The Yield of Endobronchial Ultrasound-Guided Transbronchial Needle Aspiration in Collecting Cytological Samples for Next-Generation Sequencing in Non-small Cell Cancer Patients: A Retrospective Study

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ABSTRACT **Background:** Lung cancer is a major cause of death worldwide. Accurate diagnosis and staging are essential for effective treatment. Mediastinal lymph node involvement determines the disease stage and influences treatment decisions, especially with new biological and immunotherapy options. Endobronchial ultrasound-guided transbronchial needle aspiration (EBUS-TBNA) is the main minimally invasive procedure for evaluating mediastinal and hilar adenopathy. It offers high sensitivity, specificity, and fewer complications than mediastinoscopy or video-assisted thoracic surgery. It also retrieves crucial molecular markers for guiding therapeutic decisions in non-small cell lung cancer.

Objectives: To evaluate the yield of EBUS-TBNA in molecular and genomic workup in patients with NSCLC.

Methods: This retrospective study included patients who underwent bronchoscopy with EBUS and had lymph node malignancy between 2018 and 2023. Crossmatching was conducted by pathology and genomic study results. No informed consent was required as the study was based on the hospital database.

Results Next generation sequencing was performed on 57 specimens (83%) collected via EBUS from patients with primary non-small cell lung cancer. However, 12 of the specimens (17%) were insufficient for pathological analysis. Among these, 7 (58%) were from adenocarcinomas and 5 (42%) were from squamous cell carcinoma patients.

Conclusions: The utilization of EBUS-TBNA is an effective tool for obtaining genetically profiled diagnoses by minimally invasive means. As more genetic mutations are discovered, we expect that multigene mutation analysis will gain importance in tailoring individualized treatment plans.

IMAJ 2025; 27: 783–787

KEY WORDS: endobronchial ultrasound-guided transbronchial needle aspiration (EBUS-TBNA), lung cancer, mediastinal lymphadenopathy, next-generation sequencing (NGS)

Lung cancer continues to pose a significant challenge worldwide. Despite notable progress, it still holds its position as the primary contributor to cancer-related deaths, maintaining one of the least favorable 5-year survival rates among all forms of cancer. Many cases are detected only at advanced stages. Accurate diagnosis is essential for effective treatment [1].

Mediastinal lymph node evaluation is critical for staging bronchogenic carcinomas [2]. Nodal staging helps predict prognosis and guides treatment decisions. Positive nodal tissue indicates advanced disease, typically stage II or III, warranting neoadjuvant therapy before surgery [3].

Initial staging involves imaging techniques like computed tomography (CT) and ^{18}F-fluorodeoxyglucose positron-emission tomography/computed tomography (^{18}F-FDG PET/CT) scans followed by sampling the lymph nodes using endobronchial ultrasound-guided transbronchial needle aspiration (EBUS-TBNA). EBUS-TBNA is minimally invasive, offers access to hilar nodes, reduces complications, and is cost-effective. It exhibits high sensitivity and specificity. Studies have shown that EBUS-TBNA outperforms mediastinoscopy in diagnosing mediastinal staging for lymph node stations N1-3 involvement in non-small cell lung cancer (NSCLC) [4].

EBUS-TBNA is performed using bronchoscopy and ultrasound to aspirate lesions near the trachea and major bronchi. This technology offers fewer complications, can be completed as an outpatient procedure, reduces healthcare costs, and shortens procedure times compared to surgery. It demonstrates excellent diagnostic performance with high sensitivity and specificity.

Methods for performing primary lung tumors biopsies and mediastinal staging must yield adequate tissue for genomic analysis, which are vital for personalized NSCLC treatment. To the best of our knowledge, no uniform standards exist for assessing EBUS-TBNA sample adequacy. Typically, adequacy is determined if a sample provides a diagnosis, such as granulomas or malignancy, even without lymphoid tissue, or if sufficient benign lymphoid tissue is present [5,6]. The suitability of EBUS-TBNA samples for molecular analysis depends on sample size, cellularity, tumor cell proportion, contaminants like blood or bronchial cells, and the sensitivity of the testing platform [6].

In NSCLC, treatment responses vary by subtype, necessitating accurate histological and molecular analyses. Studies confirm EBUS-FNA yields sufficient cancer cells for polymerase chain reaction detection of common driver mutations in NSCLC. A prospective study conducted in the United Kingdom reported a 99% genotyping success rate for epidermal growth factor receptor (EGFR) and ALK mutations in primary lung adenocarcinoma using EBUS-TBNA samples processed as histopathology specimens [7].

