Lung Cancer 186 (2023) 107390

Contents lists available at ScienceDirect

Lung Cancer

journal homepage: www.elsevier.com/locate/lungcan

Usefulness of bronchial washing fluid for detection of EGFR mutations in non-small cell lung cancer

Woo Kyung Ryu [a,b], Seung Hyun Yong [a], Sang Hoon Lee [a], Hye Ran Gwon [a], Hye Ryun Kim [a], Min Hee Hong [a], Go Eun Oh [a], Sehee Jung [a], Chi Young Kim [a], Yoon Soo Chang [a], Eun Young Kim [a,*]

[a] Department of Internal Medicine, Yonsei University College of Medicine, 50-1, Yonsei-ro, Seodaemun-gu, Seoul 03722, Republic of Korea
[b] Division of Pulmonology, Department of Internal Medicine, Inha University Hospital, Inha University College of Medicine, 27, Inhang-ro, Jung-gu, Incheon 22332, Republic of Korea

ARTICLE INFO

Keywords:
Bronchial washing fluid
Droplet digital polymerase chain reaction
Epidermal growth factor receptor mutation
EGFR T790M
Non-small cell lung cancer

ABSTRACT

Introduction: The implementation of bronchial washing fluid (BWF) as a diagnostic specimen may complement the low diagnostic yields of plasma in detecting EGFR mutation (mEGFR) in non-small cell lung cancer. However, the diagnostic value of BWF in detecting mEGFR has yet to be clarified.
Materials and Methods: From March 2021 to August 2022, patients with histologically confirmed NSCLC with matched tumor tissue, BWF, and/or plasma samples were enrolled. Patients were classified into either initial diagnosis or rebiopsy groups. Diagnostic yields of mEGFR in BWF and plasma were evaluated using droplet digital polymerase chain reaction and compared to mEGFR in tumor tissue as standard.
Results: The study included 123 patients (74.1 %) in the initial diagnosis and 43 patients (25.9 %) in the rebiopsy group. BWF showed higher sensitivity, specificity, and concordance rates than plasma in both the initial diagnosis (57.4 %, 96.4 %, and 74.0 % vs. 16.4 %, 96.2 %, and 53.1 %) and the rebiopsy group (87.9 %, 60.0 %, and 81.4 % vs. 25.0 %, 75.0 %, and 41.7 %). In the initial diagnosis group, mEGFR was detected in the BWF of 13 out of 16 patients, even in the absence of tumor cells in the tissue biopsy. In these cases, EGFR test results obtained from BWF showed concordance with EGFR test results from the tumor tissue obtained through repeated biopsy or surgery later. In the rebiopsy group, T790M was detected in 16 patients (37.2 %) by tissue biopsy. The combined use of tissue biopsy and BWF increased detection, confirming T790M in 22 patients (51.2 %).
Discussion: The detection of mEGFR using BWF shows higher diagnostic yields than plasma for both initial diagnosis and rebiopsy. T790M was detected earlier in BWF than in tissue rebiopsy in some cases, providing patients with an early opportunity to access third-generation EGFR-TKIs. The complementary use of BWF with tumor tissue may improve precision in EGFR-mutated NSCLC treatment strategies.

1. Introduction

The discovery of epidermal growth factor receptor (EGFR) mutations has led to a dramatic paradigm shift in treatment for advanced non-small cell lung cancer (NSCLC) [1]. As some 50 % of Asian and 20 % of Caucasian patients carry EGFR mutations, molecular testing for EGFR is recommended for treatment decisions in patients with metastatic NSCLC [2,3]. EGFR tyrosine kinase inhibitors (TKIs) are a standard treatment for EGFR-mutated advanced NSCLC [3]. Despite the strong therapeutic effects, most patients experience disease progression within 1–2 years due to acquired resistance [4]. Repeated tumor biopsy (hereafter: rebiopsy) is important for identifying resistance and choosing further treatment after EGFR-TKI treatment failure.

However, it is difficult to obtain lung tumor tissue using bronchoscopy or image-guided biopsy, and serious complications may occur with these procedures [5,6]. Limited tissue volume can mean there is inadequate tumor DNA for comprehensive molecular analysis, including assessment of EGFR mutations [7]. In particular, rebiopsy remains diagnostically challenging as there can be progressive tissue changes such as necrosis or fibrosis due to previous anti-cancer therapy [8]. There is growing interest in less invasive and high-sensitivity methods to overcome current tissue biopsy limitations.

* Corresponding author.
E-mail address: NARAE97@yuhs.ac (E.Y. Kim).

https://doi.org/10.1016/j.lungcan.2023.107390
Received 3 April 2023; Received in revised form 4 September 2023; Accepted 4 October 2023
Available online 5 October 2023

Liquid biopsy is a non-invasive method that is convenient for assessing tumor-derived material [9]. Non-invasive sampling of plasma or body fluids, including urine, pleural fluid, and bronchial washing fluid (BWF), can be applied in lung cancer diagnosis and detect targetable genomic alterations and monitor therapeutic responses [10,11]. EGFR mutation testing using plasma samples is approved by the Food and Drug Administration as a companion diagnostic test to determine treatment options in NSCLC [12]. However, the level of detectable circulating tumor DNA depends on tumor stage, tumor burden, and biological and technical factors, whereby circulating tumor DNA in plasma is typically less than 1 % [13].

Bronchoscopy is an easily performed procedure, and BWF is routinely obtained for cytology. In contrast to peripheral blood, BWF is in direct contact with the tumor and may reflect tumor heterogeneity [14]. BWF recently emerged as a sample type for the detection of cell-free DNA (cfDNA) in lung cancer. Detection of EGFR mutations in BWF is usually performed using quantitative polymerase chain reaction (qPCR), which is widely available in medical settings. Recently, more sensitive methods such as the droplet digital polymerase chain reaction (ddPCR) have been introduced and are now being utilized in clinical settings.

In this study, we aimed to evaluate the feasibility of using BWF for EGFR mutation testing by ddPCR, employing matched tumor tissues as standards. We aimed to explore whether the detection of the EGFR T790M mutation in BWF using ddPCR could be useful for guiding precise treatment in patients after EGFR-TKI treatment failure.

