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

Nathalie Cottel, MD, Sarah Saf, MD, Melisande Bourgoin-Heck, MD, Nathalie Lambert, MD, Flore Amat, MD, PhD, Pascal Poncet, PhD, Helene Senechal, PhD, Rémy Couderc, PhD, Jocelyne Just, MD, PhD, and Yannick Chantran, PharmD Paris, France

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. PA is diagnosed before the age of 6 in approximately 80% of children with PA. 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. In 2019, the incidence of food-induced anaphylaxis in Europe was higher compared with other regions in the world.
**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. 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).

The patients were stratified into 2 score severity groups for analysis*: 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*: 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–specific IgE, and Ara h 2–specific IgG4 (Ara h 2–specific IgG4 [Ara h 2–specific IgG4] 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–specific IgE 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 μL of whole blood was incubated with 50 μL of peanut extract solution at 100, 10, or 1 ng/mL for 15 minutes at 37°C. Control conditions included 50 μL of unstimulated negative activation buffer and 50 μL of anti-FceRI antibody solution or FMLP solution (positive controls with nonspecific stimulation). Basophils were gated based on CCR3+/SSClow window on the negative control, and activated basophils on CCR3+/CD63+ window on the FceRI-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 (FceRI- 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).
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).

The absolute area under the curve (AUC) of basophil activation at EC50 was calculated as follows:

\[ \text{AUC} = \frac{1}{2} \left( \frac{C_1 + C_2}{2} \right) \times \text{EC50} \]

where \( C_1 \) and \( C_2 \) are the maximum activation of a given patient.

The relative percentage of basophil activation, EC50, and AUC were 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 \(<0.05\) by likelihood ratio tests and a significant drop in the residual variance, as compared with the \( k - 1 \) variables model, using the \( \chi^2 \) test with \( P < 0.05 \). 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...
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, 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 kUa/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.

**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 noneverse peanut-allergic children.
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 = \(-10.6 + 0.099 \times \text{FceRI-positive control value} + 0.0118 \times (\text{Ara h 2-sIgE})\). 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 FceRI-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 EC\(50\) (\(P = .004\)), but also lower FceRI- 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 \log_{10}(\text{normalized basophil EC\(50\)) - 0.052 \times \sqrt{\text{fMLP} \times \text{FceRI}})-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 FceRI- 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 al\(^{19}\) 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 al\(^{22}\) 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,\(^{35}\) 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 FceRI-positive control value) or cumulative threshold doses of an allergic reaction during an OFC.

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

<table>
<thead>
<tr>
<th>Severity model (n = 55)(^*)</th>
<th>Higher risk of a severe reaction</th>
<th>(P) values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ara h 2-specific IgE (kU/mL)</td>
<td>1.01 (1.003-1.022)</td>
<td>.01</td>
</tr>
<tr>
<td>FceRI-positive control (%)</td>
<td>1.10 (1.023-1.248)</td>
<td>.047</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Cumulative threshold dose model (n = 56)</th>
<th>Higher risk of reacting at a low cumulative threshold dose(^†)</th>
<th>(P) values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normalized basophil EC(50) (\log_{10}) (ng/mL)(^\dagger)</td>
<td>0.40 (0.22-0.73)</td>
<td>.01</td>
</tr>
<tr>
<td>Age (y)</td>
<td>1.22 (1.01-1.45)</td>
<td>.04</td>
</tr>
<tr>
<td>(\sqrt{\text{FceRI} \times \text{fMLP}}) positive controls (%)</td>
<td>0.95 (0.90-0.99)</td>
<td>.02</td>
</tr>
</tbody>
</table>

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. \(OR\), Odds ratio.

\*Severity score was not recorded in 1 patient.

\†Low cumulative threshold dose: \(\leq 100\) mg of peanut (28 mg of peanut protein).

\(\dagger\text{EC}_{50}\) = allergen concentration able to activate 50% of basophils.
taking into account the nonspecific study focused on BAT parameters of basophil activation at a severity and allergen-specific level. Chinthrajah et al demonstrated that the CD63 reaction with a regression model. Finally, in a study on cow peanut/anti-IgE ratio was the best predictor of a severe allergic

<table>
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<tr>
<th>TABLE III. Descriptive analysis of parameters associated with the cumulative threshold dose of an allergic reaction during a peanut oral food challenge</th>
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<tbody>
<tr>
<td>Demographic characteristics</td>
</tr>
<tr>
<td>Age (y)</td>
</tr>
<tr>
<td>Male/female (n)</td>
</tr>
<tr>
<td>Astier score (I/II/III/IV)</td>
</tr>
<tr>
<td>Cumulative threshold dose (mg of pp)</td>
</tr>
<tr>
<td>Biological parameters</td>
</tr>
<tr>
<td>Eosinophilia (10^6 cells/mm³)</td>
</tr>
<tr>
<td>Total IgE (kU/L)</td>
</tr>
<tr>
<td>Peanut-specific IgE (kU/L)</td>
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<tr>
<td>Ara h 2-specific IgE (kU/L)</td>
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<tr>
<td>Peanut-specific IgG4 (mg/L)</td>
</tr>
<tr>
<td>Ara h 2-specific IgG4 (mg/L)</td>
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<tr>
<td>Absolute peanut BAT parameters</td>
</tr>
<tr>
<td>Negative control</td>
</tr>
<tr>
<td>FcεRI-positive control</td>
</tr>
<tr>
<td>fMLP-positive control</td>
</tr>
<tr>
<td>Absolute percentage of basophil activation at 100 ng/mL of peanut extract</td>
</tr>
<tr>
<td>Absolute percentage of basophil activation at 1 ng/mL of peanut extract</td>
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<tr>
<td>Absolute percentage of basophil activation at 10 ng/mL of peanut extract</td>
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<tr>
<td>Absolute basophil EC50 (ng/mL)</td>
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<tr>
<td>Absolute basophil activation AUC</td>
</tr>
<tr>
<td>Normalized peanut BAT parameters</td>
</tr>
<tr>
<td>Normalized percentage of basophil activation at 100 ng/mL of peanut extract</td>
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<tr>
<td>Normalized percentage of basophil activation at 10 ng/mL of peanut extract</td>
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<tr>
<td>Normalized percentage of basophil activation at 1 ng/mL of peanut extract</td>
</tr>
<tr>
<td>Normalized basophil EC50 (ng/mL)</td>
</tr>
<tr>
<td>Normalized basophil activation AUC</td>
</tr>
</tbody>
</table>

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.

LGCT: 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).

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

Contrary to our results, Song et al 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 al 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 al 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. 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, 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. 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. Santos et al also showed that CD-sens was more discriminative in predicting the threshold dose. Reier-Nilsen et al found that basophil activation was the best predictor of a very low reactivity threshold in children presenting anaphylaxis to peanut. Chapuis et al 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
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-sIgE). 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.\textsuperscript{53}

**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.\textsuperscript{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.\textsuperscript{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.

**Acknowledgments**

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**REFERENCES**


12. NIAID-Sponsored Expert PanelBoyce JA, Assa


**TABLE E1.** Astier score: systemic allergic reaction grading system

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