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Original Article

Molecular Profiling Using Next-generation Sequencing of Sufficient Endobronchial Ultrasound-guided Transbronchial Needle Aspiration and Liquid Biopsy Samples in Patients with Advanced Lung Cancer

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Abstract

Background and objectives: Tumor molecular analysis using next-generation sequencing (NGS) is the standard of care for guiding lung cancer treatment. Endobronchial ultrasound-guided transbronchial needle aspiration (EBUS-TBNA) is a minimally invasive technique used to sample mediastinal lymph nodes for diagnosing and staging lung cancer. This study aimed to determine if EBUS-TBNA provided adequate tissue samples for NGS. ***Methods:*** We evaluated EBUS-TBNA samples from adult advanced non-small cell lung cancer patients who had both EBUS-TBNA and liquid biopsy samples analyzed by NGS between July 1, 2015 and June 30, 2021. Additionally, we compared the results with those from liquid biopsies performed on these patients. ***Results:*** Among the 44 evaluated patients, 43% were male, with a median age of 66 years at diagnosis. Seventy-five percent were smokers, 79.5% were White, 6.8% were Black, and 9.1% were Asian. EBUS-TBNA samples were sufficient for NGS in 95.5% of cases. The median turnaround time for EBUS-TBNA NGS was 38.5 days compared with eight days for NGS in liquid biopsies. Actionable genetic aberrations were detected in 71% of patients. ***Conclusions:*** Our findings demonstrated that EBUS-TBNA provided sufficient tissue for identifying actionable genetic aberrations in patients with advanced non-small cell lung cancer.

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Introduction

Lung cancer is the third most common cancer and the leading cause of cancer death in both men and women worldwide, with non-small cell lung cancer (NSCLC) accounting for 85% of all lung cancer diagnoses. Survival rates for patients with advanced NSCLC are low because most patients present with advanced diseases that are minimally responsive to standard therapy. Novel treatments, such as targeted agents and immunotherapy, improve survival rates when initiated early.[1–3] Epidermal growth factor receptor (*EGFR)*, anaplastic lymphoma kinase (*ALK*), C-ros oncogene 1 (*ROS1*), and v-raf murine sarcoma viral oncogene homolog B (*BRAF*) mutations account for approximately 30% to 60% of adenocarcinomas, with overall response rates ranging from 50% to 80% for patients who receive targeted therapy. Additionally, targeted therapy is associated with an increase in overall survival from 18 to 38.6 months. Therefore, evidence-based practice guidelines recommend adequate tissue acquisition for molecular studies.[4–6]

For patients with advanced-stage NSCLC, less invasive techniques such as needle biopsy are preferred to minimize the morbidity of the diagnostic procedure. However, needle aspiration often obtains a limited amount of tissue, which may be insufficient for molecular testing.[7,8] Studies in the United States have shown that approximately one in three lung cancer patients undergo successful next-generation sequencing (NGS) testing before initial therapy.[9,10] Unfortunately, nearly half of the patients still enter treatment with inadequate biomarker testing. NGS allows parallel testing of hundreds of genetic mutations, requires smaller samples with lower concentrations of malignant cells, and can be completed more rapidly compared to multiple sequential single-gene assays.[11–13] Endobronchial ultrasound-guided transbronchial needle aspiration (EBUS-TBNA) is a minimally invasive technique for sampling mediastinal and hilar lesions and has proven to be a safe and effective method for the diagnosis and staging of lung cancer.[14–16] Core needle biopsy is preferred for obtaining larger and more adequate samples for NGS; however, EBUS-TBNA samples can be processed to produce cell blocks that replicate high-quality histologic cores. Studies have estimated that as few as two passes are sufficient to obtain adequate tissue for molecular profiling.[17–19] However, studies evaluating the adequacy of

EBUS-TBNA tissue sampling for NGS to identify targetable mutations and initiate appropriate therapy are limited. In one study, adequate tissue for cytological analysis was obtained via EBUS-TBNA in 380 out of 391 patients (97.2%), and EGFR mutation testing on EBUS-TBNA samples had a success rate of 88.8% (95% confidence interval 74.7–95.6%).[20–22]

The purpose of this study was to review our experience with EBUS-TBNA in patients with advanced lung cancer to determine if this technique provided sufficient tissue for molecular profiling with outcomes similar to core biopsies. A secondary objective was to assess whether EBUS-TBNA samples were sufficient to initiate appropriate treatment and to evaluate the turnaround time (TAT) for NGS assay via tissue and liquid biopsy (LB).

