Original Article

The Indirect Basophil Activation Test Is a Safe, Reliable, and Accessible Tool to Diagnose a Peanut Allergy in Children

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What is already known about this topic? The direct basophil activation test (BAT) is a reliable in vitro alternative to peanut oral food challenges; however, it requires fresh blood samples, and basophils unresponsive to IgE receptor–mediated signaling reduce its efficacy.

What does this article add to our knowledge? The indirect (passive) BAT has a high diagnostic accuracy, comparable to that of the direct BAT, and can reduce the number of time-consuming, patient-unfriendly, and expensive oral food challenges required. It is not affected by nonresponding basophils.

How does this study impact current management? The indirect BAT enables a peanut allergy diagnosis to be made using a serum blood sample, which can be stored for a long time and transported to a central laboratory.

BACKGROUND: The gold standard for the diagnosis of a peanut allergy is an oral food challenge (OFC), but it is a time-consuming, patient-unfriendly, and expensive test. The *in vitro direct* basophil activation test (BAT) for peanuts was shown to be a promising diagnostic tool for replacing the OFC.
OBJECTIVE: To determine the diagnostic accuracy of the *indirect* (passive) BAT. Compared with the direct BAT, the timing of the indirect BAT is more flexible, and the problem of nonresponding basophils (unresponsive to IgE receptor–mediated signaling) is circumvented.
METHODS: In 74 children, suspected of peanut allergy and eligible for an OFC, indirect BAT results for peanut extract, Ara h2, and Ara h6 were compared with the results of a double-blind placebo-controlled food challenge. The reactivity and sensitivity of the basophils in the BAT were correlated to both the allergy status and the threshold dose in the OFC.
RESULTS: The combined basophil reactivity for Ara h2 and Ara h6 showed the highest accuracy (94%) for the diagnosis of a peanut allergy, with positive and negative predictive values of 96% and 89%, respectively. The sensitivity of the basophils for Ara h2 significantly discriminates between patients who tolerated up to 0.4 g of peanut protein in the OFC and those who did not.
CONCLUSIONS: Because the indirect BAT showed a high diagnostic accuracy for peanut allergy, it is a promising alternative to the classical direct BAT and could lead to a reduction in OFC use. © 2022 American Academy of Allergy, Asthma & Immunology (J Allergy Clin Immunol Pract 2022;■:■-■)

Key words: *Food allergy; Diagnosis; Basophil activation test; Specific IgE; Peanut; Oral food challenge*

INTRODUCTION

Peanut allergy is one of most prevalent allergies in childhood (estimated 0.2%-3.0%) and can potentially induce a severe allergic reaction.[1,2] The gold standard for diagnosing a peanut allergy is an oral food challenge (OFC), preferably a double-blind placebo-controlled food challenge (DBPCFC).[3] Disadvantages of the OFC are the risk of a severe allergic reaction, the time-consuming procedure, high costs, and burden for both children and their parents and the health care system. The diagnostic value of other available tests, such as the skin prick test and allergen-specific IgE (sIgE) test using peanut extract and the major components Ara h2 and h6, is limited due to their low

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Abbreviations used
AUC- area under the curve
BAT- basophil activation test
DBPCFC- double-blind placebo-controlled food challenge
EC50- half-maximal effective concentration
n- native
NPV- negative predictive value
OFC- oral food challenge
OIT- oral immune therapy
PPV- positive predictive value
r- recombinant
sIgE- specific IgE

specificities, and unknown safe diagnostic cutoff values, which are highly dependent on population and age.[4-7]

A promising diagnostic tool is the basophil activation test (BAT). The BAT is a functional *ex vivo* assay that differentiates between sensitization and a relevant allergy, can be performed without discontinuation of antihistamine therapy, and the use of which can therefore significantly reduce the number of OFCs required.[8-15] Compared with sIgE, which is a measurement of allergic sensitization, the BAT takes into account crosslinking between sIgE molecules on their effector cells after stimulation with allergen, which is a prerequisite for triggering an allergic reaction by the degranulation of mast cells and basophils. There are 2 ways of performing a BAT. The *direct* BAT is most commonly used, and measures the activation of the patient's own basophils, and should therefore be performed within 24 hours of a blood sample collection, which poses a logistical constraint. Moreover, in around 6% to 17% of patients, basophils are unresponsive to IgE receptor—mediated signaling (so-called nonresponders).[8] The *indirect* (passive) BAT uses donor basophils sensitized with the patient's IgE. As such, the timing of the indirect BAT is more flexible and the problem of nonresponders is circumvented, although the indirect BAT is a more time-consuming procedure than the direct BAT. To our knowledge, only 1 study published results, although with a limited number of patients, on the diagnostic performance of the indirect peanut BAT (using histamine release as a read-out).[16]

The aim of this study was to determine the diagnostic value of the indirect BAT in sIgE peanut-sensitized children who were eligible for an OFC (reference test). Based on the BAT dose-response curves for peanut extract and the major components Ara h2 and Ara h6, the BAT parameters, including basophil reactivity and sensitivity, are determined and correlated with the outcome of the OFC.

