better understand the relationship between pollen exposure and risk for adverse respiratory health outcomes. Interventions to reduce pollen exposure to better manage asthma and allergies may reduce the potential for these factors to interact, and can help to prevent serious asthma exacerbations in children and adolescents.

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Alternate methods of nasal epithelial cell sampling for airway genomic studies

To the Editor:

Recent translational studies of airway inflammation have shown that nasal epithelial cells are a good surrogate for bronchial epithelial cells[1,2] in asthmatic patients.[3,4] However, the standard method of nasal sampling requires use of a nasal speculum and specialized training. In pediatric studies requiring longitudinal specimen collection, sampling by this method can be limited by subject refusal and technical challenges.

Alternate methods of nasal sampling have been proposed. Different instruments for collection have been used, ranging from polyester-tipped swabs to plastic curettes to cytology brushes. Different sampling locations have been proposed, such as beneath the inferior turbinate or the anterior nares, where respiratory epithelial cells are also located.[5] Obtaining nasal epithelial cells beneath the inferior turbinate with a cytology brush has been the most commonly used method. These cells have been validated as a surrogate for bronchial epithelial cells[2] and have been shown to be clinically important in translational asthma studies.[4] This method has also been shown in preliminary studies to be more difficult to tolerate.[6] Whether a more comfortable method of sampling exists and whether this method can provide equivalent cytologic, gene expression, and epigenetic results is undetermined.

Here we compared nasal epithelial cells sampled from the anterior nares with either a polyester swab or a cytology brush with the standard collection method of cytology brush sampling from beneath the inferior turbinate. The benefit of the former method is that it does not require the use of a speculum to visualize nasal anatomy, is technically easy to perform, and, with

TABLE I. All measured parameters comparing inferior turbinate with anterior nares sampling locations

Parameter	Inferior turbinate	Anterior nares	*P* value*
Discomfort†	3.5 (2.9-7.0)	1.0 (0.4-2.0)	<.001
Proportion of respiratory epithelial cells (%)	99.2 (92.2-100.0)	65.4 (46.8-84.7)‡	<.001
RNA yield (ng)	4620 (2364-6993)	156 (66-606)§	<.001
RNA integrity number	8.9 (8.6-9.5)	2.2 (1.0-3.9)‖	<.001
DNA yield (ng)	4288 (2081-7600)	875 (181-2375)¶	<.001
Gene expression (correlation)#	0.94 (0.93-0.96)	0.91 (0.78-0.94)	—
Gene expression (average relative error [%])#	6.1 (3.0-6.0)	9.1 (3.7-15.3)	—
Methylation, all genes (correlation)#	0.97 (0.96-0.98)	0.93 (0.81-0.98)	—
Methylation, all genes (average relative error [%])#	6.1 (5.2-7.4)	7.0 (4.7-8.8)	—
Methylation, asthma genes (correlation)#	0.98 (0.97-0.99)	0.95 (0.88-0.93)	—
Methylation, asthma genes (average relative error [%])#	5.8 (5.0-6.1)	7.6 (3.1-10.4)	—

Parameters are expressed as medians (IQRs). Forty-eight samples were collected from 12 subjects. Twenty-four samples from 8 subjects underwent downstream whole-genome expression and methylation analysis.

**P* value comparing parameters from inferior turbinate sampling versus anterior nares sampling.

†Subjects were asked to rate discomfort level on a standardized 0- to 10-point numeric scale, where 0 indicates no discomfort and 10 indicates maximum discomfort. For the anterior nares location, there were no significant differences between the brush (1.0 [0.63-2.8]) and swab (1.3 [0.3-2.0]) methods ($P = .51$).

‡For the anterior nares, there were no statistically significant differences between the brush (82.4% [59.6% to 84.9%]) and swab (50.3% [42.3% to 71.5%]) methods ($P = .28$). For the anterior nares, the brush method (11.0% [5.8% to 39.3%]) had a lower proportion of squamous epithelial cells compared with the swab method (49.7% [24.4% to 50.9%]; $P < .001$).

§For the anterior nares location, there were no statistically significant differences between the brush (192 [81-615]) and swab (156 [60-588]) methods ($P = .58$).

‖For the anterior nares location, the brush method (3.9 [2.7-5.3]) had a higher RNA integrity number compared with the swab method (1.1 [1-1.8]; $P = .047$).

