Review Article

# Endobronchial Ultrasound-Guided Transbronchial Needle Aspiration (EBUS-TBNA): An Overview and Update for the Cytopathologist

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Endobronchial ultrasound-guided transbronchial needle aspiration (EBUS-TBNA) has emerged as a minimally invasive technique for evaluating the mediastinum and staging patients with lung cancer. In the hands of an experienced operator, the procedure is safe and provides excellent sensitivity, specificity, and predictive diagnostic values. In conjunction with endoscopic ultrasound-guided fine-needle aspiration (EUS-FNA), a nearly complete mediastinal evaluation can be performed in a minimally invasive fashion. This strategy results in improved lymph node staging, markedly reduced need for mediastinoscopy, and fewer futile thoracotomies compared with a traditional surgical staging procedure. The procedure is cost effective and provides excellent cytologic specimens that have proven well suited for ancillary testing, such as immunohistochemistry and tumor genotyping. EBUS-TBNA, initially used as a tool to sample the lymph nodes adjacent to the airway walls, has now become instrumental in sampling lesions in the mediastinum, hilum, and lung parenchyma, where previously more than 1 procedure would have been necessary. Looking forward, expanded use of this procedure is likely to revolutionize the access to cytology-proven staging and restaging of lung cancer and other thoracic malignancies in a minimally invasive fashion. ***Cancer (Cancer Cytopathol)*** **2014;122:561-76.** *© 2014 American Cancer Society.*

**KEY WORDS:** endobronchial ultrasound-guided transbronchial needle aspiration; EBUS-TBNA; fine-needle aspiration; FNA; cytology; lung cancer staging; lymph node; sample adequacy; cost effectiveness.

## INTRODUCTION

Lung cancer represents a major health burden worldwide and remains the leading cause of cancer mortality for both men and women in the United States, accounting for >25% of all cancer deaths.[1] Particularly for non-small cell lung cancers (NSCLCs), recent advances in tumor classification and the identification of targetable driver mutations have revolutionized the clinical management of these patients. The cornerstone for treatment decisions in lung cancer, however, still relies on appropriate staging. The most recent edition of the widely used International Union Against Cancer tumor-lymph node-metastasis (TNM) classification scheme for lung cancer is based on the retrospective analysis of more than 80,000 patients who were treated between 1990 and 2000 and provides a unified classification scheme to help the clinician plan treatment, guide prognosis, and facilitate the continued investigation of human cancer.[2] Central to this management algorithm is the pathologic staging of patients with known or suspected lung cancer, including the mediastinal lymph node stations.

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Over the past 2 decades, endobronchial ultrasound (EBUS)-guided transbronchial needle aspiration (EBUS-TBNA) has emerged as a highly effective, minimally invasive technique for sampling peribronchial, mediastinal, and lung masses for pathologic examination. EBUS-TBNA in patients with suspected lung cancer in many instances has become the first-line approach for cytopathologic diagnosis and staging. Indeed, in a single procedure, the clinician can simultaneously establish a diagnosis, stage the disease, and acquire adequate material for ancillary testing: all in a minimally invasive manner. Therefore, the cytopathologist has assumed an increasingly central role in the management of patients with lung cancer, in part because of the development and success of EBUS-TBNA.

Although many comprehensive review articles have been written on the topic of EBUS-TBNA as a whole,[3–5] there is a relative paucity of review articles focusing specifically on the aspects of the procedure that have more immediate relevance to the cytopathology community (see the excellent review by Cameron and colleagues).[6] In this setting, the current review is specifically written for a cytopathology audience as a collaborative effort by both cytopathologists and interventional pulmonologists with the goal of helping bridge the gap in understanding between the clinical performance and application of this procedure on the one hand, with the interpretation and testing of the cellular materials by the cytopathologist on the other. Therefore, the objectives of this review are to provide the cytopathologist with a comprehensive understanding of the procedure as described from the bronchoscopist's perspective and to serve as a timely update and reference on the performance measures and applications of this diagnostic modality in pulmonary disease.

## Development of the EBUS-TBNA Tools

The use of TBNA was first described by Schieppati in 1949 with the use of a rigid bronchoscope.[7] In 1983, Wang and colleagues described the use of TBNA through a flexible bronchoscope for the staging of lung cancer.[8] Unfortunately, the lack of real-time visualization and the impression of some pulmonologists that this was a "blind" procedure, coupled with a lack of training and unfounded safety concerns, led to variable implementation and yield.[9] The development of EBUS emerged as a viable option to obtain biopsy specimens from mediastinal structures by combining the minimal invasiveness of TBNA with high-definition images provided by a 7.5-MHz convex ultrasound probe. This new tool allows the operator to localize lymph nodes and obtain samples under direct visualization.

The first application of ultrasound in the endobronchial space occurred in 1992.[10] This late application of ultrasound can be attributed to the maxim that air is an enemy of ultrasound.[11] Because sound travels at a much lower velocity through air (330 meters per second) compared with water (1497 meters per second) or soft tissues (1540 meters per second), traditionally, the lungs were not considered a primary target for ultrasound diagnostics. However, because many pathologies of the respiratory system involve the relatively solid tracheobronchial wall and the parabronchial structures, EBUS was perfected; and, in 1999 the first dedicated EBUS bronchoscope system became commercially available, augmenting access to the bronchial and mediastinal structures for diagnostic purposes. For application inside the airways, flexible catheters with a 360-degree view were developed. A balloon at the tip allowed coupling of the ultrasound probe to the tracheobronchial wall providing a 360-degree image of the surrounding structures. Under ideal conditions, a 20-MHz probe provides a high-resolution image with a depth of 4 to 5 cm. The downside to using this device was the need to remove the ultrasound probe from the bronchoscope before introducing a needle to perform the biopsy.[12] This led to the development of a smaller 7.5-MHz convex transducer at the end of a dedicated flexible bronchoscope, in which images are obtained and coupled with a special needle, thus allowing real-time visualization during the needle puncture of parabronchial structures (Figs. 1 and 2). The 7.5-MHz ultrasound probe can obtain images at a depth of penetration of up to 9 cm. There are currently 3 manufacturers of dedicated convex-probe EBUS: Olympus, Pentax, and Fuji (all located in Tokyo, Japan). The outer diameter at the tip of the bronchoscope is 6.9 mm. The angle of view is 80 degrees, and the direction of view is 35 degrees forward oblique. The inner diameter of the working channel in the bronchoscope is 2.2 mm, and a dedicated, special 21-gauge or 22-gauge needle is used to perform TBNA (Fig. 2). Currently, new needles are being developed by various manufacturers with the goal of increasing the yield of EBUS-TBNA in lymphomas, rare tumors, and applications that require larger samples. Other manufacturers have directed their efforts toward increasing the echogenicity of the needles and have introduced a 25-gauge needle. Thus, as

