A New Technique for Endobronchial Ultrasonography and Comparison of Two Ultrasonic Probes*

Analysis With a Plot Profile of the Image Analysis Software NIH Image

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Study objectives: Endobronchial ultrasonography (EBUS) is currently the sole clinical method available for delineating the bronchial wall structure; however, the image resolution is inadequate. Thus, an improved image analysis system is needed for both a more accurate and more readily interpretable endobronchial ultrasonogram.

Materials and methods: A total of 10 patients underwent pulmonary resection for lung cancer. EBUS was performed on the bronchi of the resected lungs, which had been immersed in physiologic saline solution. The same bronchial lesion in each specimen was imaged with two probes: 20 MHz and 30 MHz. The images were analyzed using the plot profile derived from freeware image analysis software: NIH Image (National Institutes of Health; Bethesda, MD). The measured echo intensity of the bronchial wall was statistically analyzed.

Results: A normal bronchial wall image consists of five layers, and the plot profile shows a W-shaped curve. The mean value of the echo intensity of each peak or trough of the W-shaped curve was calculated and compared for both probes. The differences in the mean echo intensity between both the third and fourth layer and the second and fourth layer were found to be significantly greater with the 30-MHz probe than with the 20-MHz probe. The echo intensity curve of a central-type lung cancer was not W shaped, indicating that the bronchial wall was not composed of the normal five layers.

Conclusion: We employed image analysis software and drew a plot to obtain a W-shaped curve from the EBUS image data. This enabled us to make an objective assessment of the laminar structure of the bronchial wall. In order to clearly recognize the laminar structure of the bronchial wall, the 30-MHz probe was found to be more useful than the 20-MHz probe.

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Abbreviations: EBUS = endobronchial ultrasonography; PDT = photodynamic therapy

The laminar structure of the bronchial wall as visualized by endobronchial ultrasonography (EBUS) was first described by Hurter and Hanrath in 1992. Subsequently, a number of studies have been conducted, suggesting that EBUS might be useful for imaging the laminar structure of the bronchial wall and evaluating the depth of tumor invasion in central-type bronchogenic carcinoma. EBUS is currently the sole method available for delineating the bronchial wall structure in the clinical setting; however, the image resolution is inadequate for the attainment of a clear laminated view of the bronchial wall, which has a maximum thickness of approximately 5 mm. A learning curve is required for the ultrasonic evaluation of the extent of tumor invasion into the bronchial wall. Even experienced interpreters have to rely on their subjective judgment. For more accurate and simpler interpretation,
we employed an image analysis system for EBUS that enabled us to acquire a clear laminated view of the bronchial wall and make an objective assessment of the feasibility of echo probes and the extent of tumor invasion ex vivo.

**Materials and Methods**

**Materials**

From February through March 2002, 10 patients underwent pulmonary resection for lung cancer. The operative procedure was a pneumonectomy in one case, a sleeve lobectomy in two cases, and a lobectomy in seven cases. The resected lung specimens were used for this study. The specimens were immersed in physiologic saline solution within 1 hour of the resection, in order to prevent autolytic changes and to obtain high-quality ultrasonic images without interference from air. The EBUS system used in this study was manufactured by Olympus (Tokyo, Japan), and had the following specifications: an EU-M 30 processor; a UM-BS20–26R ultrasonic probe (20-MHz radial scanner; rotation rate, 400/s); and a MAJ-643R (latex balloon sheath; outer diameter, 2.6 mm). The balloon sheath of the ultrasonic probe was filled with physiologic saline solution.

**EBUS Procedure**

EBUS was performed on the bronchus of the resected lung within 1 h of the resection. During the EBUS procedure, the resected lung was immersed in physiologic saline solution. First, the ultrasonic probe with the balloon sheath was inserted into the bronchial lumen. Second, the balloon sheath was expanded with physiologic saline solution until the lumen was obstructed. Third, EBUS images of the bronchus were captured after adjusting the brightness and contrast of the images (Fig 1). The same bronchial lesion in each specimen was observed using two different types of probes: 20 MHz and 30 MHz.

With the use of the balloon sheath, the study conditions were similar to the actual conditions in EBUS. When we perform EBUS in actual patients, the ultrasonic probe is used with the balloon sheath; for this reason, the balloon sheath was used in this study, although clearer images can be obtained if the EBUS is performed without the balloon sheath.

**Image Analysis**

The captured ultrasonogram was analyzed using freeware image analysis software (NIH Image, Version 1.62; National Institutes of Health; Bethesda, MD). The plot profile of the NIH Image was used, and the echo intensity of the ultrasonogram was measured. When an echo image was pure white (hyperechoic) in the ultrasonogram, the plot profile indicated 256 pixels; when it was pure black (hypoechoic), it indicated 0 pixels.

