Int J Clin Exp Med 2019;12(4):4184-4192
www.ijcem.com /ISSN:1940-5901/IJCEM0080537

Original Article

Endo-bronchial ultrasound-guided transbronchial needle aspiration for diagnosis of mediastinal lymphadenopathy and analysis of false negatives

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Received May 29, 2018; Accepted November 9, 2018; Epub April 15, 2019; Published April 30, 2019

Abstract: Background: Endobronchial ultrasound-guided transbronchial needle aspiration (EBUS-TBNA) is a minimally invasive option for the pathological examination of intrathoracic lymph nodes. This study aimed to assess the diagnostic accuracy of EBUS-TBNA in mediastinal lymphadenopathy. Methods: This study retrospectively reviewed 389 patients that had undergone EBUS-TBNA and were radiologically confirmed to have mediastinal and hilar lymph node enlargement over a 6-year period. Demographic and clinical data and pathology results were collected and analyzed. Results: From 389 patients (219 men) with a mean age of 55.1 years, 389 lymph nodes were punctured and 376 resulted in a diagnosis. Biopsy specimens were taken from lymph nodes in regions 2, 4, 7, 10, and 11. Mean diameter of the nodes was 22.6 ± 5.2 mm on computerized tomography (CT) and 21.5 ± 3.7 mm on EBUS. Regarding the diagnostic performance of EBUS-TBNA, sensitivity was 93.0% and specificity was 100.0%, with a diagnostic yield of 89.2%. The negative predictive value (NPV) was 45.7% and the positive predictive value (PPV) was 100.0%. A few minor complications occurred. The diagnostic yield increased over time and reached a plateau after approximately 60 performances. Conclusion: EBUS-TBNA should be considered the initial investigation for patients with mediastinal and hilar lymphadenopathy. It is safe and has high diagnostic value.

Keywords: EBUS-TBNA, lymph nodes, diagnosis, mediastinum, lymphadenopathy

Introduction

Endobronchial ultrasound-guided transbronchial needle aspiration (EBUS-TBNA) is an emerging diagnostic technique that allows bronchoscopist visual and minimally invasive sampling of the intrathoracic lymph nodes adjacent to the bronchial tree. It provides diagnostic access to most of the intrathoracic structures. Therefore, it has been widely used in lymph node staging for lung cancer and other mediastinal diseases, such as sarcoidosis and lymphoma [1-7]. The diagnostic yield of EBUS-TBNA in clinical trials is very high.

Mediastinal lymphadenopathy is a disease of the mediastinal lymph nodes in which they are abnormal in size, number, or consistency. As a common and nonspecific sign, many diseases can cause mediastinal lymphadenopathy. Common causes of this sign include sarcoidosis, lung cancer, and inflammation, as well as lymphoma [8].

Mediastinoscopy has been regarded as the standard for sampling mediastinal lesions for many years. However, it is invasive, costly, and requires general anesthesia. Furthermore, mediastinoscopy gives access to para-tracheal lymph node stations and anterior sub-carinal lymph node stations, but access to the posterior and inferior mediastinum is limited [9]. Consequently, EBUS-TBNA has been increasingly used for diagnosis of mediastinal lymphadenopathy. However, estimates of the diagnostic performance of EBUS-TBNA have not been thoroughly studied. The present study evaluated EBUS-TBNA for diagnosis of hilar and mediastinal lymphadenopathy, investigating possible factors that may influence the diagnostic performance of EBUS-TBNA.

Patients and methods

Patients selection

Clinical retrospective analysis was conducted for patients that underwent EBUS-TBNA in the Bronchoscopy Unit of Shandong Cancer Hospital Affiliated to Shandong University, between October 2010 to October 2016. Inclusion criteria: ① Mediastinal lymphadenopathy was found on CT images without other abnormal findings; and ② Peripheral lesions that could not be reached by conventional flexible bronchoscopy and CT images that demonstrated enlarged lymph nodes.

Collected data included patient characteristics and procedural and outcome-related information. The study protocol was approved by local Ethics Committee.

