

# Mucosal brush biopsy testing of the inferior turbinate to detect local, antigen-specific immunoglobulin E

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**Background:** This study investigated the ability of mucosal brush biopsy testing (MBBT) from the inferior turbinate to detect local, antigen-specific immunoglobulin E (IgE) in patients with allergic rhinitis, and these results were then compared to skin prick testing (SPT).

**Methods:** A standard cytology brush was used to harvest epithelial cells from the inferior turbinates of 20 patients who tested positive to 2 or more airborne antigens out of a panel of 9 on SPT (N = 20). These cells were lysed and the resulting solution was tested for total and antigen-specific IgE using immunofluorescence.

**Results:** Antigen-specific IgE for at least 1 antigen was detected in 15 (75%) patients on MBBT. A statistically significant association overall between the results of MBBT and SPT was present ( $p = 0.01$ ), but no significant associations were seen when examining individual antigens. Patients with positive tests on MBBT had higher amounts of IgE and total protein in biopsy samples than patients without positive tests on MBBT. The mean wheal diameter in

tests that were positive on MBBT were significantly larger than the mean wheal diameter in tests that were negative on MBBT ( $p < .0001$ ). Eight tests were positive on MBBT that were negative on SPT in the same patients.

**Conclusion:** This study demonstrates for the first time that antigen-specific IgE can be detected using MBBT in the sinonasal cavity. MBBT is more likely to be positive in higher SPT grades and in biopsy samples with more protein present. MBBT is also able to detect local IgE that is not detected on SPT. © 2011 ARS-AAOA, LLC.

## Key Words:

allergy testing; brush biopsy; epithelium; antigen; inferior turbinate; IgE

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Since the early 1970s, it has been recognized that antigen-specific immunoglobulin E (IgE) is present in the nasal secretions of patients with allergic rhinitis who are challenged with that antigen.<sup>1,2</sup> KleinJan et al.<sup>3</sup> demonstrated that antigen-specific IgE is produced locally in the sinonasal mucosa of patients with seasonal and perennial allergic rhinitis, but not in nonallergic patients. It has also been shown that locally-produced IgE can be present in nasal

secretions even though skin testing<sup>4</sup> and in vitro testing<sup>5</sup> are negative, and could potentially cause an inflammatory reaction in a specific part of the airway, without affecting the rest of the body. Ohashi et al.<sup>6</sup> demonstrated elevated IgE levels in homogenized nasal mucosa from 12 patients with symptoms suggestive of allergy who were negative on skin and radioallergosorbent (RAST) testing.

Recent evidence suggests that local IgE may also have an important role in other respiratory diseases, such as chronic rhinosinusitis with nasal polyps (CRSwNP), allergic fungal rhinosinusitis (AFRS), and asthma. In patients with CRS, the levels of *Alternaria*-specific IgE have been found to be significantly higher in nasal polyp tissue than in nonpolyp tissue, and 60% of CRS patients with nasal polyps had evidence of local IgE without evidence of *Alternaria*-specific IgE in the serum.<sup>7,8</sup> Wise et al.<sup>9</sup> demonstrated increased production of both fungal and nonfungal IgE in the sinus mucosa of patients with AFRS compared to controls. Antigen-specific IgE has also been recovered from bronchoalveolar lavage fluid in patients with asthma after antigen challenge.<sup>10</sup>

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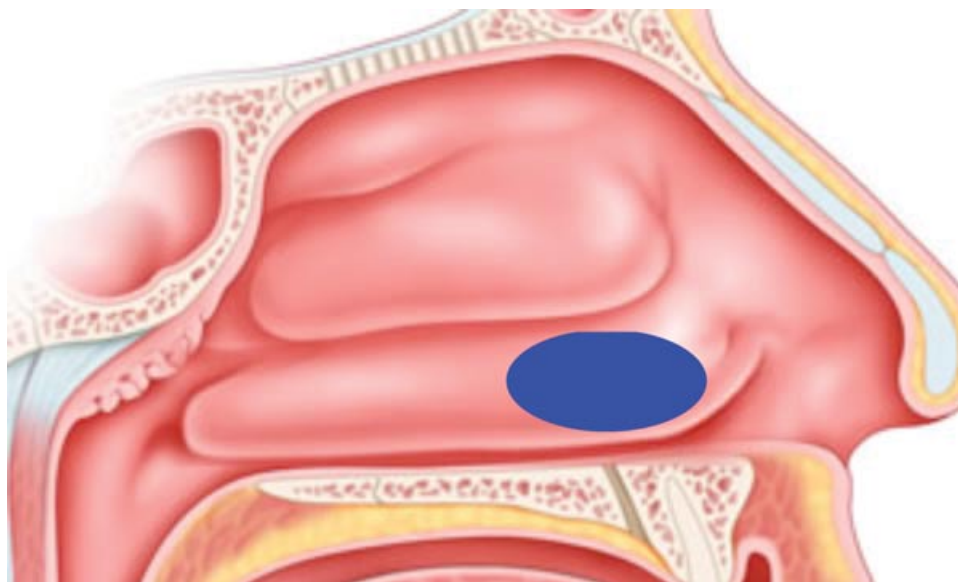


