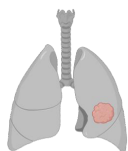# Comparison of tissue-based and plasma-based testing for *EGFR* mutation in non–small cell lung cancer patients

Yoon Kyung Kang[1], Dong Hoon Shin[1], Joon Young Park[1], Chung Su Hwang[1], Hyun Jung Lee[1], Jung Hee Lee[1], Jee Yeon Kim[1], JooYoung Na[2]

Graphical abstract

Background

- Epidermal growth factor receptor (EGFR) gene mutation testing is crucial for the administration of tyrosine kinase inhibitors to treat non-small cell lung cancer (NSCLC).
- In addition to traditional tissue-based tests, liquid biopsies using plasma are increasingly utilized, particularly for detecting T790M mutations.

This study compared tissue- and plasma-based EGFR testing methods.

Methods

EGFR test

248 patients (2018~2023) at the Pusan National University Yang-San Hospital

Tissue samples
Using PANAmutyper

Plasma samples
Using the Cobas EGFR Mutation Test v2

Results

For the activating mutation

	Tissue samples	Plasma samples
The number of tested patients	248	243
Positive ratio	98.8%	67.1%

For the T790M mutation

	Tissue samples	Plasma samples
The number of tested patients	69	207
Positive ratio	40.6%	27.5%
Combined plasma and tissue testing		
Positive ratio	56.7%	

CONCLUSIONS Although the tissue- and plasma-based tests showed a sensitivity of 38.8% and 31.3% respectively, combined testing increased the detection rate to 56.7%. Thus, neither test demonstrated superiority, rather, they were complementary.

Kang YK T et al. Journal of Pathology and Translational Medicine

Comparison of tissue-based and plasma-based testing for *EGFR* mutation in non–small cell lung cancer patients

Yoon Kyung Kang[1], Dong Hoon Shin[1], Joon Young Park[1], Chung Su Hwang[1], Hyun Jung Lee[1],
Jung Hee Lee[1], Jee Yeon Kim[1], JooYoung Na[2]

[1]Department of Pathology, Pusan National University School of Medicine, Yangsan, Korea
[2]Department of Forensic Medicine, Pusan National University School of Medicine, Yangsan, Korea

Background: Epidermal growth factor receptor (*EGFR*) gene mutation testing is crucial for the administration of tyrosine kinase inhibitors to treat non–small cell lung cancer. In addition to traditional tissue-based tests, liquid biopsies using plasma are increasingly utilized, particularly for detecting T790M mutations. This study compared tissue- and plasma-based *EGFR* testing methods. **Methods:** A total of 248 patients were tested for *EGFR* mutations using tissue and plasma samples from 2018 to 2023 at Pusan National University Yangsan Hospital. Tissue tests were performed using PANAmutyper, and plasma tests were performed using the Cobas *EGFR* Mutation Test v2. **Results:** All 248 patients underwent tissue-based *EGFR* testing, and 245 (98.8%) showed positive results. Of the 408 plasma tests, 237 (58.1%) were positive. For the T790M mutation, tissue biopsies were performed 87 times in 69 patients, and 30 positive cases (38.6%) were detected. Plasma testing for the T790M mutation was conducted 333 times in 207 patients, yielding 62 positive results (18.6%). Of these, 57 (27.5%) were confirmed to have the mutation via plasma testing. Combined tissue and plasma tests for the T790M mutation were positive in nine patients (13.4%), while 17 (25.4%) were positive in tissue only and 12 (17.9%) in plasma only. This mutation was not detected in 28 patients (43.3%). **Conclusions:** Although the tissue- and plasma-based tests showed a sensitivity of 37.3% and 32.8%, respectively, combined testing increased the detection rate to 56.7%. Thus, neither test demonstrated superiority, rather, they were complementary.