EBUS-TBNA

Standard diagnostic bronchoscopy using Olympus bronchoscopes was performed prior to all EBUS procedures. EBUS-TBNA was performed using an Olympus BF-UC260FW ultrasonic bronchoscope and either 21- or 22-gauge needles. EBUS-TBNA was performed under conscious sedation, using incremental doses of lidocaine, without anesthetic support. Sampling of lymph nodes from the esophagus was not performed and rapid onsite evaluation (ROSE) of samples was not available.

The lymph node biopsies were immersed in CytoLyt solution. After standing for 2 minutes, the supernatant was used for liquid-based cytology, while the remaining sample was used for pathological diagnosis. Adequacy of the lymph node biopsy was confirmed by the presence of lymphocytes in the cytology specimen (indicating that the sample was representative).

Complications were recorded by the primary operator following patient recovery. Complications were categorized as major or minor, based on U.K. National Guidelines [10]. Major complications included major bleeding (defined as the need for resuscitation, transfusion, critical care admission, or death), cardiac arrhythmia requiring intervention, seizure, myocardial infarction or pulmonary edema, pneumothorax requiring intercostal chest drain or aspiration, over-sedation requiring ventilatory support or sedation reversal, unplanned hospital admission, or death. Minor complications included mild or moderate bleeding (defined as the need for continual suctioning or vasoconstrictors), cardiac arrhythmia not requiring intervention, hypotension requiring intervention, and poor tolerance of the procedure requiring early termination.

Lymph nodes

For patients receiving CT scans, the images were retrospectively reviewed. Short axis diameters of lymph nodes sampled by EBUS-TBNA were measured. Lymph node locations were classified according to the IASLC lymph node map 2009 [11].

EBUS-TBNA was performed in one lymph node per patient. The short axis observed using EBUS was measured and its location was determined for each lymph node sampled. Criteria for lymph node aspiration were as follows. Subcarinal lymph nodes (station 7) were sampled before the lower paratracheal lymph nodes (stations 2 and 4), followed by the hilar nodes (stations 10 and 11) [11]. Lymph nodes with a diameter of 10 to 30 mm were sampled before those with a diameter exceeding 30 mm, followed by those with a diameter less than 10 mm.

Diagnosis

EBUS-TBNA samples were examined by a specialist thoracic pathologist and classified according to standard pathological criteria. Morphology and immunohistochemistry were used. EBUS-TBNA samples were classified as positive if malignant cells could be found and/or lymph node aspirates that were diagnostically significant could be found in the formalin-fixed paraffin-embedded section. EBUS-TBNA samples that were deemed inadequate by the reporting pathologist were also classified as negative. For liquid-based cytology, a result was considered positive only when the cytological diagnosis was 'positive for malignant cells'. All other results, such as atypical cells present, suspicion of malignant cells, or miscellaneous, were classified as negative. For both pathological and liquid-based cytological results, conclusive diagnosis, inconclusive diagnosis, and nonrepresentative samples were confirmed by further confirmative medical procedures (surgical sampling and repeated EBUS-TBNA) and/or 6 months of follow-ups (**Figure 1**).

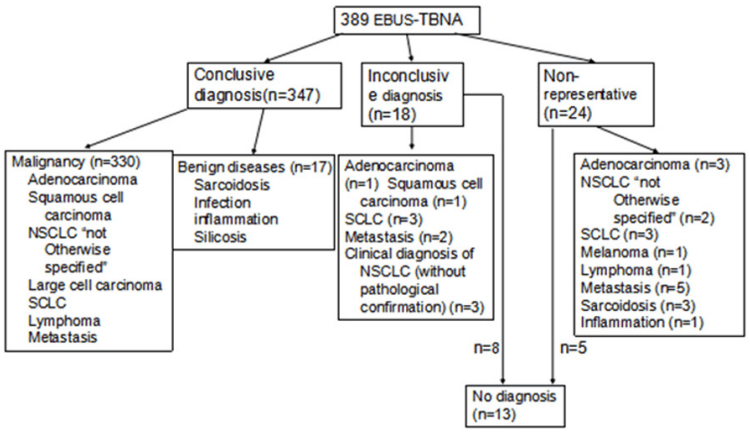

Figure 1. Diagnostic process for all patients. EBUS-TBNA: Endo-bronchial ultrasound-guided transbronchial needle aspiration; NSCLC: non-small-cell lung cancer; SCLC: small-cell lung cancer.