EGFR and anaplastic lymphoma kinase (ALK) mutations in advanced NSCLC predict better prognosis and responsiveness to tyrosine kinase inhibitors (TKIs) and ALK inhibitors, respectively. Targeted therapy is recommended over chemotherapy and immunotherapy due to its significant impact on survival compared to chemotherapy alone [8]. Moreover, a recent study discovered that a tissue surface area of at least 1 mm is necessary to facilitate tailored targeted treatments guided by next-generation sequencing (NGS) analyses. The study also reported an overall success rate of 84% for DNA sequencing using EBUS-FNA samples [9,10].

PATIENTS AND METHODS

We retrospectively analyzed medical records of patients who were referred for bronchoscopy between January 2018 and January 2023. Of 2230 bronchoscopies, EBUS-TBNA was used for 429 cases of mediastinal lymphadenopathy evaluations that were detected on computed tomography (CT) scans. These cases were categorized into three groups: known malignancy for staging, suspected malignancy for diagnosis and staging, and suspected non-malignant conditions.

Institutional review board approval was obtained (IRB no: 0010-23-SZMC). No specific informed consent was required for this retrospective study. Informed consent for each bronchoscopy was obtained prior to the procedure. Each patient underwent a standard pre-operative assessment, including physical examination, routine laboratory tests, spirometry, chest radiography, and CT scans. During the EBUS-TBNA procedure, an initial diagnostic flexible bronchoscopy was performed under general anesthesia. Induction involved administering midazolam (1–10 mg), alfentanil (0.5–1.5 mg), and propofol (0.5–1.0 mg/kg). The procedure was conducted in the bronchoscopy suite with positive pressure ventilation, using an anesthesia circuit connected to the side port of the endotracheal tube (ET) or laryngeal mask airway (LMA). The equipment included flexible bronchoscopes (FUJIFILM EB-530Tor530S video bronchoscopes: Fujifilm Europe, GmbH Duesseldorf, Germany) for the evaluation of the airways and collection of bronchoalveolar lavage specimens. An endobronchial biopsy was performed if necessary. An EBUS scope (FUJIFILM EB-530 US 12.5-megahertz convex probe linear array puncture scope) [Figure 1] was then used. Lymph nodes were sampled with a single-use aspiration needle width of 21G (Vizishot, Olympus Co. Tokyo, Japan) under real-time ultrasound guidance.

Figure1 shows an ultrasound image of a biopsy needle in an enlarged lymph node, station 7(N2) and the sampling order for known or suspected cancer begins with the opposite side Hilar and Mediastinal lymph nodes, side lymph nodes, and the N3-N2-N1 order as suggested by the 8th edition lung cancer staging system. The median number of passes performed during EBUS-TBNA procedures to achieve an adequate specimen for diagnostic purposes is 3 to 4 passes per lymph node.

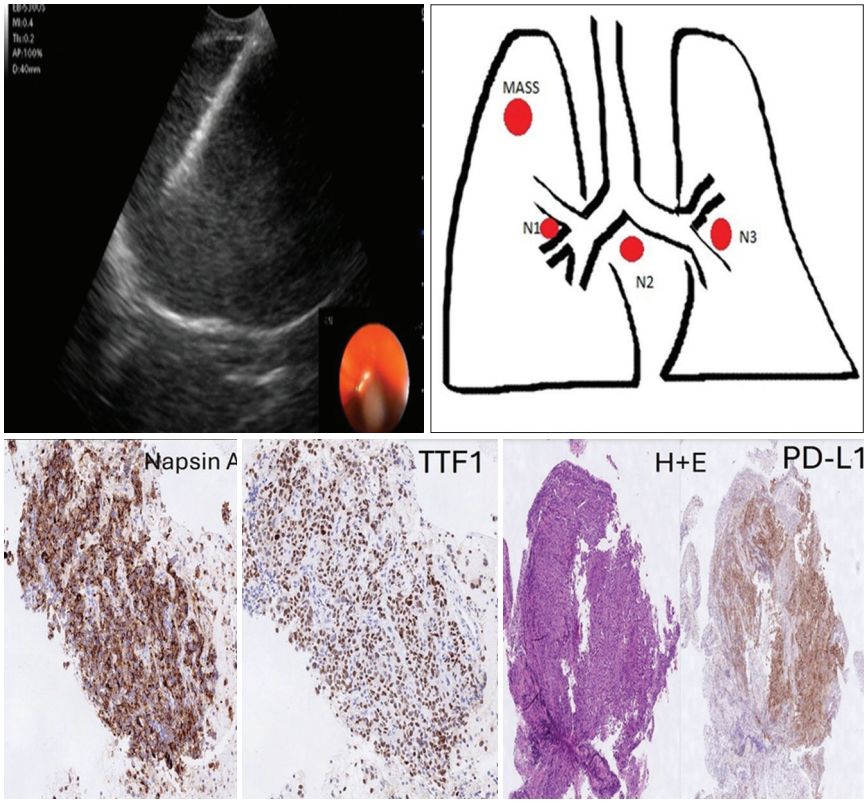
SPECIMEN PREPARATION AND HISTOLOGICAL ANALYSIS