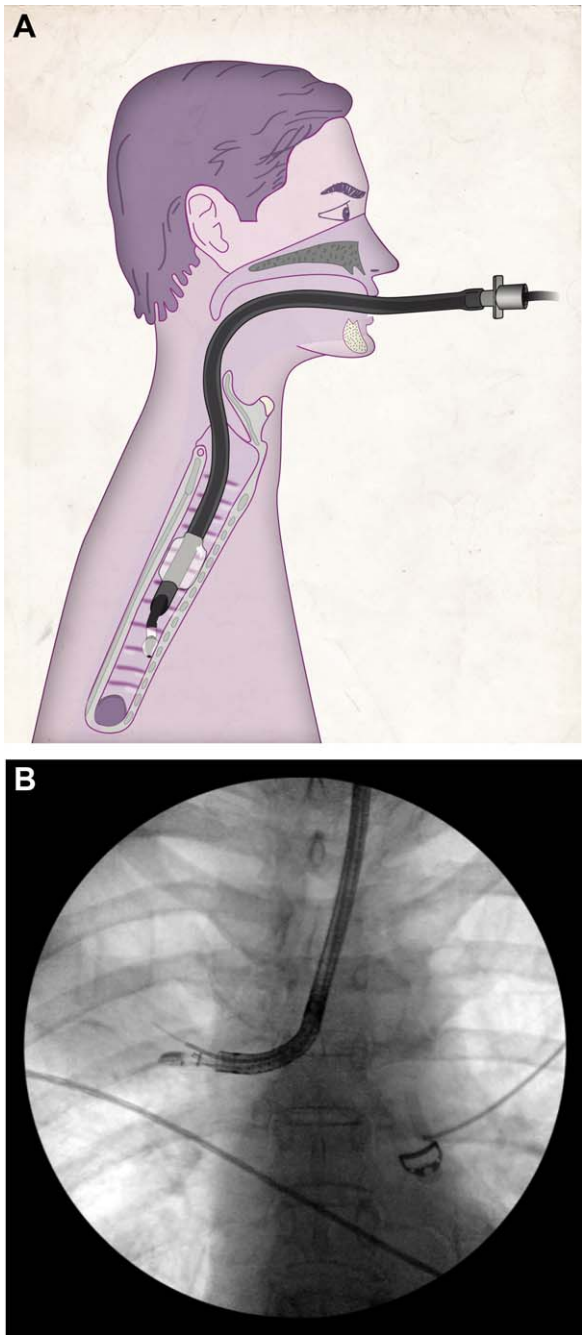


**Figure 1.** (A) This diagram depicts the use of endobronchial ultrasound for the identification of mediastinal, hilar, and parenchymal structures. The dedicated bronchoscope can be introduced through the mouth, through a laryngeal mask, or, as in this case, through an endotracheal tube. (B) This is a fluoroscopic view of a convex-probe endobronchial ultrasound (EBUS) with transbronchial needle aspiration of a peripheral parenchymal mass at the right upper lobe. The use of fluoroscopy in this case highlights the landmarks and peripheral location of the lesion. Although fluoroscopy is rarely necessary for endobronchial ultrasound-guided transbronchial needle aspiration, it is recommended for transbronchial lung biopsies without the use of real-time ultrasound guidance.

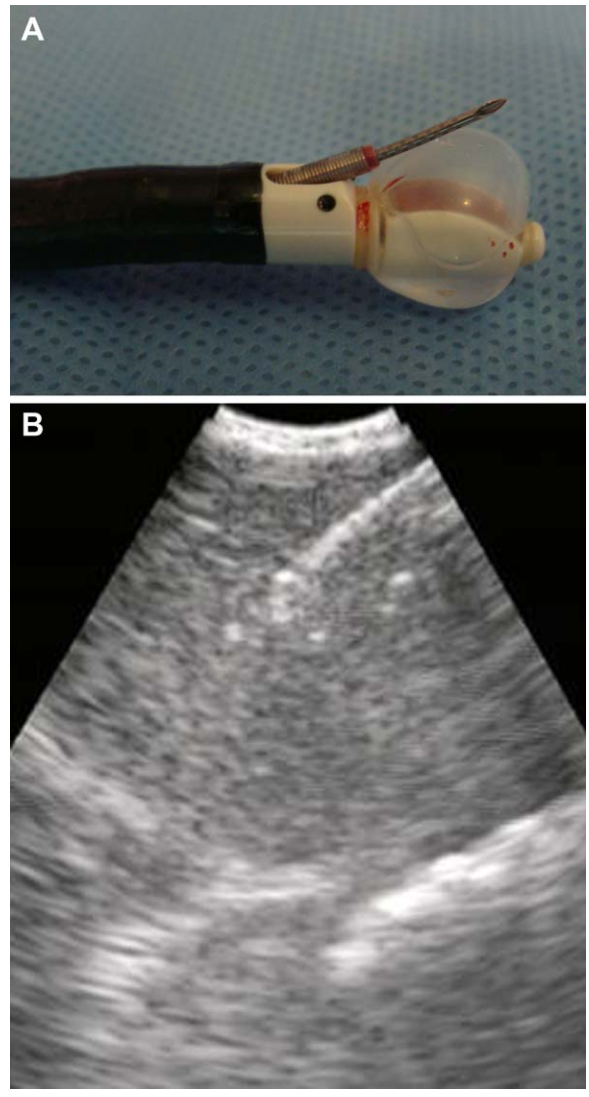


**Figure 2.** (A) The dedicated probe for endobronchial ultrasound is 6.9 mm in diameter at the distal end and has a 7.5-MHz convex transducer. The balloon is inflated with sterile water and allows for better coupling between the ultrasound probe and the airway wall. The dedicated needle is introduced through the working channel and comes out at a 35-degree angle that allows visualization in real time of the needle insertion. (B) Transbronchial needle aspiration using a dedicated 21-gauge needle is observed within the lymph node during the procedure. This real-time visualization is the biggest advantage of endobronchial ultrasound-guided transbronchial needle aspiration and the reason for its recent widespread implementation.

pulmonary application of EBUS continues to increase, it is likely that we will witness a broadening interest in the development of various needle sizes and specifications, as has been the case in the gastrointestinal arena.[13] In the last few years, a color Doppler function also has been added to the processor for improved differentiation of vascular and nonvascular structures (Fig. 3).