First, a radial line from the ultrasonic probe was drawn on the ultrasonogram, which perpendicularly intersected the bronchial wall. Second, the echo intensity on the line was plotted, and the plotted data on the total length of the line were obtained. Finally, we obtained the echo intensity curve as shown in Figure 2, top. Normally, the bronchial wall consists of five layers in an ultrasonogram: an innermost hyperechoic layer representing the balloon latex sheath and the bronchial epithelium; a hypoechoic second layer representing the submucosal layer; a hypoechoic third layer, which represents the marginal echo of the bronchial cartilaginous layer; and the innermost (fifth) lamina is composed of a hyperechoic layer representing the marginal echo of the outer cartilaginous layer and the outer margin of the bronchial wall. Numbered arrows indicate the layers of the bronchial wall as mentioned above.

The analysis was made of the image displayed on the monitor. The pixel values altered when the brightness of the image was changed. However, these alterations did not influence the results of the image analysis. First, each lamina has to be distinguished from the others in order to clearly recognize the laminar structure of the bronchial wall. If the difference of the pixel values between laminae increases, we can more clearly recognize the laminar structure. Accordingly, the most important thing to clearly recognize the laminar structure is not the pixel values of each lamina but the difference in pixel values between laminae. Furthermore, the differences of the pixel values between peaks and troughs did not vary even if the brightness of the image was changed. Because the change of each pixel value was the same as other values, the difference in the pixel values was constant.

Second, if we compare the performance of two ultrasound probes, each ultrasound image had to be obtained under the optimal conditions of that probe; specifically, each image must be obtained by the ultrasound probe, which is in accordance with its performance characteristics. The optimal conditions for one
ultrasound probe may be different than those for the other probe. We had to adjust the brightness of the image each time in order to obtain its optimal conditions.

**Statistical Analysis**

The measured echo intensity of the bronchial wall was statistically analyzed. The W-shaped echo intensity curve has five peaks and troughs, and each peak or trough represents the echo intensity of the most hyper or hypoechogenic lesion in the corresponding layer of the laminar structure of the bronchial wall. The mean value of each peak or trough of the echo intensity curve of all specimens was calculated, and statistical comparison was conducted. The paired $t$ test was used to compare the echo intensities, and $p < 0.05$ was considered as denoting significance. Informed consent was obtained from all patients before they were entered into the study group.

**Figure 2.** EBUS was performed at the same site of the bronchus with two kinds of ultrasonic probes, and ultrasonograms and their accompanying echo intensity curves were obtained (top, 30-MHz probe; bottom, 20-MHz probe). It would be subjectively understood that the ultrasonogram of the 30-MHz probe (top) shows higher resolution than that of the 20-MHz probe (bottom). However, the five-layer laminar structure might not be seen easily with the direct evaluation of the ultrasonogram. However, image analysis with NIH Image shows bronchial laminar structure as a W-shaped curve. An open rectangular lesion is analyzed with plot profile of NIH Image, and a certain plot of the rectangle is assigned from 0 to 256 pixels, according to its echo intensity. A W-shaped curve indicates a five-layer laminar structure of the bronchial wall. Echo intensity is plotted on the y-axis, and the distance from the echo probe is shown on the x-axis. Furthermore, the echo intensity curve of the 30-MHz probe shows steeper W-shaped curve than that of the 20-MHz probe. That is, the difference between peak and trough of the echo intensity curve is larger with the 30-MHz probe than the 20-MHz probe, and objectively shows that the 30-MHz probe has higher resolution than the 20-MHz probe.
RESULTS

A total of 10 normal bronchi from 10 patients with lung cancer were examined. Figure 2 presents ultrasonograms and echo intensity curves, which were obtained at the same site of the normal bronchus by two different ultrasonic probes: 30 MHz and 20 MHz. It is common knowledge that the ultrasonogram obtained with a 30-MHz probe (Fig 2, top) exhibits a higher resolution than that obtained with a 20-MHz probe (Fig 2, bottom). However, five layers of laminar structure might not be readily seen by direct assessment of the ultrasonograms. Conversely, the echo intensity curves showed five peaks and troughs, indicating that the normal bronchial wall is a laminar structure consisting of five ultrasonically distinct layers. Moreover, the echo intensity curve of Figure 2, top, showed a steeper W-shaped curve than that of Figure 2, bottom; specifically, the difference between the peak and trough of the echo intensity curve is greater with the 30-MHz probe than it is with the 20-MHz probe. This finding objectively demonstrates that the 30-MHz probe has a higher resolution than the 20-MHz probe. Figure 3 shows the mean value of the echo intensity of each layer of the laminar structure of the bronchial wall, obtained with different ultrasonic probes: 20 MHz and 30 MHz. Figure 4 shows the difference in the mean echo intensity between the third and fourth layer as well as the second and fourth layer using two different ultrasonic probes. The differences were found to be significantly greater with the 30-MHz probe than with the 20-MHz probe. Figure 5 shows the echo intensity curve of a central-type bronchogenic squamous cell carcinoma. This curve is not W-shaped, indicating that the bronchial wall is not composed of the normal five layers and the tumor has invaded beyond the bronchial wall.

