Original Article

Rapid detection of non-small cell lung cancer driver mutations using droplet digital polymerase chain reaction analysis of bronchial washings: a prospective multicenter study

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Background: Molecular profiling of non-small cell lung cancer (NSCLC) is crucial for personalized treatment, but obtaining adequate tumor tissue can be challenging. This study evaluated the utility of droplet digital polymerase chain reaction (ddPCR) analysis of bronchial washings (BWs) and serum for detecting driver oncogene mutations in NSCLC patients, comparing its performance to standard tissue genotyping methods.

Methods: In this prospective, multicenter study conducted at two university hospitals in Yokohama, Japan, 73 treatment-naïve NSCLC patients underwent bronchoscopy with BW collection and blood sampling between October 2022 and April 2024. ddPCR was performed on BW and serum samples to detect epidermal growth factor receptor (EGFR; L858R, exon 19 deletions, G719X), KRAS (G12/13), and BRAF (V600E) mutations. Results were compared with standard tissue genotyping methods, including AmoyDx and Oncomine Dx Target Test (DxTT) assays. Turnaround time (TAT) for results was also assessed. The study protocol was approved by the institutional review boards, and all participants provided informed consent.

Results: ddPCR analysis of BW samples showed high concordance with tissue genotyping, detecting EGFR mutations in 31.5% of cases (identical to tissue). For common EGFR mutations (L858R and exon 19 deletions), BW genotyping demonstrated 100% sensitivity and 98.0% specificity compared to tissue. TAT was significantly shorter for BW ddPCR compared to tissue genotyping (4.4±1.8 *vs.* 20.4±7.7 days, P<0.001). Serum ddPCR showed lower sensitivity (7.8% *vs.* 33.3% for EGFR mutations) compared to tissue genotyping, with detection associated with the presence of bone metastases. KRAS and BRAF mutations were detected at similar rates in BW and tissue samples, but at lower rates in serum.

Conclusions: ddPCR analysis of BWs demonstrates high accuracy and rapid TAT for detecting common driver mutations in NSCLC. This approach represents a promising alternative to tissue biopsy for molecular profiling, potentially expediting treatment decisions. While serum ddPCR showed limited utility, it may complement tissue genotyping in specific clinical scenarios.

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Keywords: Non-small cell lung cancer (NSCLC); molecular profiling; droplet digital polymerase chain reaction (ddPCR); bronchial washings (BWs); liquid biopsy

Submitted Aug 29, 2024. Accepted for publication Dec 24, 2024. Published online Feb 22, 2025.
doi: 10.21037/tlcr-24-772
View this article at: https://dx.doi.org/10.21037/tlcr-24-772

Introduction

Background

Lung cancer remains the leading cause of cancer-related mortality worldwide. Early and accurate diagnosis is crucial for improving outcomes in lung cancer patients (1-3). The diagnosis and molecular characterization of lung cancer relies on obtaining tumor tissue through invasive procedures such as bronchoscopy, mediastinoscopy, or surgical biopsy. These tissue biopsies confirm the presence of malignancy and provide valuable information about the histological subtype and molecular characteristics of the tumor.

In recent years, personalized medicine has transformed lung cancer treatment. The identification of specific molecular alterations, such as epidermal growth factor receptor (EGFR) mutations, anaplastic lymphoma kinase (ALK) rearrangements, and other targetable driver mutations, has led to the development of targeted therapies that have significantly improved outcomes in selected patient populations (4-6). Consequently, comprehensive molecular profiling of lung tumors has become an essential component of the diagnostic workup.

Rationale and knowledge gap

However, obtaining adequate tumor tissue for molecular testing can be challenging, particularly in advanced-stage lung cancer patients who often present with inoperable or metastatic disease. In such cases, the diagnosis and molecular characterization relies on small biopsy or cytology specimens obtained through minimally invasive procedures (7,8). These limited samples may not provide sufficient tumor content for comprehensive molecular analysis, potentially leading to suboptimal treatment decisions.

