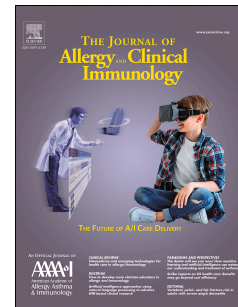


Journal Pre-proof

Biomarkers of severity and threshold of allergic reactions during oral peanut challenges

Alexandra F. Santos, MD PhD, George Du Toit, MD FRCPCH, Colin O'Rourke, MS, Natalia Becares, PhD, Natália Couto-Francisco, MSc, Suzana Radulovic, MD, Ekaterina Khaleva, MD, Monica Basting, MA, Kristina M. Harris, PhD, David Larson, PhD, Peter Sayre, MD PhD, Marshall Plaut, MD, Graham Roberts, DM FRCPCH, Henry T. Bahnson, MPH, Gideon Lack, MD FRCPCH



PII: S0091-6749(20)30490-5

DOI: <https://doi.org/10.1016/j.jaci.2020.03.035>

Reference: YMAI 14497

To appear in: *Journal of Allergy and Clinical Immunology*

Received Date: 20 September 2019

Revised Date: 10 February 2020

Accepted Date: 20 March 2020

Please cite this article as: Santos AF, Du Toit G, O'Rourke C, Becares N, Couto-Francisco N, Radulovic S, Khaleva E, Basting M, Harris KM, Larson D, Sayre P, Plaut M, Roberts G, Bahnson HT, Lack G, Biomarkers of severity and threshold of allergic reactions during oral peanut challenges, *Journal of Allergy and Clinical Immunology* (2020), doi: <https://doi.org/10.1016/j.jaci.2020.03.035>.

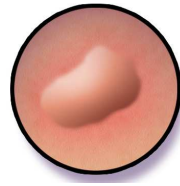
This is a PDF file of an article that has undergone enhancements after acceptance, such as the addition of a cover page and metadata, and formatting for readability, but it is not yet the definitive version of record. This version will undergo additional copyediting, typesetting and review before it is published in its final form, but we are providing this version to give early visibility of the article. Please note that, during the production process, errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

© 2020 Published by Elsevier Inc. on behalf of the American Academy of Allergy, Asthma & Immunology.



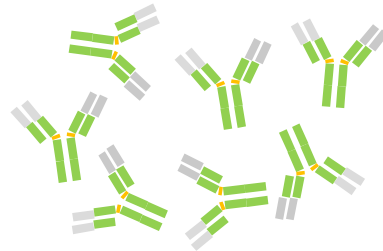
Biomarkers of severity and threshold of allergic reactions during oral peanut challenges

Skin prick test to peanut



>8mm
>6mm

Ara h 2-specific IgE

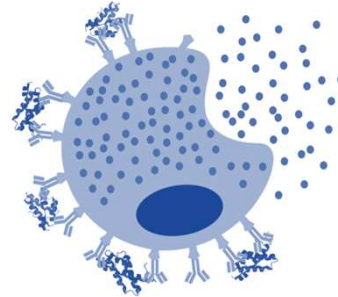


>1.4 KU/L
>0.1 KU/L

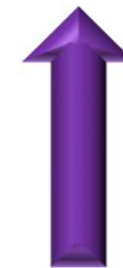


Probability of
severe or life-
threatening
reactions to
peanut

%CD63+ Basophils to peanut

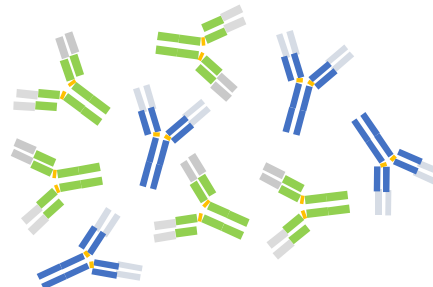


>48%
>1.7%



Probability of
threshold dose
<0.1g of peanut
protein

Ratio of IgG4/IgE to peanut



<1.6

Biomarkers of severity and threshold of allergic reactions during oral peanut challenges

Alexandra F. Santos, MD PhD^{1,2,3,4*}; George Du Toit, MD FRCPCH^{1,2,3+}; Colin O'Rourke, MS⁵⁺; Natalia Becares, PhD^{1,2,4}; Natália Couto-Francisco, MSc^{1,2,4}; Suzana Radulovic, MD^{1,2,3}; Ekaterina Khaleva, MD³; Monica Basting, MA^{1,2}; Kristina M. Harris, PhD⁶; David Larson, PhD⁶; Peter Sayre, MD PhD⁷; Marshall Plaut, MD⁸; Graham Roberts, DM FRCPCH⁹; Henry T Bahnson, MPH⁵; Gideon Lack, MD FRCPCH^{1,2,3,4*}.

+These authors contributed equally to the manuscript.

¹Department of Women and Children's Health (Pediatric Allergy), School of Life Course Sciences, Faculty of Life Sciences and Medicine, King's College London, London, UK

²Peter Gorer Department of Immunobiology, School of Immunology and Microbial Sciences, King's College London, London, UK

³Children's Allergy Service, Guy's and St Thomas' Hospital, London, UK

⁴Asthma UK Centre in Allergic Mechanisms of Asthma, London, UK

⁵Immune Tolerance Network, Benaroya Research Institute, Seattle, USA

⁶Immune Tolerance Network, Bethesda, MD, USA

⁷Division of Hematology–Oncology, Department of Medicine, University of California, San Francisco, San Francisco, USA

⁸National Institute of Allergy and Infectious Diseases, Bethesda, USA

⁹David Hide Asthma and Allergy Research Centre, St Mary's Hospital, Isle of Wight; NIHR Biomedical Research Centre, University Hospital Southampton NHS Foundation Trust and Clinical and Experimental Sciences Academic Unit, University of Southampton Faculty of Medicine, Southampton, United Kingdom

24

25 ***Corresponding authors:**

26 Name: Alexandra F. Santos

27 Postal address: Department of Pediatric Allergy, 2nd floor, South Wing, St Thomas' Hospital,

28 Westminster Bridge Road, SE1 7EH London, United Kingdom

29 Telephone: +44 (0) 20 7188 6424

30 Fax: +44 (0) 20 7403 8640

31 Email address: alexandra.santos@kcl.ac.uk

32

33 Name: Gideon Lack

34 Postal address: Department of Pediatric Allergy, 2nd floor, South Wing, St Thomas' Hospital,

35 Westminster Bridge Road, SE1 7EH London, United Kingdom

36 Telephone: +44 (0)2071889730

37 Fax: +44 (0)2071889782

38 Email address: gideon.lack@kcl.ac.uk

39

40 **Conflicts of interest statement:** Dr. Santos reports grants and personal fees from Medical Research
41 Council (MRC Clinical Research Training Fellowship G0902018, MRC Centenary Early Career Award,
42 MRC Clinician Scientist Fellowship MR/M008517/1); grants from Asthma UK and the NIHR through the
43 Biomedical Research Centre (BRC) award to Guy's and St Thomas' NHS Foundation Trust, during the
44 conduct of the study; grants from Immune Tolerance Network/National Institute of Allergy and Infectious
45 Diseases (NIAID, NIH), grants from Asthma UK; personal fees from Thermo Scientific, Nutricia,
46 Infomed, Novartis, Allergy Therapeutics, Buhlmann, as well as research support from Buhlmann and