METHODS

Study population and design

In this observational study, children (<18 years old) with a suspected peanut allergy and sensitization to peanut (sIgE peanut) were enrolled during the study period (2011-2019) at the Rijnstate Hospital in Arnhem and Canisius Wilhelmina Hospital in Nijmegen, both of which are large secondary clinics in the Netherlands. Suspected peanut allergy was based on a history of allergic symptoms after (possible) peanut ingestion and sIgE to peanut extract. All children underwent clinical evaluation (detailed medical history and physical examination), sIgE measurement to peanut extract and Ara h2/h6, and an OFC. During regular blood collection, additional blood was obtained for the BAT and the serum was stored at −80°C.

All parents of the patients gave their written informed consent to use data from the patients' files and to perform the BAT. This study was approved by the local medical ethical committee Arnhem/Nijmegen (2010-0116, WP-10-301). The procedures followed were in accordance with the Medical Research Involving Human Subjects Act, and the principles of the Declaration of Helsinki (59th WMA General Assembly, Seoul, Republic of Korea, October 2008) of the World Medical Association.

Oral food challenges

According to the Dutch food challenge test guideline, a DBPCFC should be performed when there is a reasonable risk of a positive or undecided outcome, whereas an open challenge test is recommended if the chance of a negative outcome is high or for small children (<2 years) who are not able to eat the amount of gingerbread required in the DBPCFC protocol.[17] The exclusion criteria for a challenge test were recent use of antihistamines or systemic steroids, or the presence of rhinoconjunctivitis, poorly controlled asthma, unstable atopic dermatitis, or urticaria.

Double-blind placebo-controlled food challenge

The placebo and verum challenges were administered on 2 separate days, with an interval of at least 1 week between them. The challenge procedure included 8 incremental dose steps with a starting dose of 0.001 g peanut protein and a maximum cumulative dose of 4.4 g.[18] The stop criteria were according to the Practical Allergy consensus, and the clinical symptoms were observed by a trained nurse, confirmed by a pediatric allergist, and recorded and graded as described by Sampson et al.[3] In cases of only subjective symptoms, the DBPCFC was stopped when the subjective symptoms occurred persistently/progressively following 2 consecutive doses; this differs from the Practical Allergy consensus, which recommends 3 consecutive doses. This was mainly because, in practice, it is difficult to motivate patients and their parents to continue the challenge test after repeated symptoms. The challenge was defined as positive when the symptoms on the verum day appeared without symptoms on the placebo day. A challenge was doubtful when patients had dubious symptoms on the verum day or symptoms on both challenge days. These doubtful challenge outcomes were marked as inconclusive and excluded from the analysis of the diagnostic performance of the BAT.

The step at which the test was stopped was recorded as the "threshold dose." A threshold dose of 0.4 g or more of peanut protein in this protocol is used in clinical practice as the dose at which patients are assumed to tolerate peanut traces. This is based on the fact that we have observed that patients who have been given this advice have not had any complaints.

Open challenge test

Open food challenges were performed with peanut butter, following the same dose steps and using the same safety measurements and definitions for results as the DBPCFC.

sIgE measurement

The sIgE values for peanut extract and the recombinant (r) Ara h 2 and rAra h6 were routinely measured on a Phadia ImmunoCAP 250 (Thermo Fisher Scientific, Waltham, Mass). Patients with an sIgE value of greater than or equal to 0.1 kU/L were considered IgE-sensitized.

TABLE I. Demographic and clinical data of the study participants (n = 74), categorized according to the OFC outcome

Characteristic	Total population	Peanut-allergic	Peanut-sensitized	Unknown
No. of patients	74	49	18	7
Age (y)	5.3 (3.6-8.0)	5.1 (3.7-8.0)	6.1 (3.6-8.8)	3.9 (2.7-8.4)
Male/female	56/18	36/13	13/5	7/0
sIgE (kU/L)				
Peanut extract*	6.0 (2.2-36.7)	19.5 (4.0-97.3)	1.7 (0.83-3.2)†	6.0 (5.8-24.6)
Peanut rAra h2	3.4 (1.0-33.2)	15.4 (2.5-73.9)	0.37 (<0.1-0.82)†	3.6 (2.7-14.7)
Peanut rAra h6*	3.1 (0.35-34.7)	11.5 (2.1-47.7)	0.11 (<0.1-0.20)†	2.4 (2.0-6.2)
OFC				
Double-blind placebo-controlled/open	69/5	48/1	14/4	7/0
Indication (diagnosis/follow-up)	52/22	36/15	11/7	5/2
Outcome				
Positive	49	49		
Negative	18		18	
Inconclusive	7			7
Threshold dose (g peanut protein)		0.71 (0.18-2.8)		
Sampson score 1-2/3-4		39/10		

Values are expressed as the numbers or medians (interquartile range).
*n = 3 peanut extract and n = 2 Ara h6 values were missing due to low sample volumes.
†Significant difference ($P < .001$) with peanut-allergic group.

