

Sensitivity, specificity, and predictive value of oral mucosal brush biopsy for the diagnosis of peanut allergy

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Background: Current diagnostic testing methods for peanut allergy, including serum specific immunoglobulin E (sIgE) and skin-prick testing (SPT), have low specificity, whereas oral food challenge (OFC) carries significant risk of provoking adverse events. Mucosal brush biopsy (MBB) from the oral cavity is currently being studied as a new diagnostic test for peanut allergy, but normative data is not yet available with which to begin measuring specificity and predictive value.

Methods: Twenty individuals with no history of adverse reactions from eating peanuts underwent oral MBB and serum testing for peanut sIgE. These data were then compared with previously published data from 10 individuals with a history of clinical peanut allergy, in order to generate receiver operating characteristic (ROC) curves and calculate the sensitivity, specificity, and positive and negative predictive values for both testing modalities.

Results: The optimal cutoff levels for oral MBB and sIgE testing were 0.12 kU/L and 1.0 kU/L, respectively. At 0.12 kU/L, the sensitivity of oral MBB testing was 80% and

the specificity was 85%, whereas at 1.0 kU/L, the sensitivity of sIgE testing was 50% and the specificity was 100%. From the ROC curves, the areas under the ROC curve (AUC) for oral MBB and sIgE were 0.91 ($p < 0.001$) and 0.74 ($p = 0.007$), respectively. Combination testing further increased both sensitivity and accuracy over oral MBB alone.

Conclusion: In this pilot study, oral MBB demonstrated high sensitivity and specificity for screening individuals with and without oral cavity clinical reactivity to peanuts and may represent a potentially useful testing method for the diagnosis of peanut allergy in the future. © 2019 ARS-AAOA, LLC.

Key Words:

oral mucosal brush biopsy; peanut; diagnosis; food allergy; local IgE

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Peanut allergy is 1 of the most common, and potentially fatal, food allergies. In 1997, a randomized telephone survey found that approximately 0.6% of the general U.S. population reported having peanut allergy.¹ However, data collected in 2016 now suggests that the rate of peanut

allergy in children alone has increased to 2.2%.² Problems related to peanut allergy include the severe consequences of anaphylaxis, difficulty avoiding this ubiquitous food item and the high likelihood for persistence throughout a person's lifetime.³⁻⁶ Individuals with peanut allergy, along with their caregivers, experience a decrease in quality of life and higher anxiety scores, negatively impacting their enjoyment of social activities and psychosocial development.³⁻⁶

Currently, the 3 most common methods of peanut allergy testing are serum specific immunoglobulin E testing (sIgE), skin-prick testing (SPT), and oral food challenge (OFC). The main drawback of sIgE and SPT tests is lower specificity despite higher sensitivity. One study found that sIgE and SPT have sensitivities of 75% and 86%, respectively, yet only a specificity of 46% and 67%, respectively, and an accuracy of 61% and 75%.⁷ Sampson⁸ found that a sIgE level of 0.35 kU/L in peanuts resulted in only a 20% to 30% probability of a clinical reaction.

A positive test on sIgE or SPT is often followed up by a double-blind, placebo-controlled food challenge (DBPCFC)

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for confirmation. However, this exam is time-consuming, costly, and can lead to severe systemic reactions. In a retrospective study examining OFC in 584 individuals over a 7-year period, severe reactions were reported in 25% of cases, with 10% requiring epinephrine.⁹ Similar to sIgE measurement and SPT, there was poor correlation between self-reported reactions and the severity of reactions induced by DBPCFC, along with a 3% false-negative rate.^{10,11}

Previously, mucosal brush biopsy (MBB) from inferior turbinate mucosa has been used to detect locally-present IgE to airborne allergens in individuals with allergic rhinitis (AR), confirmed by positive SPT.¹² In this study, sIgE against at least 1 environmental allergen was detected in 75% of individuals. Locally-present sIgE was also more likely to be present in those individuals with larger wheal diameters. In another study using MBB from the nasal cavity, 100% of individuals diagnosed with nonallergic rhinitis (NAR), who previously tested negative on SPT and sIgE, tested positive on MBB for at least 1 environmental allergen.¹³

