

RESEARCH ARTICLE

Endobronchial Ultrasound-Guided Transbronchial Needle Aspiration in the Diagnosis of Lymphoma

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Abstract

Background: Endobronchial ultrasound-guided transbronchial needle aspiration (EBUS-TBNA) is highly accurate in diagnosing mediastinal lymphadenopathies of lung cancer and benign disorders. However, the utility of EBUS-TBNA in the diagnosis of mediastinal lymphomas is unclear. The aim of this study was to determine the diagnostic value of EBUS-TBNA in patients with suspected lymphoma. **Materials and Methods:** Sixty-eight patients with isolated mediastinal lymphadenopathy and suspected of lymphoma were included in the study. EBUS-TBNA was performed on outpatients under moderate sedation. The sensitivity, specificity, negative predictive value and diagnostic accuracy of EBUS-TBNA were calculated. **Results:** Sixty-four patients were diagnosed by EBUS-TBNA, but four patients with non-diagnostic EBUS-TBNA required surgical procedures. Thirty-five (51.5%) patients had sarcoidosis, six (8.8%) had reactive lymphadenopathy, nine (13.3%) had tuberculosis, one (1.5%) had squamous cell carcinoma, two (2.9%) had sarcoma and fifteen (22%) had lymphoma (follicular center cell, large B-cell primary, and Hodgkin lymphomas in three, two, and ten, respectively). Of the 15 lymphoma patients, thirteen were diagnosed by EBUS and two by thoracotomy and mediastinoscopy. The sensitivity, specificity, negative predictive value, and diagnostic accuracy of EBUS-TBNA for the diagnosis of lymphoma were calculated as 86.7%, 100%, 96.4%, and 97%, respectively. **Conclusions:** EBUS-TBNA can be employed in the diagnosis of mediastinal lymphoma, instead of more invasive surgical procedures.

Keywords: EBUS-TBNA - lymphoma - mediastinal lymphadenopathy

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Introduction

Endobronchial ultrasound-guided transbronchial needle aspiration (EBUS-TBNA) is a minimally invasive technique with a high diagnostic yield in the diagnosis and staging of lung cancer (Herth et al., 2008; Cetinkaya et al., 2011; Yasufuku et al., 2011). Additionally, numerous studies have reported the successful diagnosis of sarcoidosis by EBUS-TBNA (Garwood et al 2007; Tremblay and Stather, 2009). However, similar studies in patients with tuberculous lymphadenitis are limited (Navani et al., 2011). Likewise, the utility of EBUS-TBNA in the diagnosis of mediastinal lymphoma also remains unclear.

Lymphoma, a lymphoproliferative malignancy, is a common cause of mediastinal tumors. Primary mediastinal lymphoma in adults is rare, representing only 10% of all mediastinal lymphomas (Duwe and Serman, 2005). Cervical or supraclavicular lymphadenopathy is a common presentation in patients with lymphoma, and is easily diagnosed by excisional lymph node biopsy. However,

obtaining biopsy specimens from isolated mediastinal lymphadenopathies is a challenge (Stollo and Jett, 1997). In these patients, excisional biopsies require invasive procedures such as thoracotomy, thoracoscopy or mediastinoscopy. These procedures are performed under general anesthesia, and the associated complications cannot be ignored (Smith and Besien, 2009). EBUS-TBNA has recently been considered for the diagnosis of such mediastinal abnormalities. However, few studies have examined the effectiveness of EBUS-TBNA in evaluating patients with current or previously diagnosed lymphoma. Therefore, this study aimed at evaluating the role of EBUS-TBNA in the diagnosis of isolated mediastinal lymphadenopathies in patients with suspected lymphoma.

Materials and Methods

We retrospectively evaluated our database for patients who underwent EBUS between September of 2010 and September of 2013. Patients with suspected

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lymphoma were included in the study on the basis of a history of lymphoma, or newly isolated mediastinal lymphadenopathy, identified using computed tomography or positron emission tomography-computed tomography (Figure 1a). Patients with other likely causes of lymphadenopathy (lung cancer, extrathoracic malignancy or granulomatous diseases) and those with pulmonary lesions accompanying mediastinal lymphadenopathy were excluded from the study. The study was approved by the local Institutional Ethics Committee.

