Endobronchial Ultrasound–Guided Transbronchial Fine-Needle Aspiration

The University of Minnesota Experience, With Emphasis on Usefulness, Adequacy Assessment, and Diagnostic Difficulties

Mariam Alsharif, MD,1 Rafael S. Andrade, MD,2 Shawn S. Groth, MD,2 Edward B. Stelow, MD,3 and Stefan E. Pambuccian, MD1

Key Words: Pulmonary and mediastinal cytology; Fine-needle aspiration; Endobronchial ultrasound; Adequacy

Abstract

Endobronchial ultrasound–guided transbronchial fine-needle aspiration (EBUS-TBNA) is a new technique that facilitates cytologic sampling of mediastinal lymph nodes. We describe our initial experience with this method, including adequacy assessment, impact on cytopathologists’ work, and diagnostic pitfalls. There were 229 EBUS-TBNA samples obtained from 100 patients; a mean of 22 minutes was spent with an average of 3 passes performed and 6 slides prepared per site. Of 193 aspirates, 5 were categorized as atypical, 54 as positive, and 134 as negative for malignancy; 36 (15.7%) aspirates were nondiagnostic. We found EBUS-TBNA to have a high specificity (100%) and good sensitivity (86%) in our institution, in which a cytopathologist is available on-site to ensure sample adequacy. Most true-negative samples had moderate to abundant lymphocytes, confirming lymphocyte numbers as a marker of adequacy. For pathologists, this was a relatively time-consuming procedure. Recognizing bronchial contamination, especially with metaplastic or dysplastic cells, is important for avoiding diagnostic pitfalls.

Most lung cancers can be diagnosed by a bronchoscopic or other minimally invasive procedure.1 The prognosis for these cancers depends greatly on the presence of mediastinal lymph node metastases. Imaging studies are often used for the primary staging of lung cancer. Standard imaging techniques such as computed tomography (CT) and positron emission tomography (PET) have their limitations, however, because enlarged, benign lymph nodes can be present with lung cancer and metastases can be present in small nodes. Although PET is more accurate than CT for mediastinal staging,2 pathologic confirmation is needed for PET “positive” nodes and mediastinoscopy with biopsy remains the “gold standard” for the assessment of mediastinal nodal disease.

Mediastinoscopy is an invasive surgical procedure and poses small but significant risks to patients. Transbronchial needle aspiration (TBNA), on the other hand, is safer and less expensive. It is highly specific for identifying mediastinal metastatic disease in patients with non–small cell lung cancer, and complications are uncommon.3-5 Conventional TBNA, however, is a “blind” procedure that is operator-dependent and restricted to large subcarinal nodes. Its sensitivity varies from 39% to 89%.4

Endobronchial ultrasound (EBUS)-guided TBNA (EBUS-TBNA) using a novel endobronchoscope with needle has been noted to have higher yields than those typically associated with conventional TBNA (85% vs 66%).6-22 Some of the early studies on conventional TBNA, however, did not use on-site evaluation of adequacy of the specimens,23 which may have improved the sensitivity of the procedure by increasing the rate of adequate specimens. EBUS-TBNA allows sampling of upper and lower paratracheal (stations 2 and 4), station 3, and subcarinal (station 7) lymph nodes, the nodes...
most frequently sampled during cervical mediastinoscopy. In addition, it can also reach hilar and interlobar lymph nodes (stations 10 and 11). In contrast, endoscopic ultrasound-guided fine-needle aspiration (EUS-FNA) performed through the esophagus provides access to paraesophageal and inferior mediastinal lymph nodes (stations 7-9) with limited or no access to the subaortic (station 5) and para-aortic (station 6) nodes. EUS-FNA is an established procedure with a high diagnostic accuracy that is widely used for the detection, staging, and planning of optimal management of gastrointestinal tract and pancreatic tumors. EUS-FNA has also been successfully used in sampling of mediastinal lesions and for staging of lung cancer.