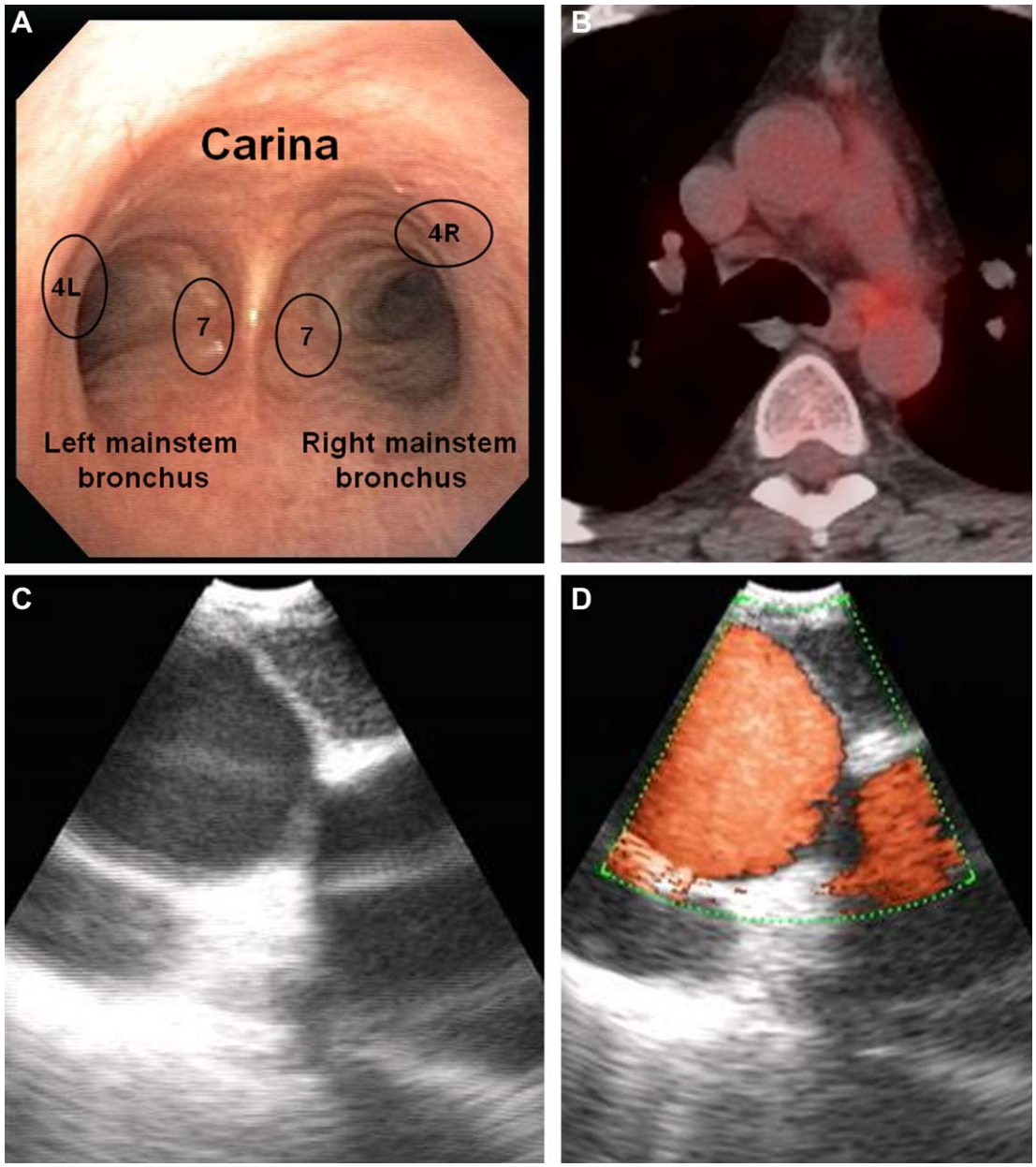


**Figure 3.** (A) This is an endobronchial view of the distal trachea. Labels indicate that the carina separates the right (R) and left (L) main bronchi. The subcarinal or lymph node station 7 can be accessed at the medial wall through either the right or left main bronchi. Station 4L is observed at the 9 to 10 o'clock position, proximal to the main carina. (B) This positron emission tomography-computed tomography image at the level of the carina demonstrates significant 2-deoxy-2-(18F)fluoro-D-glucose uptake at station 4L at the aortopulmonary window. (C) Endobronchial ultrasound images demonstrate the location of superficial lymph node station 4L and the flanking vessels: the aorta and left main pulmonary artery seen as anechoic (black) structures. (D) The use of Doppler allows the operator to clearly identify vascular structures. The fully colored vessel is the left main pulmonary artery, and the other vessel is the aorta. Lymph node station 4L can be seen clearly as a hypoechoic (gray) structure on the surface.

### *Overview of the EBUS-TBNA Procedure*

Although most cytopathologists are intimately familiar with the cytologic specimens generated by EBUS-TBNA procedure, it is important to have an understanding of just how those specimens were acquired. An appreciation for how the cellular material is obtained can aid the cytopathologist not only in the interpretation but also in potential troubleshooting when specimen adequacy is

suboptimal. Although the exact procedural features for EBUS-TBNA probably vary to a large degree from institution to institution, there are certain core steps to the procedure that should be followed in virtually every situation.

The bronchoscopist informs the patient of the indications for, risks and benefits of, and alternatives to the EBUS-TBNA procedure; and, after answering questions, the procedure is scheduled, typically at a later date and frequently in the outpatient setting. The patient typically fasts for 4 to 8 hours before the procedure to minimize the gastric contents and the risk of aspiration during sedation. During the procedure, the patient is monitored in usual fashion (heart rate, noninvasive blood pressure, and pulse oxymetry). The procedure can be done under moderate sedation or general anesthesia, depending on the local resources, purpose of the procedure, and individual patient characteristics. For example, whenever complete and systematic staging of the mediastinum is required or when endoscopic ultrasound (EUS) may be attempted, the use of general anesthesia is preferred; however, if only 1 or 2 lymph nodes will be biopsied, then moderate sedation with midazolam and fentanyl is commonly used. In both cases, local anesthetic is used to minimize coughing. The procedure can be carried out in the operating room or in a procedural suite with monitoring capabilities. Given the short half-life of midazolam and fentanyl, the pulmonologist or thoracic surgeon doing the procedure can monitor the patient and ask an assistant to provide repeat doses to maintain an appropriate level of sedation. Whenever an anesthesiologist is present, the operator can concentrate solely on the procedure. It is recommended to have an additional assistant present who can help with the processing of the obtained tissue.

The procedure always starts with a "time-out," in which all personnel involved in the case stop and confirm the identity of the patient, the procedure to be carried out, the function of everyone involved in the case, and the site and location of the intended procedure. Next, a flexible video bronchoscope is introduced through mouth, laryngeal mask, or an endotracheal tube (Fig. 1). This initial airway examination allows a detailed, direct evaluation of all bronchial segments and subsegments as well as the aspiration of secretions and the administration of endobronchial 1% to 2% lidocaine to minimize cough. Then, the flexible bronchoscope is removed, and the EBUS bronchoscope is introduced into the airway. Once the target lymph node is observed, the needle is advanced through the 2.2-mm channel of the bronchoscope (Fig. 2). By trigger activation, the needle can be extended up to a length of 40 mm, although intrinsic safety mechanisms of the device prevent excessive needle protrusion. In the bronchoscope, the needle is also encased in an internal sheath that protects the working channel of the bronchoscope. A stylet or wire is present inside the hollow needle at the time of insertion to clear any bronchial or cartilage plugs that may be collected when traversing the bronchial or tracheal wall.

