Asymmetry of the Vocal Folds in Patients With Vocal Fold Immobility

Yukio Oyamada, MD; Eiji Yumoto, MD; Koji Nakano, MD; Hidenori Goto, MD

Objectives: To measure the vocal fold length (VFL) during inspiration and phonation and to determine the vertical difference of the vocal folds during phonation in patients with unilateral vocal fold immobility.

Design: Prospective study.

Setting: University hospital.

Patients: Thirty patients with unilateral vocal fold immobility.

Interventions: Each subject was asked to sustain the vowel /a/ and, after a short rest, to inhale slowly. The region over the larynx was scanned using multislice helical computed tomography during each maneuver; 3-dimensional endoscopic, coronal, and sagittal images were produced.

Main Outcome Measures: The VFLs on each side and the vertical differences between the vocal folds were calculated.

Results: The inspiratory VFL on both sides was significantly longer in men than in women. It was significantly longer on the healthy side than on the immobile side in both groups. On the healthy side, the inspiratory VFL was significantly longer than the phonatory VFL in men, but there was no significant difference in women. In contrast, on the immobile side, the phonatory VFL was significantly longer than the inspiratory VFL in women, but there was no significant difference in men. The VFLs of the healthy and immobile sides varied in tandem. The immobile vocal fold was situated lower than the healthy vocal fold during phonation in 3 patients.

Conclusions: Multislice helical computed tomography is a novel method to measure the VFL and the vertical level difference between the vocal folds. Application of this method might facilitate further understanding of laryngeal behavior in patients with unilateral vocal fold immobility. Arch Otolaryngol Head Neck Surg. 2005;131:399-406

Patients with unilateral vocal fold paralysis often experience breathy dysphonia that may interfere with the patients' daily activities.3,4 Because electromyography is not routinely performed in clinical settings to confirm the absence of a nerve supply to the intrinsic laryngeal muscles by the recurrent laryngeal nerve, the condition is called unilateral vocal fold immobility (UVFI) in the present study. An immobile vocal fold may be fixed at a midline, paramedian, or intermediate position. It is often thinner and less tense than the healthy vocal fold. Furthermore, a difference in the vertical position of the 2 vocal folds usually exists during phonation.

Video-stroboscopy has been the method of choice for observing vocal fold vibration.3 In most patients with UVFI, this examination reveals the horizontal position of the immobile vocal fold, the flaccidity of the immobile vocal fold, and a glottal gap during phonation. Stroboscopy may also reveal vertical differences in the position of the 2 vocal folds, considering the following findings: (1) the contact pattern of the 2 vocal processes during phonation, (2) the brightness and thickening of the vocal folds during inspiration and phonation, and (3) the status of focusing with vertical movements of the telescope.3 Based on stroboscopic findings, Brewer et al3 and Woodson6 reported that during respiration the vocal fold length (VFL) on the affected side appeared to be shorter than on the normal side. Woodson et al7 stated that the length difference between the 2 vocal folds decreased during sustained phonation owing to compensatory shortening of the healthy vocal fold. The ambiguity of the findings regarding the VFL in patients with UVFI can be attributed partly to the difficulty in viewing the entire length of the vocal fold by endoscopy and to asymmetry in the vertical position of the 2 vocal folds during phonation in patients with UVFI.

A novel system was developed that uses multislice helical computed tomography (MSHCT) to virtually reconstruct 3-dimensional (3-D) images of the laryngeal lumen in patients with UVFI.8,9 The present study was designed primarily to obtain quantitative measurements of the VFL during inspiration and phonation in pa-
The lateral position were excluded from the study. Consecu-
tial fold on the same side moved during quiet inspiration. Such
not reach the lateral position to which the video-recorded vo-
ning, the vocal fold on the healthy side in some patients did
cause the patients were asked to inhale slowly during the scan-
with 3-D endoscopic images produced from the voxel data. Be-
ners in the vertical positions of the healthy and immo-
tances in the vertical positions of the healthy and immo-