Keywords: ErbB receptors; Lung cancer; Liquid biopsy

INTRODUCTION

Non–small cell lung cancer (NSCLC) is a major cause of cancer-related mortality globally [1,2]. However, the patients' prognosis and quality of life have significantly improved with the introduction of targeted therapies, including tyrosine kinase inhibitors (TKIs), which act against mutations in the epidermal growth factor receptor (*EGFR*). Despite their initial effectiveness, resistance to first- or second-generation TKIs often develops within months or years owing to various mechanisms, with the T790M mutation in the *EGFR* gene accounting for approximately 50% of these resistance cases [3,4]. To counter this, third-generation TKIs such as osimertinib have been developed

to target this specific mutation, making detection of the T790M mutation crucial for patients with NSCLC who have developed resistance to previous TKIs [3-9]. However, this detection can be challenging, especially in patients with small, hard-to-reach tumors, or when tissue biopsy is not an option.

Recent developments have underscored the potential of liquid biopsy as either an alternative or a supplementary approach to tissue biopsy for molecular diagnostics, including the detection of the T790M mutation in blood samples [5-9]. This method is particularly appealing because of its noninvasive nature, feasibility of repeated testing, fast turnaround time, and potential to reflect changes in tumor burden through serial monitoring. Unlike tissue biopsies, which only reveal mutations at the

sampling site, blood tests can detect all tumor DNA present in patient bloodstreams. Currently, the National Comprehensive Cancer Network guidelines recommend liquid biopsy as an alternative to tissue biopsy for initial T790M mutation testing [10]. The importance of accurately determining the sensitivity and specificity of liquid biopsies is paramount, given their broad applications. However, optimization of liquid biopsies is challenging, primarily because of the inability to directly quantify cell-free tumor DNA (ctDNA). This uncertainty in ctDNA levels complicates quality control compared with that of tissue biopsies [8,9]. In contrast, tissue biopsies allow pathologists to directly observe the number of tumor cells under a microscope, providing a clearer understanding of whether negative results stem from an insufficient amount of tumor DNA. Direct observation is not possible using liquid biopsies. Therefore, these differences may play a role in the diverse findings regarding the effectiveness of liquid biopsy for *EGFR* mutation detection, as reported in various studies [3-9].

In Korea, tissue-based tests are performed in the Department of Pathology, whereas blood-based tests are performed either in the Department of Pathology or the Department of Laboratory Medicine, depending on the institution [8,9,11]. However, this division may result in communication challenges between the two departments, potentially affecting the management of *EGFR* testing. The primary objective of our study was to evaluate the performance of tests performed independently in each department. Furthermore, we evaluated the effectiveness of *EGFR* assays conducted on both tissue and blood samples by examining a series of consecutive tests from each patient. This approach enabled us to ascertain the overall sensitivity and specificity on an individual basis, rather than limiting our evaluation to a per-test perspective. Consequently, our study also aimed to offer comprehensive insights into the detection of T790M mutations in both tissue and liquid biopsies. This was aimed at assisting clinicians in choosing the most appropriate testing approach for patients considering variables such as tumor burden and metastatic sites.

MATERIALS AND METHODS

Patients

Between 2018 and 2023, a total of 590 plasma *EGFR* mutation tests were conducted at Pusan National University Yangsan Hospital. Among these patients, those diagnosed with adenocarcinoma, squamous cell carcinoma, or NSCLC were selected.

Subsequently, only patients who also underwent an *EGFR* mutation test using tissue-based methods and tested positive for an *EGFR* mutation in either tissue or plasma at least once were included in the study. For analysis of T790M mutation, only the patient who received TKI treatment were considered. A comprehensive review of the longitudinal data of these patients was performed, including pathology reports with *EGFR* tests, the results of plasma *EGFR* tests, radiological imaging, and positron emission tomography scans.