Indirect BAT

Resensitized donor basophils. A 4-mL aliquot of fresh EDTA-anticoagulated blood (<24-hour old) from 8 adult nonallergic healthy blood donors with the blood group O was centrifuged for 10 minutes at 2200*g* at room temperature. Buffy coats were collected and combined, then washed with physiological salt and resuspended in a total volume of 2 mL (leucocyte count between 12.5× 10^9/L and 15 × 10^9/L). The resuspended buffy coat was centrifuged for 5 minutes at 1000*g* and 11°C after which 2 mL of cold stripping buffer (0.15 mol/L sodium dihydrogen phosphate monohydrate and 0.005/L mol potassium chloride, pH 3.55) was added to the buffy coat and the centrifuge protocol was repeated. After the stripping procedure, the buffy coat was washed with Basophil Stimulation Buffer (containing calcium, heparin, and IL-3; Bühlmann, Basel, Switzerland). A 500-μL aliquot of buffy coat was incubated with 130 μL of serum from the tested patient for 16 hours at 37°C. A BAT was performed with the resensitized donor basophils, which were separately stimulated with peanut extract, Ara h2, or Ara h6.

BAT protocol and classification of responses. The BAT was performed using a Flow2-CAST kit (Bühlmann) according to the manufacturer's instructions. Basophil activation was determined by the CD63 level of 500 basophils, as measured using flow cytometry (FACS Canto II; BD Biosciences, San Jose, Calif). The stimulation of basophils using an anti-FcR1 IgE receptor antibody was used as a positive control (a <20% difference between the positive and negative control values indicates a nonresponder; however, we did not detect any nonresponders in this study).

Dose-response curves were created using peanut extract (ALK-Abelló, Hørsholm, Denmark), or native (n) Ara h2 or nAra h6 (Bühlmann) using a final concentration range of 0.15 to 750 ng/mL for the peanut extract and 0.11 to 18 ng/mL for Ara h2 or Ara h6 to reach a plateau phase in the dose-response curve for most patients. Basophil activation was normalized as follows: %CD63-positive basophils on stimulation with the allergen relative to exposure to anti-FcεR1 IgE receptor antibody (positive control), both adjusted for the negative control.

The read-out parameters of the BAT were based on the individual dose-response curve: the basophil reactivity (area under the curve [AUC]) and sensitivity (EC50 [half-maximal effective concentration or concentration of allergen that induces a response halfway between baseline and maximum]).[8] Dose-response curves were fitted in GraphPad Prism (version 8.0.2, GraphPad Software, San Diego, Calif) using a 3-parameter logistic curve fit (hill slope 1). The result of the OFC was compared with the AUC for Ara h2 and Ara h6, and with the sum of the AUC of Ara h2 and Ara h6 (referred to as BAT Ara h2 plus Ara h6).

This indirect BAT protocol has been performed several times with 1 serum sample of 2 different patients with peanut allergy with different donor basophil pools, resulting in a mean coefficient of variation of 13% for the AUC (see Figure E1 in this article's Online Repository at www.jaci-inpractice.org).

Statistical analysis

Using GraphPad Prism, the receiver-operating characteristic curve was obtained to determine the cutoff point at a sensitivity of more than 95% (low number of false-negative BAT or sIgE test results) with the corresponding specificity. We consider this high sensitivity essential for new diagnostic tests (such as the BAT) to reliably replace the OFC, because patients with peanut allergy will not be missed with the risk of (severe) reactions on the reintroduction of peanut then. For the predictive power of the EC50, the cutoff point was taken at the highest sensitivity plus specificity (Youden index) in the receiver-operating characteristic curve.

A contingency 2 × 2 table was created to calculate the sensitivity, specificity, and positive and negative predictive values (PPVs and NPVs, respectively). The significance between the BAT parameters for patients with peanut allergy and patients tolerant of peanut was calculated using the Mann-Whitney *U* test. A *P* value of less than .05 was considered statistically significant.

TABLE II. Test characteristics of sIgE tests and BAT at a cutoff value with a >95% sensitivity

