Ann Med Res 2021;28(3):454-8

Annals of Medical Research

DOI: 10.5455/annalsmedres.2020.11.1151

Original Article

Application of endobronchial ultrasound-guided transbronchial needle aspiration for re-biopsy in lung cancer

Pinar Akin Kabalak[1], Derya Kizilgoz[1], Ulku Yilmaz[1], Funda Demirag[2]

[1]Department of Chest Disease, Ankara Ataturk Chest Disease and Thoracic Surgery Training and Research Hospital, Ankara, Turkey
[2]Department of Pathology, Ankara Ataturk Chest Disease and Thoracic Surgery Training and Research Hospital, Ankara, Turkey

Abstract

Aim: The initial mutation status of non-small cell lung cancer (NSCLC) is important for guiding treatment. However, re-biopsy is needed to determine the new mutation status in patients receiving targeted therapy or who develop resistance during first-line therapy. Endobronchial ultrasound (EBUS) is an important tool to obtain adequate tissue for molecular analyses.

Materials and Methods: We used a clinical database of 349 patients who were previously included in a multi-center study. Adequacy of tissue collected, new molecular profile, and treatment regimens were evaluated.

Results: A total of 50 patients (median age of 60 ± 9 years) received biopsy by EBUS. The success rate was 36% (n = 18), and repeat re-biopsy was performed for patients with anthracosis (n = 32). In 18 patients, only one lymph node station was sampled. Initial histopathology was adenocarcinoma in 28 patients, squamous cell carcinoma (SCC) in 18 patients, and not otherwise specified (NOS) in four patients. No correlation was found between initial treatment type and success rate of the procedure (p = 0.101). After re-biopsy in six patients (five of whom had newly detected mutations), targetable mutations were detected for T790M (n = 2), EGFR-exon 19 (n = 1), c-Met (n = 1), and EGFR-21 (n = 2).

Conclusion: : In cases of resistance to treatment, re-biopsy should be performed to detect different gene amplifications. Re-biopsy with EBUS is an effective method for an appropriate progressive lesion.

Keywords: Endobronchial ultrasound (EBUS); lung cancer; re-biopsy

INTRODUCTION

New developments in targeted therapies for non-small cell lung cancer (NSCLC) have been created to cope with resistance mechanisms. While not mandatory, it is important to obtain a new tissue sample to determine the molecular typing in cases of progression (1). Especially in NSCLC patients with epidermal growth factor receptor (EGFR) mutations, acquired resistance is attributed to gatekeeper mutations in exon 20 (EGFR-T790M mutation) (2). Third-generation EGFR-tyrosine kinase inhibitors (TKIs), such as osimertinib, have shown promise when the new molecular profile is positive for EGFR-T790M (3). Many procedures are available depending on patient consent, performance status, and availability of the progressive lesion (4-6). Endobronchial ultrasound (EBUS) is one such method. According to a recent study of 367 patients, the diagnostic accuracy of EBUS in detecting malignancy was 95.6% (overall sensitivity) with no major complications (7). The lowest sensitivity was 83.3% in the group who had previously received radiotherapy (7).

Re-biopsy in NSCLC, which is necessary to guide individual treatment, is an ongoing area of research to determine the adequacy and parallelism of biopsy methods. This study retrospectively evaluated the adequacy of EBUS in obtaining adequate tissue for molecular analyses.

MATERIALS and METHODS

Study design

The records of NSCLC patients who underwent re-biopsy between January 2017 and October 2019 at Ataturk Chest Disease and Thoracic Surgery, Thoracic Oncology Palliative Care Unit in Ankara, Turkey were retrospectively reviewed. The cohort consisted of 349 NSCLC patients with re-biopsy who had been previously included in a multi-center study. Of the 349 re-biopsy patients, 50 received re-biopsy by EBUS. These were primarily patients with a progression site in the mediastinal or hilar lymph nodes.