The samples collected were fixed in formaldehyde, embedded in paraffin, and sectioned for histological examination. hematoxylin and eosin staining, along with necessary immunohistochemical stains (TTF1, Napsin, CK5, CK6) [Figure 1]. Malignant cells were further analyzed using next generation sequencing (NGS) via the Oncomine Comprehensive Assay identifying alterations in over 500 cancer-related genes and calculating tumor mutation burden and microsatellite instability.

STATISTICAL ANALYSIS

Data collection was retrospective. Descriptive data are shown as mean ± standard deviation or median (range). Statistical analyses were conducted using PS Power and Sample Size Calculations version 3.1.6 (2018) Vanderbilt University, USA.

Figure 1. Ultrasound image of biopsy needle in an enlarged lymph node, station 7 (N2), illustrating the order of lymph node sampling by stations according to the 8th lung cancer stage classification [19]; immunohistochemical stains positive for napsin A, TTF1, and programmed death-ligand 1 (PD-L1) with standard Hematoxylin and Eosin staining from the same area



RESULTS

Demographic and pathological data were compiled from 429 patients with mediastinal lymphadenopathy who underwent EBUS-TBNA between January 2018 and January 2023. Specimens from the patients were subsequently processed and subjected to NGS, specifically for those diagnosed with NSCLC. Table 1 shows the demographic and clinical characteristics of patients who underwent EBUS-TBNA.

The mean proportion of patients with malignancy was 124 (29%), of which 69 (56%) were diagnosed with primary NSCLC (61% had adenocarcinoma and 39% had squamous cell carcinoma of all NSCLC). Among the affected patients, 33% were classified as having stage III lung cancer and 39% were classified as having stage IV lung cancer. In addition, 6% of patients were diagnosed with primary small cell lung carcinoma [Table 2]. Furthermore, 71.5% of patients presented with other etiol-

Table 1. Demographic and clinical characteristics of patients

Characteristics	N=429	%
Age in years	62 (13–95)	
Male/female	254/175	59/41
Smoker or past smoker/never smoked	219/220	51/49
Malignancy	124	28.5
NSCLC	69	16
NSCLS in smoker or past smoker/never smoked	53/16	77/23
Metastatic lung disease	30	7
Small cell lung cancer	25	6
Sarcoidosis	124	28.5
Lymphoma	12	3
Silicosis	9	2
Reactive lymph nodes	70	16
Normal lymphoid tissue	86	20

NSCLC = non-small cell lung cancer

ogies of lymphadenopathy, with 27.5% diagnosed with sarcoidosis, 3% with lymphoma, and 2% with an infectious etiology for the enlarged lymph nodes. In addition, 2.5 were diagnosed with Silicosis. It is significant to note that 36% of patients who underwent EBUS-TBNA had normal or reactive lymphoid tissue.

Table 2. Histological characteristics

Diagnosis	N=124	%	Male/ Female	%
NSCLC	69	16	50/19	59/41
Adenocarcinoma	42	39 (61% of NSCLC)	29/13	69/31
Squamous cell carcinoma	27	22 (39% of NSCLC)	21/6	77/23
Metastatic lung disease	30	7	13/17	43/57
SCLC	25	6	14/11	56/44

NSCLC = non-small cell lung cancer, SCLC = small cell lung cancer

MOLECULAR AND GENOMIC YIELD:

Next generation sequencing was performed on 57 EBUS-collected specimens from the 69 primary NSCLC patients (83%). However, 12 of these specimens (17%) were insufficient for pathological analysis: 7 adenocarcinomas (58%) and 5 squamous cell carcinoma (42%). EGFR mutations were identified in 7 of the specimens (17%), with all occurrences in patients with adenocarcinoma. Programmed cell death ligand (PDL) mutations were present in 28 of the specimens (49%), with 15 (53%) found in patients with adenocarcinoma. KRAS mutations were detected in 6 specimens (10.5%), with 3 (50%) of these mutations found in adenocarcinoma specimens. Only 3 cases (5%) had ALK mutations: two with adenocarcinoma and one with squamous cell carcinoma. Mutations were absent in 13 of NSCLC specimens (23%) suitable for analysis [Table 3].