ted vocal folds during phonation in patients with UVFI.
Assessment of such asymmetries between the vocal folds
provides useful information because various phonosur-
ical axis and the latter as parallel to the glottal axis.
Axial plane, with the former defined as perpendicular to the glot-
the anterior and sagittal images were systematically generated for all sub-
oral and sagittal planes were perpendicular to the
patient to the thyroid gland
the thyroid gland
22/F/75 L Idiopathic 2 mo
23/F/79 L Lung cancer 8 mo
24/F/73 L Idiopathic 5 mo
25/F/46 L Thyroid cancer 1 y
26/F/68 L Jugular foramen neurinoma 5 mo
27/F/59 L Thyroid surgery 8 d
28/F/64 L Heart surgery 17 d
29/F/60 L Thyroid cancer 2 mo
30/F/61 R Idiopathic 4 y 3 mo

Abbreviations: L, left; R, right.

### METHODS

Fifty patients with UVFI underwent MSHCT examination as
described herein between April 1, 1999, and August 31, 2002,
and videotransysteroscopy within 2 weeks of the computed tomog-
raphy (CT) scanning. In each patient, laryngeal images ob-
tained during phonation and quiet inspiration were compared
with 3-D endoscopic images produced from the voxel data. Be-
cause the patients were asked to inhale slowly during the scan-
ning, the vocal fold on the healthy side in some patients did
not reach the lateral position to which the video-recorded vo-
cal fold on the same side moved during quiet inspiration. Such
patients in whom the vocal fold on the healthy side did not reach
the lateral position were excluded from the study. Conse-
sequently, the subjects included 13 men and 17 women with no
history of surgical intervention for UVFI. Each subject under-
went MSHCT examination during 2 maneuvers, in which the
subject was asked to inhale slowly for 5 seconds and then to
sustain the vowel sound /a/ in his or her comfortable pitch and
intensity for 5 seconds after a short rest. Because patients with
UVFI use a larger volume of air to phonate, they often can-
not produce sustained phonation for 5 seconds. In such
cases, the subjects were asked to phonate as quietly as pos-
sible throughout the entire scan. Those who could perform
the tasks were included in the study. The ages of the subjects
ranged from 28 to 83 years (mean, 59.9 years). The

tions of the healthy and immobile vocal folds was defined as the dis-

tional procedure; y-axis, anterior to posterior direction; and z-axis, su-

tuose of the subject simultaneously (HiSpeed Advantage QX/I; GE Healthcare, Milwaukee, Wis),
using 200 mA, 120 kV, 1.25-mm slice thickness, 1 x-ray tube
rotation per 0.8 seconds, and 7.5-mm table speed per 0.8 sec-
onds. Each subject was scanned during phonation and inspira-
tion. The scan covered the region from the protrusion of the
thyroid notch to 37.5 mm below this point and included the
ventricular fold, ventricle, vocal fold, and subglottal region. All
CT variables and 3-D rendering techniques were determined as
described elsewhere.9

Axial images were generated at 1-mm intervals by overlap-
ing each adjacent image by 0.5 mm and were then sent to an
MSHCT workstation together with voxel data. A volume-
rendering technique was used with 3-D endoscopic mode. In our
study, 2 voxels of -100 Hounsfield units or less served as the
surface of the laryngeal lumen. Other voxels were eliminated.
Views from the tracheal and oral sides, as well as 2 vertically split
hemilaryngeal images, were displayed for qualitative assess-
ment. Two-millimeter-thick multiplanar reconstruction coro-
nal and sagittal images were systematically generated for all sub-
jects. The coronal and sagittal planes were perpendicular to the
axial plane, with the former defined as perpendicular to the glo-
tal axis and the latter as parallel to the glottal axis.