Procedures

Conventional flexible bronchoscopy was first performed to examine the tracheobronchial tree. Thereafter, EBUS was performed at 7.5 MHz (CP-EBUS, BF-UC160F; Olympus, Tokyo, Japan), and a dedicated ultrasound scanner (EU-C2000; Olympus, Tokyo, Japan) was used for image processing. All EBUS-TBNA procedures were performed using a 22-gauge needle (Figures 1b, c), and each nodal station was systematically imaged by EBUS and classified according to the Mountain-Dressler classification. A minimum of three needle passes were performed; the initial aspirated material was placed on glass slides and air-dried, alcohol-fixed smears were prepared, and the remaining material was placed in a formalin solution to prepare a cell block for histological evaluation and immunohistochemistry.

The needles and syringes used to obtain the fine-needle aspirates were rinsed in 10 mL of 50% ethanol in a specimen container. The material was centrifuged in a 10-mL centrifuge tube at 4,000 rpm for 6 min to obtain cell pellets. The supernatant fluid was decanted, and the cell deposit was fixed in a freshly prepared alcohol-formalin substitute (nine parts 100% ethanol and one part 40% formaldehyde). After a 45 min fixation, the fixed cell pellets were re-centrifuged at 4,000rpm for 6 min, such that they detached themselves or could be easily removed with a disposable Pasteur pipette following centrifugation. The cell pellets were wrapped in crayon paper, placed in a cassette, and stored in 80% ethanol until they were ready for processing in the automatic tissue processor, using a 13-h processing schedule.

The cell blocks were then embedded in paraffin and sectioned into 3µm-thick slices. Routine Harris hemotoxylin and eosin (H&E) staining was used for all sections. When necessary, histochemical stains for pigments, bacteria and fungi were used, and a



Figure 1. A) Contrast-enhanced Computed Tomography Scan Showing Right Hilar, Paratracheal and Subcarinal Lymphadenopathy; B) EBUS Image of an Enlarged Station 7 Lymph Node in a Patient Diagnosed with Mantle Cell Lymphoma

comprehensive range of polyclonal and monoclonal antibodies was employed in cases requiring identification or the phenotyping of tumor cells using streptavidin-biotin without prior enzymatic digestion. Immunohistochemical staining for cytokeratin, CD20, CD79a, CD3, CD5, CD10, BCL-6, BCL-2, MUM-1 and cyclin-D1 was performed on the cytological samples. The final diagnosis was based on cumulative information obtained from the cytological and histological results.

While a diagnostic sample was defined as one with a definitive benign or malignant diagnosis, an adequate sample was defined as one with abundant lymphoid cells and no bronchial epithelial cells, or a sample with a definitive diagnosis.

Statistical analysis

The sensitivity, specificity, negative predictive value and positive predictive value, as measures of diagnostic accuracy, were based on the standard definitions. The statistical analyses were performed using the Statistical Package for Social Sciences (SPSS) version 20. p<0.05 was considered to be significant.

sensitivity of 86.7%, a specificity and positive predictive value (PPV) of 100%, a negative predictive value (NPV) of 96.4%, and diagnostic accuracy of 97%.

Results

Thirty-five (51.5%) patients were male and 33 (48.5%) were female, and their median age was 50 years (range, 20-80 years). Thirty-five patients had sarcoidosis, nine had tuberculous lymphadenitis, six had reactive lymphadenopathy, two had sarcoma, fifteen had lymphoma, and one had squamous cell lung cancer (Figure 2). The patient with squamous cell lung cancer also had lymphoma in the remission phase; however, there were no parenchymal lesions in this patient. Table 1 shows the features of the 15 patients with lymphoma. Two of the 68 (2.9%) patients had a prior diagnosis of lymphoma.