¶For the anterior nares location, there were no statistically significant differences between the brush (850.0 [175.0-2343.8]) and swab (875.0 [212.5-2281.3]) methods ($P = .87$).

#Correlation and average relative error were calculated between the left and right inferior turbinate samples from the same subject and between inferior turbinate and anterior nares samples from the same subject.

the swab method, is already widely used in clinical practice for obtaining microbiologic samples.

Informed consent was obtained from 12 healthy adults. Four samples were collected from each subject; for each naris, paired inferior turbinate and anterior nares samples were collected. Inferior turbinate samples were collected with cytology brushes using nasal speculums for direct visualization. Anterior nares samples were obtained by inserting either a brush or swab inferior to the nasal bone and vigorously rubbing along the nares (see the Methods section in this article's Online Repository at www.jacionline.org). Each sample was immediately aliquoted for cytology and RNA and DNA extraction. Paired samples with sufficient nucleic acid yield for downstream microarray analysis were analyzed for whole-genome expression (Illumina HumanHT-12 v4 Expression BeadChip; Illumina, San Diego, Calif) and methylation (Illumina BeadChip Infinium HD array) levels. Subjects were asked to rate their discomfort level immediately after each collection using a numeric 0- to 10-point rating scale.

The largest observed difference between sample collection methods for measured parameters was between the location of sampling (inferior turbinate vs anterior nares) rather than the use of a cytology brush versus swab (Table I). Average discomfort levels were significantly higher for inferior turbinate compared with anterior nares sampling (median, 3.5 [interquartile range [IQR], 2.9-7.0] vs 1 [IQR, 0.4-2.0]; $P < .001$). Inferior turbinate samples had significantly more respiratory epithelial cells than anterior nares samples (median, 99.2% [IQR, 92.2% to 100.0%] vs 65.4% [IQR, 46.8% to 84.7%]; $P < .001$; see Fig E1 in this article's Online Repository at www.jacionline.org); proportions for squamous epithelial cells were reversed. Regardless of the sampling method, there were very few inflammatory cells in all samples (median, 0%; IQR, 0% to 1.2%).

Inferior turbinate samples had higher RNA yields (4620 vs 156 ng, $P < .001$) and higher RNA integrity numbers (8.9 vs 2.2, $P < .001$) than anterior nares samples (Table I). The same was true for DNA yields (4288 vs 875 ng, $P < .001$). Importantly, 91.7% of inferior turbinate samples but only 33.3% of anterior nares samples yielded sufficient RNA, and 100% of inferior turbinate samples but only 50% of anterior nares samples yielded sufficient DNA for downstream microarray analysis.

Twenty-four samples were selected for paired whole-genome expression and methylation analysis. Seven failed to hybridize to the expression microarray; of these, all were anterior nares samples with low RNA yields. Despite the low RNA integrity number in the remaining anterior nares samples, average expression intensity was highly correlated in expressed genes when comparing inferior turbinate with anterior nares samples from the same subject (correlation, 0.91 [IQR, 0.78-0.94]; Fig 1, *A*). To determine the accuracy of the 2 sampling techniques, we calculated the relative error averaged over all genes. Average relative errors comparing anterior nares with inferior turbinate samples were not significantly different from those comparing left and right inferior turbinate samples from the same subject (9.1% [IQR, 3.7% to 15.3%] vs 6.1% [IQR, 3.0% to 6.0%]; $P = .19$).

All 24 DNA samples were successfully hybridized to the methylation array. Among all variable methylation sites, methylation was again highly correlated between inferior turbinate and anterior nares samples from the same subject (correlation, 0.93 [IQR, 0.81-0.98]; Fig 1, *B*), although the average relative error was also not significantly different (6.1% [IQR, 5.2% to 7.4%] vs 7.0% [IQR, 4.7% to 8.8%], $P = .67$). When we looked only at genes previously reported to be associated with asthma (see Table E1 in this article's Online Repository at www.jacionline.org), methylation was highly correlated between inferior turbinate and anterior nares samples (correlation, 0.95 [IQR, 0.88-0.93]; see Fig E2 in this article's Online Repository at www.jacionline.org).

High-dimensional genomics studies have recently focused on the use of nasal epithelial cells as a noninvasive surrogate for bronchial epithelial cells in asthmatic patients, with the hope that prognostic biomarkers discovered can ultimately be translated to the bedside. Although nasal epithelial cell collection from beneath the inferior turbinate has been the standard collection method, it requires specialized training and equipment, can be