Thermo Scientific through a collaboration agreement with King's College London. Dr Larson, Dr Harris and Mr Bahnson report grants from the National Institute of Allergy and Infectious Diseases (NIH UM1AI109565); Dr. Du Toit reports grants from National Institute of Allergy and Infectious Diseases (NIAID, NIH), Food Allergy & Research Education (FARE), UK Dept of Health through NIHR, National Peanut Board (NPB), and UK Food Standards Agency (FSA); these grants part funded salary over period of this submitted work. Dr Lack reports grants from the National Institute of Allergy and Infectious Diseases (NIAID, NIH – NO1-AI-15416 and UM1AI109565), Food Allergy & Research Education (FARE), NIHR through BRC award to Guy's and St Thomas' NHS Foundation Trust, National Peanut Board, UK Food Standards Agency, Davis Foundation; and personal fees from Novartis, Aravax, ALK-Abello, DBV Technologies and Mighty Mission Me. The other authors have nothing to disclose.

Abstract (246 words):

Background: Oral food challenge (OFC) is the gold-standard to assess peanut allergy (PA) but involves a risk of allergic reactions of unpredictable severity.

Objective: To identify biomarkers for risk of severe reactions or low dose threshold during OFC to peanut.

Methods: We assessed LEAP, LEAP-On and PAS participants with basophil activation test (BAT), skin prick test (SPT), peanut-specific IgE (sIgE) and Ara h 2-sIgE and peanut-specific IgG4 and analyzed the utility of the different biomarkers in relation to PA status, severity and threshold dose of allergic reactions to peanut during OFC.

Results: Using a previously defined optimal cut-off, BAT diagnosed PA with 98% specificity and 75% sensitivity. BAT identified severe reactions with 97% specificity and 100% sensitivity. SPT, Ara h 2-sIgE, peanut-sIgE and IgG4/IgE ratios also had 100% sensitivity but slightly lower specificity (92%, 93%, 90% and 88% respectively) to predict severity. Participants with lower threshold of reactivity had higher basophil activation to peanut in vitro. SPT and BAT were the best individual predictors of

threshold. Multivariate models were superior to individual biomarkers and were used to generate nomograms to calculate the probability of serious adverse events during OFC for individual patients.

Conclusions: BAT diagnosed PA with high specificity and identified severe reactors and low threshold with high specificity and high sensitivity. BAT was the best biomarker for severity, surpassed only by SPT in predicting threshold. Nomograms can help estimate the likelihood of severe reactions and reactions to low dose of allergen in individual peanut allergic patients.

Key messages:

- The basophil activation test was useful to diagnose peanut allergy, predict severity and threshold of reactivity to peanut in the LEAP studies.
- Skin prick test and Ara h 2-specific IgE were also useful biomarkers of severity and threshold in the LEAP cohorts.
- Multivariate models combining basophil activation with other tests using a nomogram identified children with severe allergic reactions and low threshold of reactivity during oral peanut challenges.

Capsule Summary (32 words): Combinations of skin prick, specific IgE and basophil activation tests in multivariate models predicted severe reactions and low threshold dose during peanut challenges and were used to build nomograms for clinical practice.

Keywords: basophil, basophil activation test, diagnosis, food allergy, LEAP Study, peanut allergy, severity, threshold, adverse events

95 **Abbreviations:**

96 BAT, basophil activation test

97 CTCAE, Common Terminology Criteria for Adverse Events

98 HR, hazard ratio

99 IQR, interquartile range

100 LEAP, learning early about peanut allergy study

101 OFC, oral food challenge

102 PAS, peanut allergy sensitization study

103 sIgE, specific IgE

104 SPT, skin prick test

105

106 **Word count:** 4274107 **Table count:** 3 tables108 **Figure count:** 5 figures109 **Online repository:** 12 tables and 14 figures

110

Introduction:

Oral food challenge (OFC) is the gold-standard to diagnose and assess resolution of food allergies. OFC is also the gold-standard to confirm the eligibility of food allergic patients for clinical trials of experimental treatments for food allergy and to assess patients' clinical response to such treatments. OFC can result in potentially severe allergic reactions and requires an experienced and highly skilled clinical team with the ability and equipment needed to treat anaphylaxis. Although rare, two deaths have been reported in children undergoing OFC^{1,2}. A biomarker that could identify individuals at high-risk for severe allergic reactions and/or for reacting to small amounts of the allergens would ensure patients' safety, comfort, and improve current management of food allergic patients.

Peanut is one of the main culprits of fatal and near-fatal food-induced allergic reactions³. The prevalence of peanut allergy (PA) is increasing, and some studies have reported an increase in food-induced anaphylaxis fatalities⁴. Evidence about the utility of skin prick test (SPT) and specific IgE (sIgE) to predict the severity of allergic reactions to peanut is conflicting⁵⁻⁸. Ara h 2-sIgE is a discriminative marker to diagnose PA and an association with severity has been observed^{9,10}. The basophil activation test (BAT) has shown the highest accuracy to diagnose PA, with a higher proportion of activated basophils (as measured by %CD63-positive basophils) being associated with more severe allergic reactions during OFC^{9, 11, 12} and the cumulative threshold dose being associated with the concentration at which the basophils reacted *in vitro*^{9, 13}. Despite the need for fresh blood samples and the existence of a subset of individuals with non-responder basophils, the BAT has emerged as a promising biomarker to identify high-risk allergic patients.

The LEAP Study¹⁴ conferred a unique opportunity to perform the BAT and other tests in a large number of well-characterized children who were being assessed for PA. We aimed to assess their utility in diagnosing PA and in predicting severity and threshold dose of allergic reactions during oral peanut challenges in this cohort.

Methods:**Study cohorts**

Participants in the Learning Early About Peanut allergy (LEAP)¹⁵, LEAP-On¹⁶ and peanut allergy sensitization (PAS) studies were included (Table E1). The LEAP study was a large interventional study that assessed the effect of early peanut consumption on the development of peanut allergy by age 5 years¹⁵. LEAP participants (Groups II and III of the LEAP screening study¹⁴) were followed up after one year of peanut avoidance in the LEAP-On study¹⁶. Children who had been excluded from the LEAP Study as infants were included in the PAS Study¹⁴. An additional cohort of children recruited from specialized Paediatric Allergy clinics in London⁹ was included for external validation (Table E2). All studies were approved by the relevant Research Ethics Committees in the UK - references: 04/Q0403/13 (LEAP), 10/H0711/77 (LEAP-On), 11/LO/0045 (PAS) and 10/H0802/044 (clinic cohort).

We aimed to perform BAT in LEAP participants who showed evidence of peanut sensitization at any time point in the LEAP study either on SPT (≥ 1 mm) or serum sIgE (≥ 0.10 kU/L) to peanut, and in 50 LEAP participants who showed SPT to peanut of 0 mm and sIgE to peanut < 0.1 kU/L at all time points of the study prior to their assessment. We aimed to repeat BAT to peanut at the end of LEAP-On study in the same participants. In the PAS study, we aimed to test all participants who were assessed at approximately 60 months of age¹⁴.