**FIGURE 1.** Region of the inferior turbinate where MBBT was performed. MBBT = mucosal brush biopsy testing.

Up until the present time, methods to test for local IgE in the nose have been limited to nasal lavage<sup>1</sup> and surgical biopsy of sinonasal tissue.<sup>11</sup> However, nasal lavage is a difficult test to perform in the office setting, particularly in pediatric patients. It is also uncomfortable for the patient and has a high rate of technical variability, which limits the interpretation of the results. In addition, nasal lavage specimens can only detect IgE that is present in the mucus, but not IgE that is present in the epithelium of the sinuses as well as the inferior turbinates.<sup>11</sup> Surgical biopsy will detect epithelial IgE, but this testing is invasive and not practical to perform in the office setting solely for the purpose of allergy testing.

The purpose of this study is to investigate the feasibility of mucosal brush biopsy testing (MBBT) to detect local, antigen-specific IgE from the inferior turbinate of adult patients with allergic rhinitis who test positive to airborne antigens on standard skin prick testing (SPT). Brush biopsy testing, which is able to harvest epithelial cells using a standard cytology brush, has been used inside the nose for the study of ciliary ultrastructure<sup>12</sup> and for viral culture,<sup>13</sup> but it has never been used for this purpose. The primary hypothesis for this study is that antigen-specific IgE will be detectable in brush biopsy specimens. A secondary hypothesis is that there will be a correlation between positive reactions on MBBT and SPT.

## Patients and methods

### Patient sample

Approval for this study was obtained from the Institutional Review Board of Weill Cornell Medical College, New York City (Protocol #0910010694). Inclusion criteria for the study included a clinical history of allergic rhinitis, age 18 years or greater, and positive SPT (grade 1 or

higher) for at least 2 of 9 screening antigens: white oak, Timothy grass, ragweed, cat, dog, cockroach, *Alternaria*, *Aspergillus*, and *D. farinae*. Patients with a history of allergy immunotherapy were excluded from the study. Patients were counseled about the research protocol and informed consent was obtained prior to enrollment. All testing was performed at a single institution, Weill Cornell Medical College.

### MBBT

Prior to MBBT, the nasal cavity was decongested and anesthetized with topical 2% lidocaine and 1% phenylephrine. After waiting 5 minutes, a 7.75-inch cytology brush (Aeromed, Glastonbury, CT) was inserted along the medial surface of the anterior third of the inferior turbinate (Fig. 1). While avoiding direct pressure on the septum, the brush was rotated and moved back and forth approximately 10 times. After the procedure was repeated on the opposite side with a fresh brush, the brushes were rinsed in phosphate buffered saline (PBS) to extract mucus and epithelial cells.

### Measurement of IgE and total protein

The suspensions of epithelial cells were stored in a sterile, polypropylene tube and frozen immediately after harvest. After thawing, the cells 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 concentration for the lysates was determined using a BCA assay (Thermo Scientific, Rockford, IL). One-milliliter samples of each lysate were then sent frozen to a reference laboratory (IBT

Laboratories, Lenexa, KS) for ImmunoCAP (Phadia AB, Portage, MI) analysis to determine total IgE levels as well as antigen-specific IgE levels for the 9 study antigens.

Statistical analysis

Data are presented as mean values ± standard error of the mean (SEM). The mean wheal diameters for SPT corresponding with a positive vs negative MBBT were compared with Student *t* test using online statistical software.<sup>14</sup> After performing an F-test to determine the significance of the difference between the variances of the 2 samples, a 2-tailed *p* value was calculated, with *p* < 0.05 considered statistically significant. Similar comparisons were made between patients who had positive MBBT vs patients with no positive reactions on MBBT for mean total IgE, mean corrected IgE (total IgE divided by total protein) and mean total protein.

Chi-square analysis was performed to test the association of all positive reactions on SPT vs all positive reactions on MBBT. The *p* value as well as phi coefficient were calculated using the same software as previously cited. In order to test the strength of association for each antigen, Fisher’s exact testing was performed because of the small sample size and 2-tailed *p* values were calculated.