Characteristic	Cutoff value	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)	Accuracy*
sIgE peanut extract	0.98 kU/L	95.7 (85.8-99.2)	29.4 (13.3-53.1)	79.7 (73.8-84.5)	75.0 (39.9-93.1)	79.1
sIgE Ara h2	0.22 kU/L	95.8 (86.0-99.3)	44.4 (24.6-66.3)	82.5 (75.6-87.7)	80.0 (48.4-94.5)	82.1
sIgE Ara h6	0.25 kU/L	95.7 (85.8-99.2)	77.8 (54.8-91.0)	92.2 (83.2-96.5)	87.5 (63.8-96.5)	91.0
BAT peanut extract	24367	95.9 (86.3-99.3)	66.7 (43.8-83.7)	88.7 (80.3-93.8)	85.7 (59.8-96.0)	88.1
BAT Ara h2	156	95.9 (86.3-99.7)	72.2 (49.1-87.5)	90.4 (81.7-95.2)	86.7 (61.9-96.3)	89.6
BAT Ara h6	65.5	95.8 (86.0-99.3)	72.2 (49.1-87.5)	90.4 (81.7-95.2)	86.7 (61.9-96.3)	89.6
BAT Ara h2 plus Ara h6	479.5	**95.9 (86.3-99.3)**	**88.9 (67.2-98.0)**	**95.9 (86.4-98.9)**	**88.9 (67.1-96.9)**	**94.0**

Bold values show the highest diagnostic value.
*Accuracy: Overall probability that a patient is correctly classified.

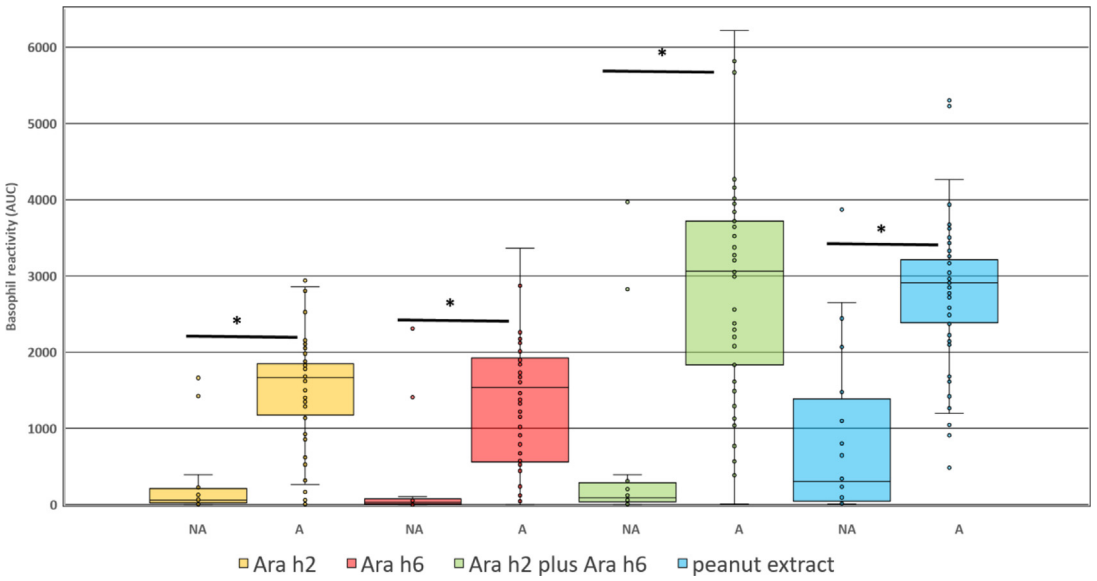

FIGURE 1. Box plots of the reactivity of the basophils (BAT AUC) for Ara h2, Ara h6, Ara h2 plus Ara h6, and the peanut extract (for the peanut extract, the AUC value is presented as AUC/25 for visual comparison with Ara h2 and Ara h6) in peanut-allergic (A) and nonallergic (NA) patients. *$P < .001$.

RESULTS

Study population

Table I presents the demographic and clinical features of the 74 study participants meeting the inclusion criteria. In 70% of these children, the indication of an OFC was the initial diagnosis, whereas the others had a confirmed peanut allergy and an OFC was executed to determine their peanut tolerance.

A DBPCFC was performed for 93% of the participants, whereas for 7% (n = 5) an open challenge test was performed. The open challenges were performed in 4 nonallergic patients and thus according to the Dutch guideline.[17] The open challenge in 1 peanut-allergic patient is a drawback of this study. However, because this patient showed a grade 1 anaphylactic reaction with itchy eyes and periorbital edema, which resolved after administration of an antihistamine drug, a false-positive outcome is highly unlikely.

According to the OFC outcome, 49 (66%) participants were labeled as peanut-allergic, 18 (24%) were peanut-sensitized but tolerant, and 7 (10%) had an inconclusive test result. The latter were due to (a) reaction at home (not clinically observed) after the completion of the challenge test (n = 1), (b) only 34% of the maximal cumulated dose of 4.4 g of peanut protein was eaten without any reaction (n = 1), and (c) the test was stopped when mild and nonspecific symptoms were observed and the child refused to continue the test (n = 5; for clinical details, see Table E1 in this article's Online Repository at www.jaci-inpractice.org).

The median levels of sIgE to peanut extract, rAra h2, and rAra h6 differed significantly between the peanut-allergic and peanut-tolerant groups ($P < .001$) (Table I); however, based on a cutoff level at more than 95% sensitivity, the specificity is limited (Table II).

