an OFC

We aimed to correlate biological parameters, including BAT parameters, with the severity of allergic reactions, in the OFC/BAT-positive group. The severe allergic reaction group presented higher total IgE, ps-IgE, and Ara h 2-sIgE than the mild-to-moderate allergic reaction group ($P = .02$, $P = .04$, and $P = .02$, respectively) (Table I).

For BAT parameters associated with severity, higher FcεRI- and fMLP-positive control values were both associated with severe allergic reactions to peanut, being higher by 10% in severe patients ($P = .01$ and $P = .04$, respectively) (Table I). Illustratively, 92% (12 of 13) of the patients in the severe allergic reaction group presented an FcεRI-positive control value above 82%, as compared with 44% (19 of 43) in the mild-to-moderate allergic reaction group (Figure 1, A).

The multivariable linear regression model revealed that higher Ara h 2-sIgE and higher FcεRI-positive control values were independently associated with the risk of a severe reaction (Table II, upper part). VIFs were below 1.1, indicating low multicollinearity. Of note, an increase in the FcεRI-positive control value by 1% increased the risk of a severe reaction in the same order of magnitude as an increase of Ara h 2-sIgE by 10 kU_A/L .

We investigated the relevance of this composite model to predict severe reactions in children with a suspicion of PA who would otherwise require an OFC by including nonallergic patients, OFC-positive BAT-negative, and OFC-negative BAT-positive patients. The performances of this model were computed by ROC curve analysis with 0.89 (0.80-0.97) AUC.

TABLE II. Multivariable logistic regression models to predict the severity and cumulative threshold dose of an allergic reaction

	OR (95% CI) per variable unit	P values
Severity model (n = 55)*		
Higher risk of a severe reaction		
Ara h 2-specific IgE (kU _A /L)	1.01 (1.003-1.022)	.01
FcεRI-positive control (%)	1.10 (1.023-1.248)	.047
Cumulative threshold dose model (n = 56)		
Higher risk of reacting at a low cumulative threshold dose [†]		
Normalized basophil EC ₅₀ × log ₁₀ (ng/mL) [‡]	0.40 (0.22-0.73)	.01
Age (y)	1.22 (1.01-1.45)	.04
√(FcεRI × fMLP) positive controls (%)	0.95 (0.90-0.99)	.02

All variables were tested by using forward multivariable logistic regression, and only variables contributing to the model ($P < .05$) were retained. P values $< .05$ are boldface. CI, Confidence interval; OR, odds ratio.

*Severity score was not recorded in 1 patient.

[†]Low cumulative threshold dose: ≤ 100 mg of peanut (28 mg of peanut protein).

[‡]EC₅₀ = allergen concentration able to activate 50% of basophils.

Best accuracy provided 92% Se (84%-97%) and 82% Sp (71%-89%) (Figure 1, B).

The bivariate composite biomarker predicting severe allergic reactions to peanut was the following formula: Severity index = $-10.6 + 0.099 \times \text{Fc}\epsilon\text{RI-positive control value} + 0.0118 \times (\text{Ara h 2-sIgE})$. An index score > -1.35 predicted severe allergic reactions to peanut.

Parameters associated with the cumulative threshold dose during an OFC

A higher age was significantly associated with the LCTG compared with the HCTG ($P = .019$) (Table III). In addition, higher ps-IgE or Ara h 2-sIgE levels were associated with a lower cumulative threshold dose ($P = .004$, and $P = .001$, respectively) (Table III).

Regarding the BAT parameters, all allergen-specific parameters indicated facilitated peanut-induced basophil activation in patients in the LCTG, with the exception of absolute percentage of basophil activation at 100 ng/mL of peanut extract, which was very close to statistical significance ($P = .06$) (Table III). Interestingly, lower P values were systematically obtained for BAT parameters after normalization against the FcεRI-positive control value.

Multivariable logistic regression models indicated a higher risk of the low cumulative threshold dose of an allergic reaction in older children ($P = .05$), patients with lower normalized EC₅₀ ($P = .004$), but also lower FcεRI- and fMLP-positive control values ($P = .015$) (Table II). Importantly, all 3 factors contributed significantly and independently to the model, with a significant drop in residual variance compared with the null or the $k - 1$ model. All VIFs were below 1.6, indicating low multicollinearity. It is worth noting that neither Ara h 2-sIgE nor ps-IgE improved the model significantly. The performances of this composite marker were computed by ROC curve analysis and reached 0.84 (0.74-0.94) AUC. Best accuracy provided 97% Se (89-99) and 61% Sp (49-71) (Figure 2).

