

Correlations between basophil activation, allergen-specific IgE with outcome and severity of oral food challenges

Ying Song, MD^{*}; Julie Wang, MD^{*}; Nicole Leung, BS^{*}; Li Xin Wang, MD, PhD^{*,†}; Lauren Lisann, BS^{*}; Scott H. Sicherer, MD^{*}; Amy M. Scurlock, MD[‡]; Robbie Pesek, MD[‡]; Tamara T. Perry, MD[‡]; Stacie M. Jones, MD[‡]; and Xiu-Min Li, MD, MS^{*}

^{*} Division of Allergy and Immunology, Department of Pediatrics, Icahn School of Medicine at Mount Sinai, New York, New York

[†] Shanghai Municipal Hospital of Traditional Chinese Medicine affiliated with Shanghai TCM University, Shanghai, China

[‡] Division of Allergy and Immunology, Department of Pediatrics, University of Arkansas for Medical Sciences, Arkansas Children's Hospital Research Institute, Little Rock, Arkansas

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ABSTRACT

Background: Double-blinded, placebo-controlled food challenges (DBPCFCs) remain the gold standard for diagnosing food allergies. Skin prick tests (SPTs) and allergen-specific IgE (sIgE) are routinely used in medical practice but are not sufficient to predict severity of clinical reactivity.

Objective: To compare the utility of SPT wheal diameter, sIgE, allergen-specific IgG4 (sIgG4), total IgE (tIgE), sIgE/sIgG4 and sIgE/tIgE ratios, peanut component-specific IgE, and basophil activation in predicting outcome and severity of reactions at DBPCFCs.

Methods: Sixty-seven subjects (12–45 years old) underwent DBPCFCs for peanut, tree nut, fish, shrimp, and/or sesame as part of screening for enrollment in a clinical trial. The SPT, sIgE, tIgE, sIgG4, and peanut component-specific IgE (if applicable) levels were measured. CD63 upregulation on basophils in response to in vitro allergen challenge was analyzed by flow cytometry. Correlations between these measurements and DBPCFC severity scores were analyzed.

Results: The SPT and sIgE showed a weak correlation with DBPCFC severity scores, but tIgE and sIgG4 did not. The sIgE/sIgG4 ratio differentiated between positive and negative reactions but did not correlate with DBPCFC severity scores. A low positive correlation was seen between DBPCFC severity score and Ara h 2 IgE, whereas a low negative correlation with Ara h 8 IgE was observed. Basophil activation was positively correlated with DBPCFC severity scores. Receiver operating characteristic curves showed basophil reactivity had the largest area under the curve at 0.904 and sIgE at 0.870.

Conclusion: These results indicate that basophil activation testing can enhance discrimination between allergic and nonallergic individuals and could serve as an additional tool to predict clinical severity.

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Introduction

Food allergies are increasing in prevalence,¹ and the ever-present possibility of severe, life-threatening anaphylaxis causes tremendous stress and negatively affects quality of life for individuals with food

allergy and their families.² Standard testing modalities include skin prick testing (SPT), measurement of allergen-specific IgE (sIgE) levels, and oral food challenges. Although SPT and sIgE are sensitive tests, they are poor predictors of clinical reactivity.² The double-blinded,

Reprints: Xiu-Min Li, MD, MS, Pediatric Allergy and Immunology, Icahn School of Medicine at Mount Sinai, One Gustave L Levy Place, New York, NY 10029-6574; E-mail: xiu-min.li@mssm.edu.

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placebo-controlled food challenge (DBPCFC) is the gold standard for the diagnosis of food allergies, but it is time and resource intensive. Furthermore, there are currently no tests that can predict the severity of food-allergic reactions.

Component-resolved diagnostics is a modality that is being examined for its potential role in food allergy diagnosis.³ It has been studied most extensively for peanut allergy. Sensitization to the major peanut protein, Ara h 2, has been shown to be the most important predictor of clinical reactivity.^{4–7} In contrast, isolated sensitization to Ara h 8, a Bet v 1 (birch pollen) homolog, is usually associated with tolerance to peanut.⁸ Although a test is commercially available for peanut components, there are fewer data regarding other food allergens, and it remains to be seen whether component testing will replace standard testing modalities or whether its role is supplementary.

Several studies have suggested a role for measurement of basophil activation in the diagnosis of food allergies.^{9–13} In a recent study of children with milk allergy, milk-specific basophil reactivity was found to be significantly greater in patients who reacted to baked milk compared with those who were allergic to milk but tolerant to baked milk, showing that basophil activation could be a marker for a more severe phenotype.¹⁰ Ciepiela et al¹¹ showed that the basophil activation test (BAT) has sensitivity similar to routine diagnostic tests and a higher specificity for milk allergy. In larger studies with children undergoing oral food challenges to milk and/or egg, high sensitivity and specificity were seen for the BAT.^{9,12} Although these data show promise, the BAT remains an investigational tool. Further evidence is needed to determine whether the BAT can be a useful addition to the currently available diagnostic tools for food allergy.

The aim of this study was to determine whether SPT, sIgE, total IgE (tIgE), allergen-specific IgG4, component testing, and basophil activation are correlated with outcomes of DBPCFCs and the severity of clinical reactions during challenge and might serve as an additional tool to predict clinical severity.