Skin prick testing and determination of IgE and IgG4 levels

SPT and BAT were performed on the same day as the OFC. Peanut-specific IgE and IgG4 levels were determined using samples collected when blood was collected for BAT. Skin prick testing was performed using a commercially available peanut extract (ALK-Abelló, Denmark), as previously described¹⁵. The

levels of sIgE to peanut and to peanut allergens rAra h 1, rAra h 2, rAra h 3, rAra h 8 and rAra h 9 and the levels of peanut-specific IgG4 in the serum were measured using an immunoenzymatic assay (ImmunoCAP, ThermoFisher).

Basophil activation test

Whole blood was collected into lithium heparin tubes and used to perform the basophil activation test to peanut extract on the same day and within 4 hours of blood collection, as previously described¹¹. Briefly, 100µl of whole blood was incubated with the same volume of peanut extract (ALK-Abello) diluted in RPMI (GIBCO, Paisley, United Kingdom), anti-IgE (1µg/ml, Sigma-Aldrich, Poole, United Kingdom), anti-FcεRI (2.5µg/ml, eBioscience, San Diego, California), formyl-methionyl-leucylphenylalanine (fMLP, 1µM, Sigma-Aldrich) or RPMI alone. Cells were stained with CD123-FITC (eBioscience), CD203c-PE, HLA-DR-PerCP, and CD63-APC (Biolegend, San Diego, Calif). Flow cytometry was performed using FACS CantoII with FACSDiva software (BD Biosciences, San Jose, Calif). A minimum of 500 basophils was acquired. The flow cytometry data were analyzed using FlowJo software (version 7.6.1; TreeStar, Ashland, Ore). Basophils were gated as SSC^{low}/CD203c+/CD123+/HLA-DR⁻^{11, 17}. Basophil activation was expressed as %CD63+ basophils. Individuals with non-responder basophils were defined as having %CD63+ basophils below 5% to both IgE-mediated positive controls (anti-IgE and anti-FcεRI) and were excluded from statistical analyses. CD-sens was calculated by for each BAT estimating the parameters of a logistic growth function, one of which is the reciprocal of CD-sens, using a non-linear model (Figure E1).

Oral peanut challenges

Oral peanut challenges were performed according to the LEAP Study protocol as previously reported^{15, 16}.

Participants who were not IgE-sensitized to peanut and had no history of reaction to peanut, no diagnosis of or suspected allergy to sesame or tree nuts and no history of anaphylaxis underwent an open challenge up to a cumulative dose of 5g of peanut protein given as a single dose. Open challenge is the gold-standard to confirm peanut tolerance and all open challenges were indeed negative for PA. All other participants underwent a double-blind-placebo-controlled-peanut challenge up to a cumulative dose of 9.35 g of peanut protein given as incremental doses (Table E3). Participants with suspected PA, history of life-threatening food-induced anaphylaxis or $SPT \geq 7$ mm received an additional lower starting active dose of 0.033g of peanut protein. Some doses may have been repeated at the discretion of the investigator performing the OFC. The challenge was considered positive only when objective signs of an allergic reaction developed (Table E4), and the symptoms were treated according to local guidelines, which follow the recommendations of the UK Resuscitation Council of administering epinephrine to patients with life-threatening airway (swelling, hoarseness, stridor) and/or breathing (tachypnoea, wheeze, fatigue, cyanosis, $SpO_2 < 90\%$, confusion) and/or circulation (pale, clammy, hypotension, faintness, drowsiness, coma) problems¹⁸. For participants for whom the outcome of OFC was inconclusive or not available, peanut allergy was assessed by a diagnostic algorithm using SPT and specific IgE to peanut. The procedure for OFC in the cohort used for external validation was similar to the described above for the majority of study subjects and was previously published¹¹.

The severity of allergic reactions during OFC were classified according to different severity scores represented in Table E5^{19, 20}. The threshold dose was determined as the cumulative amount of peanut protein (in grams) tolerated during the challenge. The cumulative tolerated dose was set to 9.35g for all participants who passed the OFC.

Statistical analyses

Continuous variables were summarized by quantiles and categorical variables were summarized by counts and percentages. Comparisons of continuous predictors use either Student's t-test or Wilcoxon's rank-sum test. ROC curve analyses were performed to assess the diagnostic performance of each individual biomarker to predict severe reactions. The performance of the optimal cut-offs was described using sensitivity, specificity, positive predictive value, and negative predictive value.

CD-sens, the reciprocal of ED_{50} , was estimated by first fitting a hierarchical Bayesian non-linear model (Figure E1) to the BAT curve for each sample. This model assumes that each BAT curve follows the form of a 3-parameter logistic growth curve. The resulting logistic curve midpoint parameter estimates from this model were used to estimate CD-sens. Model fitting was performed using MCMC, which was performed using the Stan software²¹.

Proportional odds logistic regression models were created for prediction of reaction severity. The predictors used in these models were chosen based on being established predictors of reaction to peanut and no further variable selection was done based on tests of statistical hypotheses. Model performance was described using bootstrap bias-corrected concordance probabilities (C-statistics) and calibration accuracy measurements (mean absolute prediction error and 90th percentile of absolute prediction error). External model validation was done using data available from participants in a separate clinic cohort (Table E2). Statistical analysis was done in R software (Version 3.5.2) and JMP Pro (Version 14). Datasets are available through TrialShare, a public website managed by the Immune Tolerance Network (https://www.itntrialshare.org/LEAP_JACI_BAT.url).

Results:

Study population

There were 468 individual subjects enrolled across LEAP, LEAP-On and PAS studies, and from these the BAT was performed in 706 blood samples (335 LEAP, 295 LEAP-On and 76 PAS participants) on the day of the study visits when allergic status to peanut was assessed (Tables I and E6). An additional 123 subjects were eligible for BAT but were not tested due to logistical reasons (Tables E7 and E8). On the BAT, in 93 (13.1%) samples basophils did not react to the IgE-mediated positive controls and were excluded from statistical analyses, including 11 subjects who were peanut allergic (10.8% of non-responders were PA and 6.7% of PA were non-responders); also excluded were 5 samples whose response status could not be determined due to missing data. Subjects with undetectable peanut-sIgE (<0.10 kU/L) who were not tested on the BAT ($n=373$) were imputed to have a BAT result of 0, since all patients with undetectable IgE had negative BAT both in this and in previous studies¹¹. A total of 981 performed or imputed BAT cases were available for building predictive models and 558 independent samples from LEAP and PAS were used for all other analyses. Fourteen percent were peanut allergic. The threshold cumulative dose ranged between 0 and 10.1g of peanut protein (between 5 to 10.1g in non-allergic and between 0 to 4.5g in allergic participants, respectively). The cumulative dose exceeded 9.35g in participants who were given repeat doses at the discretion of the investigators. According to the CTCAE scale, 15%, 3%, and 16% participants in LEAP, LEAP-On, and PAS studies had severe reactions, respectively. There was some degree of concordance with other severity scales (Kendall's tau = 0.12 to 0.64, Figure E2).