2. Methods

2.1. Study population

From March 2021 to August 2022, a total of 421 BWF samples were collected during bronchoscopy of patients with suspected or advanced lung cancer and screened for this study. The inclusion criteria were as follows: (1) patients with histologically confirmed NSCLC; (2) patients who underwent testing for EGFR mutations on tissue samples; and (3) patients with matched tumor tissue, BWF, and/or plasma samples. In total, 166 patients were included and classified into initial diagnosis and rebiopsy groups (Fig. 1). Among them, samples collected at the time of initial diagnosis were used for 123 patients in the initial diagnosis group, while samples collected at rebiopsy after EGFR TKI treatment failure were used for analysis in 43 patients in the rebiopsy group. The participants in the two groups did not overlap. Information on age, sex, smoking status, histology, tissue type from bronchoscopy, stage, EGFR mutations, and first-line treatment received was collected. Tumor stage was estimated using the TNM classification system (8th edition) [15]. The study was conducted in accordance with the 2008 Declaration of Helsinki, and the study protocol was approved by the independent Institutional Review Board of Severance Hospital (IRB number: 4–2021-1223). Written informed consent was obtained from all patients.

2.2. BWF processing and sample preparation

Tumor tissue, BWF, and peripheral blood samples were collected from each patient. All patients underwent bronchoscopy to obtain BWF. Bronchoscopy was performed via the nasal or oral route under local anesthesia and mild sedation with midazolam and fentanyl. A bronchoscope was placed at a wedge position within the subsegmental bronchus selected based on chest computed tomography findings for suspected lung cancer. Sterile 0.9 % saline (20 mL) was slowly injected into the segment and gently aspirated. At least 5 mL BWF was immediately collected into a disposable specimen trap. BWF was processed within 3 h by centrifugation at 3,000 rpm for 15 min at 4 °C, and the supernatant was stored at − 80 °C until analysis. cfDNA was extracted from 3 mL BWF supernatant using a QIAamp Circulating Nucleic Acid Kit (Qiagen, Hilden, Germany).

Tumor tissues were prepared by formalin fixation and paraffin embedding and cut into 10-μm sections for DNA extraction. DNA was extracted using a Maxwell CSC DNA FFPE Kit (Promega, Madison, WI). EGFR mutation analysis was performed using a GenesWell ddEGFR Mutation Test (Gencurix, Seoul, South Korea).

Peripheral blood samples (10 mL) were collected in Streck tubes (Streck, La Vista, NE) and centrifuged at 1,800 *g* for 10 min at 4 °C. Plasma was isolated within 8 h and stored at − 80 °C until DNA extraction. Plasma cfDNA was extracted from 2 mL plasma using a QIAamp Circulating Nucleic Acid Kit (Qiagen) following the manufacturer's instructions. Tumor tissue EGFR tests were performed at the hospital pathology laboratory as part of clinical diagnosis. The plasma/BWF EGFR test was conducted in our laboratory.

2.3. ddPCR method

ddPCR was performed using the QX200 Droplet Digital PCR System (Bio-Rad, Hercules, CA) and an EGFR mutation analysis kit (Droplex

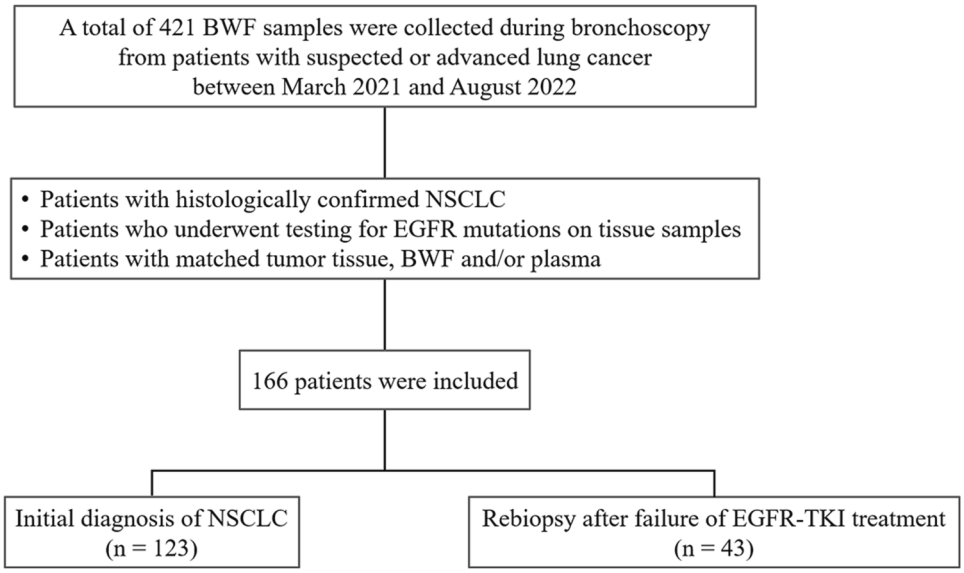

Fig. 1. CONSORT diagram.

EGFR Mutation Test v2, Gencurix) following the manufacturer's instructions. Droplex EGFR Mutation Test v2 PCR master mixture and patient DNA samples (BWF or plasma) were combined in an 8-strip PCR tube (Axygen, Tewksbury, MA) to a volume of 20 μL, and mixtures were loaded into DG8 Cartridge wells (Bio-Rad). Droplets were generated using a QX200 Droplet Generator (Bio-Rad) following the manufacturer's instructions. The droplets were transferred to a 96-well plate and the plate placed in a thermal cycler (Veriti 96-Well Fast Thermal Cycler; Thermo Fisher Scientific, Waltham, MA) for PCR. Thermocycling conditions were as follows: one cycle of 30 min at 37 °C, 10 min at 95 °C; 40 cycles of 30 sec at 94 °C; 1 min at 58 °C, and 98 °C for 10 min, with ramping at 2 °C/sec.

After thermal cycling, the droplet reader was connected to a computer running QuantaSoft Software (v1.6.6.0320; Bio-Rad), and data analysis was performed. Thresholds were set manually based on results from a positive control containing wild-type genomic DNA and standard positive DNA. Numbers of positive and negative droplets were distinguished using the threshold, and used to calculate the target concentration as copies/μL. The kit detects 107 EGFR mutations related to EGFR-TKIs using six reactions per specimen and includes primers and probes for exon 18 (L718X, G719X, G724S), exon 19 (59 deletions), exon 20 (S768I, T790M, C797X, 33 insertions), and exon 21 (L858R, L861Q). Positive controls and internal controls were included in every test.