DISCUSSION

Since its clinical introduction, EBUS-TBNA has become the preferred method for sampling hilar and mediastinal lymphadenopathy. EBUS-TBNA is primarily indicated for staging NSCLC and diagnosing mediastinal lymphadenopathy, with additional applications in benign conditions and lymphomas. It serves as a minimally invasive initial sampling procedure for suspected NSCLC, particularly in cases involving solitary hilar nodes, discrete N2 or N3 disease, or bulky mediastinal disease. Accurate genomic profiling of lung cancer patients is essential for determining eligibility for targeted therapy, necessitating tumor tissue samples that are representative of malignancy and sufficient in both quantity and quality.

In this retrospective study, we evaluated the diagnostic yield of EBUS-TBNA in patients with mediastinal lymphadenopathy. The findings demonstrated that the yield from EBUS-collected tissues was highly satisfactory, with the next generation sequencing successfully performed on 83% of the specimens.

The results indicated that EBUS-TBNA samples exhibit a high success rate in genotyping EGFR, PDL, and KRAS mutations in primary lung adenocarcinoma when processed as histopathology specimens. The combined overall success rate for genotyping EGFR, PDL, and KRAS mutations was approximately 77%, which is comparable to results from studies conducted in several international centers [10-13].

Our rate of EGFR mutation positivity in EBUS-TBNA samples was 12% (7/57) in patients diagnosed with primary lung adenocarcinoma. This prevalence rate exceeds that observed in three separate studies conducted in the United Kingdom, which reported rates of 6%, 3%, and 6% in patients with primary lung adenocarcinoma, non-squamous NSCLC, and all NSCLC types, respectively [10,12,14].

The EGFR mutation assay failure rate (0/57, 0%) was lower compared to prior studies, which reported failure

Table 3. New generation sequencing characteristics

	NGS (%)	ALK	EGFR	PDL	KRAS	Not detected	Not conducted (%)
NSCLC (n=69)	57 (83%)	3	7	28	6	13	12 (17%)
Adenocarcinoma (n=42)	35 (83%)	2	7	15	3	6	7 (7%)
Squamous cell (n=27)	22 (81%)	1	2	13	3	7	5 (9%)

ALK =anaplastic lymphoma kinase, EGFR = epidermal growth factor receptor, KRAS = Kirsten rat sarcoma virus gene, NGS = next-generation sequencing, NSCLC = non-small cell lung cancer, PDL = programmed cell death ligand

rates of 1.25%, 1.28%, 10%, and 12%, respectively [10-12,14]. PD-L1 positivity in EBUS-TBNA samples was 49% (28/57), with 60.7% of these from primary lung adenocarcinoma. This rate is like studies from Canada (48.2%) and Romania (40.9%) and exceeds most earlier results.

Our study displays significant heterogeneity relative to others, yet we consistently achieved high rates of EBUS-derived sample adequacy. Specifically, our results favorably compare to these studies regarding both mutation assay success rates and sample acquisition.

We acknowledge certain limitations to our study. First, this was a single-center study, although our numbers compare favorably with other previously published studies. Second, NSCLC can exhibit heterogeneity, with individual tumors potentially containing features of multiple cell types. This finding raise concerns that smaller cytological specimens may not fully represent the entire lesion. This limitation is relevant to the use of EBUS-TBNA cytology samples in diagnosing and treating lung cancer. However, current diagnostic and treatment protocols often rely on procedures that yield smaller samples, Third, this study was retrospective and observational, lacking histological comparisons across all specimens to confirm pathological diagnoses [15-18].

This study is highly reflective of clinical practice, and its findings are deemed applicable to other EBUS equipped centers. Consequently, these data endorse the utilization of EBUS-TBNA as an effective tool for obtaining genetically profiled diagnoses through minimally invasive means. As more genetic mutations are discovered, we expect that multigene mutation analysis will gain importance in tailoring individualized treatment plans.

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