The 3-variate composite marker predicting allergic reactions to peanut at cumulative dose < 100 mg was the following formula: LCTG index = $1.60 + 0.18 \times \text{age} - 0.85 \times \text{Log}_{10}(\text{normalized basophil EC}_{50}) - 0.052 \times \sqrt{(\text{fMLP} \times \text{Fc}\epsilon\text{RI-positive control values})}$. An index score > -0.69 predicted a low-dose allergic reaction to peanut.

DISCUSSION

This study reports the relevance of a model of different composite markers in predicting both severity and cumulative threshold dose of an allergic reaction in children with PA and, for the first time to our knowledge, the relevance of an FcεRI- and fMLP-positive control value alone.

Demographic markers related to the severity and cumulative threshold dose of an allergic reaction during an OFC

In our study, the children were older in the LCTG. These results corroborate those of the MIRABEL cohort in which an older age was identified as a predictor of low threshold reactivity.²³

Similarly, in a double-blind, placebo-controlled study, Van der Zee et al¹⁹ showed that the eliciting dose of a peanut OFC was associated with a higher age. These results are similar to previous studies that showed that adolescents experience more severe allergic reactions to peanuts than younger children in real life.¹⁹

Biological parameters related to the severity and cumulative threshold dose of an allergic reaction during an OFC

We found that Ara h 2-sIgE was related to the severity of an allergic reaction during an OFC and to a lower cumulative threshold dose. These results corroborate those of Santos et al²² where patients with severe allergic reactions were shown to have higher Ara h 2-sIgE levels. A previous study found contradictory results about the relationship between Ara h 2-sIgE and the risk of anaphylaxis,³³ whereas others found no association between this biomarker and the severity of an allergic reaction to peanut.³⁴

Furthermore, some studies found that Ara h 2-sIgE levels determined the cumulative threshold dose during an OFC, with contradictory results observed in others.^{16,33,34}

In our study, the relationship between Ara h 2-sIgE levels and lower reaction doses observed in univariate analysis disappeared in multivariable models once controlled for basophil reactivity.

Various BAT parameters were associated with severity (non-allergen-specific FcεRI-positive control value) or cumulative threshold doses of an allergic reaction during an OFC

TABLE III. Descriptive analysis of parameters associated with the cumulative threshold dose of an allergic reaction during a peanut oral food challenge

	Cumulative threshold dose groups		P value
	LGCT* (n = 26)	HCTG† (n = 30)	
Demographic characteristics			
Age (y)	8.2 (56-11.0)	12.4 (7.7-14.1)	.02
Male/female (n)	15/11	21/9	
Astier score (I/II/III/IV)	7/11/6/2	10/15/5/0	.24
Cumulative threshold dose (mg of pp)	317 (200-332)	27.5 (17-40)	—
Biological parameters			
Eosinophilia (10 ⁶ cells/mm ³)	0.47 (0.31-0.62)	0.40 (0.31-0.56)	.58
Total IgE (kU/L)	662.5 (242.5-1128.5)	618 (331-1123)	.61
Peanut-specific IgE (kU _A /L)	30.5 (5.8-253.7)	131 (78.7-219)	.004
Ara h 2-specific IgE (kU _A /L)	11.8 (3.6-115.5)	62.4 (37.7-98.9)	.001
Peanut-specific IgG4 (mg/L)	0.37 (0.1-1.6)	0.56 (0.28-1.18)	.25
Ara h 2-specific IgG4 (mg/L)	0.09 (0.05-0.36)	0.18 (0.077-0.51)	.30
Absolute peanut BAT parameters			
Negative control	0.3 (0.01-1)	0.4 (0.01-0.975)	.69
FcεRI-positive control	86.4 (76.3-92.3)	83.2 (76.9-89.8)	.73
fMLP-positive control	35.4 (23.1-46.5)	32.1 (20.8-43.9)	.33
Absolute percentage of basophil activation at 100 ng/mL of peanut extract	56.3 (36.7-85.4)	75.1 (60.6-87.0)	.06
Absolute percentage of basophil activation at 10 ng/mL of peanut extract	29.6 (2.7-62.5)	75.8 (48.5-88.3)	.002
Absolute percentage of basophil activation at 1 ng/mL of peanut extract	0.8 (0.2-6.9)	15 (4.3-51.3)	.02
Absolute basophil EC ₅₀ ‡ (ng/mL)	68.3 (23.2-109.1)	121.7 (64.5-153.3)	.004
Absolute basophil activation AUC	83.7 (8.4-1000)	5.4 (0.1-47.5)	.02
Normalized peanut BAT parameters			
Normalized percentage of basophil activation at 100 ng/mL of peanut extract	81.1 (62.6-93.8)	97.0 (86.0-100.9)	.01
Normalized percentage of basophil activation at 10 ng/mL of peanut extract	31.5 (3.9-87.0)	98.4 (71.6-106.0)	.0003
Normalized percentage of basophil activation at 1 ng/mL of peanut extract	1.1 (0.3-8.6)	24.7 (5.4-61.2)	.01
Normalized basophil EC ₅₀ ‡ (ng/mL)	74.4 (38.8-149.4)	169.0 (121.8-188.1)	.001
Normalized basophil activation AUC	43.3 (5.3-79.1)	2.8 (0.1-7.0)	.001