### DATA ANALYSIS

A monitor screen of the workstation was split into 4 windows
to display 3-D endoscopic, axial, coronal, and sagittal images
of the subject simultaneously (Figure 1). A cursor, placed on
each window, was designed to indicate a particular point of the
larynx, so that an examiner could locate the point in an xyz
coordinate system, where the x-axis indicated left to right di-
rection; y-axis, anterior to posterior direction; and z-axis, su-
rior to inferior direction. The display was designed in such
a way that the examiner could move the position of the view-
point on the 3-D endoscopic image window, with the 3 other
images depicted according to the location of the cursor. Two
of us (Y.O. and K.N.) independently identified the anterior com-
missure and the tips of the vocal processes during inspiration
and phonation in each subject in a random order. The VFL,
declared as the distance between the anterior commissure and
the tip of the vocal process, was calculated based on the xyz
coordinates obtained for the anterior commissure and the tip
of the vocal process. The vertical difference in the positions of
the healthy and immobile vocal folds was defined as the dis-
ance along the z-axis between the tips of the vocal processes
and was calculated by subtracting the z coordinate of the im-
mobile vocal fold from that of the healthy vocal fold.

### SUBJECTS

Fifty patients with UVFI underwent MSHCT examination as
described herein between April 1, 1999, and August 31, 2002,
and videotransysteroscopy within 2 weeks of the computed tomog-
raphy (CT) scanning. In each patient, laryngeal images ob-
tained during phonation and quiet inspiration were compared
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cause the patients were asked to inhale slowly during the scan-
ning, the vocal fold on the healthy side in some patients did
not reach the lateral position to which the video-recorded vo-
cal fold on the same side moved during quiet inspiration. Such
patients in whom the vocal fold on the healthy side did not reach
the lateral position were excluded from the study. Conse-
sequently, the subjects included 13 men and 17 women with no
history of surgical intervention for UVFI. Each subject under-
RELIABILITY OF THE MEASUREMENTS

To determine the reliability of vocal fold measurements using the present method, an excised canine larynx was scanned using MSHCT, and the voxel data obtained were processed in the same way as for the human subjects. Two of us (Y.O. and K.N.) independently identified the anterior commissure and the tip of the left vocal process on the monitor screen 6 times, and the VFL was calculated. The 2 examiners also independently measured the VFL on the left side of the canine larynx 6 times with the aid of vernier calipers with a precision of 0.05 mm. The reliability of the measurements was tested with the Mann-Whitney test,\textsuperscript{10} which revealed no significant differences between the measurements by the 2 examiners with MSHCT or with the vernier calipers, or between the measurements made by each examiner using these 2 methods. The measurements of the study subjects made by the 2 examiners were similar, and an average of the 2 measurements was used for all analyses.

STATISTICAL ANALYSIS

Paired or unpaired $t$ tests were performed for statistical analyses. The correlation between 2 variables was calculated using the Pearson product moment correlation coefficient. The significance level was set at $P<.05$.

RESULTS

Although minimal “staircase” motion artifacts were seen in some subjects, such artifacts occurred in the ventricle and did not interfere with further evaluation of these subjects. Table 2 summarizes the means and standard deviations of the VFL on the healthy and immobile sides, as well as the vertical differences in the positions of the vocal folds, during phonation and inspiration for men and women.
Although the VFL differences between men and women were not significant during phonation on either side, the inspiratory VFL was significantly longer in men than in women on the immobile side (\(P <.05\)) and on the healthy side (\(P <.01\)) (Figure 2). There was no significant difference in the phonatory VFL between the immobile and healthy sides in either sex. On the other hand, the inspiratory VFL was significantly longer on the healthy side than on the immobile side in both sexes (\(P <.01\)). The inspiratory VFL was significantly longer than the phonatory VFL on the healthy side in men (\(P <.01\)), whereas the phonatory VFL was significantly longer than the inspiratory VFL on the immobile side in women (\(P <.01\)) (Figure 3). No significant differences in the VFL were found between phonation and inspiration on the immobile side in men or on the healthy side in women.