Diagnostic power of the indirect BAT

Basophil reactivity (AUC of the dose-response curve). Following stimulation with peanut extract, nAra h2, or nAra h6 (see Figure E2 in this article's Online Repository at www.jaci-inpractice.org), the reactivity of the basophils differed significantly ($P < .001$) between patients who reacted to peanut and those who tolerated it in the challenge test (Figure 1).

Based on the BAT AUC cutoff at more than 95% sensitivity, the specificity, PPV, NPV, and accuracy were determined

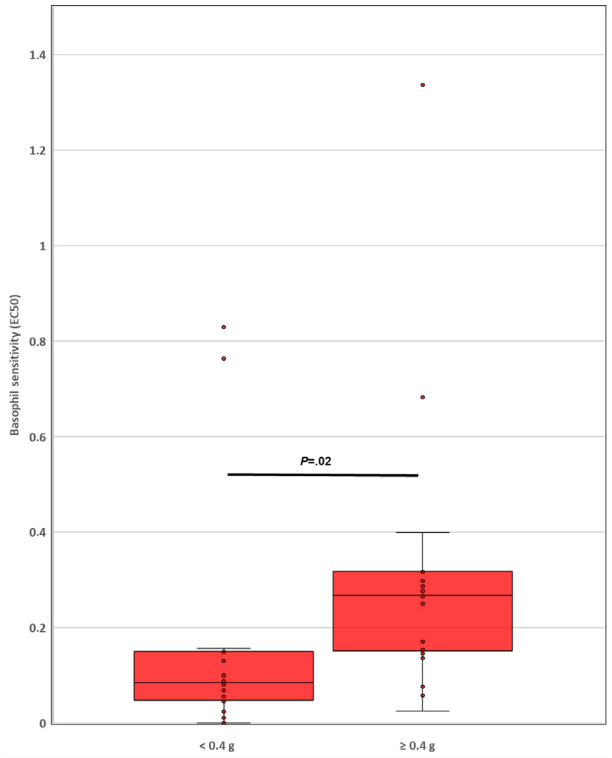

FIGURE 2. Box plots of the sensitivity of the basophils (BAT EC50) for Ara h2 in patients who (n = 18) tolerate less than 0.4 g of peanut protein vs patients (n = 20) who tolerate 0.4 g or more.

(Table II). The indirect BAT with the individual allergens was shown to be diagnostically superior to sIgE measurements (except for sIgE Ara h6). The BAT with Ara h2 plus Ara h6 showed the highest diagnostic performance, reflected in an increased specificity and PPV (decreased number of false-positive BAT results). The lower specificity for the BAT with Ara h2 or Ara h6 was due to patients (n = 4) with a 10-fold higher reaction to Ara h2 than to Ara h6 or vice versa, indicating a more Ara h2− or Ara h6−driven reaction (the median ratio in the allergic group was 1.0 [0.91-1.5]). These patients lowered the BAT AUC cutoff value for Ara h2 and Ara h6 to reach more than 95% sensitivity and consequently decreased the specificity (more false-positive BAT results).

Based on the BAT with Ara h2 plus Ara h6, 2 false-positive and 2 false-negative BAT results were obtained. For the patients with a false-positive BAT outcome, the analysis was repeated, both with another batch of donor basophils and with a serum sample from another time point (for 1 patient), showing similar results. The 2 patients with a false-negative BAT outcome reached a very high threshold value in the challenge test, at which only mild subjective symptoms (Sampson score 1) occurred and the test was stopped (at 3.3 and 4.4 g peanut protein, being 75% and 100% of maximum dose, respectively). The patient with a threshold dose of 3.3 g peanut protein was not IgE-sensitized (<0.1 kU/L) to Ara h2 or Ara h6, and thus a negative BAT result for Ara h2 or Ara h6 was expected. However, this patient had a positive BAT result for peanut extract. The patient with a threshold dose of 4.4 g peanut extract was IgE-sensitized to Ara h2 (1.1 kU/L) and Ara h6 (0.7 kU/L) but had a negative BAT result for peanut extract, Ara h2, and Ara h6.

It was not possible to correlate the severity of the allergic reaction with the reactivity of the basophils because severe, potentially life-threatening allergic reactions involving cardiovascular, neurological, bronchial, and/or laryngeal symptoms and signs were rarely observed (only 4 patients had a Sampson score of 4, and a score of 5 was not observed).

Basophil sensitivity (EC50 of the dose-response curve). Although a limited correlation between the threshold dose and the EC50 ($r = 0.4$; see Figure E3 in this article's Online Repository at www.jaci-inpractice.org) was observed only for Ara h2, a significant difference ($P = .02$) was noticed between the sensitivity of the basophils and the threshold dose for patients (n = 20) who tolerated up to 0.4 g peanut protein (high dose) and those (n = 18) who did not (low dose) (Figure 2). The sensitivity, specificity, PPV, and NPV for reacting to a dose of less than 0.4 g of peanut protein were 94.4 (95% CI, 72.7-99.9), 70.0 (45.7-88.1), 73.9 (59.0-84.8), and 93.3 (67.1-100), respectively. For all above analyses, we could not include the results of 9 patients, because their calculated EC50 values showed such a broad CI that these results had to be regarded as ambiguous (this was due to these dose-response curves showing a maximum response at low allergen concentrations) as well as the results of 2 patients who hardly reacted to Ara h2 but reacted strongly to Ara h6 stimulation (10-fold stronger reaction).

