Basophil activation test discriminates between allergy and tolerance in peanut-sensitized children

Alexandra F. Santos, MD, MSc,[a,b,c,d] Abdel Douiri, PhD,[e,f] Natalia Bécares, BSc,[a,b] Shih-Ying Wu, MSc,[a,b] Alick Stephens, PhD,[a,b] Suzana Radulovic, MD,[a,b] Susan M. H. Chan, MD,[a,b,f] Adam T. Fox, MD,[a,b] George Du Toit, MD,[a,b] Victor Turcanu, PhD,[a,b] and Gideon Lack, MD, FRCPCH[a,b] *London, United Kingdom, and Coimbra and Lisbon, Portugal*

Background: Most of the peanut-sensitized children do not have clinical peanut allergy. In equivocal cases, oral food challenges (OFCs) are required. However, OFCs are laborious and not without risk; thus, a test that could accurately diagnose peanut allergy and reduce the need for OFCs is desirable.
Objective: To assess the performance of basophil activation test (BAT) as a diagnostic marker for peanut allergy.
Methods: Peanut-allergic (n = 43), peanut-sensitized but tolerant (n = 36) and non–peanut-sensitized nonallergic (n = 25) children underwent skin prick test (SPT) and specific IgE (sIgE) to peanut and its components. BAT was performed using flow cytometry, and its diagnostic performance was evaluated in relation to allergy versus tolerance to peanut and validated in an independent population (n = 65).
Results: BAT in peanut-allergic children showed a peanut dose-dependent upregulation of CD63 and CD203c while there was no significant response to peanut in peanut-sensitized but tolerant ($P < .001$) and non–peanut-sensitized nonallergic children ($P < .001$). BAT optimal diagnostic cutoffs showed 97% accuracy, 95% positive predictive value, and 98% negative predictive value. BAT allowed reducing the number of required OFCs by two-thirds. BAT proved particularly useful in cases in which specialists could not accurately diagnose peanut allergy with SPT and sIgE to peanut and to Arah2. Using a 2-step diagnostic approach in which BAT was performed only after equivocal SPT or Arah2-sIgE, BAT had a major effect (97% reduction) on the number of OFCs required.
Conclusions: BAT proved to be superior to other diagnostic tests in discriminating between peanut allergy and tolerance, particularly in difficult cases, and reduced the need for OFCs. (J Allergy Clin Immunol 2014;134:645-52.)

Key words: *Anaphylaxis, basophil activation test, CD203c, CD63, diagnosis, flow cytometry, food allergy, peanut allergy, ROC curve*

Ten percent of North American children are sensitized to peanut,[1] but only 1.4% are clinically allergic to peanut.[2] The gold standard for the diagnosis of peanut allergy is double-blind placebo-controlled food challenge (DBPCFC); however, this is time-consuming and carries the risk of causing an acute allergic reaction.[3] Therefore, in clinical practice, whenever possible, the diagnosis of peanut allergy is based on the combination of a history of an immediate-type allergic reaction to peanut together with *in vivo* or in *vitro* measurement of sensitization.[4] Some clinics use peanut-specific IgE (P-sIgE) alone, others use peanut skin prick test (SPT) alone, and some such as ours use a combination of these tests. No clear consensus exists as to which is the best approach. The diagnosis of peanut allergy can be particularly difficult in cases in which there is no clear history of peanut consumption. With increasing awareness about food allergy and the fact that many families avoid peanut in the first few years of life, peanut-sensitized children with no history of oral exposure to peanut constitute a considerable proportion of patients seen in allergy clinics. This has resulted in a marked increase in the number of oral food challenge (OFC) requests. Thus, a test that could accurately diagnose peanut allergy reducing the need for OFC is desirable and would change clinical practice.

From [a]the Division of Asthma, Allergy & Lung Biology, Department of Pediatric Allergy, King's College London; [b]MRC & Asthma UK Centre in Allergic Mechanisms of Asthma, London; [c]the Immunoallergology Department, Coimbra University Hospital, Coimbra; [d]Gulbenkian Programme for Advanced Medical Education, Lisbon; [e]the Department of Public Health Science, School of Medicine, King's College London, London; and [f]the National Institute for Health Research, Biomedical Research Centre, Guy's and St Thomas' Hospital NHS Foundation Trust, London.