On the healthy and immobile sides, the inspiratory VFL was longer than the phonatory VFL in men, whereas the inspiratory VFL was shorter than the phonatory VFL in women, as shown in Table 2. A scatter diagram of the VFL difference between phonation and inspiration (phonatory VFL minus inspiratory VFL) on the immobile side and that on the healthy side is shown in Figure 4. The VFL on the immobile side decreased in tandem with decreases in the VFL on the healthy side during phonation in 7 of the 13 men. In 8 of the 17 women, the opposite phenomenon occurred, with the VFL on the immobile side increasing with increases on the healthy side during phonation. The Pearson product moment correlation coefficient of these 2 variables was 0.837 and was statistically significant (\(P <.001\)).

No significant differences between men and women were found in the vertical difference in the positions of women on the immobile side (\(P <.05\)) and on the healthy side (\(P <.01\)) (Figure 2). There was no significant difference in the phonatory VFL between the immobile and healthy sides in either sex. On the other hand, the inspiratory VFL was significantly longer on the healthy side than on the immobile side in both sexes (\(P <.01\)). The inspiratory VFL was significantly longer than the phonatory VFL on the healthy side in men (\(P <.01\)), whereas the phonatory VFL was significantly longer than the inspiratory VFL on the immobile side in women (\(P <.01\)) (Figure 3). No significant differences in the VFL were found between phonation and inspiration on the immobile side in men or on the healthy side in women.

Table 2. Vocal Fold Length on the Immobile and Healthy Sides and Vertical Difference During Phonation and Inspiration*

<table>
<thead>
<tr>
<th>Sex Group</th>
<th>During Phonation</th>
<th>During Inspiration</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Vocal Fold Length</td>
<td>Vertical Difference</td>
</tr>
<tr>
<td></td>
<td>Immobile Side</td>
<td>Healthy Side</td>
</tr>
<tr>
<td></td>
<td>Healthy Side</td>
<td></td>
</tr>
<tr>
<td>Men (n = 13)</td>
<td>11.7 ± 2.5</td>
<td>1.3 ± 1.5</td>
</tr>
<tr>
<td>Women (n = 17)</td>
<td>10.0 ± 2.3</td>
<td>0.7 ± 0.9</td>
</tr>
</tbody>
</table>

*Data are given as mean ± SD millimeters.

Figure 2. A schematic representation of the vocal fold length on the healthy and immobile sides during phonation and inspiration for men and women. Asterisk indicates \(P <.05\); double asterisk, \(P <.01\).

Figure 3. A schematic representation of the vocal fold length on the healthy and immobile sides during phonation and inspiration for men and women. The order of data presentation was changed for comparison. Double asterisk indicates \(P <.01\).

Figure 4. Scatter diagram of the vocal fold length differences between phonation and inspiration. The abscissa represents those on the healthy side; ordinate, those on the immobile side. The linear regression line is drawn. Open circles indicate data of women; black circles, those of men.
the healthy and immobile vocal folds during phonation or inspiration (Table 2). In both groups, the vertical difference in the positions of the healthy and immobile vocal folds during phonation was statistically lower than that during inspiration ($P<.001$). In other words, in men and women, the vocal process was situated higher on the immobile side than on the healthy side during phonation, but lower on the immobile side than on the healthy side during inspiration. However, in 3 subjects (1 man and 2 women), the immobile vocal fold was situated lower than the healthy vocal fold during phonation.

**COMMENT**

Videostroboscopy has been the standard tool for the evaluation of vocal fold vibration in patients with UVFI. It can reveal the horizontal position of the immobile vocal fold (deviation from the glottal axis), the shape and size of the glottal gap during phonation, the degree of bowing of the vocal fold, and the presence or absence of compensatory overadduction of the healthy vocal fold during phonation. However, little information is available concerning quantitative differences in the VFL and the vertical differences in the position of the tip of the vocal processes in patients with UVFI. The present study was performed to obtain objective assessments of the VFL, vertical positional differences, and dynamic changes that accompany inspiration and phonation in patients with UVFI.