DISCUSSION

This study showed that an indirect BAT using Ara h2 in combination with Ara h6 in our population had a high diagnostic accuracy (94%), with PPV and NPV scores of 96% and 89%, respectively. As such the indirect BAT is a promising test for replacing the risky, time-consuming, and expensive OFC in similar populations. Although in our population, the indirect BAT with the direct BAT could not be compared because of logistic constraints for performing the direct BAT, these diagnostic values are comparable with data published for the direct BAT for peanut and Ara h2 (taking the cutoff value at the highest published sensitivity): the PPV was 67% to 95% and the NPV was 81% to100%.[9,10,14] This indicates that the indirect BAT is a reliable alternative to the direct BAT and is potentially even superior to it because it is not affected by nonresponding basophils. Furthermore, it shows that the immunologic state of the patient's basophils (eg, IgE receptor density and IgE signaling pathway capacity) appears to be less important than the amount and type of patient's sIgE to provoke an allergic reaction. The BAT using individual allergen components performed better than that using peanut extract as shown before.[14] This might be caused by the fact that peanut extracts differ in composition due to diversity in the allergen source and protein extraction but the influence of digestion and processing on the inactivation of allergens/epitopes might also play a role.[19] The fact that the relatively stable Ara h2 and Ara h6 components performed well in the BAT advocates their use instead of the mainly unknown composition of whole peanut extracts, although peanut extract can still be useful in case patients are not sensitized to Ara h2/Ara h6 but have a suspected clinical history of peanut allergy (see below). Nevertheless, the 2 false-positive BAT outcomes could be a consequence of sIgE against an epitope that can be inactivated because of processing or digestion. Another possibility

might be that the indirect BAT protocol is less sensitive for inhibiting factors (such as $sIgG_4$) produced during the development of natural tolerance. Indications for that have been obtained by the fact that the indirect BAT result can still be positive after effective oral immune therapy (OIT). However, on changing the BAT protocol, that is, incubation of allergen with post-OIT serum before addition of resensitized donor basophils with pre-OIT plasma, the basophil activation was almost completely suppressed. Although it is unknown whether clinical tolerance induced by OIT resembles natural tolerance development, further research with this indirect BAT model for patients who are suspected of a peanut allergy might be valuable, especially for those patients with a confirmed peanut allergy who are being followed for tolerance induction.[20,21]

The 2 false-negative BAT results for Ara h2 and Ara h6 were obtained in patients who reached a very high threshold value in the OFC, at which only mild subjective symptoms occurred. In contrast to what has been suggested by others, the false-negative results in these 2 patients are not expected to be due to low sIgE values because positive indirect BAT results in our patients were observed with sIgE for Ara h2 or Ara h6 down to 0.3 kU/L.[16] However, 1 of these 2 patients was not sensitized to Ara h2 and Ara h6, but only to peanut extract, and had a positive BAT result for peanut extract. This suggests that this patient might be sensitized to another (unknown) peanut component. Nevertheless, although the challenge test is the gold standard, and the DBPCFC is superior to an open test, it is questionable whether subjective symptoms are diagnostically equivalent to at least 1 or more objective symptoms. The accuracy of an OFC has been reported to be limited when using subjective symptoms due to a high interobserver variability.[22] In addition, OFCs show a low reproducibility when it comes to determining the threshold dose.[23] This might also explain the limited correlation in this study between the sensitivity of the basophils in the BAT and the threshold dose in the OFC, as was also observed in other studies,[11,24] showing a low PPV (43%) for determining a threshold value of less than 0.1 g of peanut protein.[24] Furthermore, the threshold dose is not an independent variable because it is correlated with the severity of the allergic reaction when an OFC is started at a relatively high dose (0.1 g peanut protein) and so was mainly stopped because of objective, and thus more severe, symptoms.[24] However, the fact that only the EC50 for Ara h2 was significantly different between a low and high threshold dose in this study indicates that Ara h2 is the major component affecting the sensitivity of a peanut allergy, as suggested before.[25]

Another drawback of the OFC is the significant number of inconclusive test results generated (10% in this study, which is comparable to others[26]), but the indirect BAT did not show a single inconclusive result. Inconclusive test outcomes may result in uncertainty and anxiety in children and parents, unnecessarily restrictive diets, and increased diagnostic costs due to their lowered efficacy.[22,27]