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Corresponding author: Gideon Lack, MD, FRCPCH, Department of Pediatric Allergy, St Thomas' Hospital, 2nd Fl, Stairwell B, South Wing, Westminster Bridge Rd, London SE1 7EH, UK. E-mail: gideon.lack@kcl.ac.uk.

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Abbreviations used
BAT: Basophil activation test
CRD: Component-resolved diagnosis
DBPCFC: Double-blind placebo-controlled food challenge
fMLP: Formyl-methionyl-leucyl-phenylalanine
NA: Non–peanut-sensitized nonallergic
OFC: Oral food challenge
PA: Peanut-allergic
PPV: Positive predictive value
PS: Peanut-sensitized but tolerant
P-sIgE: Peanut-specific IgE
ROC: Receiver-operating characteristic
sIgE: Specific IgE
SPT: Skin prick test

To try to improve the utility of SPT and P-sIgE, diagnostic decision values have been determined.[5-9] However, a large proportion of peanut-sensitized children have SPT and P-sIgE results below these cutoffs, falling in the immunologic gray area[10] (see Fig E1 in this article's Online Repository at www.jacionline.org). Ara h 2 is a dominant allergen and has been proved to be particularly useful for diagnosis[11,12]; however, peanut allergy can develop in patients with undetectable specific IgE (sIgE) levels to Ara h 2 and other major peanut allergens.[12-14]

The basophil activation test (BAT) to peanut is an *in vitro* assay in which the expression of activation markers on the surface of basophils is evaluated by using flow cytometry after stimulation with peanut allergens.[15,16] It can be performed using 1 mL of blood without requiring cell separation. We sought to assess the performance of BAT in the diagnosis of peanut allergy and to compare it with existing diagnostic tests.

METHODS

Study population

Peanut-allergic (PA), peanut-sensitized but tolerant (PS), and non–peanut-sensitized nonallergic (NA) children were prospectively and consecutively enrolled from our Pediatric Allergy service on the days when the investigator (A.F.S.) was available to perform BAT. The allergic status to peanut was determined by using OFCs, except for (1) children with a convincing history of systemic reaction(s) to peanut within 1 year of their visit and (a) wheal size of SPT of 8 mm or more[8] and/or (b) P-sIgE level of 15 KU_A/L or more,[8] who were considered peanut allergic; and (2) children (15 NA and 5 PS) who were able to eat 4 g or more of peanut protein twice a week (as assessed by a validated peanut consumption questionnaire[17]) without developing allergic symptoms, who were considered peanut tolerant. Peanut sensitization was defined by a wheal size of SPT of 1 mm or more and/or P-sIgE level of 0.10 KU_A/L or more.

All children underwent clinical evaluation, SPT, P-sIgE determination, component-resolved diagnosis (CRD), and OFC, as appropriate. An additional sample of blood was drawn in lithium heparin (BD Vacutainer, Plymouth, United Kingdom) for BAT, which was performed within 4 hours of blood collection. The study was approved by the South East London Research Ethics Committee 2, and written informed consent was obtained from parents of all children.

Skin prick testing, serum-sIgE, and OFCs

SPT was performed using peanut extract (ALK-Abelló, Hørsholm, Denmark), as previously described.[18] The level of sIgE (peanut and CRD) was measured using an immunoenzymatic assay (ImmunoCAP, ThermoFisher, Uppsala, Sweden).