The external surface of the needle is grooved at the distal end, as mentioned above, rendering it more hyperechoic and, thus, improving ultrasound visualization (Fig. 2A). Immediately after puncturing the lymph node, the stylet is used to clear any bronchial or cartilage debris, and then suction is connected. At this time, the needle undergoes excursion into the lymph node (or tumor). There is no general consensus on the number of times each lymph node is punctured, the number of excursions, or the exact location within the lymph node that should be biopsied. In our experience, 3 punctures per lymph node with 15 excursions each time provide diagnostic material in >95% of patients.

After each pass, the needle is withdrawn, and a small amount of material can be applied to a slide for preparation of direct air-dried or alcohol-fixed smears (in the setting of rapid on-site evaluation [ROSE] by cytopathology). Alternatively, the aspirate can be collected directly into saline or cell culture media for allocation to flow cytometry or microbiologic cultures, or it can be collected directly into a preservative solution (such as CytoLyt [Cytyc Corporation, Marlborough, Mass]). This material, in turn, can be used to prepare a ThinPrep cytologic slide, as well as a cell block preparation for ancillary immunohistochemical or molecular studies, as is often needed in the workup of NSCLC.

The procedure duration varies and depends on the number of lymph node stations sampled. In average, it takes 45 to 60 minutes from start to finish, and the patient's recovery period is less than 1 hour. When the procedure is performed in the outpatient setting, the patient is observed until gag reflex is regained and can go home later that day. Conversely, EBUS-TBNA can be performed in the operating room with the intention of proceeding to a more invasive surgical procedure (such as cervical mediastinoscopy or surgical resection), depending on the results of the EBUS-TBNA as assessed by cytopathology using ROSE.

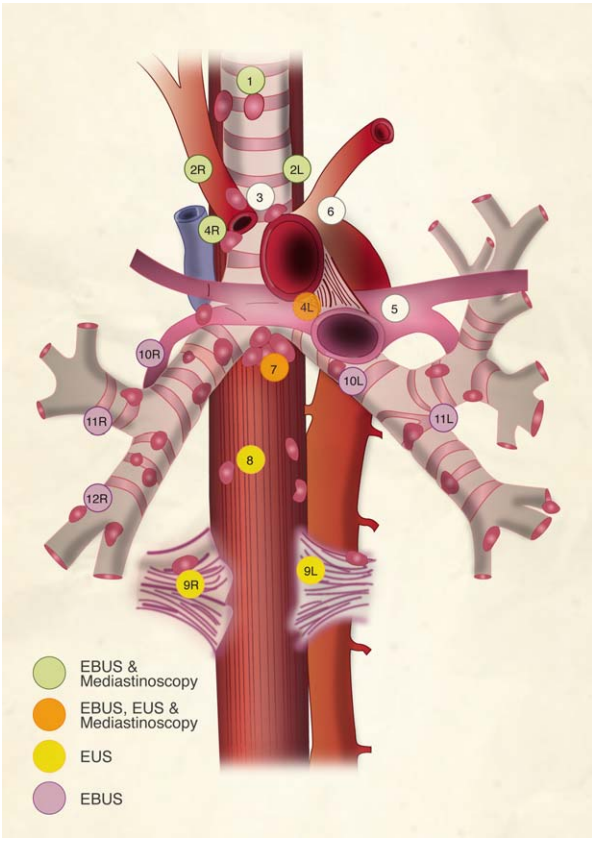


**Figure 4.** This regional lymph node map for lung cancer staging depicts the lymph node stations that can be reached by endobronchial ultrasound (EBUS), endoscopic ultrasound (EUS), and/or mediastinoscopy. It is clear that EBUS offers an advantage in reaching the hilar lymph nodes, whereas EUS is particularly useful for reaching lymph node stations 8 and 9. Evidently, mediastinoscopy is useful for mediastinal stations that also can be reached by EBUS. The close proximity of vessels highlights the importance of direct ultrasound visualization to guide the site of puncture for transbronchial needle aspiration. These same vessels are the natural landmarks that separate the lymph node stations and determine staging.

### *Which Anatomic Locations Are Suitable for EBUS-TBNA?*

Before the development of EBUS-TBNA and from the surgical perspective, the staging of the mediastinum was accomplished by cervical mediastinoscopy through an incision at the sternal notch. Conventional mediastinoscopy is able to access lymph node stations 2R and 2L (upper paratracheal), 4R and 4L (lower paratracheal), and 7 (subcarinal),[14] as illustrated in Figure 4. Video mediastinoscopy provides better visualization of the lymphoid tissue and, in some series, has yielded more lymph nodes with a lower complication rate. The accuracy of both approaches has reached approximately 84% to 88% in some series.[14,15]

Compared with the more invasive surgical procedure of cervical mediastinoscopy, EBUS-TNBA has demonstrated the potential to sample a wider range of lymph node stations. In addition to the stations sampled by mediastinoscopy, EBUS can also sample the more distal lymph node stations, including levels 10 (hilar), 11 (interlobar), and on occasion 12 (lobar), as illustrated in Figure 4. At least one case series that encompassed multiple institutions described access to station 5 (subaortic) through a transpulmonary artery route.[16] In addition to lymph nodes, several case reports and series have described EBUS-mediated access to paratracheal and parabronchial masses, including parenchymal lesions that occur close to the airway.

EUS-guided fine-needle aspiration (EUS-FNA) using a transesophageal route has a diagnostic reach complementary to that of EBUS-TBNA, because it is capable of accessing lymph node stations 8 and 9 in the lower mediastinum, as illustrated in Figure 4. In addition, EUS-FNA potentially can access the left adrenal gland, lung tumors adjacent to the esophagus, and, in selected cases, lymph node stations 5 and 6 through a transaortic route. The cases in which the combined use of EBUS and EUS is necessary for appropriate staging are few. Nonetheless, the salient point is that combined EUS and EBUS staging of the mediastinum provides a complete and systematic evaluation of virtually all mediastinal lymph nodes, extending beyond the diagnostic reach of the traditional gold standard of cervical mediastinoscopy.[17,18]