One hundred and thirty-five lymph nodes were

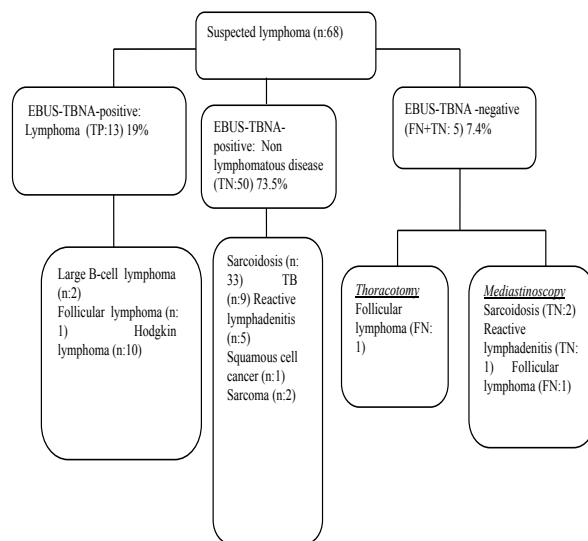


Figure 2. Diagnostic Flow Chart in 68 Patients with Isolated Mediastinal Lymphadenopathy

Table 1. Patients Diagnosed with Lymphoma (n=15)

Patient no.	Sex/age	Lymph node station and diameter	Diagnosis by EBUS	Surgical procedure	Primary or recurrent	Diagnosis by surgery
1	M/57	7:22mm 4L:20mm 11L:15mm	B-cell lymphoma	-	Primary	
2	F/35	4R:15mm 10R:13mm	Hodgkin lymphoma	-	Primary	
3	M/31	4R:25mm 7:23mm 10L:23mm	Hodgkin lymphoma	-	Primary	
4	M/35	4R:30mm	Hodgkin lymphoma	-	Primary	
5	M/70	4R:28mm	Hodgkin lymphoma	-	Primary	
6	F/43	7:18mm 11R:24mm	Hodgkin lymphoma	-	Recurrent	
7	M/43	4R:27mm 7:30mm	Hodgkin lymphoma	-	Primary	
8	F/24	4R:35mm	Diffuse necrosis and epitelioid histiocytes	Thoracotomy	Primary	Follicular lymphoma
9	F/71	7:14mm	Follicular lymphoma		Recurrent	
10	F/24	7:15mm 4R:17mm	Hodgkin lymphoma		Primary	
12	M/65	7:15mm	B-cell lymphoma		Primary	
13	F/28	10L:13mm	Hodgkin lymphoma		Primary	
14	M/32	4R:17mm 7:20mm	Hodgkin lymphoma		Primary	
15	M/57	4R:15mm 10R:17mm	Suspicious for lymphoma	Mediastinoscopy	Primary	Follicular lymphoma

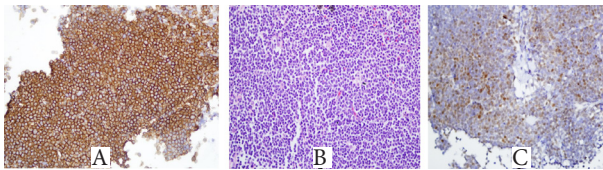


Figure 3. Homogeneous Population of Small-intermediate Size Lymphoid Cells. A) Diffuse CD20 expression immunohistochemically; B) Hematoxylin eosine; C) Nuclear staining for cyclin D1 x200

sampled by EBUS-TBNA. The median number of needle passages into a nodal site was three (range, 2-6), and the median size of the lymph nodes detected with EBUS-TBNA was 15mm (range, 5-50mm). The most frequently sampled lymph nodes were the subcarinal and right hilar lymph nodes.

Of the 15 patients with a final diagnosis of lymphoma, EBUS-TBNA correctly diagnosed lymphoma in thirteen patients; however, there were two false-negative cases that were diagnosed by thoracotomy and mediastinoscopy (Table 1). Ten patients were diagnosed with Hodgkin's lymphoma, three with follicular center cell lymphoma, and two with extranodal marginal zone B-cell lymphoma (MALTOMA) (Figures 3).

EBUS-TBNA provided a definitive diagnosis in 63 of 68 patients, but only the retrieval of adequate lymphoid tissue with no specific diagnosis in three patients (true negatives for lymphoma). Thus, in 66 of 68 patients, adequate specimens were obtained (Figure 2). The overall diagnostic sensitivity of EBUS-TBNA in detecting both malignancy and benign disorders was 94%. For the diagnosis of lymphoma, EBUS-TBNA had a sensitivity of 86.7%, a specificity and positive predictive value (PPV) of 100%, a negative predictive value (NPV) of 96.4%, and diagnostic accuracy of 97%.