Table 1. Patient characteristics

Items	Values
Age, years (mean ± SD)	55.1 ± 7.3
Sex, n%	
Male	219 (56.3%)
Female	170 (43.7%)
Complications, n%	23 (5.9%)
Major complications	0 (0.0%)
Minor complications[&]	7 (1.8%)
Previous malignancy, n%	24 (6.2%)
Final diagnosis, n%	
Malignant diseases[†]	355 (91.3%)
Benign diseases[††]	21 (5.4%)
No diagnosis	13 (3.3%)

&: Mild or moderate bleeding (defined as the need for continual suctioning or vasoconstrictors) (1 patients), cardiac arrhythmia not requiring intervention (3 patients), and poor tolerance of the procedure requiring early termination) (3 patients). †: adenocarcinoma, squamous cell carcinoma, NSCLC "not otherwise specified", large cell carcinoma, clinical diagnosis of NSCLC (without pathological confirmation), SCLC, melanoma, lymphoma, and nonprimary lung cancer. ††: Sarcoidosis, infection, inflammation, silicosis.

Statistical analysis

Data were analyzed using SPSS (version 20.0.0; IBM corporation, NY, USA). Categorical data are presented as frequencies and χ^2 tests were used to determine significance. Continuous data are presented as mean values with standard deviations and *t* tests were used to detect differences after testing for normal distribution. The diagnostic yield is defined as the proportion of patients with a conclusive cytological finding (malignant or benign) within the entire study group. The success rate of lymph node aspiration is defined as the proportion of lymph nodes with a conclusive diagnosis (malignant or benign) within the entire study group.

Results

Patient characteristics and safety profile

Three hundred and eight-nine eligible patients that underwent EBUS-TBNA during this period were enrolled. Characteristics are shown in **Table 1**. The mean age was 55.1 years and 56.3% (219/389) were male. Overall, 7 (1.8%) complications were recorded during this period, all classified as minor complications. Additionally, 24 (6.2%) subjects had previous malignancies in their history. Of the 389 patients included, 355 (91.3%) were diagnosed as having a malignant disease and 21 (5.4%) as having benign disease. In 13 cases (3.3%), a diagnosis was not established within the time frame of the study (**Table 1**). Final diagnoses of the target lesions in patients enrolled in the study are presented in **Table 2**.

Punctured lymph nodes

A total of 389 lymph nodes were sampled. The diameter of the punctured lymph nodes ranged between 7.0 mm and 45.0 mm. The mean short axis diameter was 22.6 ± 5.2 mm on CT and 21.5 ± 3.7 mm on EBUS, with 92.0% measuring 10.0 mm or above. There were no significant differences between EBUS and CT with respect to lymph node size (P=0.294, n=276). The success rate of lymph node aspiration was associated with the diameter and position of lymph nodes (P=0.021, P=0.006, respectively) (**Table 3**). The success rate for lymph nodes, with diameters ranging between 10 mm and 30 mm, was the highest (89.1%), followed by lymph nodes with diameters more than 30 mm (71.7%). Sub-carinal lymph nodes had the highest aspiration success rate (92.4%), followed by lower para-tracheal lymph nodes (81.8%). However, there was no correlation between size

Table 2. Final diagnosis

Diagnosis	n	Conclusive diagnosis by initial performance of EBUS-TBNA	Diagnosis by other methods (surgery, follow-up and other medical institutions) or repeated performances of EBUS-TBNA
Malignant diagnosis			
Adenocarcinoma	77	73	4
Squamous cell carcinoma	76	75	1
NSCLC "not otherwise specified"	8	6	2
Large cell carcinoma	3	3	0
Clinical diagnosis of NSCLC (without pathological confirmation)	3	0	3
SCLC	161	155	6
Melanoma	1	0	1
Lymphoma	2	1	1
Nonprimary lung cancer	24	17	7
Total	355	330	25
Benign diagnosis			
Sarcoidosis	16	13	3
Infection	1	1	0
Inflammation	2	1	1
Silicosis	2	2	0
Total	21	17	4
No diagnosis	13		
Total	389	347	29

EBUS-TBNA: endobronchial ultrasound-guided transbronchial needle aspiration; NSCLC: non-small-cell lung cancer; SCLC: small-cell lung cancer.