Values are expressed as numbers or medians (interquartile ranges). P values <.05 are boldface for 2 group comparisons of qualitative variables by using Fisher's exact test and for 2 group comparisons of quantitative variables by using permutation 1-way analysis tests.

AUC, Area under the curve; BAT, basophil activation test; pp, peanut protein.

*LGCT = low cumulative threshold dose group ≤100 mg of peanut (28 mg of peanut protein).

†HCTG = high cumulative threshold dose group >100 mg of peanut (28 mg of peanut protein).

‡EC₅₀ = allergen concentration able to activate 50% of basophils.

(normalized basophil Se to peanut [EC₅₀] and non-allergen-specific FcεRI- and fMLP-positive control value).

Contrary to our results, Song et al²⁰ showed a correlation between the severity of an allergic reaction during an OFC and the percentage of basophil activation at a specific concentration of peanut (200 ng/mL of allergen $r = 0.50$, $P < .0001$). This study focused on BAT parameters of basophil activation at a given concentration of peanut extract or component without taking into account the nonspecific basophil activation to an FcεRI-positive control value.

However, Santos et al²² found that the association between severity and allergen-specific BAT parameters was improved when the value of the FcεRI-positive control was taken into account. Chinthrajah et al³⁵ demonstrated that the CD63⁺ peanut/anti-IgE ratio was the best predictor of a severe allergic reaction with a regression model. Finally, in a study on cow's milk allergy, this ratio was strongly correlated with the severity but also, as in our study, with the threshold dose of the allergic reaction.³⁶ However, in our study, we found that Ara h 2-sIgE levels appear more relevant than allergen-specific BAT

parameters to predict severity, but that, similar to Santos et al's study,²² the FcεRI-positive control value added information to this allergen-specific parameter, indicating the activation potential of the patient's basophils.

Several studies have demonstrated the usefulness of the BAT to predict the cumulative threshold dose during an OFC.^{22,37,38} In our study, lower normalized basophil EC₅₀ levels were associated with the LCTG, even after controlling for age and an FcεRI-positive control value. Previous studies have already demonstrated the value of CD-sens (1/EC₅₀) for predicting the threshold dose.^{22,38} Santos et al²² also showed that CD-sens was more discriminative in predicting the threshold dose. Reier-Nilsen et al³⁷ found that basophil activation was the best predictor of a very low reactivity threshold in children presenting anaphylaxis to peanut. Chapuis et al³⁸ confirmed the close link between the percentage of CD63⁺ basophils or CD203c and threshold dose.

Our study suggests for the first time that the allergen-independent basophil parameters such as FcεRI- and fMLP-positive control values are associated with the severity of