Age, gender, Tumor-Node-Metastasis (TNM) stage, first-line treatment modalities, and mutational status (both

Received: 30.11.2020 **Accepted:** 12.01.2021 **Available online:** 18.03.2021
Corresponding Author: Pinar Akin Kabalak, Department of Chest Disease, Ankara Ataturk Chest Disease and Thoracic Surgery Training and Research Hospital, Ankara, Turkey **E-mail:** pinarakinn@yahoo.com

initial and after re-biopsy and treatments) were recorded before and after re-biopsy. In cases where cancer tissue could not be obtained by EBUS, re-biopsy results from another region were also considered.

Pathological evaluation and mutation analyses

A cell block was prepared from all samples. Aspirated materials were inserted into a 10-cc sterile saline solution and sent to the pathology laboratory. Samples were processed through filter paper and fixed in 10% buffered formalin. Cell blocks were embedded in paraffin, and 6-µ sections were made and stained with hematoxylin-eosin. At first it was as conducted to determine whether the sample contained a tumor. If it did contain a tumor, the sections were examined alongside the first biopsy samples. If the histopathological examination revealed no difference in subtype or small cell carcinoma transformation, the tissue was directed to molecular study without immunohistochemistry and histochemistry analysis. Next generation sequencing was used to study mutations in AKT1, anaplastic lymphoid kinase (ALK), BRAF, DDR2, EGFR, ERBB2/HER2, ESR1, FGFR1, KIT, KRAS, MAP2K1, MET, NRAS, NTRK1, PDFRA, PIK3CA, PTEN, RICTOR, and ROS1.

Endobronchial ultrasound procedure

EBUS was conducted using a fiberoptic ultrasound bronchoscope (Convex Probe EBUS, Fujinon SN14912K150). Needle aspiration was performed using a 22-gauge needle with a syringe connected proximally. Histological cores were placed in 10% formalin. The aspirate material was smeared onto glass slides and fixed. Cytology and histology specimens were sent for pathologic examination as soon as possible (Figure 1). The study was approved by the local ethical committee (protocol number 701-19.11.2020, Ataturk Chest Disease and Thoracic Surgery Medical Specialty Research Coordinator).

Statistical analyses

Descriptive statistics were obtained for frequency, percentage, and median (range). After the biopsy, success was coded as successful vs. unsuccessful in the database. Discrete data were compared using the chi-squared test. A p value less than 0.05 was considered to indicate statistical significance.