To reduce the number of indirect BATs in the future, a 2-step diagnostic protocol that starts with sIgE Ara h2 as a first screening step can be investigated.[10,14] However, because sIgE Ara h2 cutoff values are strongly dependent on age and population, this requires determination of these values in the own population. Because these values are not available for our population, we applied, as an example, sIgE Ara h2 cutoff values determined in a Dutch university center on our population. On the basis of a cutoff value of sIgE Ara h2 of less than 0.1 kU/L and more than 5 kU/L (with an NPV and PPV of >95%, respectively), 54% of the included patients had an sIgE Ara h2 of 0.1 to 5 kU/L.[4,13] The diagnostic power of the BAT in this latter group of children was comparable to that of the whole patient cohort and thus, if sIgE Ara h2 is used as a first screening step, 54% of the children need a BAT. Because the indirect BAT had no nonresponder results and the 2 false-negative BAT results were obtained in patients tolerating high amounts of peanut with mild symptoms, a significant reduction in OFCs is achievable and clinically justified. To justify the safe replacement of the OFC with an indirect BAT in daily clinical practice, more data in larger populations, in combination with the use of sIgE screening tests, should be obtained as well as data on the (cost)-effectiveness of these strategies. However, such a study is more effective when the applicability of the OFC outcome is more thoroughly assessed. First, what is the reproducibility of the threshold value, to what extent do patient/parents rely on this information, and will knowledge on the threshold value have a positive influence on patient quality of life? Second, how well can the OFC predict the risk of a severe reaction and need for the prescription of an epinephrine autoinjector?[28,29]

CONCLUSIONS

To diagnose a peanut allergy, the potentially life-threatening, time-consuming, and expensive OFC can be replaced with the safe, patient-friendly, rapid, and cheap indirect BAT. This indirect BAT method enabled a peanut allergy diagnosis to be made using a serum blood sample, which can be taken at any time, stored for a long time, and transported to a central laboratory. Moreover, its efficacy is higher than that of a direct BAT because it does not suffer from inconclusive results due to nonresponding basophils. The validation of this highly promising diagnostic test in other and larger populations is mandatory, with an emphasis on the (cost)-effectiveness in comparison with the (dis)advantages and applicability of the OFC.

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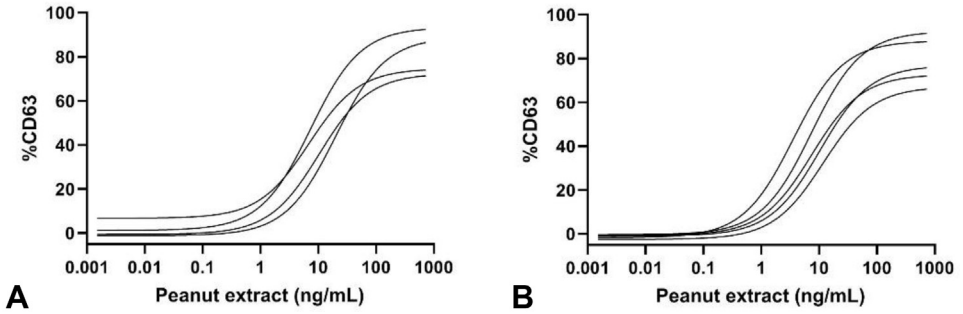

FIGURE E1. BAT dose-response curves for peanut extract with serum of 2 patients (**A** and **B**). The BAT is repeated with different donor basophil pools.

TABLE E1. Clinical symptoms of 5 patients with an inconclusive OFC outcome due to symptoms on verum that were by far less specific for allergy and patient refused to continue the test

P. no.	Symptoms during DBPCFC
1	Swollen eyes, stuffy nose, and sneezing. At home, abdominal pain and burping
2	Itchy throat, abdominal pain
3	Abdominal pain
4	Child refuses to continue eating. Half hour later vomiting
5	Itchy throat and arms, abdominal pain