Materials and methods

Record review

Records were reviewed for adult patients (≥18 years) diagnosed with stage III or IV NSCLC who had NGS performed on EBUS-TBNA samples between July 1, 2015, and June 30, 2021. The study included patients who underwent EBUS-TBNA at Memorial Cancer Institute and who began NSCLC therapy at Memorial Cancer Institute. The Epic Beacon Processing System was used to review the electronic medical records, and data were collected into a REDCap database.[23,24] Data collected included patient demographics (gender, age at diagnosis, race, ethnicity), smoking history, clinical stage, histology, detection of genomic biomarkers (e.g., *ALK, BRAF, MET, EGFR mutation, EGFR type, KRAS mutation, KRAS type, PDL1, PIK3CA, RET, ROS1, NTRK, STK11 type, TP53 mutation, TP53 type, TMB*), occurrence of insufficient tissue samples, biopsy TAT, biopsy type used for final treatment decision (tissue vs. liquid), treatment regimen initiated, number of stations sampled, NGS success using EBUS-TBNA tissue samples, and date of disease progression or death. The TAT was defined as the time between the date the NGS was ordered in the electronic medical record and the date the results were reported by the laboratory. EBUS-TBNA tissue samples were formed from multiple aspirates pooled into Roswell Park Memorial Institute cell culture solution to create a cell block, which was then sent for NGS. All collected data were stored on a secure shared drive accessible only to the research team. Records were de-identified and coded to ensure patient confidentiality. Descriptive statistics were performed for all demographic and baseline levels of clinical outcome endpoints.

EBUS-TBNA procedure

EBUS-TBNA procedures were performed using a standardized technique with moderate sedation. After administering topical lidocaine anesthesia throughout the airway and conducting an inspection bronchoscopy, the Olympus EBUS bronchoscope was advanced through the oropharynx and into the trachea. Ultrasound examination of all mediastinal stations was routinely performed. Needle aspiration was conducted with a 22 Ga needle, targeting first the mediastinal node station farthest from the lung cancer (i.e., contralateral stations first, then ipsilateral). Aspiration samples were prepared for cytologic examination by dispensing the aspirate onto a glass slide, which was immediately placed in a fixative. Depending on the clinical situation, these slides were either sent for immediate cytologic interpretation (equivalent to rapid on-site evaluation) or submitted for routine examination. At least three additional aspirates were collected and prepared for histologic and molecular analysis by dispensing them into a single vial of Roswell Park Memorial Institute. This pooled sample was then centrifuged to produce a cell pellet, which was used to create a cell block. EBUS-TBNA samples were sent to CARIS Life Sciences (https://www.carislifesciences.com/), headquartered in Irving, Texas, for NGS testing. CARIS Life Sciences is a well-known provider of DNA and RNA NGS solutions and is a national leader in molecular testing in the United States.

Table 1. Patient demographics

Characteristic	Number (%)
Median age at diagnosis (range) – yr.	66 (43–83)
Gender	
Male	19 (43.2)
Female	25 (56.8)
Race	
White/Caucasian	35 (79.5)
Black/African American	3 (6.8)
Asian	4 (9.1)
Unknown	2 (4.5)
Ethnicity	
Hispanic	16 (36.4)
Non-Hispanic	26 (59.1)
Smoking history	
Current smoker	1 (2.3)
Former smoker	33 (75.0)
Never smoker	10 (22.7)
Histology	
Squamous cell carcinoma	11 (25.0)
Adenocarcinoma	30 (68.2)
Carcinoid	1 (2.3)
Poorly differentiated carcinoma	2 (4.5)

Liquid biopsy procedure

Liquid biopsies (defined here as blood collection of free circulating tumor DNA (ctDNA)) were collected from peripheral blood and sent to Guardant Health (https://guardanthealth.com/) for ctDNA isolation and NGS of 77 genes. The usual turnaround time for most samples was eight days, and the report was emailed to the team for inclusion in the patient's medical records.

Results

Patient characteristics

Of the 44 patients reviewed, approximately half were female, and a large majority were smokers (Table 1). The median age at diagnosis was 66 years (range 43–83 years), and most patients were White/Caucasian (35, 79.5%).

Occurrence of sufficient tissue samples

EBUS-TBNA samples were sufficient for NGS in 42 patients (95.5%). In the remaining two patients, tissue biopsies were inconclusive due to insufficient sampling, but an EGFR mutation was detected by LB. LB was completed in 35 patients (79.5%) at diagnosis.