### *EBUS-TBNA Versus Other Staging Modalities*

Both the location and the number of lymph nodes affected by cancer have long been used for staging of breast, gastric, and colorectal cancers.[19] Although this duality (location and number) may be emulated for lung cancer staging in the future, currently, only the location of positive lymph nodes is important for staging. Therefore, lymph node staging of the mediastinum has been performed using multiple different but complementary modalities, including imaging (computed tomography [CT] scans and positron emission tomography [PET]-CT scans), surgical (cervical mediastinoscopy and video mediastinoscopy), and minimally invasive modalities (EUS-FNA and EBUS-TBNA). Over the past 5 years, the minimally invasive sampling techniques of EBUS-TBNA and EUS-FNA have revolutionized the acquisition of tissue for diagnosing and staging of lung cancer and have

**TABLE 1.** Comparison of Different Staging Modalities for Nonsmall Cell Lung Carcinoma[a]

| Procedure | No. of Studies | Total No. of Specimens | Cancer Prevalence, % | Sensitivity, % | Specificity, % | PPV, % | NPV, % |
|---|---|---|---|---|---|---|---|
| CT | 43 | 7368 | 30 | 55 | 51 | 58 | 83 |
| PET | 45 | 4105 | 28 | 80 | 88 | 75 | 91 |
| Integrated PET-CT | 19 | 2014 | 22 | 62 | 90 | 63 | 90 |
| Mediastinoscopy | 35 | 10,648 | 34 | 81 | 100 | 100 | 91 |
| TBNA | 27 | 2408 | 81 | 78 | 100 | 100 | 77 |
| EUS-FNA | 26 | 2443 | 58 | 89 | 100 | 100 | 86 |
| EBUS-TBNA | 26 | 2756 | 58 | 89 | 100 | 100 | 91 |
| EBUS-TBNA and EUS-FNA | 7 | 811 | 33 | 91 | 100 | 100 | 96 |

Abbreviations: CT, computed tomography; EBUS-TBNA, endobronchial ultrasound-guided transbronchial needle aspiration; EUS-FNA, endoscopic ultrasound-guided fine-needle aspiration; NPV, negative predictive value; PET, positron emission tomography; PPV, positive predictive value; TBNA, transbronchial needle aspiration.

[a] Median data values were compiled from the third edition *American College of Chest Physicians Guidelines for the Diagnosis and Management of Lung Cancer* (see Silvestri et al, 2013[20]).

become the preferred methods of tissue diagnosis in most patients.

Currently, there are insufficient data to make a recommendation regarding which staging modality is the "gold standard," because each has come with certain advantages and limitations. The most recent American College of Chest Physicians (ACCP) 2013 NSCLC guidelines pooled together numerous studies and compared the accuracy of staging tests for NSCLC.[20] Those results are summarized in Table 1. However, available data suggest that CT scan-based mediastinal lymph node evaluation has significant limitations, because from 5% to 15% of patients with clinical T1N0 (stage IA) disease have positive lymph nodes identified after surgical evaluation.[21] Therefore, there is no lymph node size that can reliably determine malignant involvement and, thus, operability.

In the case of PET imaging, a recent guideline from the ACCP reviewed 4105 patients who, together, had a median prevalence of mediastinal involvement of 28%. Those authors determined that the median sensitivity was 80%, and the specificity was 88% for mediastinal metastasis.[20] These findings suggest that PET is more accurate than traditional chest CT, although it is not quite perfect.

Therefore, noninvasive or imaging-based staging is suboptimal and can overestimate or underestimate malignant lymph node involvement. Tissue sampling, accomplished through either invasive or minimally invasive techniques, for the staging of lymph nodes in the mediastinum has proven essential, especially if molecular testing is anticipated. Cervical mediastinoscopy has been considered the standard procedure for staging. Current guidelines from the ACCP and the European Society of Thoracic Surgeons recommend that mediastinoscopy should include exploration and biopsy of representative nodes in 5 mediastinal lymph node stations (2R, 2L, 4R, 4L, and 7).[20,22] The use of needle techniques (EUS, EBUS, or combined EBUS/EUS) samples lymph nodes beyond the reach of cervical mediastinoscopy, as described above, and has been suggested as a best first test over surgical staging (grade 2B evidence). In addition, mediastinoscopy is an invasive procedure with morbidity, and even mortality. Furthermore, as indicated in Table 1, a pooled analysis of combined EBUS-TBNA with EUS-FNA reveals comparable, if not superior, sensitivity compared with cervical mediastinoscopy (91% vs 81%) while maintaining a specificity of 100%.

Overall, in many published studies, EBUS-TBNA has demonstrated superior performance measures compared with other staging modalities. A summary of the performance measures of EBUS-TBNA in a collection of more recent, larger scale studies is presented in Table 2.

### *Role of EBUS-TBNA in restaging or post-treatment biopsies*

The need for restaging of the mediastinum after induction chemotherapy and before surgical resection is frequently encountered by the treating clinician. However, repeat mediastinoscopy is fraught with complications. The EBUS bronchoscope allows for repeat biopsies of multiple lymph node stations in a minimally invasive way, as described above.

**TABLE 2.** Recent Studies Summarizing the Performance Measures for Endobronchial Ultrasound-Guided Transbronchial Needle Aspiration

| Study | No. of Patients | Total No. of Specimen (Specimen Type) | ROSE | Adequacy, % | Sensitivity, % | Specificity, % | PPV, % | NPV, % |
|---|---|---|---|---|---|---|---|---|
| Alsharif 2008[23] | 100 | 229 (219 Lymph nodes, 10 lung) | Yes | 84 | 86 | 100 | 98 | 100 |
| Feller-Kopman 2009[24] | 135 | 195 (131 Lymph nodes, 64 lung) | Yes | 95 | 95 | 100 | 100 | 97 |
| Szlubowski 2010[25] | 226 | 320 (All lymph nodes) | Yes | 96 | 89 | 100 | 100 | 84 |
| Hwangbo 2010[18] | 150 | 310 (307 Lymph nodes, 3 lung) | No | ? | 84 | 100 | 100 | 93 |
| Yasufuku 2011[26] | 153 | 426 (All lymph nodes) | Yes | 71 | 81 | 100 | 100 | 91 |
| Lee et al 2012[27] | 73 | 140 (All lymph nodes) | No | 99 | 95 | 100 | 94 | 97 |
| Nakajima 2013[28] | 438 | 965 (All lymph nodes) | Yes | 91 | 96 | 100 | 90 | 98 |
| Karunamurthy 2014[29] | 356 | 593 (553 Lymph nodes, 22 mediastinal masses, 18 lung) | Yes | 89 | 80 | 100 | 100 | 95 |

Abbreviations: NPV, negative predictive value; PPV, positive predictive value; ROSE, rapid on-site evaluation.