Results

Skin prick testing

Twenty patients were enrolled in the study. Twelve patients were male and 8 patients were female. The mean age was 33.4 years (range, 24–54 years) and the mean number of positive antigens on SPT was 4.7 (range, 2–7). Wheal diameter, in millimeters, for positive tests was recorded. A wheal diameter of 4 mm defined a grade 1 reaction, 5 to 6 mm defined a grade 2 reaction, 7 to 9 mm defined a grade 3 reaction, and wheal diameter ≥10 mm or pseudopodia of any size defined a grade 4 reaction. Pseudopodia were assigned a wheal size of 13 mm and negative tests were assigned a wheal size of 0 mm. Out of 180 tests, there were 93 positive reactions on SPT: 10 were grade 1, 32 were grade 2, 33 were grade 3, and 18 were grade 4.

Tolerance of MBBT

Patients reported mild to moderate discomfort from the procedure, but all tolerated it well. On visual inspection, there was mild erythema at the brush biopsy sites. No immediate complications occurred from the biopsy. On the day after biopsy, 1 patient experienced a brief episode of epistaxis, not requiring packing or cauterization, and another patient reported mild upper respiratory infection (URI) symptoms. No other adverse events were reported.

Antigen-specific IgE, total IgE, and total protein

Of all 20 patients, 15 (75%) had positive reactions of class 1 or higher (greater than or equal to 0.35 kU/L) to 1 or more antigens on MBBT, while 5 had no positive reactions

TABLE 1. Distribution of positive reactions on SPT and MBBT for each antigen, and Fisher’s exact testing for association between positive SPT and positive MBBT

| Antigen (n = 20 for each) | SPT positive | MBBT positive | <i>p</i> |
|---------------------------|--------------|---------------|----------|
| White oak                 | 15           | 4             | 0.53     |
| Timothy grass             | 15           | 5             | 1        |
| Ragweed                   | 12           | 3             | 0.24     |
| Cat                       | 11           | 7             | 0.37     |
| Dog                       | 1            | 4             | 1        |
| Cockroach                 | 12           | 0             | 1        |
| <i>Alternaria</i>         | 8            | 2             | 0.15     |
| <i>Aspergillus</i>        | 5            | 0             | 1        |
| <i>D. farinae</i>         | 14           | 6             | 0.61     |
| Total                     | 93           | 31            | –        |

Values of *p* are based on 2-tailed *t* test. Statistically significant values are in bold. MBBT = mucosal brush biopsy testing; SPT = skin prick testing.

on MBBT. The mean number of positive reactions for those patients with positive reactions were 2.07 (range, 1–5). Out of 180 tests, there were 31 positive reactions on MBBT: 14 were class 1 (0.35–0.70 kU/L), 15 were class 2 (0.71–3.50 kU/L), and 2 were class 3 (3.51–17.50 kU/L). No class 4 reactions (greater than 17.51 kU/L) were detected. The distribution of positive antigens for both SPT and MBBT is seen in Table 1.

Total IgE for each patient ranged from 3 to 40 IU/mL. Total protein harvested from each patient ranged from 1.21 to 2.74 mg/mL. The comparison of mean total IgE, mean corrected IgE, and mean total protein between the patients with positive reactions on MBBT vs the patients without any positive reactions on MBBT is presented in Table 2. Both total and corrected IgE was significantly higher in the positive MBBT group. Although total protein was fairly consistent from each patient, there were significantly higher amounts detected in patients who tested positive on MBBT.