EBUS-TBNA has been reported to perform very well with high sensitivities, specificities, positive predictive values, and accuracy, even when very small lymph nodes are sampled. This study reviewed the University of Minnesota (Minneapolis) experience with this technique from the cytopathologist’s perspective.

Materials and Methods

We performed a computerized search of the cytopathology electronic database from our institution for all aspirations performed by EBUS-TBNA from September 1, 2006, to February 14, 2008. In our institution, the EBUS-TBNA procedure was performed under real-time guidance in the operative suite by a thoracic surgeon. This practice enabled immediate conversion to an alternative, more invasive procedure (eg, mediastinoscopy or thoracoscopy) if indicated. An ultrasonic bronchofibervideoscope (Olympus Exera, Olympus Imaging America, Center Valley, PA) was used. Each target lymph node station was punctured with a 22-gauge needle 1 to 3 times using 15 needle revolutions. If necessary, multiple passes were done to obtain diagnostic material.

The presence of an on-site cytopathologist or cytopathology fellow was required in our institution during EBUS-TBNA procedures. He or she performed adequacy assessment and made preliminary interpretations on each sampled node or lesion. This was done using air-dried material stained with the rapid-Romanowsky method. The cytopathologist or cytopathology fellow prepared all smears. In general, depending on quantity, at least 1 air-dried smear was prepared from each FNA pass. Additional smears were also prepared and air-dried or fixed in 95% alcohol to be later stained by the Papanicolaou method; any remaining sample was placed in 10% formalin for cell block preparation. An immediate assessment was given after each pass. Multiple passes were performed for each site until on-site assessment was diagnostic of a disease process or showed an adequate amount of lymphoid material. The thoracic surgeon performing the FNA decided on when to stop collecting material despite nondiagnostic samples. The immediate interpretation was frequently noted by the thoracic surgeon on a form and subsequently entered in the spreadsheet with the final diagnosis.

Thin sections from the paraffin-embedded cell block were cut on the following day and were stained with H&E for light microscopy. The aspirate samples from the lymph node sites were categorized as “positive for malignancy” when frankly malignant cells were present, as “suspicious” when rare cells suspicious for malignancy were seen, as “atypical” when reactive or dysplastic bronchial epithelium was present, as “negative for malignancy” when lymphoid tissue without tumor cells were seen, and as “nonrepresentative/nondiagnostic” when scant or no lymphoid tissue was present and only blood, mucus, and/or benign bronchial cells were seen or when the specimen was acellular. Patients with negative or nondiagnostic cytology on EBUS-TBNA frequently underwent immediate mediastinal lymph node biopsies with an alternative sampling procedure such as mediastinoscopy or thoracoscopy.

The cytologic slides from all cases were retrieved and retrospectively reviewed by 2 pathologists (M.A. and S.E.P.) with experience in cytopathology. The slides were reassessed for the following features: presence and amount of tumor cells (including their presence in the cell block sections); presence and amount of bronchial epithelial cells; background acute inflammation, granulomas, and necrosis. A semiquantitative assessment of the number of lymphocytes was performed by one of us (M.A.), blinded to the follow-up histologic diagnosis, on an Olympus BX45 microscope (Olympus Imaging America). Lymphocyte numbers were estimated in the most cellular areas of the slides with most lymphocytes under ×40 magnification using a scoring system of 0 to 3. Scant numbers of lymphocytes were assigned a score of 0 (<40 lymphocytes), low numbers a score of 1 (41–200 lymphocytes), moderate numbers a score of 2 (>200 lymphocytes, but not confluent), and abundant numbers a score of 3 (confluent sheets of lymphocytes or germinal center fragments). A lymphocyte score of 0 was considered nonrepresentative and a score of 1 or more or the presence of clusters of anthracotic pigment-laden macrophages as representative of lymph node sampling.