Tissue *EGFR* test

The *EGFR* test in tissue was performed using the PANAMuytper, a peptide nucleic acid (PNA)–mediated, real-time polymerase chain reaction (PCR)–based assay. Mutant DNA was selectively amplified using wild-type DNA-specific PNA clamp probes, after which mutant-specific PNA detection probes were used to genotype the *EGFR* mutations using fluorescence melting curve analysis. This assay can detect and discriminate 47 types of *EGFR* mutations, including three G719X substitutions, 29 exon 19 deletions (Exon19del), one T790M substitution, one S768I substitution, 10 exon 20 insertions (Exon20ins), two L858R substitutions, and one L861Q substitution, with a high level of sensitivity [12].

Plasma *EGFR* test

Venous blood samples were collected from patients using 21G needles in a 10-mL Cell-Free DNA BCT tube (Streck, Omaha, NE, USA) per patient. For the 1-step centrifugation group, within 4 hours of room-temperature blood collection, blood samples were centrifuged at 1,600 ×g for 10 minutes, and 2 mL was dispensed into each Eppendorf tube. *EGFR* mutations were identified using the Cobas EGFR Mutation Test v2 on a Cobas z 480 analyzer (Roche Diagnostics, Pleasanton, CA, USA), according to the manufacturer's instructions [11]. This assay was designed to detect insertions and deletions in *EGFR*, such as deletions in exon 19 and insertions in exon 20, as well as nucleic acid substitutions, such as G719X, S768I, T790M, L858R, and L861Q.

Statistical analyses

Python software ver. 3.7.6 was used to perform all computation and analyses in this study. The Pandas library was used to perform basic statistical analysis of the data. All statistical tests were two-sided, and $p < .05$ was considered significant unless otherwise specified.

RESULTS

Clinical characteristics of patients

A total of 248 patients were enrolled for this analysis (106 males and 142 females) (Table 1). The average age at diagnosis was 67 years (range, 40 to 86 years). The pathological diagnoses included 238 cases of adenocarcinoma, eight of squamous cell carcinoma, and two of adenosquamous carcinoma. The distribution of patients according to stage was as follows: stage I, 16; stage II, 10; stage III, 39; and stage IV, 183. The most common sites of metastasis upon recurrence or progression were the lungs (136 patients), followed by the brain (n = 74), bone (n = 52), pleura (n = 31), lymph nodes (n = 19), liver (n = 15), and other organs (n = 10).

Activating *EGFR* mutation

All 248 enrolled patients underwent *EGFR* testing using tissue samples, including biopsy or resection, at the time of diagnosis or during the follow-up period. The results identified 245 (98.8%) positive and three (1.2%) negative cases (Table 2). For plasma *EGFR* testing, 408 tests were conducted across these patients, with 58 at the time of diagnosis and 350 following the initial diagnosis, showing 237 positive results (58.1%) and 171 negative results (41.9%). Of the patients tested, 163 (67.1%) tested positive and 80 (32.6%) tested negative in the plasma tests (Table 2). When both tissue- and plasma-based tests yielded positive results, they were consistent with exon 19 deletion, L858R, exon 20 insertion, and L861Q mutations. However,

discrepancies were observed in compound mutations. In these instances, tissue tests identifying exon 19 deletion/L861Q, L861Q/G719S, G719S/S768, and L858R/T790M mutations were detected as exon 19 deletion, L861Q, G719S/L861Q, and L858R mutations, respectively, in the plasma-based tests. The tissue-based tests did not detect activating mutations in four of the samples. In one sample, the tumor cell count ranged from 200 to 500, while the other three samples had tumor cell counts exceeding 1,000. A repeated tissue biopsy conducted after 18 months in one case revealed an exon 19 deletion mutation. Additionally, three other cases were found to have mutations in plasma-based tests: one with an exon 19 deletion and the others with the L858R mutation.