2.4. Statistical analysis

Diagnostic yields for EGFR mutation detection in BWF and plasma were compared for sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and concordance rate. McNemar's test was used to assess the significance of differences between BWF and plasma samples. The ddPCR area under the curve (AUC) for BWF and plasma was calculated, and outcomes were compared using Epi and pROC packages in R software (version 4.2.1, Institute for Statistics and Mathematics, Vienna, Austria). DeLong's test was used to compare AUC values for BWF and plasma and determine statistically significant differences. A two-sided $p < 0.05$ was considered statistically significant. Statistical analyses used SPSS software (version 19.0; SPSS, Chicago, IL).

3. Results

3.1. Patient characteristics

There were 166 patients included in the study. Baseline characteristics are summarized in Table 1. The median age was 66.6 years (range 31–84 years), and 92 patients (55.4 %) were female. More than half of the patients (57.2 %) had never smoked, and most (95.8 %) were diagnosed with adenocarcinoma.

We classified 123 patients (74.1 %) into the initial diagnosis group and 43 patients (25.9 %) into the rebiopsy group. In the initial diagnosis group, EGFR results were wild-type in 55 patients (44.8 %) and mutated in 68 patients (55.2 %). Of the patients with EGFR mutations, 34 (50.0 %) had an exon 19 deletion (E19del) while 29 (42.6 %) had an L858R mutation. In the rebiopsy group, E19del was the most common mutation (53.5 %), followed by L858R (32.6 %).

Table 1
Baseline characteristics of the study population.

Variables	All patients (N = 166)	Initial diagnosis (N = 123)	Rebiopsy (N = 43)
	N (%) or mean ± SD	N (%) or mean ± SD	N (%) or mean ± SD
Age (years)	66.6 ± 10.1	66.1 ± 9.6	68.1 ± 11.2
Gender			
Male	74 (44.6)	62 (50.4)	12 (27.9)
Female	92 (55.4)	61 (49.6)	31 (72.1)
Smoking status			
Never	95 (57.2)	63 (51.2)	32 (74.4)
Former	45 (27.1)	35 (28.5)	10 (23.3)
Current	26 (15.7)	25 (20.3)	1 (2.3)
Histology			
Adenocarcinoma	159 (95.8)	117 (95.1)	42 (97.7)
Other	7 (4.2)	6 (4.9)	1 (2.3)
Tissue type			
Bronchus	14 (8.4)	7 (5.7)	7 (16.3)
Lung	138 (83.2)	109 (88.6)	29 (67.4)
Lymph node	13 (7.8)	7 (5.7)	6 (14.0)
Other	1 (0.6)	0 (0.0)	1 (2.3)
Stage			
Early stage (I–IIIA)	93 (56.0)	86 (69.9)	7 (16.3)
Advanced stage (IIIB–IV)	73 (44.0)	37 (30.1)	36 (83.7)
EGFR mutation types at initial diagnosis			
Wild-type	55 (33.1)	55 (44.8)	0 (0.0)
EGFR mutation	111 (66.9)	68 (55.2)	43 (100)
Exon 19 deletion	57 (51.4)	34 (50.0)	23 (53.5)
L858R	43 (38.7)	29 (42.6)	14 (32.6)
Other[a]	11 (9.9)	5 (7.4)	6 (13.9)
EGFR mutation type after failure of EGFR-TKIs			
Wild-type		–	10 (23.3)
EGFR mutation		–	33 (76.7)
Exon 19 deletion/T790M		–	9 (27.2)
Exon 19 deletion		–	9 (27.2)
L858R/T790M		–	7 (21.2)
L858R		–	5 (15.3)
L861Q		–	2 (6.1)
G719X		–	1 (3.0)
First-line treatment			
Surgery	68 (41.0)	63 (51.2)	5 (11.6)
Radiation or CCRT	21 (12.7)	17 (13.8)	4 (9.3)
EGFR-TKI	53 (31.9)	19 (15.4)	34 (79.1)
Platinum-based chemotherapy	10 (6.0)	10 (8.2)	0 (0.0)
Other	14 (8.4)	14 (11.4)	0 (0.0)

Notes: [a]Two cases of exon 20 insertion, one G719X, one G719X/S768I, one G719A, two L861Q, one L747P, one L858R/T790M, one L858R/S768I, and one L858R/exon 20 insertion.
Abbreviations: CCRT, concurrent chemoradiotherapy; EGFR, epidermal growth factor receptor; SD, standard deviation; TKI, tyrosine kinase inhibitor

3.2. Diagnostic yield of using BWF for initial diagnosis

Detection of EGFR mutations in patients at initial diagnosis showed sensitivity and specificity, respectively, of 57.4 % and 96.4 % in BWF and 16.4 % and 96.2 % in plasma, compared with tumor tissue. Concordance rates for EGFR mutation detection, based on results in tumor tissue, were 74.0 % for BWF and 53.1 % for plasma. BWF thus had a higher diagnostic yield than plasma for detecting EGFR mutations ($p < 0.001$) (Table 2). The AUC for EGFR mutation detection was 0.769 (95 % confidence interval (CI): 0.704–0.833) in BWF and 0.563 (95 % CI: 0.509–0.617) in plasma (Fig. 2A). BWF predicted EGFR mutations in tumor tissue more accurately than did plasma ($p < 0.001$).

Among patients in the initial diagnosis group, 16 had no tumor cells detected on tissue biopsy, but in some patients, EGFR mutations were detected in BWF, and patients were later diagnosed with lung cancer by surgery or repeat biopsy (Table 3). The concordance rate of ddPCR EGFR test results between BWF and tumor tissues was 81.3 %. Of the 16 patients, 13 were in an early stage and three in an advanced stage at the

Table 2
Comparison of EGFR mutation detection rates in BWF and plasma.