Methods

Study Population

Patients with food sensitivity 12 through 45 years old with a convincing history of allergy to peanut, tree nut, sesame, fish, or shrimp, as documented by a positive SPT response (mean wheal diameter >3 mm than the mean of saline control) and/or food allergen-specific IgE level (peanut-, tree nut-, sesame-, fish-, or shrimp-specific IgE >0.7 kU_A/L) who were undergoing DBPCFCs as part of screening for enrollment in a clinical trial (Therapeutic Effect of Chinese Herbal Medicine on Food Allergy (FAHF-2); ClinicalTrials.gov identifier NCT00602160) were included in this study. This study was approved by the Icahn School of Medicine at Mount Sinai (New York, New York) and the University of Arkansas for Medical Sciences (Little Rock) institutional review boards. Written informed consent was obtained before enrollment.

Skin Prick Tests

End-point titrated SPTs with serial 10-fold dilutions were performed in all patients. The standard extracts (1:20 wt/vol) of stock peanut, individual tree nuts, sesame, and individual fish or shrimp (Greer Laboratories, Lenoir, North Carolina) were used. Negative controls (phenol-saline solution) and positive controls (1-mg/mL histamine base) were included. The SPTs were performed by pricking through a drop of extract with a GreerPick (Greer Laboratories). A wheal diameter larger than 3 mm compared with the negative control was considered a positive response.

Double-blinded, Placebo-controlled Oral Food Challenges

The DBPCFCs to peanut, tree nut, sesame, fish, or shrimp were performed on separate days within 1 week of one other or on the

same day at the discretion of the physicians at the Mount Sinai or Arkansas Children's Hospital clinical research units. The DBPCFCs were performed by gradually feeding increasing amounts of the food allergen to a maximum of 2 g of protein at 10- to 15-minute intervals under supervision. The 2 sites used the same procedure to prepare the challenge food materials, and a uniform approach was used for the DBPCFCs. The doses were distributed in the following manner: 1, 5, 15, 50, 75, 100, 250, 500, and 1,000 mg (cumulative dose 2 g). Frequent assessments were made for symptoms affecting the skin, gastrointestinal tract, respiratory tract, nervous system, and/or cardiovascular system.

A food challenge reaction was considered positive when a subject developed cutaneous (urticaria, angioedema, and/or flushing), gastrointestinal (abdominal cramping, vomiting, and/or diarrhea), respiratory (persistent nasal congestion, persistent rhinorrhea, persistent sneezing, tightness in the throat, dysphonia, dyspnea, and/or wheezing), central nervous system (change in activity level, confusion, and/or anxiety), and/or cardiovascular (dizziness, loss of consciousness, and/or hypotension) symptoms. Oral food challenges also were stopped if the subject had persistent subjective symptoms. Based on symptoms recorded during the DBPCFCs, the severity of the symptom was assessed according to the method used by Sampson¹⁴ (Grading of Food-Induced Anaphylaxis According to Severity of Clinical Symptoms). Severity was scored on a scale from 1 to 5, with 1 being the least severe symptoms and 5 being most severe.

Laboratory Studies

Serum sIgE, allergen-specific IgG4, tIgE, and peanut component-specific IgE measurements

Allergen-specific IgE and IgG4, tIgE, and peanut component-specific IgE levels, including recombinant Ara h 1, 2, 3, 8, and 9, were measured in serum samples using the ImmunoCAP 100 instrument (Thermo Fisher, Portage, Michigan) according to the manufacturer's instructions.¹⁵

Basophil activation test

The BATs were performed with the Flow 2 CAST kit (Alpco Diagnostics, Windham, New Hampshire) as previously described.¹⁶ Crude peanut, pecan, hazelnut, cashew, sesame, catfish, cod, salmon, and shrimp extracts were prepared using a previously described protocol¹⁷ and used as specific allergens for in vitro stimulation. A stimulation buffer containing interleukin-3 was used as the negative control. Allergens and a highly specific monoclonal antibody recognizing the high-affinity IgE binding receptor (FcεRI) was used as a positive control, and N-formyl-methionyl-leucyl-phenylalanine was used as a nonspecific positive control, according to the manufacturer's instructions. One hundred microliters of stimulation buffer containing interleukin-3, heparin, and calcium was added to 6 aliquots of 50 μL of heparinized blood. Fifty microliters of stimulation buffer, anti-FcεRI, N-formyl-methionyl-leucyl-phenylalanine, or allergen (200, 2, and 0.02 ng/mL) was added to each aliquot. Twenty microliters of staining reagent containing monoclonal antibodies labeled with anti-CCR3 and fluorescein isothiocyanate and with anti-CD63 and phycoerythrin was added. The tubes were incubated at 37°C in a water bath in the dark for 15 minutes. The red blood cells were lysed, and cells were resuspended in wash buffer and acquired on a LSR-II flow cytometer (BD Biosciences, San Jose, California). Basophils and eosinophils were gated as CCR3⁺ cells and segregated based on side scatter. CCR3⁺ cells with low side scatter were considered basophils, and among these, the CD63⁺ cells were termed *activated basophils*. Fifty thousand to 100,000 leukocytes were acquired, and at least 300 basophils were used for analysis using FlowJo 5.4 software (Tree Star, Inc, Ashland, Oregon).