To address this issue, there has been growing interest in exploring alternative methods for tumor sampling and molecular profiling. One such approach is the use of liquid biopsies, which involve analyzing circulating tumor DNA (ctDNA) or other tumor-derived components present in blood or other body fluids (9,10). While liquid biopsies offer a minimally invasive alternative to tissue biopsies, their diagnostic and predictive utility in lung cancer remains an area of active investigation.

Objective

Our study introduces a novel approach by focusing on bronchial washings (BWs), routinely collected samples during bronchoscopy procedures, as a potential source for molecular testing. We employ droplet digital polymerase

Highlight box

Key findings

- Droplet digital polymerase chain reaction (ddPCR) analysis of bronchial washings (BWs) demonstrated 100% sensitivity and 98.0% specificity for common EGFR mutations compared to tissue genotyping.
- Turnaround time for BW ddPCR was significantly shorter than tissue genotyping (4.4 *vs.* 20.4 days, P<0.001).

What is known and what is new?

- Molecular profiling is essential for personalized treatment in non-small cell lung cancer (NSCLC). Obtaining adequate tumor tissue for genotyping can be challenging, especially in advanced disease.
- This study demonstrates the high accuracy and rapid turnaround time of ddPCR analysis on bronchial washings for NSCLC driver mutation detection. It provides evidence that BW can serve as a reliable alternative to tissue biopsy for molecular profiling in NSCLC.

What is the implication, and what should change now?

- Clinical implementation of BW ddPCR could expedite molecular profiling and treatment decisions in NSCLC patients. Protocols for collecting and processing bronchial washings for molecular analysis should be standardized.
- Integration of BW ddPCR into diagnostic algorithms for NSCLC should be considered as a complementary tool to tissue biopsy, offering rapid molecular profiling results. Future research should focus on expanding the panel of detectable mutations in BW samples to provide more comprehensive molecular characterization.

chain reaction (ddPCR), a highly sensitive technique, to detect driver oncogene mutations in these samples. Additionally, we explore the utility of serum samples as a complementary liquid biopsy source.

By investigating the potential of BW and serum as sources for molecular testing, this study aims to contribute to the ongoing efforts to optimize molecular profiling in lung cancer. The successful implementation of this approach could significantly impact clinical practice by reducing the need for invasive tissue rebiopsies, providing faster turnaround times (TATs) for molecular testing results, and enabling more frequent monitoring of molecular changes during treatment. We present this article in accordance with the STARD reporting checklist (available at https://tlcr.amegroups.com/article/view/10.21037/tlcr-24-772/rc).

Methods

Study design and participants

This was a multicenter, observational study conducted at Yokohama City University Hospital and Yokohama City University Medical Center in Yokohama, Japan. Between October 2022 and April 2024, a total of 73 treatment-naïve patients with non-small cell lung cancer (NSCLC) were prospectively enrolled. The study protocol was approved by the institutional review board of Yokohama City University Hospital (No. F220900066), and the study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). Yokohama City University Medical Center was informed and agreed with this study. Written informed consent was obtained from all participants prior to their enrollment in the study. Patient anonymity was preserved throughout the study using methods approved by the Ethics Committee.

Inclusion criteria were: (I) age ≥18 years; (II) histologically or cytologically confirmed NSCLC; (III) no prior treatment such as chemotherapy or radiotherapy for NSCLC; and (IV) all stages.

Sample collection and processing

Bronchoscopy specimens were processed for both cytological examination and molecular testing. For cytological examination, BW specimens underwent standard cytological processing including slide preparation and morphological assessment. Bronchoscopy was performed under sedation, and BW samples were collected from the tumor site in patients with suspected lung cancer on the same day as the tissue biopsy. BW samples were collected by administering 10 mL of saline solution after the biopsy was completed. In some cases, samples were also obtained by washing the biopsy forceps. BW samples were stored at −80 ℃ until tested. For ddPCR analysis, 2 mL of the BW sample was centrifuged at 20,000 g for 5 minutes at 4 ℃, and the precipitate was collected. DNA extraction from the precipitate was performed using the DNeasy Blood & Tissue Kit (Qiagen, Venlo, the Netherlands) according to the manufacturer's instructions. Tissue samples were tested for oncogene mutations after the pathological diagnosis. This procedure was conducted as part of the usual clinical care.