Discussion

The present study shows that EBUS-TBNA is an accurate and safe procedure for the evaluation of primary or recurrent lymphoma. It demonstrated a sensitivity of 86.7% and an accuracy of 97%, and can be considered as an initial procedure for the diagnosis of mediastinal lymphadenopathies in patients with suspected lymphoma.

The cytological diagnosis of lymphoma based on fine needle aspiration (FNA) samples has been shown to be of limited value as a diagnostic technique. Pathologists have questioned whether an accurate definitive cytological diagnosis of follicular lymphoma and marginal zone lymphoma are difficult to make using small samples. Therefore, excisional biopsy is considered necessary by some authors to confirm these diagnoses (Metzgeroth et al., 2012); however, this is not always possible because of the poor general medical condition of these patients (Ahmed HG et al., 2013). Recent studies have indicated that an accurate diagnosis of lymphoproliferative disorders can be achieved by FNA in 85.9-87% of the cases, particularly when cytology is supported by flow cytometry and immunohistochemistry techniques, representing a powerful first-choice diagnostic technique (Mayall and Dray, 2000).

Cell block preparation is a simple method that provides important additional information after EBUS-TBNA in lymphoma. Sanz-Santos et al. (2012) reported that cell blocks improved the pathological diagnosis attained with conventional smears. The present study indicates that when cytomorphological studies were used in combination with cell blocks, we were able to provide a diagnosis from lymph node EBUS-TBNA samples with good sensitivity and specificity. Our results underscore the clinical utility of this diagnostic modality.

In the past, surgical approaches, such as mediastinoscopy, were the gold standard for the definitive diagnosis of isolated mediastinal lymphadenopathy in patients suspected of lymphoma (Zhao et al., 2011). However, this approach does not allow access to the aortopulmonary window, inferior-posterior mediastinum or perihilar lymph nodes. Moreover, it may be impossible in some circumstances because of the poor condition of patients with comorbidities (Gomez and Silvestri, 2009).

Several authors have emphasized the use of low-volume tissue samples by EBUS-TBNA. The role of EBUS-TBNA in a lymphoma diagnosis is, therefore, of uncertain value in cases of marginal zone and follicular lymphoma (Farmer and Bailey, 2007). Steinfors et al. (2010) reported the value of EBUS-TBNA in mediastinal lymphadenopathies in cases suspicious for lymphoma. They have criticized the use of EBUS-TBNA for some lymphoma subtypes, such as marginal zone lymphomas or hypocellular variants. In our study, a definitive pathological diagnosis and histological typing were achieved in thirteen of fifteen (86.7%) patients with lymphoma.

There are some articles on endoscopic ultrasound guided fine needle aspiration (EUS-FNA) in the diagnosis of lymphoma. Ribeiro et al. (2010) showed that EUS-FNA enabled the diagnosis of lymphoma in 79% of the cases, and classification in 67%. They proposed that the low rate of classification was related to 63% of the cases being low-grade or Hodgkin's lymphoma. Moonim and Breen (2013) showed that EBUS-TBNA can be successfully applied to diagnose and subtype de novo and relapsed mediastinal lymphoma. A more recent study also demonstrated that EUS-FNA achieved 83% diagnostic accuracy in lymphoma (Korenblit and Anantharaman, 2012).

Currently, the diagnosis of lymphoma is based on the World Health Organization (WHO) classification system. Yasudu et al. (2006) was able to assess the yield of EUS-guided biopsy in the determination of the lymphoma sub-classification based on the WHO classification system in 88% of the cases.

Kennedy et al. (2008) showed that ten out of 25 patients with suspected mediastinal recurrences of lymphoma or mediastinal lymphadenopathy of unknown cause were diagnosed with lymphoma using EBUS-TBNA. They reported a sensitivity of 90.9%, specificity of 100%, PPV of 100% and NPV of 92.8%, which were similar to our result.