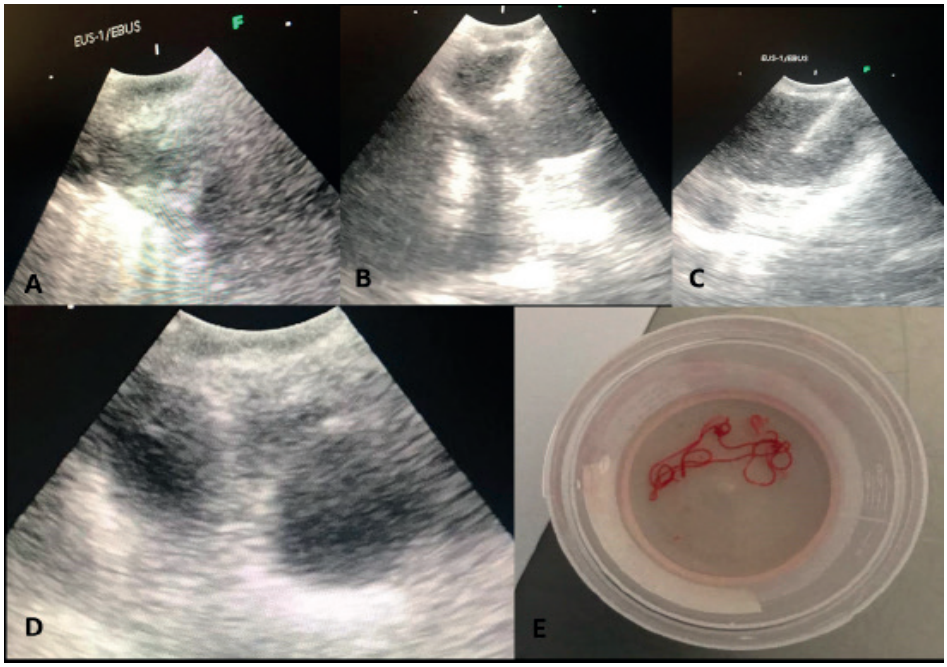

Figure 1. Examples from stations sampled with EBUS. 1A: Right interlobar lymphadenopathy+mass (11R+mass), 1B: Left lower paratracheal (4L), 1C: Subcarinal (7), 1D: Right hilar (10R) and 1E: Adequate EBUS tissue sample

RESULTS

Of the 368 re-biopsy procedure, 50 were performed by EBUS. The median age of these 50 patients was 60 ± 9 years. Previous therapies included chemotherapy (n = 13), chemo-radiotherapy (n = 17), surgery (n = 15), tyrosine-kinase inhibitors (n = 3), and immunotherapy (n = 2). Most of the initial histopathology was adenocarcinoma (n = 28), followed by squamous cell carcinoma (SCC; n = 18), and not otherwise specified (NOS; n = 4). Regarding TNM stage, 14 patients were in early stages (TNM I and II), 20 were stage III, and 16 were stage IV. EGFR exon 19 deletion was found in three patients, ALK in one patient, and BRAF-V600E mutation in one patient. Initial histopathology, mutation status, distribution of TNM stages at the beginning of therapy, type of treatment, and success rate of the EBUS procedure are summarized in Table 1.

Positive biopsy results (adequate tissue to perform molecular tests) were obtained in 18 patients (success

rate of 36%). The remaining was reported as anthracosis (n = 32). Among these 32 patients for whom no tumor tissue was obtained by EBUS, 14 underwent repeat re-biopsy at another anatomic site, and progression was detected (Figure 2). No correlation was found between initial treatment regimen and success of obtaining adequate tissue by EBUS (p = 0.101). In 18 cases, only one lymph node station was sampled (Table 1).

Table 1. General characteristics of study population

Characteristics	N (&)
Age	60±9
Gender	
Male	42 (84%)
Female	8 (16%)
Histopathology	
Adenocarcinoma	28 (56%)
Squamous cell	18 (36%)
NOS*	4 (8%)
Initial mutation profile	
EGFR 19 deletion	3
ALK	1
BRAF V600	1
Distribution of initial treatments	
Chemotherapy	13 (26%)
Chemo-radiotherapy	17 (34%)
Surgery	15 (30%)
TKIs**	3 (6%)
Immunotherapy	2 (4%)
TNM Stage	
Stage I-II	14 (28%)
Stage III	20 (40%)
Stage IV	16 (32%)
Success rate	18 (36%)
Repeat biopsy after EBUS	14 (28%)
Number of sampled LN* **	
1 station	18 (35%)
≥2 station	32 (64%)
Treatments after re-biopsy	
Chemotherapy	20 (40%)
Chemo-radiotherapy	5 (10%)
BSC****	15 (30%)
TKIs**	7 (14%)
Immunotherapy	2 (6%)

*Not-other wised specified, **Thyrosine kinase inhibitors ***Lymph node, ****Best supportive care

Molecular analysis results from samples with sufficient tumor tissue were as follows: T790M positivity was found in two cases, EGFR-exon 19 deletion in one case with SCC, c-Met positivity in one case, and exon 21 L858R mutation in two cases (Figure 2). In line with the new biopsy results, six patients (five of whom targeted therapies were newly started) continued to receive targeted therapies, and two patients were enrolled in international multi-center studies for immunotherapy. Systemic palliative chemotherapy was started in 20 patients, and the best supportive care was decided for 15 patients. There were no major or minor complications.