	Initial diagnosis group (N = 123)					Rebiopsy group (N = 43)				
	BWF		Plasma		p-value	BWF		Plasma		p-value
	EGFR mut	EGFR WT	EGFR mut	EGFR WT		EGFR mut	EGFR WT	EGFR mut	EGFR WT	
Tissue EGFR mut	39	29	10	51		29	4	2	6	
Tissue EGFR WT	2	53	2	50		4	6	1	3	
Sensitivity	57.4 % (39/68)		16.4 % (10/61)		<0.001	87.9 % (29/33)		25.0 % (2/8)		0.016
Specificity	96.4 % (53/55)		96.2 % (50/52)			60.0 % (6/10)		75.0 % (3/4)		
PPV	95.1 % (39/41)		83.3 % (10/12)			87.9 % (29/33)		66.7 % (2/3)		
NPV	64.6 % (53/82)		49.5 % (50/101)			60.0 % (6/10)		33.3 % (3/9)		
Concordance rate	74.0 % (91/123)		53.1 % (60/113)			81.4 % (35/43)		41.7 % (5/12)		

Abbreviations: EGFR, epidermal growth factor receptor; mut, mutation; WT, wild type; BWF, bronchial washing fluid; PPV, positive predictive value; NPV, negative predictive value

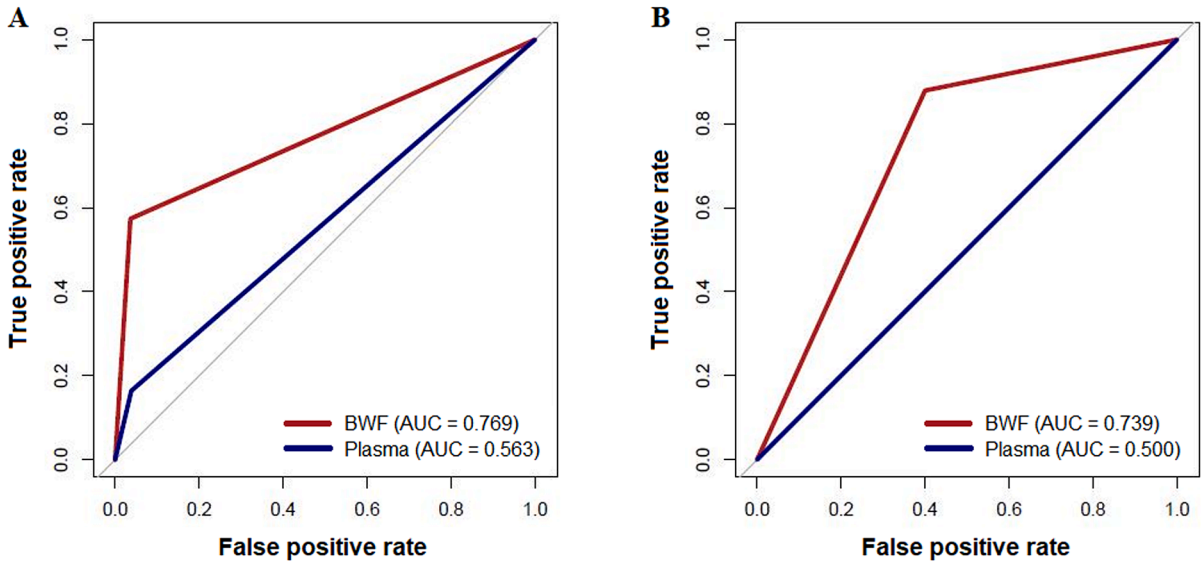

Fig. 2. Comparison of receiver operator characteristic curves in bronchial washing fluid and plasma. (A) Initial diagnosis group (bronchial washing fluid (BWF): area under the curve (AUC) = 0.769, 95 % confidence interval (CI) = 0.704–0.833; plasma: AUC = 0.563, 95 % CI = 0.509–0.617). (B) Rebiopsy group (BWF: AUC = 0.739, 95 % CI = 0.569–0.909; plasma: AUC = 0.500, 95 % CI = 0.207–0.793).

time of diagnosis. Using analysis by stage, the concordance rate of BWF EGFR results for patients in an advanced stage was 100 % (3/3), and that for patients in an early stage was 76.9 % (10/13). The findings show that BWF samples assessed by ddPCR reflect EGFR mutations in tumor tissue even when tissue biopsies did not contain adequate tumor cell numbers.

3.3. Diagnostic yield of using BWF for rebiopsy

The patient characteristics in the rebiopsy group according to the presence of EGFR T790M are shown in Supplementary Table 1. In the rebiopsy group, most patients (95.4 %) had histologically confirmed adenocarcinoma, and more than half (58.1 %) underwent rebiopsy at the primary tumor site. All patients harbored EGFR mutations at diagnosis. There were 10 patients (23.3 %) with wild-type EGFR and 33 (76.7 %) with EGFR mutations in rebiopsied tissue samples. Reflecting a change in the EGFR mutation status of tumor tissue at rebiopsy, 15 patients (34.9 %) had newly detected T790M mutations, one patient was confirmed for de novo T790M identical to the initial finding, and 18 patients (41.9 %) only harbored the initial mutation, with 9 patients (20.9 %) showing loss of activating EGFR mutations.

For EGFR mutation detection, relative to results in tumor tissue, the sensitivity and specificity, respectively, were 87.9 % and 60.0 % for BWF and 25.0 % and 75.0 % for plasma. Concordance rates were 81.4 % for BWF and 41.7 % for plasma. Similar to results in the initial diagnosis group, the diagnostic yield for EGFR mutation detection was higher in BWF than in plasma in the rebiopsy group (p = 0.016) (Table 2). The AUC for EGFR mutation detection in the rebiopsy group was 0.739 (95 % CI: 0.569–0.909) for BWF and 0.500 (95 % CI: 0.207–0.793) for plasma (Fig. 2B).

3.4. EGFR T790M detection in BWF for rebiopsy

Of 43 patients in the rebiopsy group, 16 (37.2 %) were T790M-positive in BWF and 27 (62.8 %) were T790M-negative in BWF. As compared with results in tumor tissue, sensitivity, specificity, PPV, and NPV of T790M detected in BWF were 68.8 %, 66.7 %, 55.0 %, and 78.3 %, respectively. In tumor tissue, T790M was detected in 16 patients (37.2 %). The combined use of tissue biopsy and BWF increased the detection rate for T790M compared with tissue biopsy alone, confirming T790M in 22 patients (51.2 %) (Fig. 3). Six patients were identified as T790M-negative in tumor tissue but positive in BWF (Supplementary Table 2). In two patients, EGFR mutations were detected in BWF despite the absence of malignant cells in tumor tissue. In three patients, T790M was detected earlier in BWF than in other samples, such as tumor tissue or plasma.