Remediastinoscopy is considered to be a difficult procedure because of peritracheal adhesions and fibrotic changes after induction chemotherapy and radiotherapy. Therefore, most thoracic surgeons consider this technique unsafe. After chemotherapy or radiotherapy, given the risk of fibrosis or necrosis, EBUS should be preferred for evaluating the recurrence of lymphoma (Herth et al., 2008; Call et al., 2011). In our study, three lymphoma patients were treated previously, and using EBUS-TBNA, two of them were diagnosed with recurrent lymphoma and one with primary lung cancer. Thus, these patients were spared from undergoing invasive diagnostic approaches such as mediastinoscopy.

Using EBUS as a first-choice diagnostic test for

mediastinal lymphoproliferative diseases will reduce the need for aggressive surgical processes such as mediastinoscopy (Marshall et al., 2011). However, negative results cannot exclude malignancy, whereas positive histopathological diagnosis has an important directive value. Owing to the relatively low NPV of EBUS-TBNA, tumor-negative findings should be verified by surgical or other techniques (Cetinkaya et al., 2013).

The residual risk of lung cancer has been reported after lymphoma treatment (Demirci et al., 2012). Lung malignancies developing after the treatment of lymphoma generally have a poor prognosis. However, a subset of patients who are incidentally diagnosed may have a potentially curable disease. In the present study, one patient had lymphoma in the remission phase, as well as primary squamous cell lung carcinoma. Another important result related to this patient was that enlarged lymph nodes after treatment for lymphoma are not always a harbinger of relapse. They may be coincidentally involved with primary lung cancer or granulomatous disease (Schoenfeld et al., 2012).

The limitations of the current study are that it is retrospective and has a small number of patients from a single center. A larger, prospective study could overcome both of these limitations. In our laboratory, we did not use flow cytometry; however, cell block immunohistochemistry was used for each case.

In conclusion, EBUS-TBNA is useful for the diagnosis of lymphoma in patients with mediastinal lymphadenopathy. This technique has a high sensitivity and specificity, particularly when combined with immunohistochemical analysis. EBUS-TBNA should be considered a primary diagnostic method in clinical practice for the diagnosis of mediastinal lymphoma, as it may decrease the need for more invasive procedures.

References

- Ahmed HG, Elmubasher MB, Salih RA, et al (2013). Fine needle aspiration cytopathology of pediatric lymphadenopathy among Sudanese children. *Asian Pac J Cancer Prev*, **14**, 4359-63.
- Call S, Rami-Porta R, Obiols C, et al (2011). Repeat mediastinoscopy in all its indications: experience with 96 patients and 101 procedures. *Eur J Cardiothorac Surg*, **39**, 1022-7.
- Cetinkaya E, Ozgul MA, Tutar N, et al (2013). The diagnostic utility of real-time EBUS-TBNA for hilar and mediastinal lymph nodes in conventional TBNA negative patients. *Ann Thorac Cardiovasc Surg*, 15 [Epub ahead of print].
- Cetinkaya E, Seyhan EC, Ozgul A, et al (2011). Efficacy of convex probe endobronchial ultrasound (CP-EBUS) assisted transbronchial needle aspiration for mediastinal staging in non-small cell lung cancer cases with mediastinal lymphadenopathy. *Ann Thorac Cardiovasc Surg*, **17**, 236-42.
- Demirci U, Ozdemir N, Benekli M, et al (2012). Lymphoproliferative disorders in multiple primary cancers. *Asian Pac J Cancer Prev*, **13**, 383-6.
- Duwe BV, Serman DH, Musani AI (2005). Tumors of the mediastinum. *Chest*, **128**, 2893-9.
- Farmer PL, Bailey DJ, Burns BF, Day A, LeBrun DP (2007). The reliability of lymphoma diagnosis in small tissue samples is heavily influenced by lymphoma subtype. *Am J Clin Pathol*, **128**, 474-80.