BAT confirmed its high specificity to diagnose peanut allergy

Basophil activation to peanut was higher in peanut allergic compared to non-allergic subjects (Figures 1 and E3), with risk of peanut allergy at the 75th percentile of BAT being 3.85 fold higher than the 25th percentile BAT (95% CI HR 3.2 to 4.7, $P < 0.001$). Using data from participants in the LEAP and PAS studies, we applied the optimal diagnostic cut-off previously identified for BAT to peanut¹¹ (i.e. 4.78%

CD63+ basophils for average BAT at 10 and 100ng/ml of peanut extract), which had 74.7% sensitivity, 98.7% specificity, 95.4% NPV, 91.5% PPV and 57.8 and 0.3 positive and negative likelihood ratios, respectively, to diagnose PA. Basophil activation in response to anti-IgE, anti-FcεRI and fMLP was similar across allergic status (Figure E4).

BAT and other tests identified participants with severe or life-threatening allergic reactions during oral peanut challenges with high accuracy

Participants with severe/life-threatening reactions during OFC had higher proportions of activated basophils in response to peanut compared to participants with mild or moderate reactions (Figure 2A and Table E9). Basophil activation in response to anti-FcεRI and anti-IgE (but not fMLP) was higher for severe reactors compared to non-severe reactors (Figure E4). Figure 2B-D illustrates the results for the other biomarkers according to allergic status highlighting participants with severe/life-threatening reactions.

BAT showed high accuracy in identifying subjects with severe/life-threatening allergic reactions according to the CTCAE severity score and was the single biomarker with the largest area under ROC curve (0.985 – Figure E5A) and therefore with the best discriminatory ability. The optimal cut-off for BAT had 100% sensitivity and 97% specificity to identify high-risk subjects (Table II). SPT, Ara h 2-specific IgE, peanut-specific IgE and IgG4/IgE ratios all had 100% sensitivity but lower specificity to predict severity (92%, 93%, 90% and 88% respectively). The performance of various tests to identify severe reactors was similar using different systems to classify the severity of allergic reactions (Figure E5B and Table E10). A multivariable model of reaction severity suggests that, after adjusting for SPT and Ara h 2-sIgE, BAT still contributes significantly to the prediction of reaction severity (Table E11).

SPT and BAT were the best predictors of the threshold dose of reactivity to peanut during oral challenges

Participants reacting clinically to lower doses of peanut protein had higher proportions of activated basophils (Figures 3 and E6-E9) and higher basophil sensitivity, as measured by CD-sens (Figure E8). There was no association between lower threshold dose and basophil activation to the positive controls fMLP, anti-IgE or anti-FcεRI. Figure E7 shows the estimated proportion of participants who remain reaction-free as a function of cumulative peanut dose and reinforces the concept that the higher the proportion of activated basophils, the lower the dose of peanut protein the patients react during OFC. Using ROC curve analyses, the optimal cut-offs for SPT, Ara h 2-specific IgE and IgG4/IgE ratios also had a very good performance in identifying patients with a cumulative threshold dose of 0.1g or lower (Table II).

Models combining different biomarkers to predict severity and threshold dose of allergic reactions during oral peanut challenges

We designed multivariate models combining different parameters to determine the risk of a severe reaction and, with the application of our findings to routine clinical practice in mind, we used these models to generate nomograms (Figures 4A and E10.A-H). We performed internal validation of these models using bootstrap to correct for the bias of using the same data to fit and validate the model. While the performance of both multivariate models is good (Table IIIA), based on calibration, the model including BAT predicts the probability of severe/life-threatening reactions more accurately compared to the model without BAT or to individual tests.

A multivariable model for threshold built using the best predictors for cumulative threshold dose and including BAT (SPT, BAT, Ara h 2-specific IgE, peanut-specific IgE and IgG4/IgE ratio) was superior to the model without BAT and outperformed models based on any of the predictors individually (Table III).

The nomogram in figure 4B depicts this multivariable model and can be used to make predictions of threshold of future reactions during peanut allergy testing. A similar nomogram without BAT is shown in Figure E11.

Internal validation reflects mostly the performance of the model within a population similar to the present study, and external validation is important to assess the model in more general settings. External validation of the severity and the threshold models was performed using an independent cohort recruited from two specialized Paediatric Allergy outpatient clinics in London (Table E2). While this dataset with limited by not having severe cases, the model did accurately predict those without allergy and mild/moderate reactions - for more details see results' section of online repository and Table E12.

We observed a relationship between clinical severity and cumulative threshold dose in peanut allergic patients: the lower the cumulative dose of peanut protein tolerated the greater the severity of allergic reactions to peanut during an incremental peanut challenge (Figure 5). A test of the association between the risk of having a reaction during OFC and percentage of CD63 positive basophils was found to be significant ($P < 0.001$).

Discussion:

The OFC is the gold-standard to diagnose PA, to confirm eligibility for clinical trials and to assess the response to treatments of patients who are known to be allergic. Although OFC is generally safe, the severity of allergic reactions is unpredictable and allergic symptoms can cause significant discomfort and anxiety to patients and families^{1, 2}. Furthermore, OFC are costly, time-consuming and require a lengthy visit that may be obviated by objective, safer, cheaper and more convenient biomarkers. Herein, we tested a large number of very well-characterized subjects, who participated in the LEAP and associated studies, and confirmed that the BAT is an excellent biomarker of peanut allergy and that BAT and other biomarkers are able to identify patients at risk of reacting to small amounts of peanut protein and of

developing severe symptoms during peanut OFC. The multivariate model that combines various biomarkers can be computed using nomograms to identify individuals at high-risk of a severe/life-threatening reaction or with low threshold of reactivity during OFC in clinical practice. There is very little data in the literature combining different biomarkers to determine diagnostic accuracy and we have demonstrated in this paper that a multivariate model predicts severity and threshold. We have also demonstrated an inverse correlation between threshold and severity of allergic reactions to peanut.

We show, for the first time, that SPT, specific IgE, Ara h 2-specific IgE and BAT are predictors of severity of allergic reactions during OFC. The optimal cut-offs for these tests showed 100% sensitivity to identify severe reactors, meaning that all subjects with severe reactions had results for these tests above the defined cut-off. Furthermore, this is the largest cohort tested on BAT to peanut using a method that was previously validated¹¹. Study participants were very well characterized with rigorous methodology^{15, 16}. BAT proved to be an accurate diagnostic test, able to confirm PA with high specificity, in agreement with our previous findings¹¹. Being able to identify peanut allergic patients at risk of reacting to low dose of the allergen and/or of developing life-threatening reactions can improve current management of patients with PA. Previous studies, including our own peanut study using the exact same BAT methodology⁹, have shown a direct correlation between the severity of symptoms and basophil activation and an inverse correlation between the threshold of reactivity and basophil activation^{9, 12, 13}. More recently, another study of patients undergoing OFC to peanut identified BAT as the best biomarker for severity of allergic symptoms²², using the same BAT parameter that we had previously chosen as the preferred outcome for the BAT⁹. In the present study, we also assessed basophil sensitivity as expressed by CD-sens, which previously was the marker that best reflected low threshold dose, and found that basophils of patients reacting at lower doses of peanut protein in the OFC reacted to lower concentrations of peanut extract *in vitro* thus displaying a higher CD-sens. Of note, the associations between SPT and threshold and between SPT and severity may be biased towards a stronger association given that the clinician knows the results of the SPT before doing the challenge. Conversely, since the clinician is not

aware of the BAT results, the BAT may have less of an intrinsic bias and may constitute a more robust biomarker of severity and threshold compared with for instance skin prick test that was known to the clinical team performing the OFC.