In cases with subsequent mediastinoscopic or surgical staging resections, tissues were fixed in 10% formalin and thin histologic sections were cut from paraffin-embedded tissue blocks followed by H&E staining. A retrospective review of all available histologic slides was performed and correlated with the respective cytologic diagnoses. A spreadsheet was prepared containing patient age and sex, clinical history, procedure time, number of slides examined, amount of lymphoid tissue, cytologic immediate interpretation and final diagnosis for each site sampled, and histologic correlation when
available. Then $2 \times 2$ tables were prepared to calculate the sensitivity, specificity, and positive and negative predictive values, and the Fisher exact test was used to compare differences between groups. Calculations were performed using SPSS 14.0 for Windows (SPSS, Chicago, IL). An $\alpha$ of .05 was considered significant.

**Results**

EBUS-TBNA was performed on 100 patients (52 men and 48 women; ages 27 to 91 years; mean $\pm$ SD, 61 $\pm$ 14 years). Overall, 33.0% of patients (33/100) had histologic follow-up. There were 229 samples obtained from 219 lymph nodes and 10 lung lesions. The smallest lymph node from which diagnostic tissue was obtained was 4.5 mm. The mean number of needle passes from each site was 3.

Aspirate samples from 36 sites (36/229 [15.7%]) were considered nondiagnostic. Of the nondiagnostic samples, 10 had corresponding surgical pathology follow-up. Of these, 6 were benign and 4 were malignant. Of the aspirate samples, 193 were diagnostic: 5 of these were designated as “atypical” including 2 interpreted as “radiation-induced atypia,” 2 with “reactive/dysplastic bronchial epithelium,” and 1 with a single cluster of atypical cells that ultimately correlated with a micropapillary carcinoma, metastatic from the lung. This case also showed granulomatous inflammation on cytologic and histologic examination **Image 1**.

**Image 1** Granulomatous inflammation (A, rapid Romanowsky, $\times$200) and cluster of atypical cells (B, rapid Romanowsky, $\times$400). Corresponding lymph node histologic features showing sarcoid-type granulomata (C, H&E, $\times$1) and foci of metastatic micropapillary carcinoma (D, H&E, $\times$400).
Fifty-four samples were diagnosed as positive for malignancy. Of these, 40 were from pulmonary malignancies (9 small cell carcinomas, 16 adenocarcinomas, 11 squamous cell carcinomas, and 4 non–small cell carcinomas, not otherwise specified) and 10 represented distant metastases to the lung or mediastinal lymph nodes of known extrathoracic malignancies. These were metastases from carcinomas of the breast and endometrium (2 each) and esophagus, colon, and urothelium (1 each) and from hepatocellular carcinoma (1 case) and melanoma (2 cases). Four samples showed lymphoma (3 Hodgkin lymphoma and 1 T-cell lymphoma), in which the diagnosis was made with the help of flow cytometry or immunohistochemical stains. Of the samples, 134 were interpreted as benign. Table 1 and Table 2 summarize the clinical details including indications (Table 1) and cytologic and histologic results by site sampled (Table 2) of all EBUS-TBNA cases.

Of the 193 samples that were considered diagnostic on EBUS-TBNA, 54 (28.0%) had histologic follow-up. When

<table>
<thead>
<tr>
<th>Indication</th>
<th>No. (%) of Cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary lung cancer staging: NSCC or SCLC</td>
<td>37 (37.0)</td>
</tr>
<tr>
<td>Restaging of lung cancer</td>
<td>10 (10.0)</td>
</tr>
<tr>
<td>Mediastinal lymphadenopathy with known extrathoracic malignancy</td>
<td>22 (22.0)</td>
</tr>
<tr>
<td>Mediastinal lymphadenopathy without known extrathoracic malignancy</td>
<td>30 (30.0)</td>
</tr>
<tr>
<td>Other (questionable lung lesion/nodule)</td>
<td>1 (1.0)</td>
</tr>
</tbody>
</table>

NSCC, non–small cell lung cancer; SCLC, small cell lung cancer.
histologic diagnoses were taken as the gold standard, the sensitivity, specificity, and positive and negative predictive values were 86%, 100%, 98%, and 100%, respectively. No false-positive case was encountered. The only false-negative case had a small metastatic deposit that was lying outside the node in the perinodal soft tissue.