EBUS-TBNA was used for restaging in 124 consecutive patients with stage IIIA (N2) disease who had previously received neoadjuvant chemotherapy. The results demonstrated a sensitivity of 76%, a specificity of 100%, a positive predictive value of 100%, a negative predictive value of 20%, and diagnostic accuracy of 77%.[30] Because of the low negative predictive value, it was suggested that a negative EBUS should be confirmed with surgical staging. However, another group that included some members from the first study later published a second report that included 61 patients who underwent restaging EBUS. In that report, the sensitivity of EBUS was 67%, specificity was 86%, diagnostic accuracy was 80%, the positive predictive value was 91%, and the negative predictive value was 78%. Thus, those authors concluded that, given a negative predictive value of 78%, patients who have a negative EBUS-TBNA may not need surgical restaging of the mediastinum.[25]

In patients with previously definitively treated thoracic malignancy, EBUS-TBNA can also provide pathologic diagnoses in a less invasive manner. In a retrospective review of 14 patients who had been treated for thoracic malignancy and required EBUS-TBNA evaluation for new mediastinal or hilar lymphadenopathy, all were identified as positive for malignancy. Eleven had the same pathology as their previous malignancy, and 3 patients presented with a new malignant disease according to the pathologic examination.[31]

### *Cytology of Specimens: Diagnostic Criteria*

The cytomorphologic features of cells obtained by EBUS-TBNA are no different from those obtained using traditional transbronchial needle aspiration techniques (or trans*thoracic* needle aspiration techniques, for that matter). Therefore, it is not the intent of this review to reiterate the cytologic features of the major neoplastic and non-neoplastic lesions sampled using this technique, and the reader is directed to several comprehensive publications on this topic.[23,24,32]

When evaluating EBUS-TBNA specimens, it is imperative to be aware of cellular "contaminants" that are acquired when the needle passes through the tracheal wall en route to the targeted lesion. Care must be taken not to misinterpret benign bronchial columnar cells (look for the terminal bar and cilia), histiocytes (sometimes fine-pigment laden), fragments of cartilage, mesothelial cells, or glandular cells derived from submucosal seromucinous glands as lesional cells. These tracheal/bronchial contaminants can be quite challenging when performing cytologic ROSE of air-dried smears and can often be the predominant cellular component of bloody smears.

### *Adequacy Criteria for EBUS Specimens*

When EBUS-TBNA is used to sample peritracheal and peribronchial lymph nodes for mediastinal tumor staging, an important consideration is that of specimen adequacy, especially when no tumor is present. The specificity and negative predictive value of this procedure depends on ensuring that the needle is indeed sampling the targeted lymph node, and not just airway tissue. Essentially, lymph node sampling should yield abundant lymphocytes and/or lymphohistiocytic aggregates. When lymphocytes are sparse, the qualitative question is whether or not the number of lymphocytes in the specimen is more than what

would be expected from blood contamination alone (an important distinction to make, because EBUS-TBNA specimens are often quite bloody). Although there does not appear to be a universally accepted "gold standard" for EBUS specimen adequacy (especially for lymph nodes), criteria used by various groups in the literature range from simply noting the presence of lymphocytes/lymphoid tissue[26,33–36] to more quantitative measures, such as >40 lymphocytes per high-power field[6] or >5 low-power fields with >100 lymphocytes in each *and* <2 bronchial cell groups per low-power field.[37] Some groups have also considered the presence of anthracotic pigment-laden macrophages and/or tingible body macrophages as proof of adequate lymph node sampling.[23,28,38,39] A specimen is generally considered adequate if abundant tumor is present even in the absence of lymphocytes or histiocytes, because this typically represents near total tumor replacement of the lymph node.

Adequacy assessment for parenchymal lung lesions can be more problematic, with a potentially more difficult distinction that the cytopathologist has to make between a "negative" and "nondiagnostic" classification. Broadly speaking, as with any fine-needle aspiration, the qualitative question must be posed: does the cellular (or acellular) material present explain the radiographic and clinical characteristics of the targeted lesion? Care must be taken not to over-interpret as "negative" a sparsely cellular specimen composed only of benign parenchymal elements (pneumocytes, pulmonary macrophages, bronchial columnar cells), because this may not represent lesional sampling. It is also worth mentioning that sampling of peritumoral granulomatous inflammation can be a cause of "false-negative" EBUS-TBNAs of lung parenchymal lesions. In short, to preserve the sensitivity and negative predictive value of this procedure, a "negative for malignancy" diagnosis should be approached with caution, and a finding of "nondiagnostic" is more appropriate in instances of sparse cellularity or when a mass lesion cannot be explained.

### *Learning Curve for Performing and Interpreting EBUS-TBNA Specimens*

EBUS-TBNA has proven to be a safe and reproducible procedure. It is safer than surgical mediastinoscopy, but its potential complications, although rare, include pneumomediastinum, pneumothorax, mediastinitis, bacteremia, and hemomediastinum. Large series have produced a major complication rate of approximately 0.15%,[40] and only 2 deaths have been reported to date in the literature.[41,42] Nonetheless, the safety of the procedure is maintained during the relatively steep learning curve experienced by pulmonologists or thoracic surgeons in training by the presence of an experienced operator for mentoring.[43] As of 2013, American Thoracic Society, European Respiratory Society, and ACCP guidelines suggest from 40 to 50 supervised procedures for initial acquisition of competence and 20 procedures per year for maintenance of competency. Unfortunately, most hospitals are not following such recommendations.[44–46] At this time, pulmonologists and thoracic surgeons are exposed to EBUS training by one of the following: limited training during fellowship, short courses of 1 to 3 days, self-learning, or a dedicated 1-year fellowship in Interventional Pulmonology. The actual learning curve required for EBUS is unclear and likely to be different for each individual.[47,48] However, available studies suggest that yield continues to improve up to 120 to 140 procedures.[49,50]

The expertise and skill of the bronchoscopist performing the EBUS-TBNA procedure directly impacts the cytopathologist as they interpret the cytologic materials obtained from the procedure. This task can be made more challenging when an inexperienced operator provides hypocellular specimens or when overly bloody or poorly preserved smears are prepared. Furthermore, a similar learning curve for the cytopathologist in the evaluation and mastery of the EBUS-TBNA cytologic specimens can be concluded, although scant data exist to describe the number of cases or frequency of evaluating such cases necessary to achieve and maintain competency. The learning curve of cytopathologists depends very much on the individual's prior experience with cytology, as cellular morphology varies little with collection methods. The combined skill and competency of the bronchoscopist and the cytopathologist influence the true accuracy of the procedure, in other words minimizing the risk of erroneously upstaging or downstaging an individual patient with lung cancer. In the former, the patient will be prevented from receiving potentially curative therapy; whereas, in the latter, the patient will likely undergo unnecessary surgery or therapy without benefit. Therefore, an experienced team approach between cytopathology and pulmonology/thoracic surgery is paramount in providing the patient with the most accurate diagnosis.