The observation that a higher proportion of activated basophils is associated with more severe reactions and a lower threshold raise the question as to whether severity is linked to threshold. Although clinically this association is difficult to establish due to ethical constraints of continuing an OFC after an allergic reaction has started, we have previously shown that *in vitro* BAT markers of severity and threshold are strongly correlated. This strong correlation is probably due to the ability to stimulate basophils *in vitro* with higher concentrations of allergen than is possible *in vivo* during OFC, for ethical reasons. In the LEAP protocol, the OFC started at a relatively high dose of peanut protein (0.1g in most patients and 0.033g in high-risk patients)^{15, 23}, because this was a diagnostic OFC, while most OFC start below 10mg of peanut protein and 100 mg approaches the eliciting dose for 30-50% of the peanut allergic population based on other threshold studies; thus, the LEAP study is poorly designed to look at threshold. However, because OFC started at a relatively high dose, we were able to see a strong correlation between threshold and severity that would not have been otherwise possible as the OFC would have been stopped at the first signs of an allergic reaction at lower doses. The fact that BAT in this study is related to both severity and threshold further indirectly supports a link between low threshold and increasing severity of allergic reactions.

Having a high proportion of activated basophils in response to the allergen does not necessarily mean that the patient will have a severe allergic reaction when accidentally exposed to the allergen but allows to identify high-risk patients who deserve a closer follow-up. This is in line with other studies and the notion that severity of allergic reactions depends on a multitude of factors²⁴, of which the effector cell biology is only one of them. The fact that the basophil response to IgE-mediated positive controls (but not to the non-IgE mediated positive control fMLP) was higher in severe reactors than in patients with mild-

moderate reactions may suggest that differences intrinsic to the basophil effector cells can influence the severity of symptoms. The same was not observed for threshold dose or for allergic status to peanut.

Previous studies investigating SPT and specific IgE testing as biomarkers for severity showed conflicting results, some with positive^{5, 6, 9, 25-30} and others with negative^{7, 8, 31-35} findings, possibly due to differences in study design, patients included and OFC protocols. In contrast, in our study, tests other than BAT, such as SPT, Ara h 2-sIgE, peanut-sIgE and IgG4/IgE ratios, performed well identifying severe reactors. For instance, a SPT of 8 mm or above can be useful clinically to identify patients at greater risk. SPT was in fact the best biomarker for threshold followed by BAT. However, better than any individual test were the multivariate models, as shown by the higher C-value. We would like to underscore that, statistically speaking, improving on an already high c-index is 'more significant' than the same level of improvement of a lower c-index. Multivariate models take into account the correlation between metrics and this adds robustness to predictions of individual tests. We included the results of tests in a multivariate model to generate user-friendly nomograms to predict the probability of a serious adverse event during a peanut OFC for a given patient. This may be different from allergic reactions in the community, where additional factors that are controlled for in the context of an OFC can contribute to the outcome (e.g. uncontrolled asthma, concomitant infection and other co-factors)^{5, 36} The nomograms can be very useful clinically, with the ability to identify high-risk patients who may benefit from a different OFC protocol, starting with lower doses with more careful clinical monitoring (e.g. cannulation prior to start of OFC, higher ratio of clinician: patient during OFC) or who should be dispensed of an OFC. Instead, confirmation of PA could be done with an *in vitro* test such as the BAT, or a combination of tests using the nomogram. Due to the limitations of the external validation cohort used in the present study, further rigorous external validation is needed before applying these results more broadly, namely with other food allergens and in other patient populations.

A proportion of participants could not be tested on BAT for logistical reasons, namely failure to collect blood, appointments too late in the day or too many per day to allow for performance of BAT in a timely fashion. This limitation highlights the need to simplify the BAT procedure and have an organized system to transpose these barriers in clinical practice, should BAT be used as a biomarker in a real-life scenario. The differences observed between patients who were and who were not tested on BAT reflect the fact that tested patients were more likely to be peanut allergic. As non-allergic patients do not ever have a positive response to allergen on the BAT, we believe this potential bias has not affected the results but rather granted the ability to capture a high risk population.

Fourteen percent of samples were not considered in the analyses due to non-responder basophils, which is a known limitation of BAT. However, non-responders would not lead to misdiagnosis of high-risk patients as these patients required an OFC to confirm or exclude PA and thus, we would not exclude allergy based on non-responder status. In clinical practice, there are cases for whom we do not have results for the other tests. For instance, a significant proportion of children do not react on SPT when, during the pollen season, they cannot stop anti-histamines and, in such situations, BAT is particularly useful because BAT is not affected by anti-histamines³⁷. The non-responder status was not consistent over time, with some participants having non-responder basophils at one time-point (LEAP/LEAP-On) and not the other, which is in line with experimental data showing that the non-responder status results from transient changes in cell signalling proteins and can be reversed in different culturing condition, namely in the presence of IL-3^{38, 39}. The model without BAT could be used to identify patients at risk of severe reactions and low threshold, in the case of non-responders.

In summary, using a large cohort of well-characterized patients we confirmed that BAT is a biomarker of PA and of the severity and threshold of allergic reactions to peanut during OFC. All other markers had a very good sensitivity but lower specificity to predict severity whereas SPT was a better biomarker for threshold. The best predictive approach both for severity and for threshold was to combine tests in a

multivariate model. Using novel models that integrate various biomarkers, we are able to generate nomograms that could be used in clinical practice to identify allergic patients at higher risk of experiencing severe allergic reactions or of reacting to low dose of peanut protein and offer them a more personalized management plan and follow-up.

Acknowledgments:

Research reported in this publication was sponsored by the Immune Tolerance Network and supported by the National Institute of Allergy and Infectious Diseases of the National Institutes of Health under Award Number UM1AI109565. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health. Additional support came from the Medical Research Council (MRC Clinical Research Training Fellowship G090218, MRC Clinician Scientist Fellowship MR/M008517/1 and MRC Centenary Early Career Award awarded to A. F. Santos), the Asthma UK Centre and the UK Department of Health through the National Institute for Health Research (NIHR) comprehensive Biomedical Research Centre award to Guy's & St. Thomas' NHS Foundation Trust, in partnership with King's College London and King's College Hospital NHS Foundation Trust. The Paediatric Allergy clinical trials unit is supported by the National Peanut Board, Atlanta, Ga and The Davis Foundation. The UK Food Standards Agency provided additional support for the costs of phlebotomy. We thank Dr Susanne Heck and her team at the BRC Flow Cytometry Core Laboratory of the NIHR GSTT/KCL Comprehensive Biomedical Research Centre at Guy's & St. Thomas' NHS Foundation Trust and Dr Alastair Wilson for technical assistance and the many nurses, dietitians, doctors and administrative staff of the Guy's and St Thomas' NHS Foundation Trust Children's Allergy Service for clinical and logistical assistance over the period of the study. We acknowledge Rho, Inc. for data management, cleaning, derivation and discussion. Above all, we are indebted to all of the children and their families who generously took part in the ITN032AD LEAP, ITN049AD LEAP-On and PAS studies.