Statistical Analysis

Data were analyzed using the SigmaStat statistical software package (Systat, Chicago, Illinois). The Bonferroni *t* test was used for comparisons when the data were approximately normal. Differences between groups were analyzed by the Mann-Whitney rank sum test if the data were not normally distributed. Nonparametric (Spearman) correlation was used for correlation analysis. Analysis of receiver operating characteristic curves was performed of SPT, sIgE, and BATs at different doses to calculate the area under curve (AUC) to obtain the most accurate measurement compared with

the gold-standard DBPCFCs. *P* values less than .05, based on 2-tailed tests, were considered statistically significant.

Results

Subject Characteristics

Sixty-seven subjects were enrolled. The median age was 16 years (range 12–43 years), and 56% were male. In this cohort, 88% of study subjects exhibited other allergic diseases, including asthma (59%), allergic rhinitis (72%), and atopic dermatitis (46%).

Table 1
Age, sex, wheal size of skin prick test, sIgE to food allergens, and results of double-blinded, placebo-controlled food challenge per subject followed by ED, CD, and score—characteristics of subjects with positive reactions

Pt	Age (y)	Sex	Ag	Wheal (mm)	sIgE (kU _A /L)	Symptoms	Other allergic diseases	ED (mg)	CD (mg)	Score
1	14	M	PN	15	>100	S, R, GI, CNS	AR, AD, AS	6	496	3
2	17	F	PN	10.5	37.3	S, R, GI	AR, AS	6	71	2
3	35	F	PN	15	5.07	S, R, GI, CNS	AR	6	996	2
4	18	F	PN	13	>100	S, R, GI, CNS	AR, AS	246	246	3
5	13	M	PN	19.5	94.6	S, R, GI, CNS	AR, AD, AS	1	6	3
6	28	F	PN	26	85.2	S, R, GI, CNS	AS	71	246	3
7	15	M	PN	28	25.4	S, GI, CNS	AR, AD, AS	6	146	3
8	12	M	PN	22.75	78.4	GI, CNS	AR, AD, AS	21	246	2
9	16	M	PN	9	20.6	S, R, GI	AS, AR, AD	1	246	2
10	19	M	PN	12	6.42	GI	AS, AR, AD	146	246	4
11	19	M	PN	5	14.9	S, R, C, GI	AS, AD	71	996	3
12	19	M	PN	2	23.3	S, R	AR, AD	496	2,000	4
13	16	M	PN	12	23.6	S, R, C, GI, CNS	AS	246	246	3
14	27	M	PN	10	100	S, R, GI, CNS	AS	71	946	4
15	19	M	PN	9	4.44	S, GI, CNS	AS, AD	1	71	3
16	20	M	PN	7	78.5	S, R, GI, CNS	AS	71	146	3
17	24	F	PN	10.5	9.83	S, R	AS, AR	1	146	4
18	13	F	PN	7	100	S, R, C, GI	AS	6	196	4
19	15	F	PN	13	100	R, GI	AS, AR, AD	6	6	2
20	19	M	PN	13	1.82	S, GI	AS, AR, AD	256	1,000	3
21	13	M	PN	9.5	100	S, R, GI	none	1	6	3
22	15	M	PN	9.5	8.24	GI	AS, AR	1	21	1
23	14	F	PN	5	100	R, GI	AS, AR	1	1	4
24	14	M	PN	10.5	>100	S, R, GI	AD	1	6	3
25	13	F	PN	11	>100	GI	AS, AR	1	21	2
26	15	F	PN	12	100	GI	AR	15	71	3
27	17	F	PN	11	>100	R, GI	AS, AR, AD	6	246	4
28	13	M	PN	8	9.92	R, GI	AR, AD	0	21	3
29	13	M	PN	6.5	24.4	R, GI	AR	1	1	4
30	13	F	PN	10	98.7	GI	AR	1	1	2
31	13	F	PN	16	79.6	R, GI	AS, AD	1	21	3
32	20	F	PN	17.5	>100	S, R, GI	AS, AR	6	156	4
33	14	F	PN	12.5	26.9	GI	AS, AR, AD	496	496	3
34	13	M	PN	8	27.60	GI	AS, AR	6	6	3
35	25	F	PN	10.5	38.90	GI	AR, AD	1	71	2
36	15	M	PN	9.5	0.59	R, GI	AR, AD	1	496	3
37	13	F	PN	8.5	12.90	GI	AS, AR, AD	1	6	3
38	42	F	PN	7	1.83	R, GI	AR, AD	1	246	2
39	12	M	PN	8	16.8	R, GI	AS, AR	1	6	3
40	14	M	PN	8	1.42	S, GI	AS	71	996	4
41	20	M	PN	10	>100	GI	none	21	21	2
42	15	M	PN	5.5	100	S, GI	AS, AD	21	71	3
43	13	M	CSH	10.5	1.27	R, GI, CNS	AR	246	996	2
44	16	F	CSH	5	50.1	S, R, GI, CNS	AS, AR, AD	71	71	4
45	13	F	CSH	14.75	>100	S, GI, CNS	AR	21	21	2
46	13	M	CSH	15	4.05	S, GI	none	1	1	4
47	40	F	HZT	2	6.66	S, R, GI	AR, AD	1	496	3
48	15	M	HZT	8	26.6	S, R, GI, CNS	AR	71	2,000	3
49	43	F	PC	11	8.27	S, R, GI, CNS	none	1	71	4
50	18	M	WT	8.5	50.50	GI, CNS	none	1	1	2
51	21	F	SES	6.5	88	S, GI	AS, AR, AD	1	1	3
52	26	M	SES	7	28.4	S, R, GI	AS, AR, AD	1	21	2
53	21	F	SES	6	37.7	GI	AS, AD	1	6	4
54	23	F	SES	5.5	1.81	S, GI, CNS	AS, AR	6	171	3
55	32	F	CTF	7	1.07	S, GI	AR	71	496	1
56	31	M	CDF	8.5	1.94	S, C	AS, AR	86	2,000	3
57	26	M	SN	7	3.82	GI	AS, AD	6	71	3
58	16	M	SHR	10.5	20.2	S, GI, CNS	none	6	996	3