Table 3. Lymph nodes

Items	N	Sampled lymph nodes by EBUS-TBNA		Success rate	F	*p*
		Positive	Negative			
Diameters of Lymph nodes						
<10 mm	31	19	12	61.3%		
10-30 mm	266	237	29	89.1%	3.924	0.021
>30 mm	92	66	26	71.7%		
Positions of lymph nodes						
Subcarinal	172	159	13	92.4%		
Paratracheal	137	112	25	81.8%		
Hilar	48	31	17	64.6%	4.228	0.006
Others	32	20	12	62.5%		
Model of Aspiration needle						
21 G	186	147	39	79.0%	2.164	0.142
22 G	203	175	28	86.2%		

of the aspiration needle and success rates (P=0.142) (**Table 3**).

Diagnostic performance

Liquid-based cytology was performed in each of the 389 patients. A total of 278 samples were reported as positive for malignant cells. For patients with definite diagnosis (376 patients), sensitivity of liquid-based cytology in detecting malignancy of lymph nodes was 74.9% and specificity was 42.9%. The NPV was 9.2% and the PPV was 95.7% (**Table 4**).

Sensitivity of initial EBUS-TBNA in detecting malignancy in lymph nodes was 93.0% and specificity was 100.0%, with a diagnostic yield of 93.0%. The NPV was 45.7% and the PPV was 100.0% (**Table 5**). Additionally, 347 patients (330 true positive and 17 true negative) were diagnosed correctly among all of the initial EBUS-TBNA, with the diagnostic yield being 89.2% (347/389) (**Figure 1**).

Diagnostic ability of EBUS-TBNA with time

Outcomes for EBUS-TBNA performance according to the study period (per year) are presented in **Table 6**. This table demonstrates that the

Table 4. Contingency table comparing the results of liquid-based cytology to the final diagnosis

Cytology	Final diagnosis		Σ
	Positive	Negative	
Positive	266	12	278
Negative	89	9	98
Σ	355	21	376

Table 5. Contingency table comparing the results of initial EBUS-TBNA to the final diagnosis for malignancy

EBUS-TBNA	Final diagnosis		Σ
	Positive	Negative	
Positive	330	0	330
Negative	25	21	46
Σ	355	21	376

diagnostic ability increasingly improved, with a diagnostic plateau after approximately 60 performances of EBUS-TBNA. Additionally, the rate of nonrepresentative samples also declined gradually and reached a plateau.

Discussion

Results from the present study suggest that the success rate of the lymph node sampling was correlated with diameters and locations of lymph nodes. Sub-carinal lymph nodes, with a size of 10 to 30 mm, were the best choice for sampling. The diagnostic performance of initial EBUS-TBNA after a CT for mediastinal lymphadenopathy was impressive, with a diagnostic yield for malignancy of 89.2%. For skilled flexible bronchoscopy examiners without previous experience in EBUS-TBNA, the diagnostic yield and rate of representative increased, reaching a plateau after 60 performances.

In theory, numerous thoracic lymph node stations could be identified and reached by EBUS-TBNA, such as stations 1, 2, 3, 4, 5, 7, 10, and 11. However, for safety and practical reasons, stations 2, 4, 7, 10, and 11 are more frequently sampled [12]. The present study identified and aspirated lymph nodes from stations 2, 4, 7, 10, and 11, with a sampling success rate of 93.8%. It is obvious that selection of proper sites for needle aspiration can increase the diagnostic yield of EBUS-TBNA. Additionally, at least two satisfactory specimens should be obtained from each location and multiple passes may be required to increase the diagnostic yield. According to previous studies, ROSE can increase diagnostic yield [13, 14]. Since present researchers were not equipped with facilities for ROSE, the present study did not involve ROSE.