Impact of SPT grade on MBBT

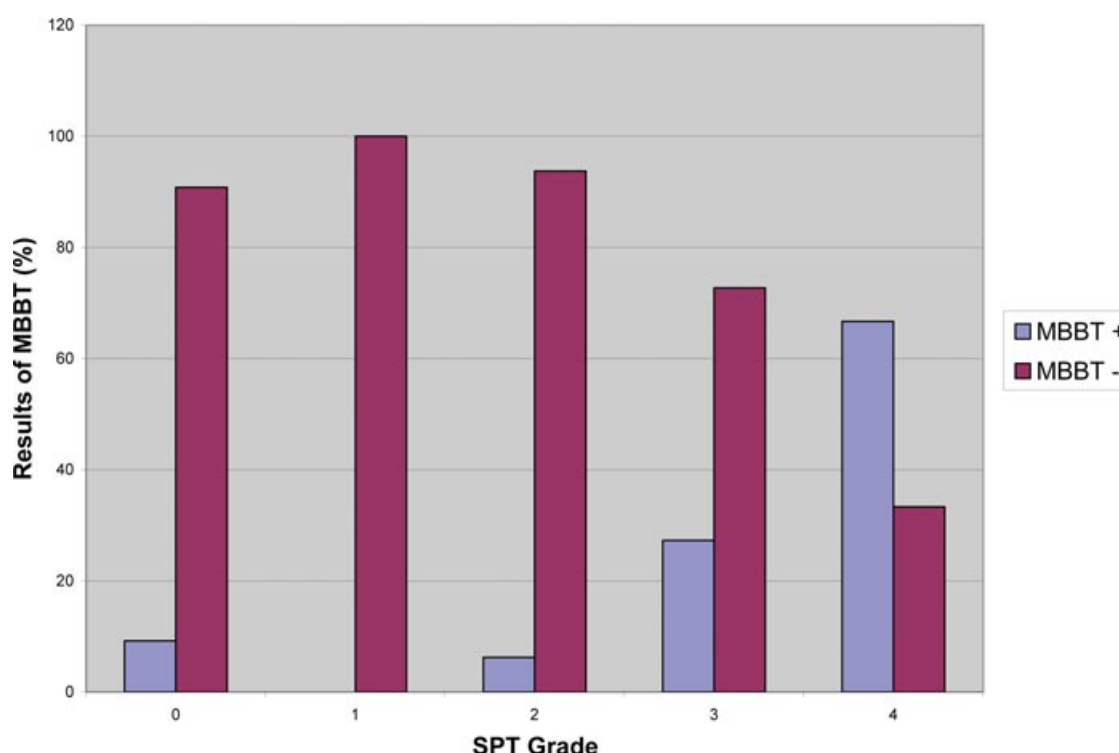
The mean SPT wheal diameter for negative reactions on MBBT was 2.99 ± 0.29 mm, while the mean SPT wheal diameter for positive reactions on MBBT was 7.5 ± 0.94 mm (*p* < 0.0001). The distribution of positive vs negative MBBT based on the grade of SPT is presented in Figure 2. When SPT grade was 1, there were no positive tests on MBBT. However, as the SPT grade increased, the ratio of positive to negative reactions on MBBT increased until, for grade 4 reactions on SPT, MBBT was more likely to be positive than negative. Of note, there were 8 positive tests on MBBT that were negative (grade 0) on SPT for the same patients: 4 were dog, 2 were cat, 1 was *D. farinae*, and 1 was Timothy grass.

**TABLE 2.** Mean total IgE, mean IgE corrected for total protein, and mean total protein in MBBT specimens from patients with positive results on MBBT vs patients with no MBBT positivity

|                       | MBBT + (n = 15)       | MBBT – (n = 5)        | p            |
|-----------------------|-----------------------|-----------------------|--------------|
| Total IgE (IU/mL)     | 13.6 ± 2.79 (4–40)    | 4.6 ± 1.03 (3–8)      | <b>0.007</b> |
| Corrected IgE (IU/mg) | 7.23 ± 1.7 (1.9–20.4) | 3.13 ± 0.56 (2.1–4.6) | <b>0.013</b> |
| Total protein (mg/mL) | 1.89 ± 0.1 (1.3–2.7)  | 1.44 ± 0.1 (1.2–1.8)  | <b>0.02</b>  |

Values of *p* are based on 2-tailed *t* test. Statistically significant values are in bold. All other values are mean ± SEM (range).

IgE = immunoglobulin E; MBBT = mucosal brush biopsy testing; SPT = skin prick testing.

**FIGURE 2.** Percentages of positive and negative MBBT results based on SPT grade. MBBT = mucosal brush biopsy testing; SPT = skin prick testing.

### Tests of association between SPT and MBBT

Chi-square testing demonstrated an overall significant association between positive reactions on SPT vs positive reactions on MBBT ( $p = 0.01$ ) with a phi coefficient of 0.21, which indicated a weak association. The results of Fisher's exact testing are presented in Table 1. None of the antigens tested individually demonstrated a significant association between the results of SPT and MBBT.

### Discussion

This study demonstrates, for the first time, that MBBT is able to detect antigen-specific IgE from the inferior turbinate epithelium of patients with allergic rhinitis. Based on probability analysis, there is a positive association between the results of MBBT and SPT, which was likely driven by the high number of tests that were both negative on SPT as well as MBBT. A positive association was not seen when individual antigens were examined separately. To-

tal protein harvested from MBBT of the inferior turbinates was very consistent among the samples (Table 2), which were all deemed adequate for analysis. Levels of total and antigen-specific IgE were more variable, which were felt to reflect actual differences in antibody levels rather than just the amount of tissue harvested.