Additionally, blood samples were obtained from each patient before treatment initiation. Serum was separated by centrifugation of the whole blood at 3,000 rpm for 10 minutes at room temperature. Serum aliquots of 600 µL were stored at −80 ℃ until cell-free DNA (cfDNA) extraction, which was carried out using the Qiagen Circulating Nucleic Acids kit (Qiagen, Venlo, the Netherlands) following the manufacturer's protocol.

ddPCR analysis

ddPCR was performed using the QX200 Droplet Digital PCR System (Bio-Rad, California, USA). Driver oncogene mutations were detected using probes specific for EGFR L858R, EGFR exon 19 del screen, EGFR G719 multi, EGFR G465 screen, KRAS G12/G13 screen, and BRAF V600 screen (Riken Genesis, Tokyo, Japan).

Droplet generation, PCR amplification, and droplet reading were performed using the QX200 droplet generator, thermal cycler, and droplet reader, respectively (Bio-Rad). Data analysis was carried out using the QuantaSoft software (Bio-Rad) (11). The ddPCR results were considered valid when the number of droplets exceeded 9,000. The presence of more than three positive droplets was defined as a positive result for the respective driver oncogene mutation (12).

Study outcomes

The primary outcomes were the sensitivity, specificity, and concordance rate of driver oncogene mutation detection using ddPCR in BW samples compared to standard genotyping methods in tumor tissue biopsy samples. Additionally, the TAT was compared between the two methods. For both methods, TAT was defined as the time

Table 1 Patient characteristics

Characteristics	Value (n=73)
Age, years	74 [38–87]
Sex	
Female	27 (37.0)
Male	46 (63.0)
Smoking status	
Never/former	16 (21.9)
Current	57 (78.1)
Pack-years	44.8±29.3
Histology	
Adenocarcinoma	53 (72.6)
Squamous cell carcinoma	17 (23.3)
Others	3 (4.1)
Stage	
I	2 (2.7)
II	0
III	15 (20.5)
IV	55 (75.3)
Recurrence	1 (1.4)
Cytology of BW	
Positive for carcinoma	58 (79.5)
Negative for carcinoma	15 (20.5)
PD-L1 tumor proportion score	
<1%	19 (26.0)
1–49%	25 (34.2)
≥50%	23 (31.5)
Unknown	6 (8.2)
Diagnostic assays in tumor tissue	
AmoyDx	32 (43.8)
Oncomine DxTT	27 (37.0)
Others	14 (19.2)

Data are presented as median [range], mean ± standard deviation, or n (%). BW, bronchial washing; PD-L1, programmed death-ligand 1; DxTT, Dx Target Test.

from sample collection to the availability of mutation results. While cytological confirmation was required for BW samples before ddPCR testing, the rapid nature of ddPCR analysis (typically completed within hours) allowed results to be available on the same day as cytological confirmation.

The secondary outcome was the sensitivity, specificity, and concordance rate of driver oncogene mutation detection using ddPCR in serum (liquid biopsy) samples compared to standard genotyping methods in tumor tissue biopsy samples.

Statistical analysis

All statistical analyses were performed with EZR version 1.53 (13), a modified version of R commander with additional biostatistical functions. Continuous variables were compared using *t*-tests, the Mann-Whitney *U* test, paired-sample *t*-tests, or the Wilcoxon signed-rank test, as appropriate. Categorical variables were compared using Pearson's Chi-squared test or Fisher's exact test. A P value <0.05 was considered statistically significant, and all tests were two-tailed. Missing data were handled using complete case analysis. The sample size was determined based on feasibility and the expected number of eligible patients during the study period, rather than formal power calculations.

Results

Patient characteristics

The study included a total of 73 patients. The median age of the study patients was 74 (range, 38–87) years, 46 (63%) patients were males, and 27 (37%) patients were females (*Table 1*). Regarding smoking status, 16 (21.9%) patients were never or former smokers, while 57 (78.1%) were current smokers, with a mean pack-years of 44.8±29.3. The predominant histological subtype was adenocarcinoma, accounting for 53 (72.6%) cases, followed by squamous cell carcinoma in 17 (23.3%) cases, and non-small cell lung carcinoma in 3 (4.1%) cases. The distribution of clinical stage was as follows: stage III in 15 (20.5%) patients, and stage IV in 55 (75.3%) patients. One patient (1.4%) had recurrent disease.