The diagnostic criteria for pulmonary and other organ metastases to the mediastinal lymph nodes are not significantly altered in specimens obtained by EBUS-TBNA. Unique features associated with this technique included the presence of fragments of cartilage and variable numbers of normal, reactive, metaplastic, or even dysplastic bronchial epithelial cells. Image 3 and Image 4. Bronchial cells were seen in more than 80% of all cases. These features are expected because the mediastinal lymph node lesions were approached transbronchially.

Of the benign cytologic samples, 34 showed granulomatous inflammation; this was confirmed on histologic examination in all 13 cases in which follow-up was available. In addition, 3 of the patients had etiologic agents for the granulomatous disease confirmed by special stains, including 1 case of histoplasmosis, 1 with acid-fast bacilli, and 1 of blastomycosis.

Table 2
Cytologic and Histologic Diagnoses by Site

<table>
<thead>
<tr>
<th>Location (Lymph Node Station, Lung)</th>
<th>Cytologic Diagnosis</th>
<th>Histologic Diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nondiagnostic</td>
<td>Negative for Malignancy (Granulomata)</td>
<td>Atypical</td>
</tr>
<tr>
<td>2R</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>4R</td>
<td>8</td>
<td>23 (5)</td>
</tr>
<tr>
<td>4L</td>
<td>9</td>
<td>15 (3)</td>
</tr>
<tr>
<td>3</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>7</td>
<td>8</td>
<td>51 (14)</td>
</tr>
<tr>
<td>10R</td>
<td>0</td>
<td>6 (3)</td>
</tr>
<tr>
<td>10L</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>11R</td>
<td>2</td>
<td>23 (6)</td>
</tr>
<tr>
<td>11L</td>
<td>4</td>
<td>10 (3)</td>
</tr>
<tr>
<td>12R</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>12L</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>RUL</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>RLL</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>LUL</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>LLL</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>36</td>
<td>134 (34)</td>
</tr>
</tbody>
</table>

L, left; LLL, left lower lobe; LUL, left upper lobe; R, right; RLL, right lower lobe; RUL, right upper lobe.

Image 3 Cytologic features of metaplastic bronchial cells in a patient treated with chemotherapy and radiation (rapid Romanowsky, ×1,000).

Image 4 Large sheet of reactive bronchial cells. Note the presence of focal cilia (left inset) and nuclear atypia (right inset) (rapid Romanowsky, ×200; insets, ×1,000).
In all cases with granulomatous inflammation, we obtained material for microbiologic cultures that allowed testing for infectious etiologies and further treatment.

Immediate on-site interpretations correlated well with final cytologic interpretations in the 221 samples for which this information was recorded. Immediate on-site interpretation predicted a final malignant diagnosis in 49 of 50 cases (4 interpreted as atypical/suspicious and 45 as positive for malignancy). Of the 42 cases interpreted as nondiagnostic on rapid on-site evaluation, additional material identified on final evaluation of fixed smears and cell block preparations allowed a change to a diagnosis of benign in 6 cases and to atypical in 1. Of the 8 cases interpreted as atypical on the immediate assessment, 4 were classified as malignant on final diagnosis.

The false-negative rate (4/10) of aspirates that were considered nondiagnostic was statistically significantly higher than that of FNAs that were considered negative for malignancy (1/48) \( (P = .002) \).

Most lymph node aspirates (41/47 [87%]) interpreted as negative for malignancy on cytologic examination and later confirmed as negative on histologic examination had moderate to abundant numbers of lymphocytes (score 2 or 3) in the aspirate smears and/or cell block.

The procedure time for EBUS-TBNA ranged from 10 to 200 minutes for each location sampled. A mean of 22 minutes and a median of 15 minutes per site were calculated. The average time spent in the operative suite for any given case was 35 minutes. On average, 6 smears were prepared per site. One

**Image 5**. In all cases with granulomatous inflammation, we obtained material for microbiologic cultures that allowed testing for infectious etiologies and further treatment.