It was concluded that sampling success rates were correlated with the size and location of targets. If a lymph node was not big enough, it was difficult to put the needle into the lymph node exactly. As patients breathed, the changing position of a small lymph node would increase the risk of injury. Additionally, necrosis commonly occurs in larger lymph nodes, in which inflammatory elements predominate and the cancerous contribution is relatively small [15]. For the relationship between location and sampling success rates, differences in breath movement, vascular pulsation, and proficiency of EBUS-TBNA in the various locations may partially explain the variations. It has been reported that lymph node characteristics may suggest a possible entity but do not allow for a definitive conclusion [16, 17]. Since this was a retrospective analysis, details regarding punctured lymph nodes under EBUS were not recorded. This will be a focus of future research, however.

The present study demonstrated that the sensitivity of initial EBUS-TBNA in detecting malignancy of lymph nodes was 93.0% and the NPV was 45.7%, excluding the missing patients. Taking all patients (missing and conclusive) into account, the diagnostic yield of EBUS-TBNA in diagnosing both benign and malignant disease was 89.2%. In the study of Herth et al., 93.5% of lymph nodes were successfully biopsied and a specific diagnosis was established. Other studies have come to similar conclusions, indicating that the sensitivity, specificity, and accuracy of EBUS-TBNA in distinguishing benign from malignant lymph nodes were very high, usually more than 90% [18-22]. However, there are differences in opinion regarding the diagnostic performance of EBUS-TBNA. Lange et al. reported that a diagnosis could be established by EBUS-TBNA in only 27%, including four patients with sarcoidosis. They also stated that sensitivity and NPV were low at 61.4% and 76.7%, respectively [22]. In these studies, including the present study, patients that were investigated were usually preselected [18-22].

Table 6. Diagnostic ability of EBUS-TBNA over time

	Year 1	Year 2	Year 3	Year 4	Year 5	Year 6
Conclusive diagnosis (%)	55 (83.3)	42 (89.4)	66 (91.6)	55 (91.7)	70 (90.9)	62 (92.5)
Inclusive diagnosis (%)	3 (4.5)	3 (6.3)	3 (4.2)	2 (3.3)	4 (5.2)	2 (3.0)
Nonrepresentative (%)	8 (12.1)	2 (4.3)	3 (4.2)	3 (5.0)	3 (3.9)	3 (4.5)
Total	66	47	72	60	77	67

Before EBUS-TBNA, most patients had been examined with CT and some of them with PET-CT or other examinations. Lange et al. thought that preselection and publication bias might be responsible for the reports of high diagnostic yield of EBUS-TBNA [22]. However, not all people are willing to accept highly expensive medical techniques and regard them as their first choice, such as EBUS-TBNA and PET-CT, especially in developed countries. Thus, it is likely that preselection could improve the diagnostic performance of EBUS-TBNA and that it is necessary to improve the diagnostic skills of clinicians to avoid futile additional biopsies. Furthermore, few complications have occurred when EBUS-TBNA is performed [23].

Mediastinoscopy has long been the mainstay of mediastinal lymph node sampling. The diagnostic accuracy of mediastinoscopy is high and complications are rare [24, 25]. However, it is also difficult to perform this procedure a second time in patients. The reach of the procedure is limited, as lymph nodes may not be accessible. Ernst et al. compared mediastinoscopy to EBUS-TBNA in patients with suspected lung cancer and mediastinal lymphadenopathy, finding that the diagnostic yield of EBUS-TBNA was higher than that of mediastinoscopy. They concluded that EBUS-TBNA was preferred in the histologic sampling in patients with lung cancer [26]. Mediastinoscopy is currently the gold standard for cancer staging, perhaps because comparative studies of EBUS-TBNA and mediastinoscopy are rare and there is a substantial learning curve effect for EBUS-TBNA. EBUS-TBNA could be the first choice for histologic sampling of paratracheal and subcarinal mediastinal lymphadenopathy.