Antigen-specific IgE for each individual antigen is detected by MBBT with a frequency of approximately 25% to 50% that of SPT. However, no IgE specific to cockroach or *Aspergillus* is detected in any of the MBBT samples. Of note, IgE to dog dander was detected in 4 MBBT samples compared to only 1 positive test for dog dander on SPT. Overall, SPT was 3 times more likely to detect antigen-specific IgE compared to MBBT. It is possible that this is due to the low amount of IgE which can be harvested by MBBT. However, it is also possible that the results of MBBT reflect the antigens most responsible for nasal symptoms in the patient suffering from allergic rhinitis, and this should be examined in future studies.

It is also interesting to note that for 8 tests, antigen-specific IgE was detected on MBBT, but not on SPT in the same patients: 4 for dog dander, 2 for cat dander, 1 for *D. farinae*, and 1 for Timothy grass. The significance of this is unknown, but reinforces the idea that local IgE can be present in the sinonasal cavity without systemic evidence. SPT is a reliable method to detect sensitization to airborne allergens, but the site of testing is distant from the region of initial sensitization and subsequent symptom production. If MBBT is able to measure local sensitization, which SPT is not able to do, then the results of MBBT might clarify the diagnosis for patients with clinical allergic rhinitis who are placed into the vast category of "nonallergic rhinitis" based solely on negative skin and blood testing.<sup>15</sup>

Two of the factors associated with a higher rate of positivity on MBBT are high sensitivity to the antigen, as represented by higher SPT grade, and larger amounts of IgE harvested. A major concern for MBBT is the ability to harvest sufficient IgE in order to perform a meaningful analysis. In this study, the mean total IgE (corrected for total protein in the biopsy specimens) is 7230 mIU/mg in the patients who had at least 1 positive test on MBBT and 3130 mIU/mg for patients with no positive reactions on MBBT (Table 2). According to the study by Ahn et al.,<sup>11</sup> surgical biopsies from the inferior turbinate yielded approximately 839 mIU/mg IgE in control patients and approximately 20,259 mIU/mg IgE in patients with AFRS. Therefore, it is estimated that MBBT is able to harvest up to 36% of the amount of IgE that is seen on surgical biopsy, although the patients in this study have allergic rhinitis, not AFRS.

Because MBBT is essentially a new test for detecting IgE, normal values for the population must be determined. However, based on data from Ahn et al.,<sup>11</sup> total IgE on MBBT from the inferior turbinates of patients with allergic rhinitis is approximately 8 times the level of IgE measured from surgical biopsies of the inferior turbinate in a normal, control population. The study patients had both positive and negative SPT, but a true control group of patients with no history of allergic rhinitis and no positive reactions on SPT should be included in future studies. Efforts must also continue to improve and standardize the technique of MBBT to maximize the amount of IgE that is collected and measured. This study demonstrated that a much smaller percentage of IgE is detected in epithelial lysate from the inferior turbinate vs the epidermis. Future studies must also establish ranges of total and antigen-specific IgE for other regions in the sinonasal cavity, as well as for other diseases such as AFRS and CRS with or without nasal polyps.

In addition to determining normal values in the population, future studies of MBBT with larger patient samples must attempt to calculate the sensitivity and specificity of the test. However, in order to determine these values, the rate of "true positives" and "true negatives" must be known. MBBT is a test of local IgE and this must be kept in mind when comparing it to measures of systemic IgE, such as SPT and in vitro testing. The findings of this study suggest that MBBT can detect IgE which is not detected on systemic testing, and in this situation, which result would be considered "true"? The most accurate way to determine the predictive value of MBBT is to compare the results against other methods of local IgE detection, such as surgical biopsy or nasal challenge.

MBBT has the potential to accelerate research in the area of local immunity. Because it is a well-tolerated, technically straightforward, and minimally-invasive test, it expands the range of patients who can be enrolled in studies, such as children and patients who are not undergoing surgical procedures. Other IgE-mediated, inflammatory conditions outside the sinonasal cavity may also benefit from the use of MBBT to study local antibodies without the risk and discomfort of surgical biopsy. This includes food allergy, oral allergy syndrome, asthma, and eosinophilic esophagitis. MBBT may also be applied to harvest non-IgE antibodies or surface proteins in order to study infectious diseases or neoplastic processes in the future.

## Conclusion

MBBT is a natural extension of a simple, noninvasive, and well-established method of tissue sampling that fills the growing need among otolaryngologists to better understand the immune system on the local level. It is able to detect antigen-specific IgE in a measurable fashion with currently available assays. With further improvements, MBBT may become a valuable tool for the diagnosis and subsequent management of IgE-mediated diseases in the sinonasal cavity as well as in other areas of the aerodigestive tract. ③

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