Table 2. Actionable genetic aberrations (AGA) detected by next-generation sequencing (NGS)

	N	*EGFR* n (%)		*KRAS* n (%)	*MET* n (%)	*BRAF* n (%)	*ALK* n (%)	*NTRK1* n (%)	*PDL1*$^{+}$ >1% n (%)
		exon 19	*exon 21*						
EBUS-TBNA	30	9 (30)		9 (30)	5 (16)	1 (3)	0	1 (3)	24 (80)
		6 (67)	1 (11)						
Liquid biopsy	22	11 (50)		9 (41)	5 (23)	2 (9)	1 (5)	0	-
		5 (45)	2 (18)						

EBUS-TBNA, endobronchial ultrasound-guided transbronchial needle aspiration; *EGFR*, epidermal growth factor receptor; *KRAS*, Kirsten rat sarcoma viral oncogene; *MET*, mesenchymal epithelial transition; *BRAF*, v-raf murine sarcoma viral oncogene homolog B; *ALK*, anaplastic lymphoma kinase; *NTRK1*, neurotrophic tyrosine receptor kinase; *PDL1*, programmed death ligand 1.

TAT

The median TAT for EBUS-TBNA was 38.5 days, compared to eight days for LB NGS. Most delays in obtaining NGS results were due to insurance authorization issues.

Actionable genetic aberrations and treatment decision

Thirty patients (71%) had an actionable genetic aberration (AGA) detected by NGS from EBUS-TBNA, while twenty-two patients (63%) had an AGA detected by NGS from liquid biopsies. AGAs identified by NGS from EBUS-TBNA and liquid biopsy samples are shown in Table 2. Final treatment decisions were based on EBUS-TBNA results for 37 patients (84.1%) and on LB results for seven patients (15.9%). Of the five patients with both EBUS-TBNA and LB results, but whose treatment decision was based on LB, four had LB results available earlier, and one had an EGFR mutation detected only by LB. A list of the treatment regimens is provided in Supplementary Table 1.

Among seven studies evaluating NGS with EBUS-TBNA, all studies analyzed the feasibility of detecting EGFR mutations in samples obtained by EBUS-TBNA, while three also detected *KRAS* mutations (Table 3).[7,8,25–29] The baseline characteristics of our study, in terms of age, were similar to those in previous literature, with a median age ranging between 60 and 70 years. Our patient population also had a gender distribution similar to that in other studies, although some reported a higher percentage of men (greater than 70%). Detection rates for *EGFR* and *KRAS* mutations ranged from 5–26% and 5–37%, respectively, in previous studies. Our study detected a higher percentage of *EGFR*

Table 3. Previous literature

Study	Methods	Eligible for analysis (N)	EBUS *EGFR* positive, Number (%)	EBUS *KRAS* Positive, Number (%)	EBUS *ALK* Positive, Number (%)
Nakajima *et al*, 2007[7]	Analyzed the feasibility of detecting *EGFR* mutations in samples obtained by EBUS-TBNA in NSCLC patients	43	11 (25.6)	–	–
Garcia-Olivé *et al*, 2010[8]	Assessed the usefulness of EBUS-TBNA for the detection of *EGFR* mutations in NSCLC patients	40	2 (5)	–	–
Navani *et al*, 2012[25]	Determined whether cytology specimens obtained from EBUS-TBNA in routine practice are suitable for phenotyping and genotyping in NSCLC patients	64	7 (10.9)	–	–
Billah *et al*, 2011[26]	Analyzed the clinical utilization of routinely prepared cytology specimens for molecular testing to detect *EGFR* or *KRAS* mutations in NSCLC patients	175 (EGFR)/174 (KRAS)	34 (19.4)	41 (23.6)	–
Schuurbiers *et al*, 2010[27]	Investigated the yield and applicability of molecular testing for *KRAS* and *EGFR* mutations in cytologic specimens obtained by Transesophageal Ultrasound (EUS) or EBUS-FNA in NSCLC patients	27	2 (7.4)	10 (37)	–
Kang *et al*, 2012[28]	Evaluated the usefulness of bronchoscopic biopsy and EBUS-TBNA biopsy for detecting *EGFR* and *KRAS* mutations in routine practice	201 (EGFR)/196 (KRAS)	30 (14.9)	11 (5.6)	–
Jeyabalan *et al*, 2016[29]	Evaluated the adequacy of EBUS-TBNA samples for *EGFR* and *ALK* genetic mutation analysis in confirmed primary lung adenocarcinomas	80 (EGFR)/21 (ALK)	5 (6.3)	–	0

ALK, anaplastic lymphoma kinase; EBUS-TUNA, endobronchial ultrasound-guided transbronchial needle aspiration; *EGFR*, epidermal growth factor receptor; EUS, endoscopic ultrasound; FNA, fine needle aspiration; *KRAS*, Kirsten rat sarcoma viral oncogene; NSCLC, non-small cell lung cancer.

mutations (30%) and similar *KRAS* mutations (30%). Additionally, our study highlighted a significant proportion of populations with *EGFR* mutations, including Asians and Hispanics.