Measurement of the VFL in patients with UVFI in vivo is difficult in a clinical setting because of the deep location of the vocal fold in the neck and the presence of the gag and airway protective reflexes. Therefore, we used MSHCT to obtain $xyz$ coordinates to identify certain points within the larynx in the $xyz$ coordinate space. Two of us (Y.O. and K.N.) independently identified the anterior commissure and the tips of the 2 vocal processes of each patient under each condition. The VFL was calculated as the distance between the anterior commissure and the tip of the vocal process. Wang and Vannier$^{11}$ recommended production of 5 or more slices per table increment to obtain better longitudinal resolution in helical CT and concluded that helical CT is superior in applications requiring a high longitudinal resolution. Burke et al$^{12}$ evaluated airway obstructive lesions in patients with a rigid bronchoscope system under general anesthesia and with virtual endoscopy images produced from the voxel data by helical CT. The stenosis-to-lumen ratios from the virtual and actual endoscopies were compared and were within 10% measurement error. They concluded that evaluation using virtual endoscopy is accurate in assessing stenosis width and the length of fixed airway lesions. We evaluated interexaminer reliability of the measurements of an excised canine larynx made using MSHCT and compared these with direct measurements using vernier calipers. The results showed that interjudge reliability was excellent and that the measurements using MSHCT were not significantly different from those made with vernier calipers.

Apparent mucosal surfaces depicted in the produced image can vary with threshold setting because the image was artificially built based on the 2 extracted voxels. As exemplified in **Figure 5**, CT endoscopic images produced with the present method coincided well with those obtained through a conventional rigid oblique-view endoscope during phonation and inspiration. Therefore, although CT scanning was not repeated, to avoid unnecessary radiation exposure to the subjects, the present method is considered to be reliable for the measurements of the anterior commissure and vocal processes.

In the present study, we defined the VFL as the length of the membranous vocal fold, that is, the distance between the anterior commissure and the tip of the vocal process. As shown in **Figure 1**, the anterior commissure and the tip of the vocal process were easy to identify using a cursor designed to indicate a particular point in the 4 windows displayed on the monitor screen. Hirano and Kurita$^{13}$ proposed that the posterior end of the vocal fold is located at the posterior end of the laryngeal ventricle. According to this definition, the vocal fold includes a part of the vocal process of the arytenoid cartilage. However, the membranous part of the vocal fold plays an essential role in phonation. Sawashima et al$^{14}$ and Nishizawa$^{15}$ measured the VFL as the distance between the anterior commissure and the tip of the vocal process with the aid of a rigid stereoeendoscope system. Therefore, our definition of the VFL seems reasonable from the clinical and physiological perspectives.

Fujimura et al$^{16}$ devised a stereofiberscope system for quantitative observation of the larynx. Two fiberscopes were inserted through each nostril and connected with a magnetic bridge in the mesopharynx to maintain a set distance between the objective lenses at the tips of the fiberscopes. Two images viewed through the separate lenses were recorded, and the actual length of the vocal fold was calculated. However, bridging the 2 tips of the fiberscopes at an appropriate place was often unreliable for precise measurements. Subsequently, the system was refined by using a rigid stereoeendoscope$^{14}$. Using this system, Nishizawa$^{15}$ measured the VFL during inspiration and phonation in 7 adults (3 men and 4 women) without any laryngeal disorders. During phonation at the habitual pitch, the VFL in men ranged from 8.9 to 11.3 mm, while in women it ranged from 7.0 to 10.1 mm. In the present study, the mean VFL during phonation in men was 11.7 mm on the immobile side and 11.6 mm on the healthy side. In women, the mean VFL was 10.0 mm on the immobile and healthy sides. Therefore, our measurements of the VFL are similar to those reported by Nishizawa.$^{15}$ Also similar to Nishizawa’s findings, the vocal fold was lengthened following the widening of the glottis during inspiration among men in our study. However, the reverse was observed among women in our study. The VFL in women decreased during inspiration compared with that during phonation. The subjects in the present study experience dysphonia due to UVFI and may have adopted uncommon laryngeal adjustments for phonation. Even in normal subjects, Nishizawa$^{15}$ observed a greater range of changes in the VFL during phonation than during respiration and noted that the VFL was not always longer during respiration than during phonation. Woodson et al$^{17}$ measured the VFL before and after arytenoid adduction procedures using 4 cadaver larynges. They found that the VFL did not change significantly, although there was a tendency for the VFL to increase after the arytenoid adduction procedure.
The vocal folds were significantly longer during inspiration in men than in women, on the healthy and immobile sides. The mean VFLs in men were 12.5 mm on the immobile side and 13.7 mm on the healthy side, while those in women were 8.6 mm on the immobile side and 9.6 mm on the healthy side (Table 2). Kurita,17 using excised larynges, reported that the membranous vocal folds were longer in adult male larynges (range, 12-18 mm) compared with adult female larynges (range, 8-12 mm). The mean VFLs in men and women obtained in the present study had a tendency to be short, although they lie within the ranges of VFLs reported by Kurita.17 This can be explained by the fact that, as Nishizawa15 reported, the VFL increases as the glottal width increases. The vocal fold of the excised larynx is known to be located at an intermediate position (the so-called cadaver position), while the subjects in the present study inhaled slowly during the scanning so that the vocal fold on the healthy side did not abduct beyond the intermediate position. The immobile vocal fold was fixed at the paramedian position in most subjects.

