Rapid On-Site Cytologic Evaluation During Endobronchial Ultrasound-Guided Transbronchial Needle Aspiration for Nodal Staging in Patients With Lung Cancer

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Background. **The utility of rapid on-site evaluation (ROSE) during endobronchial ultrasound-guided transbronchial needle aspiration (EBUS-TBNA) for lymph node staging in lung cancer is still controversial. The aim of this study was to assess the role of ROSE during EBUS-TBNA and the interpretation of its results.**

Methods. **We performed a retrospective chart review of patients with suspected or diagnosed lung cancer who underwent EBUS-TBNA for lymph node staging. The slides were air-dried and Diff-Quik (American Scientific Products, McGaw Park, IL) staining was used for ROSE. Additional smears were prepared for Papanicolaou staining and any remaining sample was placed in 10% formalin for histologic evaluation. The results of ROSE were compared with the results of the final pathologic diagnosis.**

Results. **EBUS-TBNA was performed in 438 patients on 965 lymph nodes. Eighty-four lymph nodes (8.7%) were determined insufficient for definitive diagnosis by final cytologic evaluation. However 45 of the 84 lymph nodes were able to be diagnosed by histologic examination. The non-diagnostic sampling rate was 4.0%. There were no false-positive results on ROSE; however 25 cases (5.7%) were falsely evaluated as negative on ROSE. The concordance rate for staging between ROSE and final pathologic diagnosis was 94.3%. The sensitivity, specificity, negative predictive value, and diagnostic accuracy rate of EBUS-TBNA for correct lymph node staging was 96.5%, 100%, 89.8%, and 98.2%, respectively.**

Conclusions. **ROSE during EBUS-TBNA for material adequacy showed a low rate of non-diagnostic sampling. There was a high agreement between the on-site and final pathologic evaluation during EBUS-TBNA; however immediate diagnosis should be approached with caution.**

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Endobronchial ultrasound-guided transbronchial needle aspiration (EBUS-TBNA) is a minimally invasive modality for lymph node staging in patients with lung cancer. The pooled sensitivity and specificity is reported to be 85% to 100% and 100%, respectively [1–3]. Moreover, a recent prospective trial showed that EBUS-TBNA has similar diagnostic yield to mediastinoscopy in patients with potentially resectable non-small cell lung cancer [4].

The presence of a cytopathologist during the procedure for rapid on-site evaluation (ROSE) seemed to reduce futile additional biopsies without loss in diagnostic yield [5]. The results of a randomized trial that evaluated the utility of ROSE for the diagnosis of adenopathy using TBNA showed that ROSE significantly reduces the number of TBNAs and the complication rate of the bronchoscopy [6]. ROSE during endoscopic ultrasound guided fine needle aspiration (EUS-FNA) has been reported to achieve high diagnostic yield and it is highly recommended that resources be allocated to it [7–9]. A high concordance rate is reported between cytologic results of EBUS-TBNA with ROSE and referenced histologic diagnosis [10]. Furthermore, to confirm the quality of the specimen obtained during EBUS-TBNA, it is important to apply the samples for several kinds of molecular analysis [11]. However there may be some difficulties in ROSE of aspirates from EBUS-TBNA because of the contamination of background material [12]. In addition, a recent study raised a question about the utility of ROSE for the evaluation of EBUS-TBNA sam-

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ples because there was no difference for clinical values, including diagnostic yield [13]. The interpretation of ROSE results on EBUS-TBNA samples and its role in nodal staging using EBUS-TBNA in patients with lung cancer remains controversial. This study focused on the correlation between ROSE and final pathologic diagnosis for lymph node staging in patients with lung cancer.

Patients and Methods

Patients

A retrospective chart review was completed for 438 patients with suspected or diagnosed lung cancer who underwent EBUS-TBNA for purposes of lymph node staging from April 2005 to March 2008 at the Department of General Thoracic Surgery, Graduate School of Medicine, Chiba University. All patients were evaluated by computed tomography (CT) of the chest and upper abdomen. Chest and upper abdominal CT was performed with contrast single injection and multidetector-row CT (Light Speed; GE Medical Systems, Milwaukee, WI). EBUS-TBNA was performed in patients with radiologically defined mediastinal and/or hilar lymph nodes with a short axis of 5 mm or more on enhanced chest CT [14]. The primary tumor and lymph node status was classified according to the international TNM staging system reported by Mountain and Dressler [15].