However, the disadvantages of EBUS-TBNA cannot be ignored. The major technical disadvantages are the high expense and required high level of experience of the operator. When dealing with a malignancy, specimens may not be enough to identify diseases, forcing clinicians to perform an open biopsy. Inflammation may turn out to be malignant process. However, cancer may also present as inflammation, e.g., lymphoma. Additionally, verification of rare and more complex tumors may not always be possible [27]. Additionally, numerous studies have demonstrated that the NPV of EBUS-TBNA is very low, which means that a negative TBNA should be treated carefully. Patients with a negative TBNA should be referred for a further confirmatory diagnostic procedure, such as mediastinoscopy or repeated EBUS-TBNA, or followed up until a definitive final diagnosis, such as malignancy, is confirmed.

When the pathological diagnosis reports a negative TBNA, there are a few possible scenarios: (1) Inadequate or non-representative specimen; (2) True negative (the lymph node is free from cancer cells); (3) Lymph nodes contain cancer cells which were missed by the EBUS-TBNA; and (4) Lymph nodes contain cancer cells that were missed by the pathologist. It has been reported that ROSE could reduce the percentage of inadequate and nonrepresentative specimens [28]. Although the latter two scenarios are both false negatives, they are in practice indistinguishable. In clinical practice, false negative results usually refer to the third scenario, which means that the estimate of the false negative rate of EBUS-TBNA may be high. Therefore, excellent and experienced pathologists are necessary to improve the diagnostic yield of EBUS-TBNA.

In other published clinical trials concerning this subject, many of the EBUS-TBNA examiners were familiar with this medical modality over time. This could have influenced the diagnostic performance. Steinfort et al. reported improvements in diagnostic performance of EBUS-TBNA after 20 and even 50 examinations, whereas others described the learning curve to require only approximately 10 procedures [21, 29]. A retrospective analysis by Kemp et al. found wide variability in the learning curves for EBUS-TBNA but showed a steady increase in diagnostic performance over 100 procedures

in three of five operators [30]. The EBUS-TBNA examiner in the current study was experienced in performing flexible bronchoscopies but had no experience in EBUS-TBNA. The current study demonstrated that the rate of nonrepresentative samples decreased over time. The increased success rate in obtaining representative material with EBUS-TBNA during these time frames can probably be attributed to a learning curve effect. Lymph node selection, patient selection, and technical proficiency of EBUS-TBNA and ROSE are factors which can increase the performance of EBUS-TBNA.

The present study had several limitations. First, the high rate of malignancy influenced the diagnostic yield of EBUS-TBNA. Furthermore, analysis was performed retrospectively. Thus, there were many confounding factors that could not be standardized that may have influenced EBUS-TBNA results, e.g., the anesthesia method and the frequency of passes per lymph node. Additionally, in some cases where EBUS-TBNA results were negative or nonrepresentative and further confirmatory tests were not performed, a final definitive diagnosis could not be attained. Therefore, the possibility of a false negative EBUS-TBNA result could not be ruled out. According to records, a few of the procedures were performed under general anesthesia, which may have increased the diagnostic accuracy. Finally, other diagnostic procedures were not performed on all patients. A few patients accepted mediastinoscopy after a negative EBUS-TBNA. Therefore, the comparison between EBUS-TBNA and mediastinoscopy was limited.

Conclusion

EBUS-TBNA has a high diagnostic yield in mediastinal and hilar lymphadenopathy. When performed by skilled investigators, it can be the first choice after patient selection. Subcarinal and paratracheal lymph nodes, with diameters in the range of 10 to 30 mm, are the first choice for sampling. More importantly, it is necessary for EBUS-TBNA examiners to improve their proficiency.

Acknowledgements

The present study was funded by the National Natural Science Foundation of China (Grant Number: 81770029), Key Research Project of Shandong Province (2016GSF201028), Key Research Project of Shandong Province (2017GSF218056), and Jinan Science and Technology Development Plan (201805063).

This retrospective study was approved by Institutional Review Board of Qilu Hospital of Shandong University. Written informed consent was obtained from patients willing to participate.

Disclosure of conflict of interest

None.

Abbreviations

EBUS-TBNA, Endobronchial ultrasound transbronchial needle aspiration; CT, computerized tomography; NPV, negative predictive value; PPV, positive predictive value; ROSE, rapid onsite evaluation.

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