Abbreviations: A, almond; AD, atopic dermatitis; Ag, food allergen; AR, allergic rhinitis; AS, asthma; C, cardiovascular; CD, cumulative dose at the end of challenge; CDF, codfish; CNS, central nervous system (neurologic); CSH, cashew; CTF, catfish; ED, eliciting dose at first symptom; F, female; GI, gastrointestinal tract; HZT, hazelnut; M, male; PC, pecan; PN, peanut; Pt, patient ID; R, respiratory; S, skin; SES, sesame; SHR, shrimp; sIgE, allergen-specific IgE; SN, salmon; WT, walnut.

Table 2

Characteristics of subjects with negative double-blinded, placebo-controlled food challenge reactions

Pt	Age (y)	Sex	Ag	Wheal (mm)	slgE (kU _A /L)	Symptoms	Other allergic diseases	ED (mg)	CD (mg)	Score
1-C1	16	M	CSH	0	0.78	none	AR	N/A	N/A	0
1-C2 ^a	16	M	A	2.5	1.68	R	AR	N/A	N/A	1 ^a
2	42	M	A	6	0.96	none	AR	N/A	N/A	0
3-C1	13	F	A	5	0.83	none	AS, AR, AD	N/A	N/A	0
3-C2	13	F	HZT	5	9.29	none	AS, AR, AD	N/A	N/A	0
4	18	M	CDF	5.5	2.3	none	none	N/A	N/A	0
5-C1	40	M	CDF	8.25	0.54	none	AR	N/A	N/A	0
5-C2 ^a	40	M	CTF	8.5	1.55	R	AR	N/A	N/A	1 ^a
6-C1	15	M	PN	7.25	20.5	none	AS, AR, AD	N/A	N/A	0
6-C2	15	M	SES	8.25	7.14	none	AS, AR, AD	N/A	N/A	0
7	17	F	PN	4	5.34	none	AR, AD	N/A	N/A	0
8	13	F	SES	4	3.00	none	none	N/A	N/A	0
9	43	M	SHR	7	6.06	none	AR	N/A	N/A	0

Abbreviations: A, almond; AD, atopic dermatitis; Ag, food allergen; AR, allergic rhinitis; AS, asthma; CD, cumulative dose at end of challenge; CDF, codfish; CSH, cashew; CTF, catfish; ED, eliciting dose at first symptom; F, female; HZT, hazelnut; M, male; N/A, not applicable; PN, peanut; Pt, patient ID; R, respiratory; SES, sesame; SHR, shrimp; slgE, allergen-specific IgE.

^aSubjects 1-C2 and 5-C2 had transient symptoms that self-resolved and did not preclude completion of the double-blinded, placebo-controlled food challenge so they were deemed negative reactions.

Seventy-one challenges were conducted in 67 subjects, with 4 subjects each having undergone 2 DBPCFCs. Characteristics of study subjects with 58 positive DBPCFC reactions are listed in Table 1 and those with 13 negative DBPCFC reactions are listed in Table 2.

Of the 71 challenges, positive reactions were found for peanut (42 of 44, 95%), tree nuts (8 of 13, 62%), sesame (4 of 6, 67%), fish (3 of 6, 50%), and shrimp (1 of 2, 50%). Symptoms during the DBPCFCs are listed in Table 1. Cutaneous symptoms were observed for 59% of subjects with positive DBPCFC reactions, gastrointestinal symptoms occurred for 95%, respiratory symptoms occurred for 55%, central nervous system symptoms occurred for 33%, and cardiovascular symptoms occurred for 7%. Of negative reactions, 15% were for peanut, 38% were for tree nuts, 15% were for sesame, 23% were for fish, and 8% were for shrimp.

The median DBPCFC severity score in subjects with positive reactions was 3 (range 1–4). For positive reactions, there were no significant correlations between the eliciting dose at the first symptom, the cumulative dose at the end of challenges (Table 1), and DBPCFC severity scores (correlation data not shown).

SPT Wheal Size Differentiates between Positive and Negative Reactions but Has a Negligibly Weak Correlation to DBPCFC Severity Scores

The SPT wheal sizes were significantly different ($P < .001$; Fig 1A) between positive and negative DBPCFC reactions. The median wheal measurement for positive reactions was 10 mm (range 2–28 mm), whereas the median for negative reactions was 5.5 mm (range

0–8.5 mm). The wheal measurements were negligibly correlated with DBPCFC severity scores ($r = 0.24$, $P = .05$; Fig 1B).

slgE Differentiates DBPCFC Outcomes and Is Positively Correlated with DBPCFC Severity Scores

The median slgE level from subjects with positive DBPCFC reactions was 26.9 kU_A/L (range 0.59–100 kU_A/L), whereas the median slgE level from negative DBPCFC reactions was 2 kU_A/L (range 0.54–20.5 kU_A/L). The slgE levels differed significantly in subjects with positive vs negative DBPCFC reactions ($P < .001$; Fig 2A) and were weakly correlated with DBPCFC severity scores ($r = 0.33$, $P = .0049$; Fig 2C).