References

1. Pouessel G, Beaudouin E, Tanno LK, Drouet M, Deschildre A, Labreuche J, et al. Food-related anaphylaxis fatalities: Analysis of the Allergy Vigilance Network((R)) database. *Allergy* 2019; 74:1193-6.
2. Upton J, Alvaro M, Nadeau K. A perspective on the pediatric death from oral food challenge reported from the Allergy Vigilance Network. *Allergy* 2019; 74:1035-6.
3. Turner PJ, Gowland MH, Sharma V, Ierodiakonou D, Harper N, Garcez T, et al. Increase in anaphylaxis-related hospitalizations but no increase in fatalities: An analysis of United Kingdom national anaphylaxis data, 1992-2012. *J Allergy Clin Immunol* 2014.
4. Mullins RJ, Wainstein BK, Barnes EH, Liew WK, Campbell DE. Increases in anaphylaxis fatalities in Australia from 1997 to 2013. *Clin Exp Allergy* 2016; 46:1099-110.
5. Hourihane JO, Grimshaw KE, Lewis SA, Briggs RA, Trewin JB, King RM, et al. Does severity of low-dose, double-blind, placebo-controlled food challenges reflect severity of allergic reactions to peanut in the community? *Clin Exp Allergy* 2005; 35:1227-33.
6. Wainstein BK, Studdert J, Ziegler M, Ziegler JB. Prediction of anaphylaxis during peanut food challenge: usefulness of the peanut skin prick test (SPT) and specific IgE level. *Pediatr Allergy Immunol* 2010; 21:603-11.
7. Ta V, Weldon B, Yu G, Humblet O, Neale-May S, Nadeau K. Use of Specific IgE and Skin Prick Test to Determine Clinical Reaction Severity. *Br J Med Med Res* 2011; 1:410-29.
8. Summers CW, Pumphrey RS, Woods CN, McDowell G, Pemberton PW, Arkwright PD. Factors predicting anaphylaxis to peanuts and tree nuts in patients referred to a specialist center. *J Allergy Clin Immunol* 2008; 121:632-8 e2.
9. Santos AF, Du Toit G, Douiri A, Radulovic S, Stephens A, Turcanu V, et al. Distinct parameters of the basophil activation test reflect the severity and threshold of allergic reactions to peanut. *J Allergy Clin Immunol* 2015; 135:179-86.
10. Klemans RJ, Liu X, Knulst AC, Knol MJ, Gmelig-Meyling F, Borst E, et al. IgE binding to peanut components by four different techniques: Ara h 2 is the most relevant in peanut allergic children and adults. *Clin Exp Allergy* 2013; 43:967-74.
11. Santos AF, Douiri A, Becares N, Wu SY, Stephens A, Radulovic S, et al. Basophil activation test discriminates between allergy and tolerance in peanut-sensitized children. *J Allergy Clin Immunol* 2014; 134:645-52.
12. Song Y, Wang J, Leung N, Wang LX, Lisann L, Sicherer SH, et al. Correlations between basophil activation, allergen-specific IgE with outcome and severity of oral food challenges. *Ann Allergy Asthma Immunol* 2015; 114:319-26.
13. Rubio A, Vivinus-Nebot M, Bourrier T, Saggio B, Albertini M, Bernard A. Benefit of the basophil activation test in deciding when to reintroduce cow's milk in allergic children. *Allergy* 2011; 66:92-100.
14. Du Toit G, Katz Y, Sasieni P, Mesher D, Maleki SJ, Fisher HR, et al. Early consumption of peanuts in infancy is associated with a low prevalence of peanut allergy. *J Allergy Clin Immunol* 2008; 122:984-91.

15. Du Toit G, Roberts G, Sayre PH, Bahnson HT, Radulovic S, Santos AF, et al. Randomized trial of peanut consumption in infants at risk for peanut allergy. *N Engl J Med* 2015; 372:803-13.
16. Du Toit G, Sayre PH, Roberts G, Sever ML, Lawson K, Bahnson HT, et al. Effect of Avoidance on Peanut Allergy after Early Peanut Consumption. *N Engl J Med* 2016; 374:1435-43.
17. Santos AF, Becares N, Stephens A, Turcanu V, Lack G. The expression of CD123 can decrease with basophil activation: implications for the gating strategy of the basophil activation test. *Clin Transl Allergy* 2016; 6:11.
18. Soar J, Pumphrey R, Cant A, Clarke S, Corbett A, Dawson P, et al. Emergency treatment of anaphylactic reactions--guidelines for healthcare providers. *Resuscitation* 2008; 77:157-69.
19. .] Available from <https://evs.nci.nih.gov/ftp1/CTCAE/About.html>.
20. Ewan PW, Clark AT. Long-term prospective observational study of patients with peanut and nut allergy after participation in a management plan. *Lancet* 2001; 357:111-5.
21. Bob Carpenter, Andrew Gelman, Matthew D. Hoffman, Daniel Lee, Ben Goodrich, Michael Betancourt, et al. Stan: A probabilistic programming language. *Journal of Statistical Software* 2017; 76.
22. Chinthrajah RS, Purington N, Andorf S, Rosa JS, Mukai K, Hamilton R, et al. Development of a tool predicting severity of allergic reaction during peanut challenge. *Ann Allergy Asthma Immunol* 2018; 121:69-76 e2.
23. Sampson HA, Gerth van Wijk R, Bindslev-Jensen C, Sicherer S, Teuber SS, Burks AW, et al. Standardizing double-blind, placebo-controlled oral food challenges: American Academy of Allergy, Asthma & Immunology-European Academy of Allergy and Clinical Immunology PRACTALL consensus report. *J Allergy Clin Immunol* 2012; 130:1260-74.
24. Dubois AEJ, Turner PJ, Hourihane J, Ballmer-Weber B, Beyer K, Chan CH, et al. How does dose impact on the severity of food-induced allergic reactions, and can this improve risk assessment for allergenic foods?: Report from an ILSI Europe Food Allergy Task Force Expert Group and Workshop. *Allergy* 2018; 73:1383-92.
25. Neuman-Sunshine DL, Eckman JA, Keet CA, Matsui EC, Peng RD, Lenehan PJ, et al. The natural history of persistent peanut allergy. *Ann Allergy Asthma Immunol* 2012; 108:326-31 e3.
26. Flinterman AE, Pasmans SG, Hoekstra MO, Meijer Y, van Hoffen E, Knol EF, et al. Determination of no-observed-adverse-effect levels and eliciting doses in a representative group of peanut-sensitized children. *J Allergy Clin Immunol* 2006; 117:448-54.
27. Eller E, Bindslev-Jensen C. Clinical value of component-resolved diagnostics in peanut-allergic patients. *Allergy* 2013; 68:190-4.
28. Asarnoj A, Moverare R, Ostblom E, Poorafshar M, Lilja G, Hedlin G, et al. IgE to peanut allergen components: relation to peanut symptoms and pollen sensitization in 8-year-olds. *Allergy* 2010; 65:1189-95.
29. Astier C, Morisset M, Roitel O, Codreanu F, Jacquenet S, Franck P, et al. Predictive value of skin prick tests using recombinant allergens for diagnosis of peanut allergy. *J Allergy Clin Immunol* 2006; 118:250-6.