MBB of the oral cavity has also been studied in 10 individuals with a reported history of oral cavity itching/swelling when ingesting peanuts.¹⁴ Using a cutoff level of 0.10 kU/L, all 10 individuals were found to have sIgE to peanut in the oral cavity (100% sensitivity), whereas only 5 individuals were positive on serum sIgE testing (50% sensitivity). However, because there was no control group in this study, specificity and predictive value could not be determined. Therefore, the purpose of this study is to collect a control group of 20 individuals without reported reactivity to consuming peanuts and combine their oral MBB and serum sIgE data with the previously published data in order to calculate the specificity and predictive value for both testing modalities at various cutoff levels. The results of this study will help determine the feasibility of oral MBB for the diagnosis of peanut allergy, and guide the design of subsequent studies to evaluate this test in larger populations.

Subjects and methods

Study population

Approval for this study was obtained from the Institutional Review Board of Weill Cornell Medical College, New York City (Protocol #1206012451). The inclusion criteria included healthy individuals, 18 years of age or older, who were willing to participate in the study. These individuals were recruited from both the staff and patient populations of the Department of Otolaryngology–Head & Neck Surgery. Individuals with a self-reported history of chronic rhinitis (allergic or nonallergic), chronic rhinosinusitis (with or without nasal polyps), allergic fungal rhinosinusitis, asthma, eczema or immediate pruritus, or edema (local, distant, or systemic) after consuming any type of food product were excluded from the study. Individuals who were uncertain about their allergic status on initial interview were also excluded from participating in the study.

All testing and data collection was performed at a single institution, Weill Cornell Medical College, during the month of August 2014.

Oral MBB

For the oral MBB, a 7.75-inch cytology brush (Aeromed, Glastonbury, CT) was inserted along the gingivobuccal sulcus, rotated, and moved back and forth approximately 10 times as described.¹⁴ This procedure was performed in the upper left quadrant, lower left quadrant, upper right quadrant, and lower right quadrant using a separate brush for each side. After the procedure, the 2 brushes were rinsed in phosphate buffered saline (PBS) to extract mucus and epithelial cells and frozen immediately. All samples were collected by the same investigator (W.R.R.).

Measurement of total and peanut sIgE from oral MBB

The suspensions of oral mucosal cells from each participant were lysed using an ultrasonicator at 45% amplitude for 2 minutes at 15-second intervals (Branson Digital Sonifier, Danbury, CT). Up to 4 additional 15-second pulses were performed until the lysates appeared clear and Trypan Blue exclusion test demonstrated that no viable cells were present. Total protein concentrations for the lysates were determined using a bicinchoninic acid (BCA) assay (Thermo Fisher Scientific, Rockford, IL) and total IgE for each sample was corrected for total protein. Each lysate was sent frozen to a reference laboratory for immunofluorescent assay to determine total IgE levels as well as IgE levels specific to peanut (PiRL Laboratories, Portage, MI). Total IgE levels were standardized for total protein (corrected total IgE). Samples were run in triplicate and cutoff levels chosen for oral MBB were 0.05, 0.1, 0.11, 0.12, 0.13, 0.15, 0.17, 0.2, 0.26, and 0.28 kU/L.

Measurement of total IgE and peanut sIgE

Venipuncture was also performed on all participants, and serum samples were sent frozen to a reference laboratory for immunofluorescent assay to determine total IgE levels and IgE levels specific to peanut (PiRL Laboratories, Portage, MI). Samples were run in triplicate and the cutoff levels chosen for sIgE were 0.05, 0.1, 0.35, 1.0, 2.0, 3.0, 7.0, and 8.0 kU/L.