The tIgE levels were not different between the 2 groups, and there was no significant correlation with DBPCFC severity scores (Fig 2B, D). The median tIgE level was 582.5 kU_A/L (range 13–4,379 kU_A/L) in subjects with a positive reaction and the median level was 568 kU_A/L (range 132–5,000 kU_A/L) in those with a negative

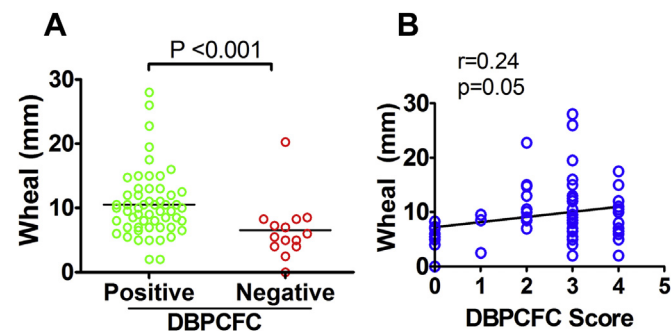


Figure 1. (A) Wheal diameter skin prick test levels in subjects with positive and negative double-blinded, placebo-controlled food challenge reactions. (B) Correlation between wheal diameter (millimeters) and double-blinded, placebo-controlled food challenge severity scores ($n = 71$). Horizontal bar indicates the mean.

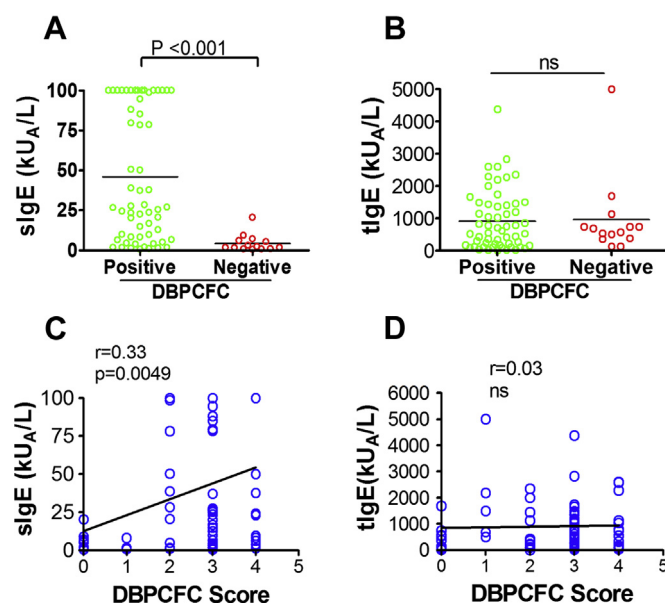


Figure 2. (A) Whole allergen extract-specific IgE (slgE) and (B) total IgE (tIgE) levels in subjects with positive and negative double-blinded, placebo-controlled food challenge reactions. Correlation between (C) whole allergen extract-specific IgE or (D) total IgE levels and double-blinded, placebo-controlled food challenge severity scores ($n = 71$). Horizontal bar indicates the mean.

reaction. The sIgE/tIgE ratios also were not significantly different between groups.

sIgE/sIgG4 Ratio Differentiates Positive and Negative Reactions but Does Not Correlate with DBPCFC Severity Scores

Allergen-specific IgG4 levels were not different between positive and negative reactions (median 0.5 mgA/L, range 0.06–7.9 mgA/L; median 0.4 mgA/L, range 0.010–4.7 mgA/L, respectively; Fig 3A).

As shown in Figure 3B, the median sIgE/sIgG4 ratio was 29.9 (range 1.8–995) for positive reactions and the ratio was 10.0 (range 1.4–230) for negative reactions. The sIgE/sIgG4 ratios effectively distinguished between positive and negative reactions ($P < .05$) but did not significantly correlate with DBPCFC severity scores (Fig 3B, C).

For Peanut Allergy, Ara h 2 IgE Is Positively Correlated with DBPCFC Severity Scores, Whereas Ara h 8 IgE Is Negatively Correlated with DBPCFC Severity Scores

Specific IgE levels to peanut components (Ara h 1, 2, 3, 8, and 9) were measured in 44 subjects with peanut allergy, including 42 subjects with positive and 2 subjects with negative DBPCFC reactions. There was a weak positive correlation between IgE to Ara h 2 and DBPCFC severity scores ($r = 0.311$, $P = .038$; Fig 4A) and a weakly negative correlation between IgE to Ara h 8 and DBPCFC severity scores ($r = -0.3681$, $P = .0139$; Fig 4B). There were no significant correlations found between the other peanut components and DBPCFC severity scores (data not shown).