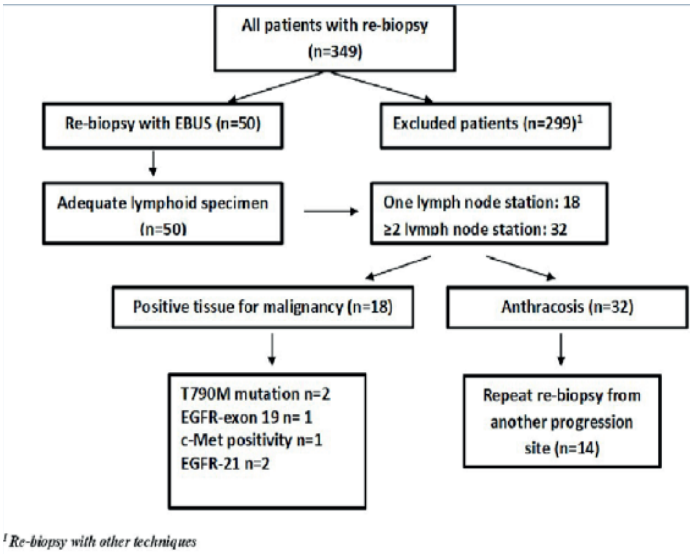

Figure 2. Flowchart of endobronchial ultrasound procedure

DISCUSSION

Re-biopsy is still relatively new in NSCLC, especially in patients who develop resistance to targeted therapy. New mutations can be detected by re-biopsy, even in cases that were initially negative for targetable mutations and thus did not receive targeted therapy as a first-line therapy (4). To avoid major complications, re-biopsy procedures should be determined according to the availability of a targetable lesion, patients' medical condition, and the institute's experience (6). Therefore, it is not possible to discuss the superiority of the different procedures. In appropriate cases, EBUS should be considered as a valuable procedure. A retrospective study of 1700 EBUS samples revealed a 79.8% success rate for adequacy of specimens obtained (7).

Izumo et al. (9) reported 79.2% positive results by EBUS among patients with initial TKI therapy. Although our rate of success in obtaining sufficient tissue for molecular analysis was low (36%), new targetable mutations were detected in five patients out of 50 who underwent EBUS re-biopsy (in a patient using TKI, the same mutation was detected as a result of re-biopsy; see Table 2). Thoracic radiotherapy, which has been applied in first line therapy, is one of the factors affecting EBUS success (8). In our study, the success rate was lowest in the chemo-radiotherapy group (23.5%) and was higher in the TKI (66.6%) and chemotherapy (61.5%) groups (Table 2).

Although two or more stations were sampled in our study, anthracosis rate was high (32 of 50 patients). It should be taken into consideration that anthracotic lymph nodes

Table 2. Characteristics of 18 patients who were successful in re biopsy with EBUS

Variable	Chemotherapy (n=8)	Chemo-radiotherapy (n=4)	Surgery (n=4)	TKIs* (n=3)	p value
Success rate (%)	61.5	23.5	26.6	66.6	0.101
Subsequent TKIs* after re-biopsy (n)	1	1	3	2	0.123
Number of sampled lymph-node station 1 vs ≥2 stations	3 vs. 5	1 vs. 3	3 vs. 1	1 vs. 1	0.511
New molecular profile	-	EGFR 21 mutation (n=1)	EGFR 19 deletion (n=2) c-MET (n=1)	T790M (n=2) EGFR 21 mutation (n=1)**	<0.001

* Tyrosine-kinase inhibitors
** In a patient using TKI, the same mutation was detected as a result of re-biopsy

can cause high standardized uptake value (SUV) uptake, especially in countries where biomass exposure is still ongoing, such as Turkey (10). Thus, this was a confounding factor for our study group. A study investigating multi-slice computed tomography to differentiate anthracotic lymph nodes from malignant lymph node enlargement using EBUS revealed some clues for identifying if a tumor is benign or malignant (11). One limitation of our study was that mean SUVmax values of lymph nodes were not available in our database.