DBPCFC consisted of 6 verum doses and 3 placebo doses randomly interspersed with verum doses up to a cumulative dose of 9.35 g of peanut protein (see Table E1 in this article's Online Repository at www.jacionline.org). Children of 1 to 3 years were given 1 placebo and 5 verum doses up to a cumulative dose of 4.35 g of peanut protein. In infants (≤1 year), the OFCs were open up to a cumulative dose of 4.35 g of peanut protein. Nine older children also received an open OFC for logistical reasons. OFCs were considered negative when all doses were tolerated. If an allergic reaction developed at any stage after a verum dose, the OFC was considered positive (see Table E2 in this article's Online Repository at www.jacionline.org) and the symptoms treated. If a reaction followed a placebo dose, the patient was brought in for 2-day challenge (1 day placebo and 1 day verum).[19]

Basophil activation test

Heparinized whole blood was stimulated for 30 minutes at 37°C with peanut extract (ALK Abelló) diluted in RPMI medium (GIBCO, Paisley, United Kingdom) at serial 10-fold dilutions from 10 μg/mL to 0.1 ng/mL. For details about the extract and allergen concentrations, see this article's Online Repository at www.jacionline.org.[20] Polyclonal goat antihuman IgE (1 μg/mL, Sigma-Aldrich, Poole, United Kingdom), monoclonal mouse antihuman FcεRI (2.5 μg/mL, eBioscience, San Diego, Calif), formyl-methionyl-leucyl-phenylalanine (fMLP, 1 μM, Sigma-Aldrich), or RPMI medium alone were used as controls. Before erythrocyte lysis, cells were stained with CD123-FITC (eBioscience), CD203c-PE, HLA-DR-PerCP, and CD63-APC (Biolegend, San Diego, Calif). Basophils were gated as SSC^{low}/CD203c+/CD123+/HLA-DR− (see Fig E2 in this article's Online Repository at www.jacionline.org). Basophil expression of CD63 and CD203c was evaluated using FACS CantoII with FACSDiva software (BD Biosciences, San Jose, Calif). The flow cytometry data were analyzed using FlowJo software (version 7.6.1; TreeStar, Ashland, Ore) by an investigator who was blinded to the clinical features of the participants. Basophil activation was expressed as $\%CD63^+$ basophils and as the stimulation index of the mean fluorescence intensity (MFI) of CD203c.

Statistical analysis

We estimated that a sample of 32 PA and 32 PS children would give us 99% power, at a 2-sided type I error probability of 0.05, to detect a significant difference in the $\%CD63^+$ basophils after peanut stimulation between PA and PS on the basis of data from a previous study.[21]

Qualitative variables were compared between PA and PS children using the Fisher exact test or χ^2 tests, and continuous variables were compared using the Mann-Whitney U test or the Kruskal-Wallis test.

The performance of allergy tests was examined against the allergic status to peanut using receiver-operating characteristic (ROC)-curve analyses. The cutoffs to predict peanut allergy and peanut tolerance for BAT and the various allergy tests with optimal accuracy were determined and validated. We performed internal validation using repeated random subsampling validation (bootstrap) and "leave-one-out" methodologies.[22] Both methodologies produced similar results in estimating the optimal cutoff points, and the former methodology is reported. The 95% CI was constructed using bootstrapping methodology with 1000 replications to reflect on the reproducibility.[23] An external validation study was also conducted using a new cohort of 65 subjects (25 PA, 24 PS, and 16 NA) mainly recruited from the Peanut Allergy Sensitization study, a group of patients from all over the country who were excluded from the Learning Early About Peanut Allergy study,[18] and from a private Pediatric Allergy clinic in London. The cutoffs previously determined in the primary study population were applied to this validation study population and sensitivity, specificity, predictive values, likelihood ratios, and accuracy were calculated.

Three Pediatric Allergy specialist attending physicians were asked to classify 44 equivocal cases from the primary study population as peanut allergic or tolerant on the basis of history and results of SPT, P-sIgE, and CRD. The agreement between physicians was calculated as percentages and assessed with κ statistics.[24]

Statistical analyses were performed with SPSS 20.0 and STATA 12.1 for Windows. Significance was determined using a 2-sided α level of 0.05.

Combination of BAT with other diagnostic tests

In the primary study population, after ROC-curve analyses, we compared the performance of BAT with SPT, P-sIgE, and Arah2-sIgE using conventional