Ratios of sIgE to Ara h 1, 2, and 3 were positively and significantly correlated with IgE to whole peanut ($r = 0.8452$, $P < .0001$; $r = 0.8915$, $P < .0001$; $r = 0.6450$, $P < .0001$; Fig 4C, D, E, respectively). There were no significant correlations found between IgE to Ara h 8 and 9 and whole peanut-specific IgE (data not shown). With only 2 negative reactions, the differentiation test for peanut components was not conducted.

Basophil Activation Is Positively Correlated with DBPCFC Severity Scores

To investigate whether the BAT could distinguish subjects with positive from those with negative DBPCFC reactions, levels of basophil activation from the 2 populations were compared under in vitro stimulation with 3 doses of allergen. It is very important to determine the optimal concentrations that provoke the maximum cellular activation for each allergen.¹⁸ Therefore, 3 concentrations were used for each allergen to challenge in vitro the basophils from patients with allergy. As shown in Figure 5, subjects with positive reactions had significantly higher levels of basophil activation than

those with negative reactions (200 ng/mL, $P < .0001$, Fig 5A; 2 ng/mL, $P < .001$, Fig 5B; 0.02 ng/mL, $P = .009$, Fig 5C).

The BAT levels were significantly correlated with DBPCFC severity scores at 200 ng/mL of allergen at in vitro stimulation ($r = 0.50$, $P < .0001$; Fig 5D), whereas BAT levels were weakly correlated with severity scores at 2 ng/mL ($r = 0.35$, $P = .0055$; Fig 5E) and 0.02 ng/mL ($r = 0.32$, $P = .01$; Fig 5F).

BAT at Highest Dose Was the Best Predictor of Clinical Reactivity at DBPCFC

Receiver operating characteristic curves were used to compare the performance of SPT wheal diameter, sIgE, and BAT in predicting challenge outcomes (Fig 6). The AUC indicated how well a test method could distinguish between 2 diagnostic groups (allergy vs non-allergy). The AUC for Ara h 2 IgE levels was not calculated owing to the smaller number of subjects who underwent peanut challenges ($n = 44$). The BAT at the highest dose (200 ng/mL [$n = 71$], AUC 0.904, 95% confidence interval 0.821–0.98, $P < .001$) had the largest AUC compared with sIgE (AUC 0.87, 95% confidence interval 0.779–0.961, $P < .001$) and SPT (AUC 0.765, 95% confidence interval 0.635–0.877, $P < .01$). The BAT at 200 ng/mL had the highest overall accuracy and best diagnostic performance. Although the SPT is used routinely to predict a patient's allergic status, measurement of the SPT had a much lower predictive power than the BAT. Measurement of sIgE had a similar predictive power as the BAT but a greater predictive power than the SPT.

Discussion

Currently, the gold standard for food allergy diagnosis is the DBPCFC.² This procedure is time and labor intensive and carries the risk of severe allergic reactions. Unfortunately, there are no reliable biological markers for clinical reactivity that can replace DBPCFC at this time. Although SPTs and measurement of food-specific IgE levels are not predictive of clinical symptom severity,² recent data have suggested that component-resolved diagnostics could be a better predictor of clinical reactivity and could have some utility in predicting severity of symptoms.^{6,7} The BAT also has been explored as a novel diagnostic tool for food allergies, and early results have indicated the BAT can enhance discrimination between allergic and nonallergic individuals in a small group of young children with peanut allergies.¹⁹

In this study, the utility of SPT, sIgE, sIgG4, sIgE/IgG4 ratio, sIgE/tIgE ratio, component-resolved diagnostics (for peanut in the subset of subjects with peanut allergy), and the BAT was compared for predicting the outcome of DBPCFC and severity of symptoms at challenge for various allergens, including peanut, tree nuts, sesame, fish, and shrimp. Significantly larger SPT reactions and higher sIgE levels were noted in those with positive DBPCFC reactions

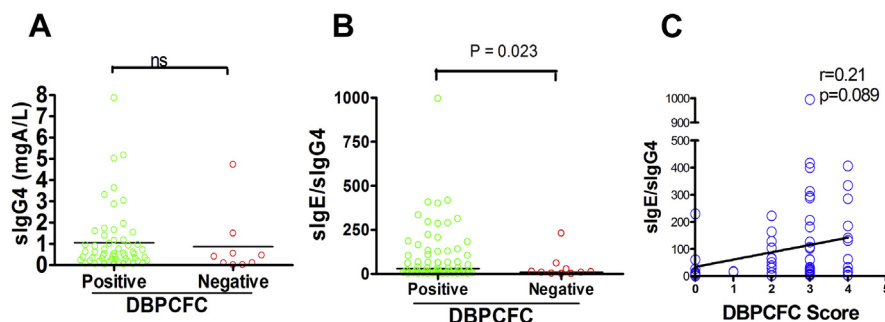


Figure 3. (A) Whole allergen extract-specific IgG4 (sIgG4) levels in subjects with positive and negative double-blinded, placebo-controlled food challenge reactions. (B) Ratios of whole allergen extract-specific IgE vs IgG4 (sIgE/sIgG4) in subjects with positive and negative double-blinded, placebo-controlled food challenge reactions. Horizontal bar indicates the mean. (C) Correlation between ratios of whole allergen extract-specific IgE vs IgG4 and double-blinded, placebo-controlled food challenge severity scores ($n = 71$).