A representative case of a patient testing negative for T790M in tumor tissue but positive in BWF is shown in Supplementary Fig. 1. A 56-year-old woman was diagnosed with stage IV lung adenocarcinoma with bone and brain metastases. She was confirmed to have an E19del and was administered gefitinib. Two years later, the primary lung tumor

Table 3
Detailed information for initial diagnosis group patients with no tumor cells in tissue biopsy and EGFR mutation detected in BWF.

No.	Stage[a]	Initial diagnostic test results				Final diagnostic test results			
		Modality	Tissue type	Pathologic result	EGFR mutation in BWF	Modality	Tissue type	Pathologic results	EGFR mutation in tissue sample
1	Early	TBLB	Lung	No evidence of malignancy	Exon 19 deletion	CT-guided gun biopsy	Lung	Adenocarcinoma	Exon 19 deletion
2	Early	TBLB	Lung	No evidence of malignancy	Exon 19 deletion	CT-guided gun biopsy	Lung	Adenocarcinoma	Exon 19 deletion
3	Advanced	TBLB	Lung	No evidence of malignancy	L858R	RP-EBUS-TBLB	Lung	Adenocarcinoma	L858R
4	Early	TBLB	Lung	No evidence of malignancy	G719X, S768I	Surgery	Lung	Adenocarcinoma	G719X, S768I
5	Early	TBLB	Lung	No evidence of malignancy	Wild-type	CT-guided gun biopsy	Lung	Adenocarcinoma	L858R
6	Advanced	TBLB	Lung	No evidence of malignancy	Wild-type	CT-guided gun biopsy	Lung	Sarcomatoid carcinoma	Wild-type
7	Early	TBLB	Lung	No evidence of malignancy	Wild-type	RP-EBUS-TBLB	Lung	Adenocarcinoma	Wild-type
8	Early	TBLB	Lung	No evidence of malignancy	Wild-type	Surgery	Lung	Adenocarcinoma	Wild-type
9	Early	TBLB	Lung	No evidence of malignancy	Wild-type	Surgery	Lung	Adenocarcinoma	Wild-type
10	Early	TBLB	Lung	No evidence of malignancy	Wild-type	Surgery	Lung	Adenocarcinoma	L858R
11	Early	TBLB	Lung	No evidence of malignancy	Wild-type	Surgery	Lung	Adenocarcinoma	Wild-type
12	Early	RP-EBUS-TBLB	Lung	No evidence of malignancy	Wild-type	Surgery	Lung	Adenocarcinoma	Wild-type
13	Early	RP-EBUS-TBLB	Lung	A few atypical cells	Wild-type	Surgery	Lung	Adenocarcinoma	Exon 19 deletion
14	Early	EBUS-TBNA	Lymph node	No evidence of malignancy	Exon 19 deletion	Surgery	Lung	Adenocarcinoma	Exon 19 deletion
15	Advanced	EBUS-TBNA	Lymph node	No evidence of malignancy	Wild-type	Surgery	Lung	Adenocarcinoma	Wild-type
16	Early	CT-guided gun biopsy	Lung	Material insufficiency	Wild-type	Surgery	Lung	Adenocarcinoma	Wild-type

Notes: [a]Early stage refers to stages I–IIIA, and advanced stage refers to stages IIIB–IV
Abbreviations: CT, computed tomography; EBUS-TBNA, endobronchial ultrasound-guided transbronchial needle aspiration; RP-EBUS-TBLB, radial probe endobronchial ultrasound-guided transbronchial lung biopsy; TBLB, transbronchial lung biopsy.

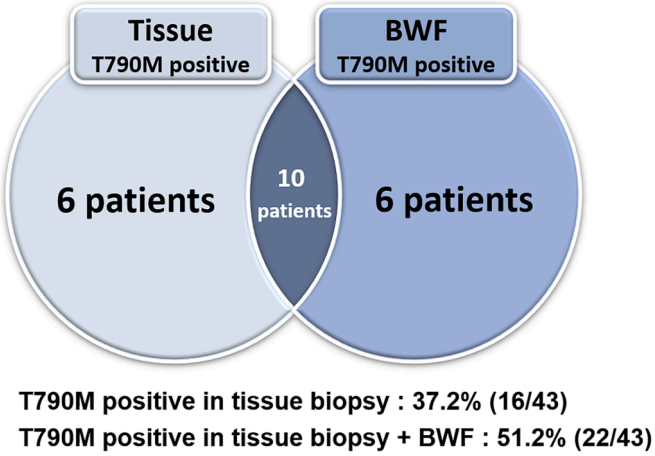

Fig. 3. Number of patients with T790M detected by sample type in the rebiopsy group. Of the 43 patients in the rebiopsy group, 10 had a T790M mutation detected in both tumor tissue and BWF. Six patients were T790M-positive in tumor tissue only, and six were T790M-positive in BWF only. The combined use of tissue biopsy and BWF increased the detection rate for T790M compared with the use of tissue biopsy alone, confirming T790M in 22 patients (51.2 %).

progressed, and leptomeningeal metastases developed. The patient underwent bronchoscopy for rebiopsy; however, it was difficult to obtain tumor tissue because of bronchial stenosis caused by fibrotic changes after EGFR-TKI treatment. On tissue biopsy, only E19del was detected in the rebiopsied tumor tissue, but both E19del and T790M were detected in BWF. A plasma EGFR mutation test at the time of rebiopsy performed at the clinic showed loss of E19del and no T790M by Cobas EGFR Mutation Test v2 (Roche Molecular Systems Inc., Branchburg, NJ). As leptomeningeal metastasis progressed, treatment was switched from gefitinib to osimertinib. Four months after initiation of osimertinib treatment, the leptomeningeal metastases had almost disappeared, and the primary lung tumor size decreased. Depending on the response to osimertinib, the detection of T790M in BWF using ddPCR can be considered a reliable true-positive result. This case suggests that detection of T790M in BWF using ddPCR may be helpful in guiding treatment decisions when tumor tissue acquisition is difficult.

4. Discussion

Here, we investigated the detection of EGFR mutations in BWF using ddPCR, which was found to give high diagnostic yields for both initial diagnosis and rebiopsy of NSCLC. We found that using ddPCR, T790M was detected earlier in BWF than in tumor tissue, being detected even when biopsied tissue did not have adequate tumor cell numbers. Our results suggest that BWF can compensate for the low sensitivity of rebiopsy in EGFR-mutated NSCLC on progression after EGFR-TKI therapy.