**Image 5**. Blastomycosis. **A**, Pyogenic granulomatous inflammation (Papanicolaou, ×1,000). **B**, *Blastomyces dermatitidis* organism in histiocyte (Papanicolaou, ×1,000). **C**, *B dermatitidis* organisms with budding in giant cell (H&E, ×1,000). **D**, Silver-stained *B dermatitidis* organism with budding (Gomori-methenamine-silver, ×1,000).
of the earlier cases had 67 slides from multiple passes done to obtain diagnostic material. Most of the smears were air dried, stained using rapid Romanowsky stain, and evaluated by the on-site cytopathologist.

**Discussion**

EBUS-TBNA is a relatively new modality for sampling mediastinal lymph nodes, and there are few studies published in the United States on its clinical usefulness, with most reports coming from specialized centers in Europe, the United Kingdom, and Japan. These studies showed high diagnostic rates for EBUS-TBNA with sensitivities and positive predictive values of more than 90% and specificities of 100%. EUS-FNA through the esophagus is a well-established, safe, and relatively cost-effective sampling modality for the posterior/inferior mediastinum. EBUS-TBNA may be used complementary to EUS-FNA in the cytologic examination of different regions of the mediastinum, and, together, they can replace more invasive methods for evaluating patients with lung cancer with suspected mediastinal metastases and patients with other mediastinal disease.

EBUS-TBNA was introduced in recent years to the thoracic surgery practice at our institution as a new modality to diagnose and stage lung cancer and to evaluate mediastinal disease. Early experience with EBUS-TBNA by the thoracic surgery department in our institution focusing on the learning curve for performing this procedure concluded that good sensitivity and diagnostic accuracy could be achieved after performing 10 EBUS-TBNA procedures. In the present study, which includes more patients, diagnostic material was obtained in 84.3% of sites sampled (193/229). The unsatisfactory specimen rate was 15.7% (36/229). An accurate diagnosis was made in 53 (98%) of 54 satisfactory aspirates with histologic follow-up.

One of the limitations of our study is that only about 28% of the diagnostic samples (33% of patients) had histologic follow-up. We have attempted to correlate the cytologic findings with follow-up histologic findings because potential false-positive and false-negative results are easier to explain and reconcile. However, other studies have used a combination of histologic follow-up and clinical follow-up data and have found higher sensitivity rates. A recent meta-analysis has shown that the sensitivity of the EUS-FNA procedure was 79% (95% confidence interval [CI], 70%-85%) when histopathologic findings were used as the gold standard but increased to 93% (95% CI, 88%-96%) when a combination of histopathologic findings and clinical follow-up was used.

We observed that the presence of moderate to abundant numbers of lymphocytes or pigmented histiocytes was a good indicator of adequate sampling of lymph nodes free of metastasis. Forty lymphocytes per high-power field in the more cellular areas of the smear and/or the presence of clusters of pigmented macrophages were good predictors of final adequacy assessment. It should be noted, however, that in certain cases of nodal replacement by granulomatous or metastatic disease, lymphoid tissue might not be seen. The statistically significant difference that we found between nondiagnostic and negative sample false-negative rates reemphasizes the need for careful assessment of specimen adequacy during on-site evaluation.

Adequacy criteria are not well established and may vary among pathologists. Baker et al reported a significant difference in the predictive values of negative transbronchial aspirates with and without lymphocytes (78% vs 36%). Similar to Baker et al, we found that the presence and quantity of bronchial cells had no bearing on adequacy because these cells were found in the majority of our samples, without correlation with the number of lymphocytes.