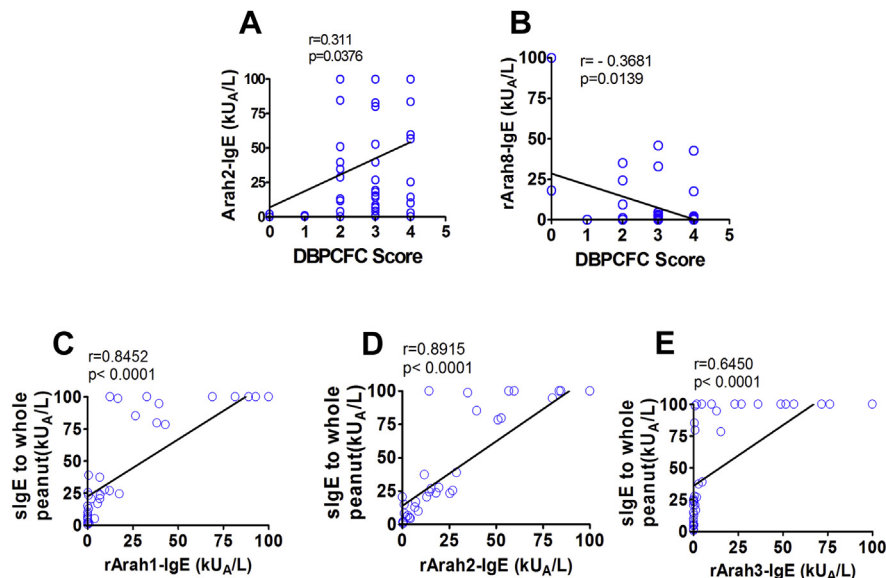


Figure 4. Correlation between (A) Ara h 2 IgE levels, (B) Ara h 8 IgE levels and double-blinded, placebo-controlled food challenge severity scores. Correlation between (C) Ara h 1 IgE levels or (D) Ara h 2 IgE levels or (E) Ara h 3 and whole peanut-specific IgE ($n = 44$).

compared with those with negative DBPCFC reactions, supporting the utility of the SPT and sIgE in predicting the presence or absence of food allergy. There were weak or negligible correlations between the SPT and sIgE with DBPCFC severity scores. Furthermore, sIgE alone was more strongly correlated with clinical reactivity than was the sIgE/IgG4 or sIgE/tIgE ratio. This difference has been noted in prior studies,¹⁰ suggesting that IgG4 levels might be more indicative of recent allergen exposure, rather than contributing to the severity of a patient's reactivity to the allergen.

Several studies have demonstrated that component testing increases the specificity of peanut allergy testing. IgE sensitization to Ara h 2 has been associated with severe symptoms at exposure to peanut, whereas isolated Ara h 8 sensitization has been associated with no or mild symptoms.^{8,20} Because most DBPCFCs were for

peanut (63%), peanut component testing was performed for this subset. Consistent with published reports,^{8,20} the present results show that detectable IgE to Ara h 2 was positively correlated with DBPCFC severity scores, whereas detectable IgE to Ara h 8 was negatively correlated with DBPCFC severity scores. Although the utility of peanut-specific IgE for predicting DBPCFC outcome was similar to that reported in other studies, in the present study, in contrast to other reports, whole peanut-specific IgE (Fig 2C and 4A) was a better predictor of challenge outcome than IgE to Ara h 2.^{6,21,22} This difference might be due to different subject selection criteria and the age groups included in different studies. The present study included adolescents and adults (12–45 years old), reflecting persistent food allergy. The study by Dang et al⁶ focused on infants. The study by Eller and Bindslev-Jensen²¹ included

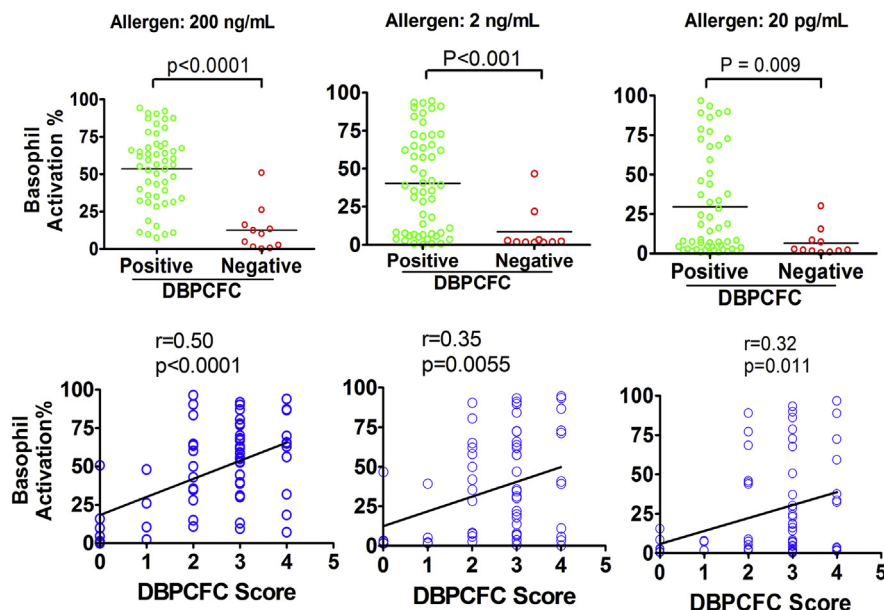


Figure 5. Basophil activation test levels with 3 dilutions of multiple allergens in subjects with positive challenge and negative double-blinded, placebo-controlled food challenge reactions. Correlations between basophil activation test levels and double-blinded, placebo-controlled food challenge severity scores ($n = 71$). Horizontal bar indicates the mean.