Third-generation EGFR-TKIs are the standard regimen for subsequent therapy when EGFR-mutated NSCLC patients acquire a T790M mutation with resistance to first-line EGFR-TKIs [16]. T790M detection has been reported in 55–60 % of patients with disease progression after first-line EGFR-TKIs [17,18]. Patients with T790M who are treated with osimertinib have a progression-free survival (PFS) of 10.1–14.2 months [19–21]. However, the median PFS of T790M-negative patients treated with platinum-based chemotherapy was only 4.4 months in the AURA3

phase III clinical trial [19], indicating that the presence of T790M is crucial for survival benefit when disease progresses during first-line EGFR-TKI therapy.

T790M molecular testing is mainly performed in tumor tissue or plasma using real-time PCR. In reports on T790M tests in rebiopsy and plasma, T790M detection was 30–40 % in tumor tissue/cytology samples when adequate numbers of tumor cells were acquired, and approximately 20 % in plasma samples [22]. For tumors located in the center of the lung and accessible only by transbronchial lung biopsy (TBLB), the accuracy and T790M detection rate are lower than for tumors in other locations. For these difficult-to-access tumors, cell-free tumor DNA within BWF, obtained from areas containing the tumor, can be easily collected during bronchoscopy, even without precise tumor targeting.

BWF and BALF (bronchoalveolar lavage fluid) have been routinely obtained during bronchoscopy using standardized methods and have long been used in the diagnosis of respiratory diseases. Coupled with highly sensitive assays such as PCR, BWF testing is widely used for accurate and rapid diagnosis of lung diseases including tuberculosis [23] and pneumonia and can be used to guide treatment decisions [24].

BWF and BALF are emerging as enriched cfDNA sources with advantages over plasma for identifying lung cancer-derived mutations [25]. Several studies have shown that BWF and BALF have better yields than plasma in liquid biopsy for detection of EGFR mutations including T790M [26–28]. In our study, the allele frequency of mutant EGFR in each sample was generally higher in BWF compared to the paired plasma (Supplementary Fig. 2). In addition, BWF better reflects tumor heterogeneity than does small-biopsy tissue. Given these advantages, studies have been conducted to determine the feasibility of identifying EGFR mutations in BWF and BWF/BALF using diverse methods.

In this study, we employed ddPCR for the detection of EGFR in BWF. Generally, ddPCR is known as a more sensitive method for detecting variants, even when allele frequencies are low. Previous studies have reported that ddPCR can rapidly and accurately detect EGFR mutations in circulating tumor DNA in lung cancer, demonstrating high sensitivity and specificity [29–31]. Enhanced detection of EGFR-TKI-sensitizing mutations using ddPCR in BWF, as compared to plasma, has been reported in previous studies [13]. In the present study, we focused on T790M detection by ddPCR in rebiopsy after EGFR-TKI therapy. As discussed above, rebiopsy through TBLB is performed in poorly accessible tumors, and tumors are surrounded by dense fibrosis after EGFR-TKI treatment, making tissue biopsy difficult. Owing to the limitations of rebiopsy, it is advantageous to obtain tumor DNA from a range of sources and use highly sensitive assays that can detect EGFR mutations even with small amounts of tumor DNA. We consider that BWF ddPCR using cfDNA is a powerful complementary test in bronchoscopic rebiopsy. The frequency of T790M in tissues obtained by TBLB is approximately 30–40 % in clinical practice, but additional use of BWF increased the detection rate of T790M to more than 50 % in the current study. The strength of our study is that BWF ddPCR using cfDNA can be applied without additional equipment in institutions where bronchoscopy is currently performed and where tumor DNA is analyzed by ddPCR.

This study has some limitations. First, BWF acquisition by bronchoscopy was performed by several doctors, including trainee pulmonologists at a university-affiliated training hospital. Second, this was a retrospective study conducted at a single institution. While BWF acquisition is a simple procedure, if BWF is collected prospectively by experienced pulmonologists, better diagnostic yields can be expected.

5. Conclusions

In conclusion, we demonstrated that detection of EGFR mutations in BWF using ddPCR showed a high diagnostic yield for both initial diagnosis and rebiopsy of NSCLC. In cases of EGFR-TKI treatment failure, T790M can, in some cases, be detected earlier in BWF than in tissue rebiopsy, providing patients with an early opportunity to access third-generation EGFR-TKIs. Given the ease of acquiring BWF and the accuracy of ddPCR results, the complementary use of BWF along with tumor tissue samples will improve precision in EGFR-mutated NSCLC treatment strategies.

Funding

This work was supported by National Research Foundation of Korea grants funded by the Ministry of Science and ICT of the Korean government [Nos. 2020R1C1C1007850 and 2023R1A2C1004922] to EY Kim.

CRediT authorship contribution statement

Woo Kyung Ryu: Conceptualization, Methodology, Formal analysis, Data curation, Writing – original draft, Visualization. **Seung Hyun Yong:** Investigation, Resources. **Sang Hoon Lee:** Conceptualization, Methodology, Validation, Supervision. **Hye Ran Gwon:** Investigation, Resources. **Hye Ryun Kim:** Investigation, Resources. **Min Hee Hong:** Investigation, Resources. **Go Eun Oh:** Investigation, Resources, Data curation. **Sehee Jung:** Investigation, Resources, Data curation. **Chi Young Kim:** Conceptualization, Methodology, Validation, Supervision. **Yoon Soo Chang:** Conceptualization, Methodology, Validation, Supervision. **Eun Young Kim:** Conceptualization, Writing – original draft, Writing – review & editing, Supervision, Project administration, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

The authors would like to thank all participants who enrolled in this study.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.lungcan.2023.107390.

References

[1] A. Russo, T. Franchina, G.R.R. Ricciardi, A. Picone, G. Ferraro, M. Zanghì, G. Toscano, A. Giordano, V. Adamo, A decade of EGFR inhibition in EGFR-mutated non small cell lung cancer (NSCLC): Old successes and future perspectives, Oncotarget 6 (29) (2015) 26814.