T790M mutation

Of the 248 enrolled patients, 210 received TKI treatment. Among these, 69 underwent a tissue biopsy, 207 had a plasma test to detect the T790M mutation, and 66 underwent both tests. Tissue biopsies were performed 87 times in 69 patients to detect the T790M mutation, which resulted in 30 (34.1%) positive results. Of the 69 patients, 28 (40.6%) harbored the T790M mutation. Plasma tests for the T790M mutation were

Table 1. Characteristics of patients

Parameter	Value
Age (yr)	67 (40–86)
Sex	
Male	106 (42.7)
Female	142 (57.3)
Pathologic diagnosis	
Adenocarcinoma	238 (96.0)
Squamous cell carcinoma	8 (3.2)
Adenosquamous carcinoma	2 (0.8)
Stage	
I	16 (6.5)
II	10 (4.0)
III	39 (15.7)
IV	183 (73.8)

Values are presented as mean (range) or number (%).
SCC, squamous cell carcinoma.

Table 2. *EGFR* mutations detected in tissue and plasma

	Tissue	Plasma
Activating mutation		
Exon 19 deletion	139 (56.0)	102 (41.1)
L858R	87 (35.1)	53 (21.4)
L861Q	4 (1.6)	5 (2.0)
S768I	1 (0.4)	0
G719C	1 (0.4)	0
T790M	1 (0.4)	0
Exon 20 insertion	4 (1.6)	1 (0.4)
G719X/L861Q	3 (1.2)	1 (0.4)
19del/L861Q	2 (0.8)	0
L858R/ T790M	1 (0.4)	0
S768I/G719S	1 (0.4)	0
L858R/S768I	1 (0.4)	1 (0.4)
Negative	3 (1.2)	85 (34.3)
Total	248	248
Resistance mutation		
T790M	28 (40.6)	57 (27.5)
Negative	41 (59.4)	150 (72.5)
Total	69	207

Values are presented as number (%).
EGFR, epidermal growth factor receptor.

conducted 333 times in 207 patients, with 62 positive results (18.6%). Of 271 negative test results, activating *EGFR* mutations were not detected in 148 patients. Among the 207 patients, 57 (27.5%) were confirmed to harbor the T790M mutation by plasma testing. Of these, 42 (20.3%) tested positive in the first plasma test, 10 out of 83 patients (12%) tested positive in the second test, and five out of 33 patients (15.2%) tested positive in the third test. Regarding tissue biopsies, 53 patients underwent the procedure once, 14 patients underwent it twice, and two patients underwent it thrice. For plasma tests during the treatment course, 124 patients were tested once, 50 patients twice, 24 patients thrice, eight patients four times, and one patient five times.

Sixty-six patients underwent both tissue- and plasma-based *EGFR* testing for T790M mutation (Table 3, Fig. 1). In nine (13.4%) of these patients, the T790M mutation was detected in both tests. The mutation was identified exclusively in the tissue samples of 17 patients (25.4%) and in the plasma samples of 12 patients (17.9%). In 28 patients (43.3%), neither test detected the T790M mutation. These cases are illustrated in Fig. 1.

Correlation of *EGFR* mutation detection with clinical parameters

In patients who underwent plasma *EGFR* testing at the time of histological diagnosis, a positive plasma test result for activating mutations was associated with the presence of nodal or distant metastases (Table 4). Conversely, the detection of the T790M mutation in the plasma did not exhibit a correlation with either nodal status or distant metastasis (Table 4).