- 522 30. Peeters KA, Koppelman SJ, van Hoffen E, van der Tas CW, den Hartog Jager CF, Penninks AH, et
523 al. Does skin prick test reactivity to purified allergens correlate with clinical severity of peanut
524 allergy? *Clin Exp Allergy* 2007; 37:108-15.
- 525 31. Blumchen K, Beder A, Beschorner J, Ahrens F, Gruebl A, Hamelmann E, et al. Modified oral food
526 challenge used with sensitization biomarkers provides more real-life clinical thresholds for
527 peanut allergy. *J Allergy Clin Immunol* 2014.
- 528 32. Perry TT, Matsui EC, Conover-Walker MK, Wood RA. Risk of oral food challenges. *J Allergy Clin*
529 *Immunol* 2004; 114:1164-8.
- 530 33. Rance F, Abbal M, Lauwers-Cances V. Improved screening for peanut allergy by the combined
531 use of skin prick tests and specific IgE assays. *J Allergy Clin Immunol* 2002; 109:1027-33.
- 532 34. Clark AT, Ewan PW. Interpretation of tests for nut allergy in one thousand patients, in relation to
533 allergy or tolerance. *Clin Exp Allergy* 2003; 33:1041-5.
- 534 35. Ballmer-Weber BK, Lidholm J, Fernandez-Rivas M, Seneviratne S, Hanschmann KM, Vogel L, et al.
535 IgE recognition patterns in peanut allergy are age dependent: perspectives of the EuroPrevall
536 study. *Allergy* 2015; 70:391-407.
- 537 36. Chan JCK, Peters RL, Koplin JJ, Dharmage SC, Gurrin LC, Wake M, et al. Food Challenge and
538 Community-Reported Reaction Profiles in Food-Allergic Children Aged 1 and 4 Years: A
539 Population-Based Study. *J Allergy Clin Immunol Pract* 2017; 5:398-409 e3.
- 540 37. Sturm GJ, Kranzelbinder B, Sturm EM, Heinemann A, Groselj-Strele A, Aberer W. The basophil
541 activation test in the diagnosis of allergy: technical issues and critical factors. *Allergy* 2009;
542 64:1319-26.
- 543 38. Lavens-Phillips SE, MacGlashan DW, Jr. The tyrosine kinases p53/56lyn and p72syk are
544 differentially expressed at the protein level but not at the messenger RNA level in nonreleasing
545 human basophils. *Am J Respir Cell Mol Biol* 2000; 23:566-71.
- 546 39. Kepley CL, Youssef L, Andrews RP, Wilson BS, Oliver JM. Multiple defects in Fc epsilon RI
547 signaling in Syk-deficient nonreleaser basophils and IL-3-induced recovery of Syk expression and
548 secretion. *J Immunol* 2000; 165:5913-20.

Tables:

Table I. Demographic and clinical characteristics of participants in the LEAP, LEAP-On and PAS studies included in the analyses. OFC, oral food challenge; CTCAE, common terminology criteria for adverse events¹⁸.

	LEAP			LEAP-On			PAS			
	Peanut Avoid (N = 252)	Peanut Consume (N = 222)	Overall (N = 474)	Peanut Avoid (N = 219)	Peanut Consume (N = 204)	Overall (N = 423)	Group I (N = 36)	Group IV (N = 45)	Group V (N = 3)	Overall (N = 84)
Male gender	164 (65%)	119 (54%)	283 (60%)	136 (62%)	112 (55%)	248 (59%)	24 (67%)	31 (69%)	1 (33%)	56 (67%)
Atopic eczema ¹	104 (41%)	80 (36%)	184 (39%)	81 (37%)	79 (39%)	160 (38%)	32/35 (91%)	44/44 (100%)	2 (67%)	78/82 (95%)
Participants with peanut allergy diagnosed	46/250 (18%)	8 (4%)	54/472 (11%)	42 (19%)	9/203 (4%)	51/422 (12%)	0 (0%)	36/44 (82%)	1 (33%)	33/79 (42%)
Participants with positive OFC to peanut	42/248 (17%)	7/221 (3%)	49/469 (10%)	26/202 (13%)	7/200 (4%)	33/402 (8%)	0 (0%)	34/42 (81%)	1 (33%)	35/81 (43%)
Ewan severe	12/41 (29%)	2/7 (29%)	14/48 (29%)	1/25 (4%)	1/7 (14%)	2/32 (6%)	(N = 0)	8/32 (25%)	0/1 (0%)	8/33 (24%)
Medication severe	7/41 (17%)	2/7 (29%)	9/48 (19%)	1/25 (4%)	0/7 (0%)	1/32 (3%)	(N = 0)	7/32 (22%)	0/1 (0%)	7/33 (21%)
CTCAE severe	7/41 (17%)	0/7 (0%)	7/48 (15%)	1/24 (4%)	0/7 (0%)	1/31 (3%)	(N = 0)	5/31 (16%)	0/1 (0%)	5/32 (16%)
Threshold dose of reaction during OFC (grams) ²	0.1 [0.0, 0.8] (N = 42)	0.1 [0.0, 0.1] (N = 7)	0.1 [0.0, 0.8] (N = 49)	0.8 [0.0, 4.3] (N = 26)	1.2 [0.4, 3.1] (N = 7)	0.8 [0.0, 4.3] (N = 33)	(N = 0)	0.0 [0.0, 0.1] (N = 34)	0.4 [0.4, 0.4] (N = 1)	0.0 [0.0, 0.2] (N = 35)

¹ Eczema based on SCORAD score of more than zero² Median [IQR]

Table II. Optimal cut-offs to classify subjects at high-risk for severe allergic reactions and for reacting to low dose (0.1g or less) of peanut protein during the oral peanut challenges. Severity was assessed according to the CTCAE scale¹⁸. Optimal cut-offs were determined based on the Youden's index which is the distance between the point of inflexion of the ROC curve and the reference line (see E-methods in the supplementary material). Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) with 95% confidence intervals are indicated for each cut-off.