Discussion

The primary aim of this study was to determine if EBUS-TBNA lung cancer samples were adequate for molecular analysis by NGS. We found that EBUS-TBNA samples were sufficient in 95% of patients and that therapeutic decisions were based on AGA results from EBUS samples in 84% of patients. In the remaining 16%, therapeutic decisions were based on LB results. Furthermore, because our study cohort was more ethnically diverse than those in similar reports, we believe our findings provide important evidence supporting the use of EBUS specimens for NGS in a broader population. This diversity was a characteristic of our patient population rather than an intentional aspect of the study design. Although there was a small number of Black patients in our cohort, this study included a higher proportion of Black patients compared to previous studies. For example, Navani and colleagues reported on 774 patients with known or suspected lung cancer, of whom 88% were Caucasian and less than 1% were African, Caribbean, or other non-Asian.[25] Folch and colleagues reported on 42 patients, 81% of whom were white, 4.8% were Asian, and only 4.8% were Black (9.5% were listed as other).[6] While the proportion of White/Caucasian patients in our study was slightly lower (79.5%), a higher percentage were Asian (9.1%) or African American (6.8%). Additionally, 36% of our patients were Hispanic, highlighting an underrepresented population in previous trials. Our study is notable for including a broad range of genetic aberrations. Xie *et al*.[5] reported 41 driver mutations in 77 patients, including 32 *EGFR* mutations, eight *ALK* rearrangements, and one *ROS1* rearrangement. In contrast, our study identified mutations in five genes (*BRAF, EGFR, KRAS, MET, and NTRK1*) and expression of PDL1. Furthermore, our study is unique in that it also included LB, allowing us to compare the diagnostic value of EBUS-TBNA with LB.

The EGFR mutation rate in our study was higher than the US average, where EGFR positivity is approximately 10% in White patients with NSCLC and up to 50% in Asian patients.[30] Of the four Asian patients in our study, one had an EGFR exon 19 deletion detected by both EBUS and LB, one had an EGFR exon 21 mutation detected by LB, and one had an *EGFR* exon mutation detected by both EBUS and LB. Among the three African American patients, one had an EGFR exon 19 deletion detected by both EBUS and LB, and one had an *EGFR exon 19 deletion* detected by EBUS only.

The main limitation of this study is its relatively small sample size. Contributing factors include some liquid biopsies being performed outside the specified timeframe and some patients being diagnosed with early-stage lung cancer.

Overall, the patient population, the variety of mutations included, and validation with LB make our results relevant to current practice.

Conclusions

EBUS-TBNA can obtain sufficient tissue samples for identifying AGA by NGS in patients with advanced NSCLC. Based on our study results, additional biopsies are necessary in only a small minority of patients when EBUS-TBNA is performed properly. This includes creating cell blocks and obtaining material sufficient for molecular diagnostics, not just cytologic diagnosis.

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Conflict of interest

Luis Raez: Astrazeneca, Lilly Oncology, LOXO, Genentech, Novocure, Merck. Mark Block: Southern Thoracic Surgical Association. Additional authors declare no conflict of interest.

Author contributions

Conceptualization (MB, LR), methodology (MB, LR, CA, KB, KD), software (CA), validation (MB, LR, CA, KB, KD), formal analysis (MB, LR, CA, KB, KD), investigation (MB, LR, CA, KB, KD), resources (MB, LR, CA, KB, KD), data curation (CA), original draft preparation (MB, LR, CA, KB, KD), review and editing (MB, LR, CA, KB, KD), visualization (MB, LR, CA, KB, KD), supervision (MB, LR, KB, KD), and project administration (CA). All authors have read and agreed to the published version of the manuscript.

Ethical statement

Ethical review and approval were waived for this study as outlined in 45 CFR 46.101 or 21 CFR 56.104. The research involved only the collection and analysis of information using identifiable health data regulated under 45 CFR parts 160 and 164, subparts A and E [HIPAA], for "health care operations" or "research" as defined in 45 CFR 164.501, or for "public health activities and purposes" as described under 45 CFR 164.512(b). The protocols adhered to the ethical guidelines of the most recent version of the Declaration of Helsinki. Patient consent was waived under 45 CFR 164.512 (i) (2) (ii).

Data sharing statement

The dataset used to support the findings of this study have not been made available because of privacy and/or ethical restrictions.

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