### *Role of ROSE in EBUS-TBNA*

ROSE of specimen adequacy by a cytopathologist/cytotechnologist is a prime example of this type of interaction with the bronchoscopist. The concept of ROSE in general has become a topic of discussion and the focus of many studies of late. In general, the real-time evaluation of a cytologic specimen by an experienced cytopathologist or cytotechnologist is believed to improve patient care, mainly by increasing diagnostic yield by providing immediate feedback to the operator as well as appropriately triaging materials for necessary ancillary testing (including microbiologic cultures, flow cytometry, and/or cell block material for immunohistochemical and molecular testing). Although recently covered extensively by other publications,[51–54] it merits a brief discussion of the benefits and drawbacks to consider when using ROSE in EBUS-TBNA.

ROSE, the cytologic equivalent to the surgical pathology frozen section used in the operative setting, is intended to provide real-time information to the bronchoscopist that will influence what is done next. For staging procedures in which EBUS immediately precedes a possible surgical cervical mediastinoscopy, the latter surgical procedure can be averted with the identification of metastatic disease in the paratracheal lymph nodes on EBUS-TBNA. The real-time feedback can prompt the bronchoscopist to obtain additional passes in the setting of nondiagnostic smears, thereby potentially lowering nondiagnostic rates. This immediate feedback can also prove useful for clinicians in the early training phase when learning the EBUS procedure. ROSE can reduce the number of passes or sites sampled as well as the total number of slides generated per procedure.[55] Although many EBUS-TBNA procedures are performed in the context of mediastinal staging for a suspected lung cancer, the sampling of a mediastinal or lung mass may reveal an inflammatory/infectious or lymphoproliferative process; ROSE during the procedure can help ensure that materials are appropriately handled and triaged for ancillary tests, including microbiologic cultures or flow cytometry.

Despite these benefits, ROSE in the setting of EBUS-TBNA poses some potential drawbacks. Like the surgical pathology frozen section, interpretative challenges and technical limitations of the cytologic preparation can result in false-positive and false-negative diagnoses.[28,54] In addition, performing ROSE entails the necessary allocation of sampled tissue for air-dried direct smears, material that, in a sense, is "used up" and is not available for traditional modes of ancillary testing that could better characterize the lesion, such as through cell block preparations (immunohistochemistry or molecular studies), microbiologic cultures, or flow cytometry. Data on EUS-FNA indicate that, with experienced operators, ROSE does not change the diagnostic rate.[56] Some randomized controlled trials of ROSE have failed to demonstrate any significant differences in sensitivity, diagnostic yield, or complication rate in the setting of TBNA with or without EBUS.[57,58] Providing routine ROSE for EBUS-TBNAs can represent a significant diversion of personnel resources for a cytopathology department, and cytopathologists tend to be inadequately compensated for time spent performing on-site assessment.[52,59] The process of staining and evaluating the cytologic smears is time consuming and may prolong procedure/anesthesia time. Although a positive ROSE result for an N3 or N2 lymph node station could end the procedure early (obviating the need for sampling additional N1 lymph node stations), there is some evidence that the total number of lymph node stations involved—and not just involvement of the highest lymph node station—provides prognostic information in patients with lung cancer,[60,61] as does potentially identifying patients who have involvement of a single N2 lymph node or those who have lymph node "skip metastasis" (ie, N2 lymph node involvement with negative N1 lymph nodes).[62] More complete mapping of the pattern and extent of lymph node involvement preoperatively can potentially influence treatment options. If future studies confirm that more extensive lymph node station sampling provides better risk stratification without significantly increasing risk to the patient, then the benefit of reducing the number of sites sampled by ROSE would be annulled if standardized sampling of multiple lymph node stations is recommended.

In total, there does not appear to be a "one size fits all" solution for the implementation of ROSE in the setting of EBUS-TBNA. Should ROSE be performed for every EBUS-TBNA procedure or for select procedures (such as a repeat procedure after a nondiagnostic result or when clinical pretest probability would suggest a need for on-site triaging for flow cytometry or microbiologic cultures)? Can ROSE be incorporated less frequently when the bronchoscopist gains procedural expertise over time? Can telepathology be used effectively to provide "rapid

off-site assessment" for EBUS-TBNA procedures, thereby minimizing the time constraints for the cytopathologist? Clearly, each practice setting has different needs and challenges that may favor or dissuade the routine use of ROSE for EBUS-TBNA procedures. Effective communication between the bronchoscopist and the cytopathologist is essential to coordinating this multidisciplinary approach, which aims to provide the most efficient and highest quality care to the patients we serve.

### Ancillary Testing of Materials Obtained by EBUS-TBNA

It has been demonstrated that EBUS-TBNA is a versatile diagnostic modality for a variety of thoracic disease processes; and, as alluded to above, more often than not, materials obtained in this fashion require ancillary testing. Because the large majority of lung cancer patients present at an advanced stage and are not surgical candidates, the diagnostic material is frequently limited to cytologic and small biopsy specimens, oftentimes obtained by EBUS-TBNA. The recent International Association for the Study of Lung Cancer/American Thoracic Society/European Respiratory Society proposed classification of lung adenocarcinomas addresses this issue and not only stresses the importance of morphologic features of lung carcinoma subsets but also provides guidance on the use of ancillary immunohistochemical and histochemical stains to further differentiate subsets of NSCLC (adenocarcinoma vs squamous cell carcinoma).[63,64] In general, when cytomorphology alone is insufficient for definitive characterization, an initial limited panel of immunohistochemical markers, including one adenocarcinoma marker (thyroid transcription factor 1 [TTF-1] or Napsin A) and one squamous marker (p63, p40, or cytokeratin 5/6) is recommended.[64] By using this type of approach, a recent analysis of EBUS-TBNA materials from 81 patients with lung cancer demonstrated that 85% of the cases were able to be classified as either adenocarcinoma or squamous cell carcinoma.[65] Although immunohistochemical stains are typically performed on cell block material, success has been reported using direct smears derived from EBUS-TBNA as the source material for immunohistochemical characterization of lung adenocarcinoma versus squamous cell carcinoma.[66]

The need to further subcategorize NSCLC into adenocarcinoma versus squamous cell carcinoma is crutial because of implications for the selection of appropriate chemotherapy as well as identifying cases that should be tested for the presence of a driver mutation (such as epidermal growth factor receptor [*EGFR*], anaplastic lymphoma receptor tyrosine kinase [*ALK*], or c-ros oncogene 1 receptor tyrosine kinase [*ROS1*]) that can be specifically treated with a targeted agent. The use of EBUS-TBNA for successfully acquiring adequate cellular materials for tumor genotyping in NSCLC has been reported by several groups.[41,67–73] In those studies, successful testing of at least one target (such as *EGFR* or Kirsten rat sarcoma viral oncogene homolog [*KRAS*] mutation analysis or *ALK*-fluorescence in situ hybridization) was observed in 72% to 98% of samples. In our experience, cell blocks prepared from EBUS-TBNA–derived material have a molecular testing success rate in excess of 90%, which is significantly higher than that from CT-guided percutaneous core-needle biopsies at our institution.[67] Therefore, cytologic materials obtained by EBUS-TBNA can clearly be a reliable source of tumor for molecular testing. Although most molecular testing from EBUS-derived specimens is performed on formalin-fixed, paraffin-embedded cell block materials, success has been described using the air-dried Diff-Quik–stained smears that may be generated during immediate on-site evaluation.[74]

Several studies have demonstrated that EBUS-TBNA is an adequate option for diagnosing lymphoma, with an overall sensitivity of approximately 76%.[34,75,76] The cytologic recognition of high-grade lymphomas, such as diffuse large B-cell lymphoma, are typically relatively straightforward; however, the diagnostic sensitivity falls for Hodgkin lymphoma and for subtyping lower grade lymphomas, including marginal zone lymphoma and follicular lymphoma.[77–79] Therefore, allocating fresh materials for flow cytometry as well as immunohistochemical profiling on cell block material can prove extremely useful in characterizing an atypical lymphoid population that is recognized cytologically.