Combination of Oral MBB and sIgE cutoff data

Based on the optimal cutoff levels for both oral MBB and sIgE, a dual criterion was applied to combine both of these tests. The participant was considered to have a positive test whenever they tested positive for either oral MBB or sIgE, using the 0.12-kU/L and 1.0-kU/L cutoff levels, respectively, whereas a negative test was defined as both tests being negative.

Statistical analysis

Total IgE data are presented as mean values $\pm$ standard error of the mean (SEM). The nonparametric Mann-Whitney *U* test was used to compare total corrected IgE levels between the study population and the peanut-sensitive comparison group, with respect to both oral MBB as well as sIgE. Fisher's exact probability testing was used to test the associations between a positive test and the presence of clinical reactivity to consuming peanuts, for both oral MBB and sIgE. Two-tailed *p* values were calculated using online statistical software and a value of *p* < 0.05 was considered statistically significant.¹⁵ Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and the percent correctly classified were calculated for each cutoff level. A test was counted as correctly classified if it represented either a true positive or a true negative result. Receiver operator characteristic (ROC) curves were created using Stata 15 software (StataCorp, LLC, College Station, TX), using the nonparametric ROC curve function to compare IgE levels detected via oral MBB and serum sIgE at the various cutoff levels.¹⁶

Results

Study population

All of the participants tolerated the oral MBB and serum sIgE testing well with no adverse events. Of the 20 participants, 7 (35%) were male and 13 (65%) were female, with a mean age of 29.2 years (range, 19 to 54 years). This is similar to the population demographics from the peanut-sensitive comparison group previously reported, which had 3 (30%) males and 7 (70%) females, with a mean age of 37.7 years (range, 18 to 53 years).¹⁴

Oral MBB peanut IgE and total corrected IgE levels

Ten out of 20 non-peanut-allergic participants (50%) had detectable IgE to peanut on MBB testing (mean 0.12 kU/L; range, 0.1 to 0.19 kU/L). Table 1 lists the sensitivity, specificity, PPV, NPV, and percent correct classified at each cutoff point. To achieve the highest percent correctly classified, the optimal IgE cutoff level for oral MBB was 0.12 kU/L. The mean total corrected IgE from the oral MBB samples of the study population was 2.16 ± 0.11 kU/ μ g, which was significantly lower than the mean total corrected IgE from the peanut-sensitive comparison group, which was 4.58 ± 0.35 kU/ μ g (*p* < 0.0001).¹⁴

Serum sIgE and total IgE

One non-peanut-allergic individual (0.05%) was positive for peanut-specific IgE on serum testing, and that positive level was 0.77 kU/L. Table 2 contains the sensitivity, specificity, PPV, NPV, and percent correctly classified at each cutoff point. To achieve the highest percent correctly classified, the optimal IgE cutoff level for sIgE was 1.0 kU/L. The mean total IgE from the serum

TABLE 1. SN, SP, PPV, NPV, and correct classification rate using several cutoff levels for oral MBB testing in peanut-allergic and non-peanut-allergic individuals

Cutoff level (kU/L)	SN (%)	SP (%)	PPV (%)	NPV (%)	Correctly classified (%)	<i>p</i> ^a
0.05	100	0	33	–	33	1.0
0.1	100	50	50	100	67	0.01
0.11	100	55	53	100	70	0.004
0.12 ^b	80	85	73	89	83	0.001
0.13	60	95	86	83	83	0.002
0.15	50	95	83	79	80	0.008
0.17	40	95	80	76	77	0.03
0.2	30	100	100	74	77	0.03
0.26	10	100	100	69	70	0.33
0.28	0	100	–	67	67	1.0

^aValues of *p* are based on Fisher's exact testing. Statistically significant values are in bold.

^bOptimal cutoff level.

MBB = mucosal brush biopsy; NPV = negative predictive value; PPV = positive predictive value; SN = sensitivity; SP = specificity.