Although there was a significant VFL difference between the immobile and healthy sides during inspiration in men and women, no significant difference was detected during phonation in either group (Table 2 and Figure 2). A possible explanation is that the affected vocal fold was fixed at a paramedian position in most subjects and it was not elongated during inspiration. Woodson6 reported that the healthy vocal fold in patients with UVFI often shortened during phonation and suggested that such a laryngeal adjustment was the result of compensatory adjustment of the healthy vocal fold to achieve an approximation of the vocal folds.

Figure 5. An example of the laryngeal images observed through a conventional rigid oblique-view endoscope (upper half) and those produced from the voxel data (lower half) during phonation (left) and inspiration (right).
The VFL on the immobile side in UVFI has not been of major interest among laryngologists because the immobile vocal fold is often fixed at a certain position in the lateral direction and because the VFL on the immobile side was believed to remain constant regardless of laryngeal activity. In the present study, although the VFL on the immobile side in men did not differ significantly between phonation and inspiration, women had significantly longer vocal folds on the immobile side during phonation. In contrast, on the healthy side, the VFL in women did not differ significantly between phonation and inspiration, whereas men had significantly shorter vocal folds during phonation. These findings suggest that men and women might have adopted different laryngeal adjustments to compensate for asymmetries due to UVFI. Another possibility for these different patterns is that the present study included patients with UVFI resulting from various causes and with a wide range of time from onset. Consequently, the innervation of the larynges ranged from partial or misdirected reinnervation to total denervation, and compensatory movements of the vocal fold on the healthy side appeared in some patients. Further study is necessary to scrutinize the effect of site and duration of the nerve injury on laryngeal behavior in patients with UVFI.

Figure 4 shows a scatter diagram of the VFL difference between phonation and inspiration (phonatory VFL minus inspiratory VFL) on the immobile side vs the healthy side. The correlation between these 2 variables was significant ($r=0.837$, $P<.001$; Pearson product moment). This result indicates that the VFL on the immobile side changes as the VFL on the healthy side changes. In another words, as the healthy vocal fold lengthens, the immobile vocal fold also lengthens. The reverse is likewise true, with the immobile vocal fold shortening as the healthy vocal fold shortens. There are 2 possible explanations for this finding. One is that the VFL on the immobile side is passively changed in relation to changes on the healthy side. The anterior end of the vocal fold is the anterior commissure, and the posterior ends of the vocal folds are connected with the arytenoid cartilage and posterior wall of the glottis. Therefore, when the VFL on the healthy side changes, the immobile side is affected to a certain degree by these shared structures. Another explanation is that the contraction of the extrinsic laryngeal muscles, including the inferior pharyngeal constrictor muscle (ie, the thyropharyngeal and cricopharyngeal muscles) and the sternothyroid, sternohyoid, and thyrohyoid muscles, might affect the length of the vocal folds indirectly.