The study was approved by the ethical committee of Chiba University, Graduate School of Medicine (No. 220). This study was a retrospective review of diagnostic results and clinical history and additional informed consent was not necessary.

EBUS-TBNA

The convex probe endobronchial ultrasound-equipped bronchoscope with a 7.5 MHz convex scanning probe on its tip (BF-UC260F-OL8, Olympus, Tokyo, Japan) with the dedicated 22-gauge needle (NA-201SX- 4022, Olympus) was used for EBUS-TBNA. EBUS-TBNA was performed on an outpatient basis using conscious sedation. Local anesthesia was achieved with nebulized 1% lidocaine solution (5 mL) in the pharynx. Bolus doses of 2 mL of 2% lidocaine were used during the procedure [14]. The bronchoscope was inserted orally using conscious sedation with midazolam. Patients were monitored with electrocardiography, pulse oximetry, and blood pressure measurements without the presence of an anesthesiologist. All mediastinal lymph node stations accessible by convex probe EBUS (stations 2, 4, and 7) as well as the hilar lymph nodes (stations 10 and 11) were assessed. Lymph nodes with a short diameter of more than 5 mm were sampled with a dedicated 22-gauge TBNA needle under direct EBUS guidance [14].

Specimen Handling

The aspirated material was smeared onto glass slides. Smears were air dried as well as fixed in 95% ethanol. Air-dried smears were stained using Diff-Quik stain (American Scientific Products, McGaw Park, IL) and were used for ROSE. Additional smears were also prepared and fixed in 95% alcohol for Papanicolaou staining. Any remaining samples were placed in 10% formalin for histologic evaluation. The formalin-fixed paraffin-embedded samples were made and tissue diagnosis was performed by hematoxylin and eosin (H&E) staining.

ROSE and Final Pathologic Diagnosis

As a result of ROSE, the aspiration samples were categorized as positive for malignancy, negative for malignancy, or non-diagnostic. ROSE was performed after each pass. Multiple passes were performed for each site until on-site evaluation was diagnostic of a disease process or showed an adequate amount of lymphoid material ($>$ 40 lymphocytes in high power field or the presence of clusters of anthracotic pigment-laden macrophages [16]). The results of ROSE were compared with the corresponding results of the final pathologic diagnosis based on Papanicolaou staining plus H&E staining.

Statistical Analysis

The sensitivity, specificity, positive predictive value, negative predictive value, and diagnostic accuracy rate for prediction of lymph node staging was calculated using the standard definitions.

Results

Patients

EBUS-TBNA was performed in 438 patients. There were 331 men and 107 women with an average age of 70 years (28–87 years). The primary tumor diagnoses were as follows: 221 cases of adenocarcinoma, 131 cases of squamous cell carcinoma, 14 cases of large cell carcinoma (including large cell neuroendocrine carcinoma), 10 cases of other histologic types (including mucoepidermoid carcinoma, pleomorphic carcinoma, and basaloid carcinoma), 20 cases of non–small-cell carcinoma, 25 cases of small-cell carcinoma, and 17 cases of suspected lung cancer (final diagnosis of cases of 14 pneumonia and 3 cases of sarcoidosis).

EBUS-TBNA and Result of ROSE for Each Lymph Node

Nine hundred sixty-five lymph nodes (707 mediastinal and 258 hilar lymph nodes) were evaluated by EBUS-TBNA. On average, 2.2 lymph nodes per patient were punctured (1–8 lymph nodes per patient). The median size of the assessed lymph node short axis was 8.7 mm (range, 2.1–42.7 mm) on EBUS image. Multiple passes were performed until on-site evaluation was judged as diagnostic. Compared with the final cytologic diagnosis, 84 lymph nodes (8.7%) were judged as insufficient for definitive diagnosis. However 45 of 84 lymph nodes were able to be diagnosed by histologic examination. Thirty-nine of 84 lymph nodes remained non-diagnostic by both cytologic and histologic evaluation, even though ROSE

Table 1. Comparison of the Result of Rapid On-Site Evaluation (Diff-Quick) and Papanicolaou Staining

Rapid On-Site Evaluation (Diff-Quik)	Papanicolaou Staining		
	Positive	Negative	Total
Positive	190	4	194
Negative	18	226	244
Total	208	230	438

judged them as diagnostic. The non-diagnostic sampling rate of EBUS-TBNA with ROSE in this study was 4.0%. Concerning the non-diagnostic 39 lymph nodes, all lymph nodes were confirmed as nonmetastatic by surgical staging or clinical follow-up.