- Garwood S, Judson MA, Silvestri G, et al (2007). Endobronchial ultrasound for the diagnosis of pulmonary sarcoidosis. *Chest*, **132**, 1298-304.
- Gomez M, Silvestri GA (2009). Endobronchial ultrasound for the diagnosis and staging of lung cancer. *Proc Am Thorac Soc*, **6**, 180-6.
- Herth FJ, Annema JT, Eberhardt R, et al (2008). Endobronchial ultrasound with transbronchial needle aspiration for restaging the mediastinum in lung cancer. *J Clin Oncol*, **26**, 3346-50.
- Kennedy MP, Jimenez CA, Bruzzi JF, et al (2008). Endobronchial ultrasound-guided transbronchial needle aspiration in the diagnosis of lymphoma. *Thorax*, **63**, 360-5.
- Korenblit J, Anantharaman A, Loren DE, Kowalski TE, Siddiqui AA (2012). The role of endoscopic ultrasound-guided fine needle aspiration (eus-fna) for the diagnosis of intra-abdominal lymphadenopathy of unknown origin. *J Interv Gastroenterol*, **2**, 172-6.
- Marshall CB, Jacob B, Patel S, et al (2011). The utility of endobronchial ultrasound-guided transbronchial needle aspiration biopsy in the diagnosis of mediastinal lymphoproliferative disorders. *Cancer Cytopathol*, **119**, 118-26.
- Mayall F, Dray M, Stanley D, Harrison B, Allen R (2000). Immunoflow cytometry and cell block immunohistochemistry in the FNA diagnosis of lymphoma: a review of 73 consecutive cases. *J Clin Pathol*, **53**, 451-7.
- Metzgeroth G, Schneider S, Walz C, et al (2012). Fine needle aspiration and core needle biopsy in the diagnosis of lymphadenopathy of unknown a etiology. *Ann Hematol*, **91**, 1477-84.
- Moonim MT, Breen R, Fields PA, Santis G (2013). Diagnosis and subtyping of de novo and relapsed mediastinal lymphomas by endobronchial ultrasound needle aspiration. *Am J Respir Crit Care Med*, **188**, 1216-23.
- Navani N, Molyneaux PL, Breen RA, et al (2011). Utility of endobronchial ultrasound-guided transbronchial needle aspiration in patients with tuberculous intrathoracic lymphadenopathy: a multicentre study. *Thorax*, **66**, 889-93
- Ribeiro A, Pereira D, Escalón MP (2010). EUS-guided biopsy for the diagnosis and classification of lymphoma. *Gastrointest Endosc*, **71**, 851-5.
- Sanz-Santos J, Serra P, Andreo F, et al (2012). Contribution of cell blocks obtained through endobronchial ultrasound-guided transbronchial needle aspiration to the diagnosis of lung cancer. *BMC Cancer*, **21**, 12-34.
- Schoenfeld JD, Mauch PM, Das P, et al (2012). Lung malignancies after Hodgkin lymphoma: disease characteristics, detection methods and clinical outcome. *Ann Oncol*, **23**, 1813-8.
- Smith S, Besien K (2009). Diagnosis and Treatment of Mediastinal Lymphomas. In Shields TW, LoCicero J, Reed Carolyn E, Feins RH. ed General Thoracic Surgery vol 2, 7th. ed. Philadelphia: Lippincott Williams and Wilkins, 2379-88.
- Steinfort DP, Conron M, Tsui A, et al (2010). Endobronchial ultrasound-guided transbronchial needle aspiration for the evaluation of suspected lymphoma. *J Thorac Oncol*, **5**, 804-9
- Strollo DC, Rosado de Christenson ML, Jett JR (1997). Primary mediastinal tumors. part 1: tumors of the anterior mediastinum. *Chest*, **112**, 511-22.
- Tremblay A, Stather DR, Maceachern P, Khalil M, Field SK (2009). A randomized controlled trial of standard vs endobronchial ultrasonography-guided transbronchial needle aspiration in patients with suspected sarcoidosis. *Chest*, **136**, 340-6.
- Yasuda I, Tsurumi H, Omar S, et al (2006). Endoscopic ultrasound guided fineneedle aspiration biopsy for lymphadenopathy of unknown origin. *Endoscopy*, **38**, 919-24.
- Yasufuku K, Pierre A, Darling G, et al (2011). A prospective controlled trial of endobronchial ultrasound-guided transbronchial needle aspiration compared with mediastinoscopy for mediastinal lymph node staging of lung cancer. *J Thorac Cardiovasc Surg*, **142**, 1393-400.
- Zhao H, Wang J, Zhou ZL, et al (2011). Application of endobronchial ultrasound-guided transbronchial needle aspiration in the diagnosis of mediastinal lesions. *Chin Med J*, **124**, 3988-92.