[2] Y.-L. Zhang, J.-Q. Yuan, K.-F. Wang, X.-H. Fu, X.-R. Han, D. Threapleton, Z.-Y. Yang, C. Mao, J.-L. Tang, The prevalence of EGFR mutation in patients with non-small cell lung cancer: a systematic review and meta-analysis, Oncotarget 7 (48) (2016) 78985.

[3] D.S. Ettinger, D.E. Wood, D.L. Aisner, W. Akerley, J.R. Bauman, A. Bharat, D. S. Bruno, J.Y. Chang, L.R. Chirieac, T.A. D'Amico, Non-small cell lung cancer, version 3.2022, nccn clinical practice guidelines in oncology, J. Natl. Compr. Canc. Netw. 20 (5) (2022) 497–530.

[4] G. Recondo, F. Facchinetti, K.A. Olaussen, B. Besse, L. Friboulet, Making the first move in EGFR-driven or ALK-driven NSCLC: first-generation or next-generation TKI? Nat. Rev. Clin. Oncol. 15 (11) (2018) 694–708.

[5] S.A. Ensminger, U.B. Prakash, Is bronchoscopic lung biopsy helpful in the management of patients with diffuse lung disease? Eur. Respir. J. 28 (6) (2006) 1081–1084.

[6] E.A. Ruud, K. Stavem, J.T. Geitung, A. Borthne, V. Søyseth, H. Ashraf, Predictors of pneumothorax and chest drainage after percutaneous CT-guided lung biopsy: a prospective study, Eur. Radiol. 31 (6) (2021) 4243–4252.

[7] L. Kim, M.S. Tsao, Tumour tissue sampling for lung cancer management in the era of personalised therapy: what is good enough for molecular testing? Eur. Respir. J. 44 (4) (2014) 1011–1022.