Cytological examination of BW revealed malignant cells in 58 (79.5%) patients and no malignant cells in 15 (20.5%) patients. All patients with positive cytology findings were subsequently confirmed to have carcinoma on histological examination. Among the 15 patients with negative cytology findings, none showed evidence of lung carcinoma in the concurrent histological specimens. However, all 15 of these patients were later diagnosed with lung carcinoma through

rebiopsy.

Regarding programmed death-ligand 1 (PD-L1) tumor proportion score, 19 (26.0%) patients had a score of <1%, 25 (34.2%) had a score of 1–49%, and 23 (31.5%) had a score of ≥50%, while the score was unknown for 6 (8.2%) patients.

The diagnostic assays used in tumor tissue included AmoyDx in 32 (43.8%) patients, Oncomine Dx Target Test (DxTT) in 27 (37.0%) patients, and other assays in 14 (19.2%) patients.

Comparison of driver oncogene genotyping between tissue biopsy and BWs

Table 2 presents the genotyping results of driver oncogenes in tumor tissue and BWs in this study. Overall, 23 (31.5%) patients harbored EGFR mutations, with 12 (16.4%) patients having the L858R mutation and 10 (13.7%) patients exhibiting exon 19 deletions. Additionally, 1 (1.4%) patient had a G719X mutation. KRAS G12/13 mutations were detected in 8 (11.0%) patients in tissue and 7 (9.6%) patients in BW samples by ddPCR. BRAF V600E mutations were identified in 1 (1.4%) patient in both tissue and BW samples.

Comparing the genotyping results between tissue and BW samples, a high concordance rate was observed. Out of the 73 patients, only 3 patients had discordant gene mutation results between tissue by the standard method and BW genotyping by ddPCR. Among these 3 patients, 2 had no malignant findings on cytology with BW, and the driver oncogene was detected in another specimen. The remaining patient had a negative oncogene mutation result on the Oncomine DxTT assay in the tissue sample but was positive for the L858R mutation in the BW genotyping. Excluding the first two cases of discordance likely attributable to sample inadequacy or testing limitations, the sensitivity of BW genotyping compared to tissue genotyping was 100%, and the specificity was 97.6%, with an overall concordance rate of 98.6%.

Table 2 Comparison of driver oncogene mutation detection in tumor tissue and BW

Mutation	BW (n=73)	Tissue (n=73)
EGFR	23 (31.5)	23 (31.5)
L858R	12 (16.4)	12 (16.4)
Exon 19 deletion	10 (13.7)	10 (13.7)
G719X	1 (1.4)	1 (1.4)
KRAS G12/13	7 (9.6)	8 (11.0)
BRAF V600E	1 (1.4)	1 (1.4)
Other mutations	0	1
Negative	42	40

Among the EGFR L858R positive cases, each group contains one case that was negative by the other testing method (i.e., one case was positive in tissue but negative in BW, and one case was positive in BW but negative in tissue). Data are presented as n (%) or number. BW, bronchial washing.

Comparison of TAT between BW by ddPCR and tissue by standard method

The TAT for diagnostic testing was compared between BW samples analyzed by ddPCR and tumor tissue samples analyzed by standard methods (*Table 3*). For BW samples analyzed by ddPCR (n=51), the TAT was defined as the time from biopsy to the availability of cytology results. In contrast, for tumor tissue samples analyzed by standard methods (n=51), the TAT was defined as the time from biopsy to the availability of mutation results, since the mutation results were available prior to the cytology results.

The analysis revealed a significantly shorter TAT for BW samples analyzed by ddPCR compared to tumor tissue samples analyzed by standard methods (4.4±1.8 *vs.* 20.4±7.7 days, P<0.001). The rapid TAT achieved with the BW ddPCR approach represents a substantial advantage, potentially enabling earlier initiation of targeted therapy or enrollment in clinical trials for patients harboring actionable mutations.