Eventually, pathologic nodal staging for mediastinal lymph nodes could be achieved in all cases. Histologic diagnosis could be obtained in 384 of 438 patients (87.7%). There were no complications related to the EBUS-TBNA procedure.

ROSE and Correlation to Final Pathologic Results

ROSE was performed in all cases. There was a discrepancy between the results of ROSE and Papanicolaou staining in 22 cases (Table 1). The concordance rate between ROSE and final cytologic diagnosis based on Papanicolaou staining was 95.0%. Four of 22 cases were positive for ROSE and negative for Papanicolaou staining; however all these cases were diagnosed as positive for metastasis by histologic diagnosis using histologic cores (Table 2).

The results of ROSE correlated well with the final pathologic diagnosis based on Papanicolaou/H&E evaluation. There were no false-positive results with ROSE; however 25 cases were falsely evaluated as negative with ROSE, which accounted for 5.7% of the cases (false-negative rate of 5.7%) (Table 3). Eighteen of 25 cases were diagnosed as positive by Papanicolaou/H&E evaluation, and 7 cases were diagnosed as positive by histologic examination only. The sensitivity and specificity of ROSE compared with the Papanicolaou/H&E evaluation was 88.6% and 100%, respectively (Table 4).

Table 2. Final Results of Rapid On-Site Evaluation (Diff-Quick)–Positive and Papanicolaou Staining–Negative Cases

Case	Rapid On-site Evaluation (Diff-Quik®)	Papanicolaou	Histological Dx (H&E)
Case 1	Atypical cells	No metastasis	Squamous cell cancer
Case 2	Atypical cells	No metastasis	Adenocarcinoma
Case 3	Adenocarcinoma	Insufficient specimen	Adenocarcinoma
Case 4	Atypical cells	No metastasis	Carcinoma

H&E = hematoxylin and eosin.

Table 3. Comparison of EBUS-TBNA Results With Final Diagnosis

EBUS-TBNA Results	Final Diagnosis		
	Malignant	Benign	Total
Malignant	219	0	219
Benign	8	211	219
Total	227	211	438

Sensitivity = 96.5% (219/227 cases), specificity = 100% (211/211 cases), and diagnostic accuracy = 98.2% (430/438 cases).

EBUS-TBNA = endobronchial ultrasound-guided transbronchial needle aspiration.

EBUS-TBNA Results for Nodal Staging of Lung Cancer

Nodal staging based on EBUS-TBNA results was confirmed by open thoracotomy, thoracoscopy, or clinical follow-up. In patients with malignant lymph nodes, the determination was based on malignant cytologic and/or histologic results at EBUS-TBNA or surgical/pathologic confirmation. In patients with benign lymph nodes, this determination was based on surgical pathologic confirmation of EBUS-TBNA targeted nodes by lymph node dissection of the lymph node station of interest or by results of clinical follow-up for at least 6 months demonstrating a lack of clinical or radiologic disease progression [14]. The sensitivity, specificity, negative predictive value, and diagnostic accuracy rate of EBUS-TBNA for lymph node staging in this study was 96.5%, 100%, 89.8%, and 97.9%, respectively (Table 3).

Comment

EBUS-TBNA is a minimally invasive and accurate modality for mediastinal lymph node staging in lung cancer. ROSE during EBUS-TBNA for material adequacy was considered to reduce the rate of non-diagnostic samples; however very few studies have investigated ROSE during the procedure and reported contrary results. The interpretation of the result of ROSE remains unclear. In this study, the non-diagnostic sampling rate of EBUS-TBNA was only 4.0% and the nodal staging based on ROSE results correlated well with the final results based on

Table 4. Comparison of the Result of Rapid On-Site Evaluation (Diff-Quick) and Final Diagnosis

Rapid On-Site Evaluation (Diff-Quik)	EBUS-TBNA Results (H&E/Papanicolaou)		
	Positive	Negative	Total
Positive	194	0	194
Negative	25	219	244
Total	219	219	438

Concordance = 94.3%, sensitivity = 88.6%, specificity = 100%, negative predictive value = 89.8%.