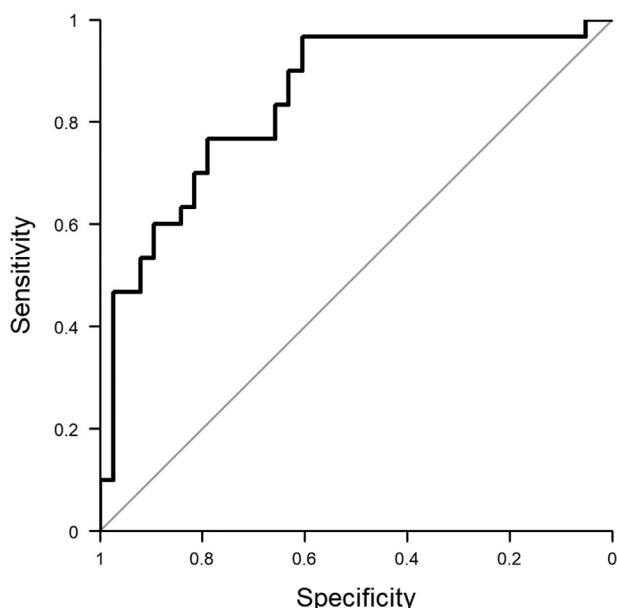


FIGURE 2. Receiver operating characteristic curve of the multivariable logistic regression model presented in Table II (lower part) comparing the performances of this model in discriminating peanut-allergic children reacting to low or high dose of peanut proteins.

peanut-allergic reactions, independently of Ara h 2-sIgE levels. This result is concordant with a decrease in BAT FcεRI-positive control during peanut oral immunotherapy.^{39,40} The value of the FcεRI-positive control probably reflects the potential of maximum percentage of nonspecific basophil activation, similar to serum tryptase levels in severe hymenoptera venom allergy in patients with mastocytosis.⁴¹

On the other hand, allergen-specific basophil reactivity is an independent factor and is related to the threshold dose of an allergic reaction, as can be observed during oral immunotherapy.

Composite markers most accurately predict the severity and cumulative threshold dose of an allergic reaction during an OFC

In our 2 models, different composite markers predicted severity and the cumulative threshold dose. This difference is probably because severity and cumulative threshold dose are not related either in our study or in the literature.

However, only 1 parameter—a higher FcεRI-positive control value—was common to both composite markers linked to severity and cumulative threshold. This factor (FcεRI-positive control value) probably reflects an “intrinsic severity” linked to the fact that oral immunotherapy is more easily performed in young children perhaps in relationship to immune plasticity.⁴²

In our study, composite markers including age and FcεRI-positive control value were the best predictor of the severity of an allergic reaction during an OFC. Similarly, Chinthrajah et al³⁵ showed that a history of exercise-induced asthma and CD63 ratio were statistically significant predictors of challenge severity score using a LASSO (least absolute shrinkage and selection operator) regression model.

Furthermore, the best model to predict cumulative threshold included both demographic (age) and biological parameters (normalized basophil activation at 1 ng/mL of peanut extract, FcεRI-positive control value, and Ara h 2-sIgG4). In the same manner, in the MIRABEL cohort, composite parameters including gender, prick test size, and Ara h 2-sIgE were found to predict the threshold dose.²³

Strengths and limits

The strength of our study lies in the nature of our well-characterized sample. Furthermore, the OFCs were performed during 1 day and with similar doses to other studies.^{22,23,37,38} However, the study was retrospective and the OFCs (as used in clinical practice) were not performed in a double-blind placebo-controlled manner.⁴³ Another limit is that allergic comorbidities, such as asthma or atopic dermatitis, were not included in the model as in other studies. However, we did not see any relationship between levels of total IgE and the FcεRI-positive control value (data not shown). Finally, the sample size and the absence of an independent validation cohort are also limitations.

Perspective

Multivariable BAT is increasingly recognized as a surrogate marker for an OFC. Kits for performing the BAT are now readily available making this analysis feasible for any medical laboratory with a flow cytometer. Thus, the BAT can now be routinely performed in any allergy clinic with access to a laboratory service, and we suggest that the BAT is offered to all children with ps-IgE sensitization and suspected PA. The multivariable biomarkers we present here could therefore be used in clinical practice to determine the severity and threshold dose of an allergic reaction, without the need of an OFC. These results must now be validated with other food allergens in larger prospective cohorts.

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TABLE E1. Astier score: systemic allergic reaction grading system

Grade	Symptoms
0	No symptoms
1	Abdominal pain that resolved without requiring medical treatment, rhinoconjunctivitis, urticaria fewer than 10 papulas, rash (eczema onset)
2	One organ involved, abdominal pain requiring treatment, generalized urticaria, nonlaryngeal angioedema, mild asthma (cough or fall of peak expiratory flow <20%)
3	Two organs involved
4	Three organs involved or asthma requiring treatment or laryngeal edema or hypotension
5	Cardiac and respiratory symptoms requiring hospitalization in intensive care