DISCUSSION

Liquid biopsy is an emerging and highly valuable tool for detecting actionable mutations. Numerous studies have demonstrated its utility and yielded promising results [13-17]. One of the most widely used liquid biopsy methods is the plasma-based *EGFR* test for patients with lung cancer, particularly to guide TKI treatment decisions. Therefore, evaluating the efficacy of this test is of paramount importance as it directly influences treatment choices. Although several studies have demonstrated that the plasma-based *EGFR* test is comparable to the tissue-based test, the reported detection rates of the T790M mutation in these studies vary, ranging from 19.2% to 47.1% [13-16]. In this comprehensive study, data from 248 patients who underwent both tissue- and plasma-based *EGFR* tests to detect *EGFR* mutations were analyzed. To identify T790M mutations, 333 plasma tests were performed on 207 patients treated with TKI, yielding positive results in 62 tests (18.6%) and confirming the T790M mutation in 57 patients (27.4%). Conversely, tissue-based tests were performed 87 times in 69 patients, resulting in 30 positive tests (34.1%), indicating the presence of the T790M mutation in 28 patients (38.6%). In 66 patients subjected to both tissue- and plasma-based *EGFR* tests, tissue-based tests demonstrated a marginally higher sensitivity, yielding positive results in 26 patients (38.8%) compared with 21 patients (31.3%) for plasma-based tests. Nevertheless, the sensitivity of both methods was relatively low considering the estimated prevalence of the T790M mutation in 50% of TKI resistance cases [3-6]. Interestingly, when the results from both tests were combined, the positive rate increased to 38 patients (56.7%), exceeding the expected prevalence of 50%. These findings suggest that tissue- and plasma-based tests complement each other.

In our study, 408 plasma assays were conducted to detect *EGFR*-activating mutations in a cohort of 248 patients with confirmed mutations. Of these, only 237 (58.1%) were positive. Remarkably, 85 of the 248 patients tested negative for these mutations in the plasma, despite the ability of plasma testing to facilitate repeated measurements. Furthermore, an examination of the 272 assays that were negative for the T790M muta-

Table 3. Patients who underwent both tissue- and plasma-based tests

	Activating *EGFR* mutation			T790M mutation		
	Tissue		p-value	Tissue		p-value
	Positive	Negative		Positive	Negative	
Plasma						
Positive	159 (65.4)	4 (1.6)	.371	9 (13.4)	12 (17.9)	.898
Negative	85 (31.6)	0		17 (25.4)	28 (43.3)	

Values are presented as number (%).
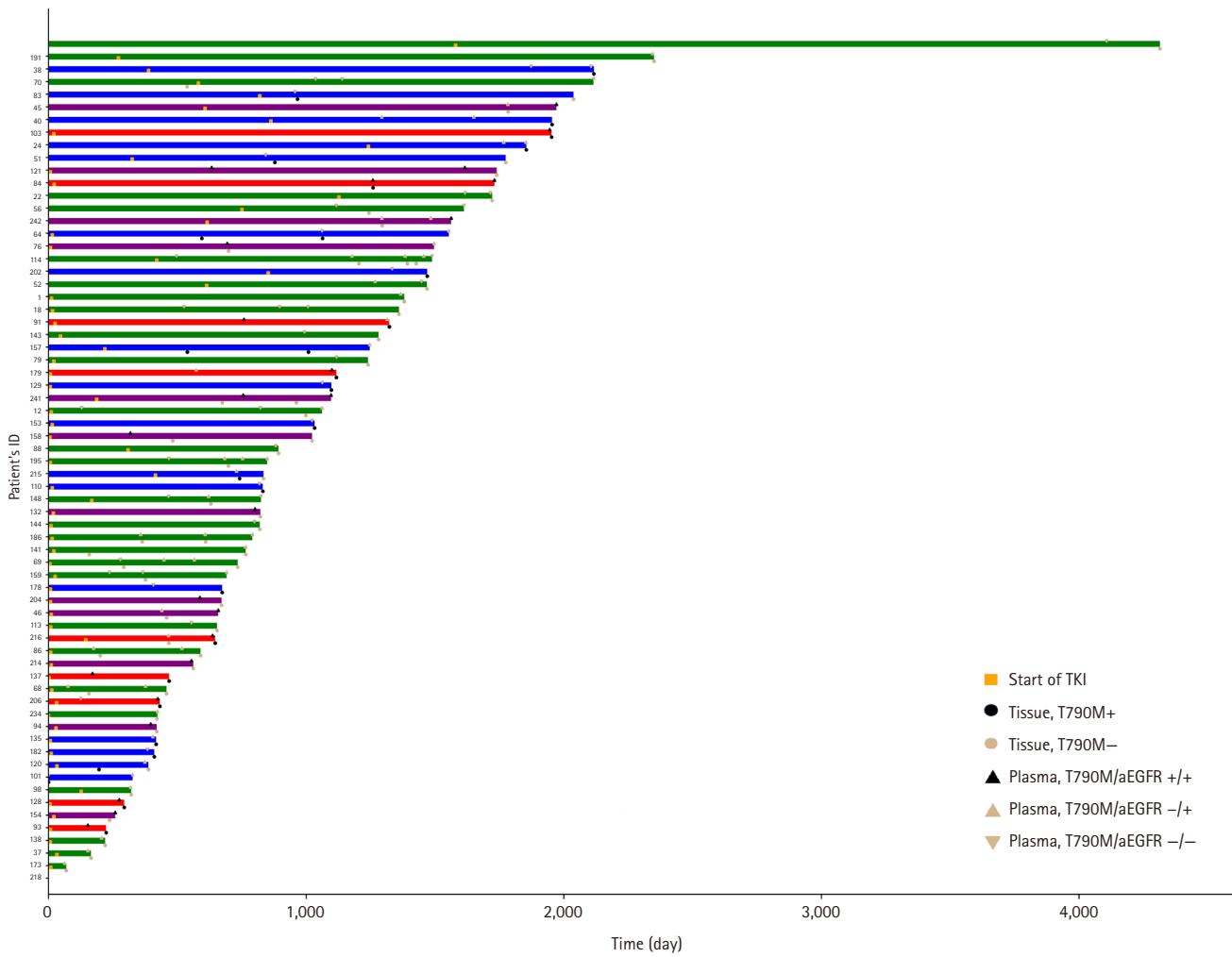EGFR, epidermal growth factor receptor.