Similar to the findings in previous reports, the present study showed that, during phonation in men and women, the tip of the vocal process on the immobile side was situated in a higher position than that on the healthy side. The reverse was true during inspiration, in which the tip of the vocal process on the immobile side was situated in a lower position than that on the healthy side. However, these results were derived from a statistical analysis. In 3 patients, the tip of the vocal process on the immobile side was situated lower than that on the healthy side during phonation. Blitzer et al noted that some patients with UVFI had immobile vocal folds that were situated lower than the healthy vocal folds during phonation and that these patients experienced breathy hoarseness. This configuration was considered to result from a loss of posterior support from the posterior cricoarytenoid muscle. Hong and Jung also examined the differences in the vertical position of vocal folds in patients with UVFI and found that there was significant variation among the patients. Therefore, the differences in the vertical position of vocal folds during phonation vary among patients with UVFI. This finding should be considered when planning phanosurgery to correct dysphonia due to UVFI.

In conclusion, we developed a novel system using MSHCT to measure the VFL and the vertical level difference between the 2 vocal folds. Application of this system might facilitate further understanding of laryngeal behavior in patients with UVFI, as exemplified by the finding that the VFL on the immobile side varies with the active changes on the healthy side.

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REFERENCES


Errors in Text and Figures. In the Original Article by VanderMeer et al titled “Innate Immunity of the Sinonasal Cavity: Expression of Messenger RNA for Complement Cascade Components and Toll-like Receptors,” published in the December issue of the ARCHIVES (2004;130:1374-1380), there were errors in the text and figures. The first paragraph of the “Results” section should have appeared as follows: “Sha et al previously detected mRNA for all 10 known TLRs in human airway epithelial cells, leading us to expect that human sinonasal tissue samples may also contain mRNA for TLRs. Therefore, we tested tissue from human sinonasal mucosa for the presence of mRNA for TLR1-TLR10. Detectable levels of all 10 TLRs were found on screening the sinonasal tissue samples with standard reverse transcription PCR. To begin to assess the relative levels of TLRs, we developed TaqMan probes and primer sets for human TLRs (Table 1). Figure 1 shows the threshold cycle values for levels of mRNA for all 10 human TLRs quantified in 5 patients with sinusitis. Values were relatively consistent among the 5 subjects, and the SEM for each TLR was 0.67 or fewer PCR cycles. Among the TLRs, relatively high values were detected for TLR1, TLR2, TLR3, and TLR5; intermediate values were detected for TLR6, TLR7, TLR8, TLR9, and TLR10; and low values were detected for TLR4.”

In addition, Figure 1 and Figure 2 should have appeared as follows. The ARCHIVES regrets the errors.

Figure 1. Real-time polymerase chain reaction (PCR) analysis of expression of toll-like receptors (TLRs) in human sinonasal tissue. Average cycle threshold (Ct) for messenger RNA (mRNA) of TLRs in human sinonasal tissue. In real-time PCR, fluorescence values are recorded during every PCR cycle and represent the amount of product amplified to that point in the amplification reaction. The more mRNA for a given gene that is present at the beginning of the reaction, the fewer number of cycles it will take for the fluorescent signal to reach a point that is statistically significant above background. The Ct is the cycle at which the fluorescent signal first increases above background. The total number of cycles performed in each experiment is 40. A Ct of 40 means that the fluorescence never reached above background level, therefore translating to an undetectable starting quantity of mRNA. The lower the Ct is below 40, the more mRNA is present in the sample, in an exponential fashion. Error bars represent SEM.

Figure 2. Agarose gel electrophoresis of polymerase chain reaction amplification products encoding complement components in human liver (A) and human sinonasal tissue (B). Top columns: ladder; complement factors B, H, and I; and properdin. Bottom columns: ladder, mannose-binding lectin-associated serine protease 1 (MASP1), MASP2, mannose-binding lectin, C3, and β-actin. Representative of 9 separate samples (sinonasal) or a single sample (liver).