EBUS-TBNA = endobronchial ultrasound-guided transbronchial needle aspiration; H&E = hematoxylin and eosin.

Papanicolaou/H&E evaluation. High agreement between the on-site and final cytologic evaluation may be applicable to early therapeutic decisions when ROSE results are positive. Conversely, negative results by ROSE need further cytologic as well as histologic evaluation for accurate diagnosis because there is a substantial false-negative rate. The false-negative rate of EBUS-TBUA was 5.7% in this study. These results may suggest that EBUS-TBNA is best performed before a confirmed final diagnosis and planned surgical resection.

The utility of ROSE during TBNA has been reported. It is reported that at least 5 to 7 TBNA procedures would be needed to achieve a plateau diagnostic yield [17, 18]. ROSE during TBNA significantly improves the diagnostic yield [19, 20] and also reduces the number of passes necessary for achieving a high diagnostic yield [5, 6]. Similar results have been reported for EUS-FNA, which is performed in a fashion similar to that of EBUS-TBNA [8, 9]. Conversely, EBUS-TBNA samples are often difficult to interpret because of the quality of the sample. The amount of lymphoid tissue depends not only on the technical aspect but also on cellularity of the lymph node [21]. EBUS-TBNA allows the evaluation of smaller lymph nodes than does conventional TBNA; therefore it may result in marginal diagnosis such as a "suspicious" negative result [21].

ROSE has been used to determine adequacy of EBUS-TBNA samples by the presence of normal lymphocytes, especially for non-metastatic lymph nodes. As noted by us and others, ROSE of EBUS-TBNA samples could help determine the need to collect additional samples for pathologic diagnosis. To create an algorithmic approach is warranted and may avoid false-negative and false-positive diagnoses [16, 22, 23]. The results of this study showed no false-positive results for ROSE for the nodal staging in patients with lung cancer. Therefore the result of this study may suggest that ROSE can be used to determine a definitive malignant diagnosis. In contrast, the negative predictive value of ROSE was 89.8% and there were 5.7% false-negative results. Hence cases in which a diagnosis of "negative for malignancy" is not rendered at on-site interpretation need further pathologic evaluation.

Rapid on-site assessment during EBUS-TBNA determines if sampling of target nodes has been achieved. This is important not only for improving the yield but also for improving the skills of the bronchoscopist. ROSE also enables triage of material to secondary investigations (ie, culture, flow cytometry) [14, 22]. Recently, lung cancer is often diagnosed by small biopsied cytologic samples. Conversely, clinical requirement for further classification and detailed biological characteristic analysis is increasing [24]. From this point of view, ROSE is an important part of the EBUS-TBNA procedure for tissue diagnosis and staging of lung cancer.

The limitation of this study is that this is not a prospective randomized trial. There remains the question about whether we could achieve a low rate of non-diagnostic sampling and high diagnostic yield without ROSE. The feeling of bronchoscopists is that ROSE is an important step to optimize the sampling procedures during EBUS-TBNA, but we will need a well-designed controlled study to support this consideration. Another limitation is that only Diff-Quik evaluation was performed for ROSE. Diff-Quik staining is based on a modification of the Wright-Giemsa stain; therefore differentiation between lymphocytes and other cells is relatively easy. Diff-Quik staining does not require ethanol fixation and the samples can be stained rapidly. Conversely, it was reported that false-negative diagnoses were more frequent with the rapid Wright-Giemsa stain than with the rapid Papanicolaou stain for the diagnosis of TBNA aspirates [25]. The sensitivity of ROSE may be improved by the use of rapid Papanicolaou staining. However the high concordance rate for the diagnosis of malignant cells in our current study is promising for ROSE using Diff-Quik staining.

In conclusion, ROSE during EBUS-TBNA for material adequacy showed a very low rate of non-diagnostic sampling. Positive results of ROSE during EBUS-TBNA are highly accurate, and the results produced by ROSE may help initiate the treatment process in patients with lung cancer. However negative results produced by ROSE should be interpreted with care. Such results need to be followed with a final pathologic diagnosis because there is a substantial false-negative rate with ROSE.

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