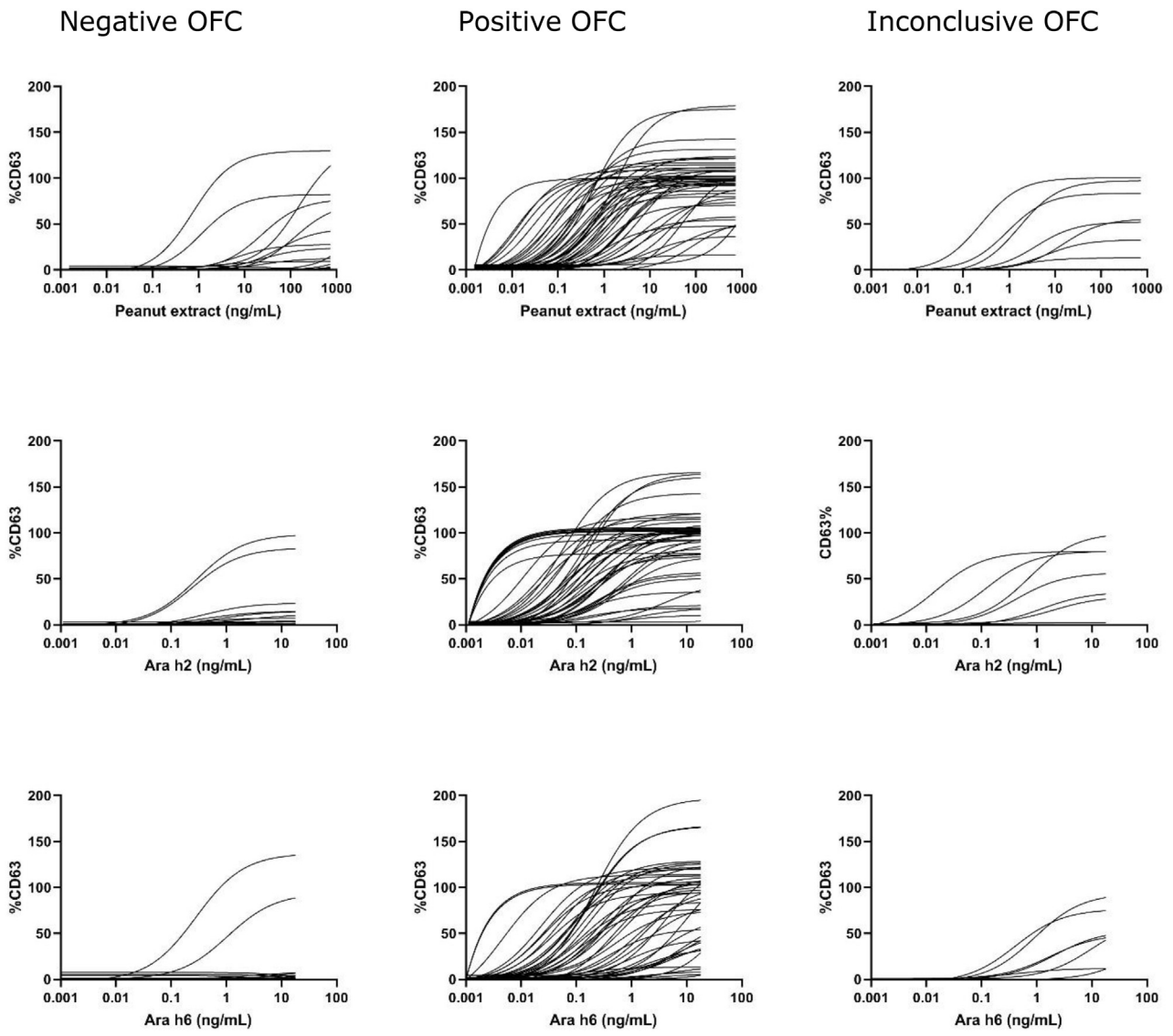

FIGURE E2. BAT dose-response curves for peanut extract, Ara h2, and Ara h6. Results are categorized for patients with a negative (n = 18), positive (n = 49), or inconclusive (n = 7) OFC.

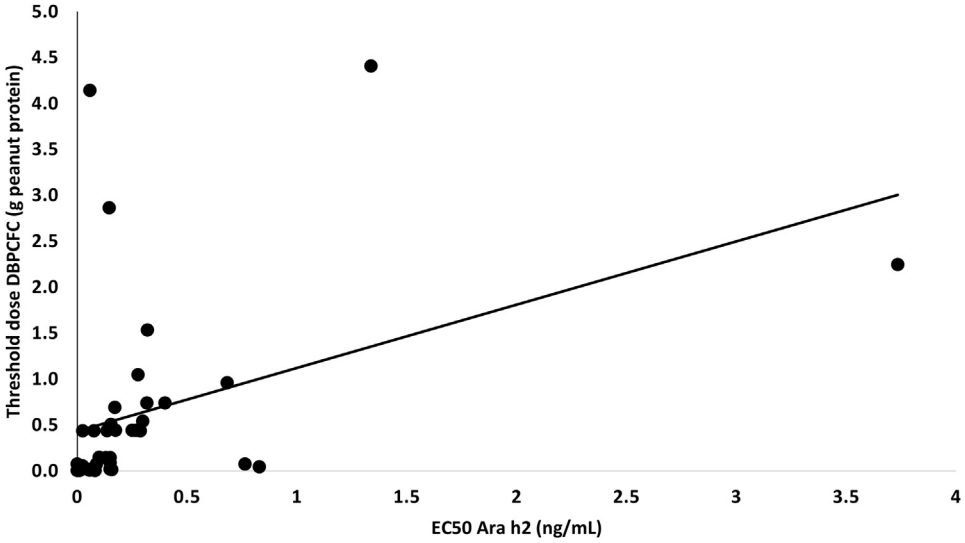

FIGURE E3. Correlation between EC50 Ara h2 and the threshold dose in DBPCFC ($r = 0.4$).