TABLE 2. SN, SP, PPV, NPV, and correct classification rate using several cutoff levels for serum sIgE testing in peanut-allergic and non-peanut-allergic individuals

Cutoff level (kU/L)	SN (%)	SP (%)	PPV (%)	NPV (%)	Correctly classified (%)	<i>p</i> ^a
0.05	100	0	33	–	33	1.0
0.1	50	95	83	79	80	0.009
0.35	50	95	83	79	80	0.009
1.0 ^b	50	100	100	80	83	0.002
2.0	40	100	100	77	80	0.008
3.0	20	100	100	71	73	0.1
7.0	10	100	100	69	70	0.33
8.0	0	100	–	67	67	1.0

^aValues of *p* are based on Fisher's exact testing. Statistically significant values are in bold.

^bOptimal cutoff level.

NPV = negative predictive value; PPV = positive predictive value; sIgE = specific immunoglobulin E; SN = sensitivity; SP = specificity.

samples of this study population was 117.98 ± 26.38 kU/L, which was also significantly lower than the mean total IgE from the peanut-sensitive comparison group, which was 149.10 ± 47.15 kU/L (*p* = 0.02).¹⁴

ROC analysis

ROC curves are shown in Figures 1 and 2. For MBB, the area under the ROC curve (AUC) was 0.91 ± 0.05 (95% confidence interval [CI], 0.74 to 0.98; *p* < 0.0001),

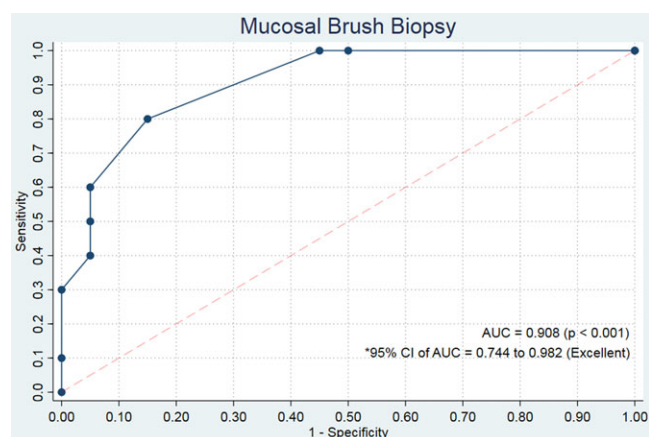


FIGURE 1. Receiver-operator curve for oral MBB testing in peanut-allergic and non-peanut-allergic individuals. MBB = mucosal brush biopsy.

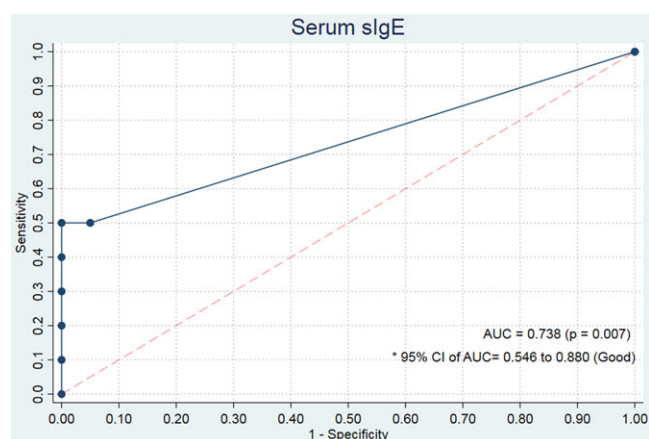


FIGURE 2. Receiver-operator curve for serum sIgE testing in peanut-allergic and non-peanut-allergic individuals. sIgE = specific immunoglobulin E.

categorized as “excellent.” For serum sIgE, the AUC was 0.74 ± 0.09 (95% CI, 0.55 to 0.88; $p = 0.007$), categorized as “good.”

Combination of oral MBB and sIgE cutoff data

Table 3 shows a comparison of sensitivity, specificity, PPV, NPV, and percent of participants correctly classified when the dual criterion was applied. The combination of oral MBB and sIgE data increased the sensitivity to 90%, and the percent correctly classified to 87%.