Although EGFR exon 19 deletion was detected in one case, the inclusion of 18 SCC cases was another factor that lowered the success rate. Although not as much as adenocarcinomas, in patients SCC patients who is non-smoker or quits smoking for long time ago, up to 5.2% EGFR positivity can be detected in Asian patients (12).

CONCLUSION

In summary, re-biopsy by EBUS is a safe and useful sampling method to obtain tissue for molecular analyses. Although initial therapy affects the success of the procedure, it should be preferred for re-biopsy in patients with mediastinal recurrence.

Competing interests: The authors declare that they have no competing interest.

Financial Disclosure: There are no financial supports.

Ethical approval: The study was approved by the local ethical committee (protocol number 701-19.11.2020, Ataturk Chest Disease and Thoracic Surgery Medical Specialty Research Coordinator).

REFERENCES

1. Besse B, Adjei A, Baas P, et al. 2nd ESMO Consensus Conference on Lung Cancer: non-small-cell lung cancer first-line/second and further lines of treatment in advanced disease. Ann Oncol 2014;25:1475-84.
2. Ko R, Kenmotsu H, Serizawa M, et al. Frequency of EGFR T790M mutation and multimutational profiles of rebiopsy samples from non-small cell lung cancer developing acquired resistance to EGFR tyrosine kinase inhibitors in Japanese patients. BMC Cancer 2016; 16: 864.
3. Yang JC, Ahn MJ, Kim DW et al. Osimertinib in pretreated T790M-positive advanced non-small-cell lung cancer: AURA study phase II extension component. J Clin Oncol 2017;35:1288-96.
4. Chouaid C, Dujon C, Do P, et al. Feasibility and clinical impact of re-biopsy in advanced non small-cell lung cancer: a prospective multicenter study in a real-world setting (GFPC study 12-01). Lung Cancer 2014;86:170-3.
5. Nosaki K, Inamasu E, Shimamatsu S, et al. Re-biopsy in advanced non-small cell lung cancer patients after the development of 3rd generation EGFR-TKI and new targeted therapies. J Clin Oncol 2015;33:19081.
6. Hata A, Katakami N, Nanjo S, et al. Rebiopsy of Histological Samples in Pretreated Non-small Cell Lung Cancer: Comparison Among Rebiopsy Procedures. In Vivo 2017; 31:475-9.
7. Ece D, Keser SH, Cağlayan B, et al. Endobronchial Ultrasound-Guided Transbronchial Fine Needle Aspiration: Determinants of Adequacy. Turk Gogus Kalp Damar Cerrahisi Derg 2018 26:123-31.
8. Kim J, Kang HJ, Moon SH, et al. Endobronchial Ultrasound-Guided Transbronchial Needle Aspiration for Re-biopsy in Previously Treated Lung Cancer. Cancer Res Treat 2019;51:1488-99.
9. Izumo T, Matsumoto Y, Chavez C, et al. Re-biopsy by endobronchial ultrasound procedures for mutation analysis of non-small cell lung cancer after EGFR tyrosine kinase inhibitor treatment. BMC Pulm Med 2016;16:106.
10. Demirci NY, Alici IO, Yilmaz A, et al. Risk factors and maximum standardized uptake values within lymph nodes of anthracosis diagnosed by endobronchial ultrasound-guided transbronchial needle aspiration. Turk J Med Sci 2015;45:984-90.

11. Kirchner J, Broll M, Müller P, et al. CT differentiation of enlarged mediastinal lymph node due to anthracosis from metastatic lymphadenopathy: a comparative study proven by endobronchial US-guided transbronchial needle aspiration. Diagn Interv Radiol 2015;21:128-33.

12. Ho HL, Kao HL, Yeh YC, et al. The importance of EGFR mutation testing in squamous cell carcinoma or non-small cell carcinoma favor squamous cell carcinoma diagnosed from small lung biopsies. Diagn Pathol 2019;14:59.