[8] T.O. Kim, I.J. Oh, B.G. Kho, H.Y. Park, J.S. Chang, C.K. Park, H.J. Shin, J.H. Lim, Y. S. Kwon, Y.I. Kim, Feasibility of re-biopsy and EGFR mutation analysis in patients with non-small cell lung cancer, Thoracic Cancer 9 (7) (2018) 856–864.
[9] C. Rolfo, P. Mack, G.V. Scagliotti, C. Aggarwal, M.E. Arcila, F. Barlesi, T. Bivona, M. Diehn, C. Dive, R. Dziadziuszko, Liquid biopsy for advanced NSCLC: a consensus statement from the international association for the study of lung cancer, J. Thorac. Oncol. 16 (10) (2021) 1647–1662.
[10] K.L. Reckamp, V.O. Melnikova, C. Karlovich, L.V. Sequist, D.R. Camidge, H. Wakelee, M. Perol, G.R. Oxnard, K. Kosco, P. Croucher, A highly sensitive and quantitative test platform for detection of NSCLC EGFR mutations in urine and plasma, J. Thorac. Oncol. 11 (10) (2016) 1690–1700.
[11] J.S. Ryu, J.H. Lim, M.K. Lee, S.J. Lee, H.J. Kim, M.J. Kim, M.H. Park, J.S. Kim, H. S. Nam, N. Park, Feasibility of bronchial washing fluid-based approach to early-stage lung cancer diagnosis, Oncologist 24 (7) (2019) e603–e606.
[12] U. Food, D. Administration, cobas EGFR mutation test v2, Summary of safety and effectiveness data. Available online: http://www. accessdata. fda. gov/cdrh_docs/pdf12/P120019S007B. pdf (2016).
[13] S.H. Lee, E.Y. Kim, T. Kim, Y.S. Chang, Compared to plasma, bronchial washing fluid shows higher diagnostic yields for detecting EGFR-TKI sensitizing mutations by ddPCR in lung cancer, Respir. Res. 21 (1) (2020) 1–8.
[14] X. Zhang, C. Li, M. Ye, Q. Hu, J. Hu, Z. Gong, J. Li, X. Zhao, Y. Xu, D. Zhang, Bronchial washing fluid versus plasma and bronchoscopy biopsy samples for detecting epidermal growth factor receptor mutation status in lung cancer, Front. Oncol. 11 (2021), 602402.
[15] P. Goldstraw, K. Chansky, J. Crowley, R. Rami-Porta, H. Asamura, W.E. Eberhardt, A.G. Nicholson, P. Groome, A. Mitchell, V. Bolejack, The IASLC lung cancer staging project: proposals for revision of the TNM stage groupings in the forthcoming (eighth) edition of the TNM classification for lung cancer, J. Thorac. Oncol. 11 (1) (2016) 39–51.
[16] D.S. Ettinger, D.E. Wood, D.L. Aisner, W. Akerley, J.R. Bauman, A. Bharat, D.S. Bruno, J.Y. Chang, L.R. Chirieac, T.A. D'Amico, M. DeCamp, T.J. Dilling, J. Dowell, S. Gettinger, T.E. Grotz, M.A. Gubens, A. Hegde, R.P. Lackner, M. Lanuti, J. Lin, B. W. Loo, C.M. Lovly, F. Maldonado, E. Massarelli, D. Morgensztern, T. Ng, G.A. Otterson, J.M. Pacheco, S.P. Patel, G.J. Riely, J. Riess, S.E. Schild, T.A. Shapiro, A. P. Singh, J. Stevenson, A. Tam, T. Tanvetyanon, J. Yanagawa, S.C. Yang, E. Yau, K. Gregory, M. Hughes, Non-Small Cell Lung Cancer, Version 3.2022, NCCN Clinical Practice Guidelines in Oncology, J Natl Compr Canc Netw 20 (5) (2022) 497-530. https://doi.org/10.6004/jnccn.2022.0025.
[17] T. John, H. Akamatsu, A. Delmonte, W.C. Su, J.S. Lee, G.C. Chang, X. Huang, S. Jenkins, Y.L. Wu, EGFR mutation analysis for prospective patient selection in AURA3 phase III trial of osimertinib versus platinum-pemetrexed in patients with EGFR T790M-positive advanced non-small-cell lung cancer, Lung Cancer 126 (2018) 133–138, https://doi.org/10.1016/j.lungcan.2018.10.027.
[18] G.J. Riely, H.A. Yu, EGFR: the paradigm of an oncogene-driven lung cancer, Clin. Cancer Res. 21 (10) (2015) 2221–2226, https://doi.org/10.1158/1078-0432.Ccr-14-3154.
[19] T.S. Mok, Y.L. Wu, M.J. Ahn, M.C. Garassino, H.R. Kim, S.S. Ramalingam, F. A. Shepherd, Y. He, H. Akamatsu, W.S. Theelen, C.K. Lee, M. Sebastian, A. Templeton, H. Mann, M. Marotti, S. Ghiorghiu, V.A. Papadimitrakopoulou, Osimertinib or platinum-pemetrexed in egfr t790m-positive lung cancer, N. Engl. J. Med. 376 (7) (2017) 629–640, https://doi.org/10.1056/NEJMoa1612674.
[20] F. Marinis, Y.L. Wu, G. de Castro Jr., G.C. Chang, Y.M. Chen, B.C. Cho, H.C. Freitas, L. Jiang, S.W. Kim, C. Martin, G. Metro, M. Provencio, J. Vansteenkiste, D. Vicente, Q. Zhou, M.F. Miranda, N.A. Bakker, J.R. Rigas, P.K. Cheema, ASTRIS: a global real-world study of osimertinib in >3000 patients with EGFR T790M positive non-small-cell lung cancer, Future Oncol. 15 (26) (2019) 3003–3014, https://doi.org/10.2217/fon-2019-0324.
[21] J.H. Lee, E.Y. Kim, C.K. Park, S.Y. Lee, M.K. Lee, S.H. Yoon, J.E. Lee, S.H. Lee, S. J. Kim, S.Y. Lee, J.H. Lim, T.W. Jang, S.H. Jang, K.Y. Lee, S.H. Lee, S.H. Yang, D. W. Park, C.K. Park, H.S. Kang, C.D. Yeo, C.M. Choi, J.C. Lee, Real-world study of osimertinib in korean patients with epidermal growth factor receptor t790m mutation-positive non-small cell lung cancer, Cancer Res. Treat. (2022), https://doi.org/10.4143/crt.2022.381.
[22] T. Seto, N. Nogami, N. Yamamoto, S. Atagi, N. Tashiro, Y. Yoshimura, Y. Yabuki, H. Saka, Real-world egfr t790m testing in advanced non-small-cell lung cancer: a prospective observational study in japan, Oncol Ther 6 (2) (2018) 203–215, https://doi.org/10.1007/s40487-018-0064-8.
[23] C. Tueller, P.N. Chhajed, C. Buitrago-Tellez, R. Frei, M. Frey, M. Tamm, Value of smear and PCR in bronchoalveolar lavage fluid in culture positive pulmonary tuberculosis, Eur. Respir. J. 26 (5) (2005) 767–772, https://doi.org/10.1183/09031936.05.00046105.
[24] A.M. Darie, N. Khanna, K. Jahn, M. Osthoff, S. Bassetti, M. Osthoff, D. M. Schumann, W.C. Albrich, H. Hirsch, M. Brutsche, L. Grize, M. Tamm, D. Stolz, Fast multiplex bacterial PCR of bronchoalveolar lavage for antibiotic stewardship in hospitalised patients with pneumonia at risk of Gram-negative bacterial infection (Flagship II): a multicentre, randomised controlled trial, Lancet Respir. Med. 10 (9) (2022) 877–887, https://doi.org/10.1016/s2213-2600(22)00086-8.
[25] V.S. Nair, A.B. Hui, J.J. Chabon, M.S. Esfahani, H. Stehr, B.Y. Nabet, L. Zhou, A. A. Chaudhuri, J. Benson, K. Ayers, H. Bedi, M. Ramsey, R. Van Wert, S. Antic, N. Lui, L. Backhus, M. Berry, A.W. Sung, P.P. Massion, J.B. Shrager, A.A. Alizadeh, M. Diehn, Genomic profiling of bronchoalveolar lavage fluid in lung cancer, Cancer Res. 82 (16) (2022) 2838–2847, https://doi.org/10.1158/0008-5472.Can-22-0554.
[26] J.Y. Hur, J.S. Lee, I.A. Kim, H.J. Kim, W.S. Kim, K.Y. Lee, Extracellular vesicle-based EGFR genotyping in bronchoalveolar lavage fluid from treatment-naive non-small cell lung cancer patients, Transl. Lung Cancer Res 8 (6) (2019) 1051–1060. https://doi.org/10.21037/tlcr.2019.12.16.
[27] Y.J. Kim, W.J. Ji, J.C. Lee, S.M. Chun, C.M. Choi, Assessment of anti-tumor efficacy of osimertinib in non-small cell lung cancer patients by liquid biopsy using bronchoalveolar lavage fluid, plasma, or pleural effusion, Cancer Res Treat 54 (4) (2022) 985–995, https://doi.org/10.4143/crt.2021.857.
[28] C.K. Park, H.J. Cho, Y.D. Choi, I.J. Oh, Y.C. Kim, A Phase II trial of osimertinib in the second-line treatment of non-small cell lung cancer with the egfr t790m mutation, detected from circulating tumor dna: liquidlung-o-cohort 2, Cancer Res. Treat. 51 (2) (2019) 777–787, https://doi.org/10.4143/crt.2018.387.
[29] W.N. Feng, W.Q. Gu, N. Zhao, Y.M. Pan, W. Luo, H. Zhang, J.M. Liang, J. Yang, Y. M. Deng, Comparison of the superarms and droplet digital pcr for detecting egfr mutation in ctdna from nsclc patients, Transl. Oncol. 11 (2) (2018) 542–545, https://doi.org/10.1016/j.tranon.2018.02.007.
[30] A.G. Sacher, C. Paweletz, S.E. Dahlberg, R.S. Alden, A. O'Connell, N. Feeney, S. L. Mach, P.A. Jänne, G.R. Oxnard, Prospective validation of rapid plasma genotyping for the detection of egfr and kras mutations in advanced lung cancer, JAMA Oncol. 2 (8) (2016) 1014–1022, https://doi.org/10.1001/jamaoncol.2016.0173.
[31] K.S. Thress, R. Brant, T.H. Carr, S. Dearden, S. Jenkins, H. Brown, T. Hammett, M. Cantarini, J.C. Barrett, EGFR mutation detection in ctDNA from NSCLC patient plasma: a cross-platform comparison of leading technologies to support the clinical development of AZD9291, Lung Cancer 90 (3) (2015) 509–515, https://doi.org/10.1016/j.lungcan.2015.10.004.