Fig. 1. Swimmer plot for test time points for enrolled patients. TKI, tyrosine kinase inhibitor; EGFR, epidermal growth factor receptor.

Table 4. Relationship between stage categories and plasma-based *EGFR* test results in patients

	Activating mutation		p-value	T790M		p-value
	Positive	Negative		Positive	Negative	
T category						
T1, T2	71	50	.052	27	75	.901
T3, T4	88	35		29	74	
N category						
N0	34	34	.013	42	111	.992
N1–N3	124	50		14	37	
M category						
M0	30	35	.012	13	45	.299
M1	133	50		44	5	

EGFR, epidermal growth factor receptor.

tion indicated that 148 (54.4%) failed to detect the activating mutation, suggesting that over half of the tests that failed to detect the activating *EGFR* mutation may not reliably indicate a true-negative result for the T790M mutation. Considering that there were no instances in which the T790M mutation was detected without the concurrent detection of the activating mutation, the presence of the activating mutation was a reliable indicator of ctDNA levels. Our findings suggest that ctDNA levels frequently fall below the detection threshold of the assay, indicating that liquid biopsies for other genes may also face similar challenges and thus require high sensitivity for effective clinical application.

The prevalent understanding is that significant tumor mass is crucial for the abundant presence of ctDNA in the bloodstream. Our study showed that higher N and M statuses at initial diagnosis were associated with the detection of activating *EGFR* mutations in the plasma, with a notably stronger correlation observed for M status. This relationship continues even after the initiation of TKI therapy, where the original M status significantly predicts the presence of activating mutations, suggesting that an increased tumor burden may lead to higher ctDNA levels. However, no significant link was found between T790M mutation detection and T, N, and M statuses. To explore the factors influencing the detection of the T790M mutation, we analyzed the organs that were predominantly affected as the cancer progressed. Our data showed that the lungs were the most affected organs, followed by the brain, bones, pleura, lymph nodes, liver, and other organs. Moreover, there was no association between the presence of the T790M mutation and specific organs affected by metastasis or the overall number of involved organs. While TNM status and the number of affected organs might reflect the patient tumor load, which is expected to correlate with ctDNA levels, the absence of a connection with the T790M mutation indicates a potential need for more sensitive detection techniques, such as digital droplet PCR or next-generation sequencing (NGS), or that factors other than tumor load might affect the detectability of the mutation in plasma. Regarding tissue-based T790M testing, among the 30 positive cases, 22 originated from the lungs or bronchi. In contrast, of the 60 negative cases, 33 samples were collected from these locations, suggesting that the biopsy site did not significantly affect the test results.