DISCUSSION

In almost all reports discussing EBUS, the ultrasound images were compared using subjective evaluation; thus, the results might vary between examiners. For example, when one examiner states that a particular bronchial wall consists of four layers, another may state that it has five layers. When one examiner states that a tumor has invaded beyond the cartilaginous layer, another might state that the tumor is within the cartilaginous layer. When one examiner states that a particular image is clearer than another, a different examiner might state the reverse. These discrepancies are due to inadequate image resolution. If EBUS had a resolution as fine as abdominal ultrasonography, all examiners would arrive at the same conclusion, merely by subjectively evaluating the raw image data.

In cases with early central-type lung cancer, it may be essential for making the appropriate therapeutic decision to know the depth of tumor invasion into the bronchial wall at the laminar level. When a tumor penetrates the cartilaginous layer, photodynamic therapy (PDT) may no longer effective. To obtain an optimal therapeutic outcome, the physician needs to know whether the lesion has invaded the cartilaginous layer. Currently, EBUS is the only clinically available method for imaging the laminar structure of the bronchial wall; however, the currently available EBUS apparatus does not yield a sharp enough image to permit accurate evaluation of the depth of tumor invasion into the bronchial wall.
In this study, we employed image analysis software to digitize the EBUS image. This allowed us to draw a plot of the EBUS data from the bronchial wall and perform statistical analysis for an objective assessment. The laminar structure of the normal bronchial wall is represented by a 5-point W-shaped curve, from which the status of each layer can be estimated. When the digitized data did not form a W-shaped curve in this study, the bronchial wall was considered to be abnormal and presence of a tumor or other lesion was assumed. The degree of deviation from W-shaped curve reflected the extent of lesion invasion into the bronchial wall.

Few studies have subjectively compared the image quality of the bronchial wall layers using EBUS transducer probes of different frequencies. In this study, we examined the ultrasound images of the bronchial wall structure using both the 20-MHz and 30-MHz transducers. The ultrasound image data were digitized, and the images were statistically compared. The results revealed that the 30-MHz transducer was significantly more useful for delineating the bronchial wall layers than the 20-MHz probe. The 30-MHz transducer achieved excellent results, particularly in regard to the echo contrast between the second and fourth layers as well as between the third and fourth layers. For therapeutic decisions for to use PDT, it is crucial to determine whether the fourth layer is invaded by the tumor; the 30-MHz transducer appeared to be significantly superior to the 20-MHz transducer for the assessment of the extent of tumor invasion into the bronchial wall.

In summary, EBUS has been used commonly in the assessment of tumor invasion in early central-type lung cancers. However, it is often difficult to interpret the ultrasound images of the bronchial wall, which has a maximal thickness of 5 mm, and consists of five layers. Although accurate evaluation of tumor invasion of the fourth (cartilaginous) layer is required for a therapeutic decision for PDT or other treatment modalities, raw ultrasound images are often too unclear to distinguish each layer. In this study, we employed image analysis software and drew a plot to obtain a W-shaped curve from the EBUS image data. This enabled us to make an objective assessment of the laminar structure of the bronchial wall. Data distribution also showed a significant difference in the delineation of the bronchial wall structure between the two transducers: 20 MHz and 30 MHz. Our image analysis is an objective evaluation, and any examiner should arrive at the same results. If we cannot clearly image the laminar structure, we cannot accurately evaluate the extent of tumor invasion. For therapeutic decisions for PDT, evaluation of tumor invasion of the fourth layer (the cartilaginous layer) is considered to be crucial. We cannot perform PDT effectively if the tumor has invaded beyond the cartilaginous layer. In this study, we showed the statistically significant superiority of the 30-MHz probe in the imaging of the laminar structure.
structure of the bronchial wall. Specifically, by using the 30-MHz probe, we can more accurately image the cartilaginous layer. Accordingly, the 30-MHz probe was found to be more useful than the 20-MHz probe for recognition of the extent of tumor invasion into the bronchial wall. Until a high-resolution EBUS system permitting direct assessment of an ultrasonogram of the bronchial wall is developed, our approach should remain useful for the detection and evaluation of the laminar structure of the bronchial wall and the extent of tumor invasion. Our image analysis is an objective evaluation, and any examiner should arrive at the same results. Our next goal is to apply this analytical method to EBUS data in clinical practice.

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REFERENCES