	Severe versus non-severe allergic reactions					Threshold ≤ 0.1 g versus > 0.1 g of peanut protein				
	Cut-off	Sensitivity	Specificity	PPV	NPV	Cut-off	Sensitivity	Specificity	PPV	NPV
BAT (%CD63+ Basophils)	48	100 (100 - 100)	97 (95 - 98)	41 (32 - 60)	100 (100 - 100)	1.7	95 (89 - 100)	91 (86 - 96)	43 (35 - 62)	100 (99 - 100)
Ara h 2-sIgE (kU/L)	1.4	100 (100 - 100)	93 (91 - 98)	22 (18 - 48)	100 (100 - 100)	0.1	97 (90 - 100)	89 (87 - 95)	38 (34 - 57)	100 (99 - 100)
Peanut-sIgE (kU/L)	5	100 (100 - 100)	90 (87 - 98)	18 (15 - 48)	100 (100 - 100)	0.4	95 (88 - 100)	79 (76 - 86)	25 (23 - 34)	100 (99 - 100)
Peanut- sIgG4 (μg/L)	175	75 (17 - 100)	46 (30 - 99)	3 (2 - 29)	99 (98 - 100)	75	82 (67 - 89)	34 (32 - 45)	8 (8 - 10)	96 (94 - 98)
IgG4/IgE Ratio	1.6	100 (92 - 100)	88 (86 - 98)	16 (14 - 60)	100 (100 - 100)	2.1	91 (86 - 100)	87 (78 - 92)	35 (25 - 46)	99 (99 - 100)
SPT Peanut (mm)	8	100 (100 - 100)	92 (89 - 94)	21 (17 - 27)	100 (100 - 100)	6	98 (97 - 100)	95 (91 - 96)	59 (45 - 66)	100 (100 - 100)

Table III. Model performance measures for (A) reaction severity and (B) cumulative dose threshold. The C-index measures the model's rank-discrimination, the ability of the model to correctly rank subjects, with 1 being perfect discrimination and 0.5 indicating a coin-toss model. The calibration error measures how far apart in percentage points model-based predictions are from true predictions, as in a calibration curve, for each ordinal model cut-point. The mean absolute calibration error is the absolute prediction errors averaged across all predictions made, with an analogous definition for the 90th percentile of absolute prediction error. In each case, the smaller the error the better.

A. Severity

Model	C-Index	Absolute calibration error			
		Moderate or higher		Severe/Life-threatening	
		Mean	90th %ile	Mean	90th %ile
Multivariable (BAT)	0.991	0.5	0.9	0.3	0.3
Multivariable (No BAT)	0.985	0.3	1	0.5	0.3
SPT	0.985	0.5	1.6	0.5	0.6
Ara h 2-specific IgE	0.951	4.1	7.3	0.3	0.1
BAT	0.951	1.6	4	0.3	0.2
IgE to peanut	0.914	4.4	14.3	0.6	1.7
IgG4 to peanut	0.563	1.1	1.7	0.4	1

B. Threshold

	C-index	Absolute Calibration Error					
		0.1 mg		5.0 mg		9.35 mg	
		Mean	90th %ile	Mean	90th %ile	Mean	90th %ile
Multivariable	0.981	1.6	5.6	0.8	2.7	0.3	0.2
Multivariable (no BAT)	0.98	2.2	5.1	1	3	0.7	1.2

SPT	0.972	1.4	8.9	0.8	3.2	0.6	2
BAT	0.938	1.8	7.8	1	2	0.9	2.8
Ara h 2-specific IgE	0.938	3.6	13.4	6.2	9.6	6.9	12.6
IgG4/IgE ratio to peanut (log 10)	0.932	3.1	8	3.6	6.4	2.1	2.8
IgE to peanut	0.899	4	10.1	6	16.8	6.6	20.7
IgG4 to peanut	0.565	1.7	2.1	2.6	4.8	3	4.2

Figure legends:

Figure 1. Mean basophil activation (measured as %CD63+ basophils corrected for spontaneous activation, i.e. minus %CD63+ basophils in the absence of *in vitro* stimulation) to increasing concentrations of peanut extract in LEAP, LEAP-On, and PAS study participants by allergic status. BAT and allergic status were determined at approximately age 5 in LEAP and PAS studies and at approximately age 6 in the LEAP-On study. Each regression line represents a smoothed mean dose response curve using a cubic spline with 95% bootstrapped confidence intervals for each combination of allergic status and reaction severity group.

Figure 2. Basophil activation to peanut in peanut allergic versus non-allergic participants (Median [IQR] 58.1 [51.2 – 74.2] vs 10.3 [1.9 – 24.2], respectively, $p < 0.001$) in the PAS and LEAP studies (A) and receiver operator characteristic (ROC) curve for the basophil activation test (BAT) and other biomarkers to identify LEAP and PAS subjects at high-risk of developing severe or life-threatening reactions during oral food challenges, using the CTCAE score (B), Ewan & Clark grading (C) or medication grading (D).

Figure 3. Threshold dose of peanut protein. Extinction curve relating the cumulative peanut threshold dose with the proportion of activated basophils following peanut stimulation (A), showing the estimated mean (and 95% confidence band) cumulative amount of peanut tolerated as a function of BAT measurement, and ROC curve for different biomarkers for predicting threshold < 0.1 g of peanut protein (B).

Figure 4. Nomogram for predicting reaction severity using BAT, SPT and Ara h 2-specific IgE (A) and nomogram for predicting cumulative dose threshold using BAT, SPT, Ara h 2-specific IgE and IgG4/IgE ratios, based on the LEAP and PAS studies at approximately age 5. Predictions from the models can be made in a clinical setting by simply adding up points earned from each of the variable axes and then using that total to read estimated probabilities from the probability axes.

Footnote for figure 4A: For example, if a participant is encountered with an rAra h 2 of 1.5, a peanut SPT of 8, and a BAT of 53, we first find the value 1.5 along the axis associated with rAra h 2 (second from the top) and read vertically up to the corresponding point along the top Points axis (blue arrow). Similarly, we find the value 8 along the Peanut SPT axis and follow vertically to the Points axis to find that a Peanut SPT of 8 earns about 76 points (red arrow). Similarly, a BAT value of 53 earns about 78 points (green arrow). Totalling the points earned from each variable gives 155 points for this participant. We find this total points value on the Total Points axis (fourth from the bottom) and imagine a vertical line extending down from that point intersecting each of the probability axes. These points of intersection are the predicted probabilities of falling into each of the severity categories. Given the values from the hypothetical participant above, we estimate a <10% chance of having a severe reaction, an 90% chance of having a moderate reaction and < 10% chance of no reaction.

Footnote for figure 4B: For example, if we suppose that in addition to the biomarker values seen in figure 4A, the participant has a $\log_{10}$ -IgG4/IgE ratio to peanut of 1.6. The nomogram above can be used if we wanted to estimate the mean cumulative tolerated dose, or the probabilities of having mean cumulative doses greater than 0.1 g or 9.35 g given these biomarker values. With a BAT of 53 we accrue about 62 points, with an arah2 of 1.5 about 5 points, a peanut wheal of 8 gives about 85 points, and an IgG4/IgE ratio of 1.6 gives about 95 points. This individual then has about 247 total points, which gives an estimated mean cumulative tolerated dose of less than 1 g, a 45% chance of tolerating more than 0.1 g peanut, and less than a 10% chance of tolerating more than 9.35 g peanut. In fact, this individual had a severe reaction during the peanut DBPCFC and tolerated 0 g of peanut.

Figure 5. Relationship between severity and threshold of allergic reactions to peanut. Points represent individual subjects submitted to either double-blind placebo-controlled (blue points) or open (red points) peanut challenges in the LEAP studies. The Spearman rank correlation between cumulative tolerated dose and CTCAE graded reactions severity is -0.96 ($P < 0.001$) at 60 months and -0.94 ($P < 0.001$) at 72 months. When computing these correlations open challenges not ending in a reaction were given an imputed tolerated cumulative dose of 9.35 g.