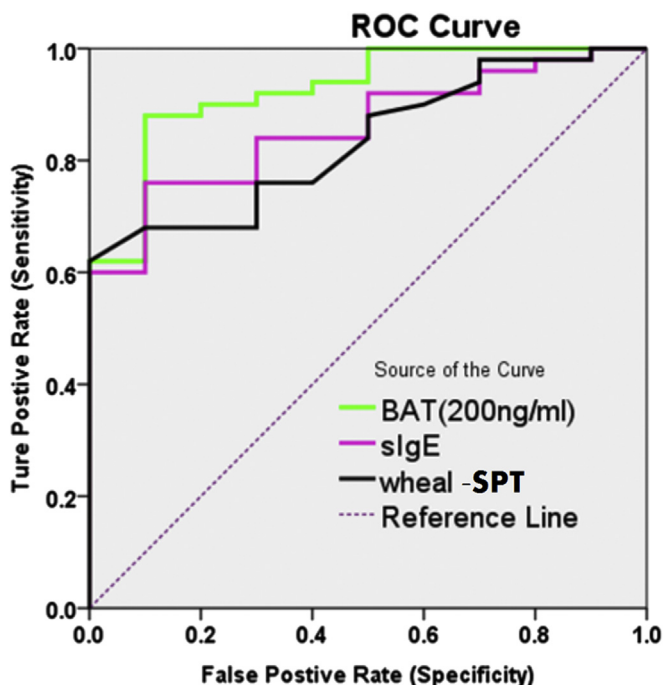


Figure 6. Comparison of basophil reactivity, allergen-specific IgE levels, and wheal size of skin prick test result in predicting the outcome of double-blinded, placebo-controlled food challenges. Receiver operating characteristic curves comparing basophil reactivity, allergen-specific IgE levels, and wheal size. Basophil reactivity, allergen-specific IgE levels, and wheal size were measured in 69 subjects with 71 challenges. The basophil activation test used 200 ng/mL of allergen.

subjects 2 to 25 years old and showed that peanut-specific IgE levels in the older age group were better correlated with severity of clinical symptoms. Further study is required to confirm this possibility.

In the present study, basophil activation measured by the BAT was an accurate predictor of clinical reactivity to food allergens and was significantly correlated with DBPCFC severity scores for multiple allergens, including peanut, tree nuts, sesame, fish, and shrimp. The BAT results were dose dependent; stimulation with 200 ng/mL of allergen had the most significant correlation not only with outcome of DBPCFC but also with the severity of symptoms. Based on data from the authors' group and previous publications,^{10,23} BATs could be an important addition to the tools available for the diagnosis of food allergy.

The BAT is an *in vitro* study that closely reflects the IgE-mediated pathophysiology of food allergy. The moderate correlation with clinical reactivity observed in this study is in line with results from prior studies demonstrating the sensitivity of the BAT.^{24,25} This correlation is not unexpected because IgE has been demonstrated to have a role in regulating basophil responses by regulating FcεRIα expression.²⁶ CD63 detection is highly specific for IgE-mediated basophil activation.⁹ In resting basophils, CD63 is anchored to the intracellular granules and is only barely detectable on the surface membrane in healthy subjects and in allergic patients. At challenge with specific allergens that cross-link FcεRI and induce granule exocytosis, CD63 becomes highly expressed at the activated basophil cell surface. CD63 expression has been noted to correlate with histamine release during anaphylaxis²⁷; thus detection of CD63 by flow cytometry could be a useful biomarker for predicting food challenge outcomes and severity.

A comparison of the various diagnostic techniques was performed using receiver operating characteristic curves. In the present study, the BAT was the best predictor of clinical reactivity as

determined by the DBPCFC reactions. Notably, the AUCs for the BAT in the present study were larger than those found in prior BAT studies on cow milk allergy.¹⁰ This is likely due to the selected nature of the present study population, which was selected to have a positive reaction to peanut. The improved performance of the BAT in this study might be due to the fact that peanuts and tree nuts normally have higher basophil activation levels than other allergens, leading to a greater range of reactivity and accuracy.^{9–13} These data provide further support that the BAT can provide valuable clinical information for food allergy.

The BATs have been shown to be an important addition to the tools available for the diagnosis of food allergy. However, use of the BAT in routine practice remains limited. This is perhaps due to the technical difficulties of the BAT because basophils are fragile and have short life spans.²⁸ It is also relatively expensive. Perhaps further studies will provide support for the use of the BAT in routine allergy practice. Furthermore, future directions for research using the BAT should be focused on an expansion of the understanding of basophil differentiation, maturation, migration, adhesion, survival, and releasability and the mechanisms underlying food allergy. Increased knowledge of the *in vivo* role of basophils and the stages of their growth and activation will complement the current knowledge on allergy testing and diagnosis. The present study represents a clinically oriented approach to research on basophils, with the ultimate goal of introducing a novel test into routine allergy diagnosis to improve patient outcomes and quality of life.

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