The primary advantage of plasma-based testing is its noninvasive nature, which allows for repeated testing and increases the likelihood of a positive result. In our investigation, initial plasma screening revealed the T790M mutation in 42 of 207 patients, yielding a positivity rate of 20.2%. A subsequent plasma test of 83 patients identified 10 more individuals (12.0%) harboring this mutation. In the third round of testing involving 33 patients, the mutation was found in five individuals, resulting in a positivity rate of 15.2%. In contrast, the first round of tissue-based testing demonstrated a considerably higher positivity rate, with 25 of the 69 patients (35.7%) testing positive for the T790M mutation. However, a follow-up tissue-based assessment of 18 patients showed a reduced positivity rate of 11.1%, with just two patients testing positive. This trend suggests a decrease in positivity rates for both plasma- and tissue-based tests in subsequent rounds compared with initial testing. Owing to the noninvasive nature of plasma-based testing, it is recommended to start with this method for detecting the T790M mutation. If the initial plasma test result is negative, a tissue-based test should be considered owing to its higher success rate. In contrast to the ease of plasma testing, tissue biopsies offer additional advantages, including the ability to identify resistance mechanisms beyond the T790M mutation, such as histological transformation. The transition to small cell carcinoma is a well-established example of such a transformation.

Although a limitation of this study was that tissue- and plasma-based tests were performed on different platforms, previous studies have shown that both platforms have similar overall performance in terms of *EGFR* mutation detection [18]. In conclusion, this study suggests that both methods are complementary for detecting the T790M mutation, as neither method was completely satisfactory. Plasma-based tests frequently fail to detect activating mutations; therefore, clinicians should be aware of this drawback and perform tissue biopsy in such cases. Currently, the selection of test modalities, timing, and biopsy sites depends solely on the judgment of the clinician, with a lack of available tools to predict the outcome of these decisions. Recent advancements in highly sensitive techniques, including NGS and digital droplet PCR, have the potential to enhance the success rates of detecting T790M mutations in both tissue- and plasma-based tests.

Ethics Statement

This study was approved by the Institutional Review Board (IRB) of Pusan National University Yangsan Hospital (No. 2024-0021). The requirement for informed consent was waived by the IRB.

ORCID

Yoon Kyung Kang   https://orcid.org/0009-0002-1746-6467
Dong Hoon Shin   https://orcid.org/0000-0002-4980-9295
Joon Young Park   https://orcid.org/0000-0002-4193-2520
Chung Su Hwang   https://orcid.org/0000-0001-9520-0301
Hyun Jung Lee   https://orcid.org/0000-0002-2995-6060
Jung Hee Lee   https://orcid.org/0000-0003-3003-2217
Jee Yeon Kim   https://orcid.org/0000-0002-0503-984X
JooYoung Na   https://orcid.org/0000-0003-1138-433X

Author Contributions

Conceptualization: DHS. Data curation: YKK. Formal analysis: YKK, JYP, CSH. Funding acquisition: DHS. Supervision: DHS. Writing—original draft: YKK, HJL. Writing—review & editing: JHL, JYK, JN, DHS. Approval of the final manuscript: all authors.

Conflicts of Interest

The authors declare that they have no potential conflicts of interest.

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