The presence of bronchial cells, however, may pose difficult differential diagnostic problems, especially during rapid on-site evaluation, when finding the characteristic cilia may be difficult. This is even more of a problem because many of the patients sampled by this procedure have metaplastic and dysplastic changes of the bronchial epithelium and/or have been treated with chemotherapy and radiation, which can give rise to severe alterations of bronchial lining cells. Bronchial contamination can produce a diagnostic challenge for cytopathologists and may result in an “atypical” diagnosis or even false-positive interpretations. This finding is analogous to the interference observed by normal duodenal and gastric epithelial cells in samples of pancreatic lesions obtained by EUS-FNA using the transgastric or the transduodenal approach. Excess contamination with normal cells can obscure the scant malignant cells or, in cases with reactive respiratory cells, mimic a well-differentiated adenocarcinoma. Marked reactive atypia and metaplastic changes in bronchial epithelium and dysplasia of squamous cells can potentially cause false-positive diagnoses in pulmonary cytology. Careful attention to the presence of cilia, cellular uniformity, and absence of mitoses and abnormal nucleoli should lead one to favor a benign diagnosis.

Attention should also be given to morphologic changes caused by air drying and poor fixation and/or poor preservation. In our experience, atypical bronchial epithelium and reserve cell hyperplasia can be sampled as tight cohesive clusters with nuclear molding and without apparent cilia, making them extremely difficult to differentiate from small cell carcinoma. We found the cell block to be extremely helpful in such cases and perform immunohistochemical analysis when necessary.

The presence of granulomata, while most often an indicator of benign disease, does not entirely exclude coexistent
malignancy because lymph nodes harboring both necrotizing and nonnecrotizing granulomas and metastatic malignancies have been reported.56-58 The current series also includes such a case. In this case, sarcoid-like granulomata were found in association with metastatic malignancy. It should be also noted that granulomata may rarely mimic malignancy and may pose difficult differential diagnostic considerations.59

We did not encounter any false-positive cases. The single false-negative case was explained by the presence of a small metastatic deposit in the perinodal soft tissue rather than the lymph node itself.

The presence of an on-site cytopathologist is often advocated in ultrasound-guided FNA procedures for the determination of material adequacy by rapid on-site evaluation to reduce the rate of nondiagnostic samples and for preliminary diagnoses.45,60-64 High agreement between the on-site and final cytologic diagnoses of EUS-FNA specimens was reported.65,66 We showed this to be true for EBUS-TBNA in which the cytopathologist ensures optimal specimen preparation and accurate preliminary assessment. This high level of agreement is important because clinicians will base their therapeutic decisions on the rapid on-site interpretation (eg, culture of material in cases with granulomatous disease; flow cytometry in cases with atypical-appearing lymphocytes; mediastinoscopy in patients with negative results). The reproducibility of the diagnosis on EBUS-TBNA and EUS-FNA is excellent among pathologists with experience in these types of cytologic samples.45

EBUS-TBNA, however, can be a time-consuming procedure, and a cytopathologist may spend anywhere between 10 minutes and an hour in the operative or bronchoscopy suite for each case. A significant minority of cases take much longer when multiple sites are sampled. These prolonged procedure times can add a great deal to the daily workload of cytopathologists and need to be adequately compensated. Currently, intraprocedural consultations by cytopathologists for CT-guided, ultrasound-guided, bronchoscopic, or endoscopic procedures are compensated insufficiently by Medicare compensation schedules compared with routine surgical pathology.67 As a result, some institutions defer on-site FNA interpretation to cytotechnologists. When endoscopists try to perform immediate cytologic interpretations, they do so with less accuracy than cytotechnologists.68 Some other institutions place the entire specimen in a liquid-based cytologic container.69 The negative predictive value reported in these cases appears inferior to the one obtained by us and other groups using on-site interpretation. A recent meta-analysis48 showed that with on-site cytologic interpretation, sensitivity is 88% (95% CI, 80%-93%), whereas without it, sensitivity is 80% (95% CI, 72%-86%). Reduced procedure time and improved yield can be expected with increased experience in performing the procedure, similar to conventional TBNA.70,71

EBUS-TBNA is an accurate, minimally invasive method for diagnosing and staging lung cancer and other mediastinal disease. Performance of immediate on-site cytologic interpretation and assessment of adequacy by the cytopathologist can be time-consuming but significantly improves the diagnostic yield.

References