### Other Applications of EBUS-TBNA

EBUS-TBNA has proven useful in diagnosing and subclassifying lymphomas as well as other non-neoplastic processes involving mediastinal lymph nodes.[80] It was able to confirm the clinical suspicion of sarcoidosis with a sensitivity of 85% in patients with stage I or II disease.[81] It appears that EBUS-TBNA can outperform both conventional TBNA and transbronchial lung biopsies in diagnosing sarcoidosis.[82,83] When comparing EBUS-TBNA

with conventional TBNA technique in a randomized trial, the diagnostic yield was 83.3% versus 53.8%, respectively.[84] Although the non-necrotizing, well formed granulomas of sarcoidosis can be observed in other diseases, clinical and radiologic correlation helps with the interpretation of such findings to reach a final definitive diagnosis.

EBUS-TBNA has also been used for detecting infections. In patients with isolated tubercular mediastinal lymphadenitis, EBUS has a high diagnostic yield.[85,86] The fungal infection of histoplasmosis is rarely encountered outside of endemic areas or activities that are likely to expose the patient. The reports of histoplasmosis in EBUS-TBNA samples are limited but well described in the literature.[87,88] When acute inflammation or granulomas are identified on cytologic material, special stains for micro-organisms in conjunction with microbiologic cultures can be useful in identifying an infectious agent.

Finally, the ability of EBUS to visualize peribronchial structures in real time increases the potential applications for this procedure going forward. EBUS bronchoscopy can be used for the placement of fiducial markers to guide stereotactic radiation for patients with early NSCLC who are not candidates for surgical resection (Fig. 5). Recent studies have produced excellent results in placing fiducial markers in central and peripheral locations guided by EBUS.[89] EBUS has also been used to guide the placement of airway stents and to identify and drain mediastinal bronchogenic cysts.[90] We have also used EBUS-TBNA for parenchymal lung biopsies with excellent results. Over time, with advances in imaging modalities and collection tools, the numbers and types of applications for this minimally invasive procedure are sure to increase.

**Figure 5.** This is a fluoroscopic image of endobronchial ultrasound used for the placement of a fiducial marker. An arrow indicates the radiopaque marker used for the precise targeting and delivery of stereotactic body radiation therapy.

### *Cost Analysis of EBUS-TBNA*

Clinicians traditionally have avoided the use of economic considerations to guide individual patient care; however, policy makers frequently use single-weighted measures or summary scores to compare changes in quality of life in participants. A commonly used measure is quality-adjusted life years (QALYs), in which death is assigned a score of zero, and health is assigned a score of 1.[91] The use of QALYs or other similar measures to establish a threshold for the type of health care that is either cost effective or recommended is now forbidden in the United States under the Patient Protection and Affordable Care Act.[92,93] Nevertheless, their use in guiding health care decisions around the world is prevalent.[94] Recently, a cost-effectiveness analysis of the Assessment of Surgical Staging versus Endoscopic Ultrasound in Lung Cancer: a randomized clinical trial (the ASTER Study) from 3 European countries was published by Rintoul and colleagues.[95] In all 3 countries, the use of EBUS/EUS staging had lower mean cost and greater mean QALYs. These differences held at 6 months despite variations in treatment and local costs. Furthermore, a cost-effectiveness acceptability curve revealed that, if there were no increases in the cost of staging, then the probability that EBUS/EUS would be more cost effective was 55% for the Netherlands, 60% for Belgium, and 82% for the United Kingdom. All 3 countries demonstrated a mean cost saving for the EBUS/EUS staging strategy.

In a probabilistic model designed to inform the Danish National Health Service about cost-effective strategies for staging NSCLC, only 2 of 6 potential strategies were identified as cost-effective: the use of PET-CT with confirmation by EBUS-TBNA and sending patients directly to EBUS-TBNA.[96] At a commonly accepted threshold of 30,000 Euros per life year,[97] PET-CT with EBUS-TBNA had a probability of 80% of being cost effective. It is noteworthy that, when we applied that

threshold to the previously mentioned study by Rintoul and colleagues, the probability that EBUS/EUS would be cost effective was approximately 68% for the Netherlands, 70% for Belgium, and 90% for the United Kingdom.

A cost-effectiveness study in Singapore indicated that lung cancer staging with EBUS-TBNA was approximately 25% less expensive compared with surgical mediastinoscopy.[98] A recent Australian study has demonstrated that a staging strategy for suspected lung cancer using the less invasive EBUS-TBNA modality followed by surgical staging (only if EBUS-TBNA was negative) is more cost effective than surgical staging alone.[99] Analysis of the ASTER cohort produced similar findings, in which a EBUS-TBNA approach (with subsequent surgical staging if the EBUS-TBNA was negative) had a higher sensitivity and a higher negative predictive value, led to fewer unnecessary thoracotomies, had a higher QALY score, and was slightly less expensive than relying on surgical staging alone.[100]

### *Conclusions*

In summary, EBUS-TBNA over the past 20 years has rapidly become a widely performed procedure for evaluating a variety of pulmonary and mediastinal diseases. EBUS-TBNA has been used most frequently for the diagnosis and staging of lung cancer, and a growing body of evidence supports the choice of this minimally invasive modality as a first-line choice in such clinical situations. The procedure has proven safe in the hands of an experienced operator, and it is cost-effective relative to more invasive surgical procedures. EBUS-TBNA has demonstrated excellent sensitivity, specificity, and positive and negative predictive values and furthermore has proven more than adequate to obtain sufficient materials for diagnostic workup and the increasingly important ancillary testing for lung cancer. Looking forward, EBUS-TBNA will most certainly play an increasingly important role in the diagnosis, staging, and management of patients with lung cancer and other thoracic/mediastinal pathologies.

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Dr. Folch has served as an education advisor for Olympus and as a scientific advisor for Boston Scientific. Dr. Majid has served as an education advisor for Olympus.

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