Discussion

This is the first study using normative data to estimate the specificity and predictive value of using oral MBB for detecting peanut allergy sensitization in a population of limited size. Oral MBB has an advantage over SPT or serum sIgE by measuring sIgE directly from the symptomatic area. Compared to previously reported data from other testing modalities, oral MBB at its optimal cutoff level demonstrated higher specificity and percent

TABLE 3. Comparison of SN, SP, and accuracy for peanut testing methods from prior studies with MBB and MBB+sIgE using the optimal cutoff level for each test

Method (study)	SN (%)	SP (%)	PPV (%)	NPV (%)	Correctly classified (%)
sIgE (DunnGalvin et al. ⁷)	75	46	–	–	61
SPT (DunnGalvin et al. ⁷)	86	67	–	–	75
sIgE+SPT (DunnGalvin et al. ⁷)	88	75	–	–	81
MBB (Reisacher and Cohen ¹⁴)	80	85	73	89	83
MBB+sIgE (Reisacher and Cohen ¹⁴)	90	85	75	94	87

MBB = mucosal brush biopsy; NPV = negative predictive value; PPV = positive predictive value; sIgE = specific immunoglobulin E; SN = sensitivity; SP = specificity; SPT = skin-prick test.

correctly classified than sIgE, SPT, or sIgE + SPT. Moreover, when oral MBB was combined with serum sIgE, no other testing modality or combination scored higher with respect to sensitivity, specificity, or percent correctly classified.

Generally, SPT and serum sIgE testing reported in the literature has higher sensitivity and lower specificity.⁷ However, for oral MBB and serum sIgE testing in our study population, the specificity is higher while the sensitivity is lower. This discrepancy may be due to the small size and high prevalence of peanut-allergic individuals in our total population of 30 individuals, as well as the strict inclusion criteria we used. The widespread use of allergy testing in the general population may result in testing for certain allergens, such as peanut, even when there is not a high suspicion for that particular allergen. Subclinical sensitization to peanuts may result in a high false-positive rate, and subsequently low specificity. On the other hand, we found a high false-negative rate, and thus low sensitivity, at many of the higher cutoff points because our study population had an artificially high rate of clinical peanut reactivity (33%) compared with the general population.

The size of our normative study population was limited to 20 because of the exploratory nature of the study and to more closely mirror the size of the comparison population with clinical peanut allergy. The results of this pilot study justify further research on oral MBB as a screening test for peanut allergy and even suggest optimal cutoff points. MBB should ideally be evaluated in a large, representative population in which testing is obtained based primarily on clinical suspicion. The population in this study, as well as the participants in the peanut-allergic comparison group, were comprised of adults only, so future studies

must also include children and adolescents. Different food allergens, as well as non-food allergens, should be evaluated separately, because the optimal cutoff levels might be different for each. The effects of coexisting sensitivity to cross-reacting airborne allergens on sensitivity and specificity should also be examined. The combination of oral MBB with serum sIgE seems to be a promising protocol for diagnosis, but the added benefit must be evaluated against the increased cost of performing 2 separate tests instead of 1.

Future applications for oral MBB, using the information gained in this study, include stratifying the risk and predicting the outcome for OFC testing. MBB can also be used to study locally-present IgE in other areas of the aerodigestive tract, such as the sinonasal cavity, esophagus, intestines, and colon. Finally, oral MBB may be used to investigate ongoing topics related to peanut-allergy testing research, such as the lack of association between sIgE, SPT, and allergic reaction severity, the challenges in determining who

will outgrow their peanut allergy, and who might develop a recurrence of symptoms as an adult.^{17–22}

Conclusion

This pilot study shows the feasibility of oral MBB as a screening tool for diagnosing peanut allergy. It compared well to SPT and serum sIgE testing, the current standards of peanut-allergy testing, with higher specificity and percent correctly classified. This information supports the need for continued investigation of this method of testing in a larger population of peanut-allergic and peanut-non-allergic individuals to further refine its sensitivity, specificity, and predictive value. 

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