Table 3 Comparison of TAT between BW by ddPCR and tissue by standard method

Outcome	BW by ddPCR (n=51)	Tissue by standard (n=51)	P value
TAT (days)	4.4±1.8	20.4±7.7	<0.001

TAT definitions—BW by ddPCR: turnaround time is defined as the time from biopsy to the time of knowing the cytology result, as the mutation results were known prior to the cytology results; tissue by standard: turnaround time is defined as the time from biopsy to the time of knowing the mutation result. Data are presented as mean ± standard deviation. TAT, turnaround time; BW, bronchial washing; ddPCR, droplet digital polymerase chain reaction.

Table 4 Comparison of driver oncogene mutations between serum by ddPCR and tissue by standard method

Mutation	Serum by ddPCR (n=51), n (%)	Tissue by standard (n=51), n (%)
EGFR	4 (7.8)	17 (33.3)
L858R	2 (3.9)	11 (21.6)
Ex19del	2 (3.9)	5 (9.8)
G719X	0	1 (2.0)
KRAS G12/13	2 (3.9)	5 (9.8)
BRAF V600	0	1 (2.0)
Total	6 (11.8)	23 (45.1)

ddPCR, droplet digital polymerase chain reaction.

Comparison of driver oncogene mutations between serum by ddPCR and tissue by standard method

To develop a less invasive method for detecting driver oncogene mutations, ddPCR using patient serum was examined and compared to the standard method using tumor tissue samples. A total of 51 patients were analyzed for driver oncogene mutations using both methods. *Table 4* presents the comparison of driver oncogene mutations detected in serum by ddPCR and tissue by standard method among the 51 patients. The results indicate a significant difference in mutation detection rates between the two methods. EGFR mutations were detected in 4 patients (7.8%) using serum by ddPCR, compared to 17 patients (33.3%) using tissue by standard method. Specifically, the L858R mutation was found in 2 patients (3.9%) with serum by ddPCR and in 11 patients (21.6%) with tissue by standard method. The exon 19 deletion mutation was detected in 2 patients (3.9%) using serum by ddPCR and in 5 patients (9.8%) using tissue by standard method. The G719X mutation was detected in 1 patient (2.0%) using tissue by standard method but was not detected in any patients using serum by ddPCR.

KRAS G12/13 mutations were identified in 2 patients (3.9%) using serum by ddPCR, whereas 5 patients (9.8%) were found to have these mutations using tissue by standard method. The BRAF V600 mutation was detected in 1 patient (2.0%) using tissue by standard method but was not detected in any patients using serum by ddPCR.

Overall, 6 patients (11.8%) were found to have driver oncogene mutations using serum by ddPCR. These findings indicate that the serum ddPCR method can detect driver oncogene mutations only in a very limited subset of cases.

Factors related to the detection of driver oncogenes in serum by ddPCR

Identifying the specific cases in which driver oncogene mutations can be detected using serum by ddPCR is crucial for recommending this less invasive testing approach to appropriate patients. To this end, we compared the 6 cases in which mutations were detected by serum ddPCR to the 17 cases that were negative, among the 23 patients found to have driver oncogene mutations by the standard tissue method (*Table 5*).

The clinical and molecular characteristics of the patients who were positive or negative for any mutation in serum by ddPCR are presented in *Table 5*. No significant differences were observed between the two groups in terms of age, sex, smoking habit, or pack-years of smoking history. Regarding the specific mutations, the frequencies of L858R, exon 19 deletion, G719X (EGFR), KRAS G12/13, and BRAF V600E were comparable between the positive and negative groups.

The distribution of T stage (1–2 *vs.* 3–4) and N stage (0–1 *vs.* 2–3) did not differ significantly between the two groups. However, a higher proportion of patients in the serum ddPCR-negative group had non-metastatic disease (M0–1a) compared to the positive group, although the difference did not reach statistical significance (41.2% *vs.* 16.7%, P=0.34).

Interestingly, bone metastases were more frequent in the serum ddPCR-positive group compared to the negative group (83.3% *vs.* 29.4%, P=0.05), suggesting a potential association between the presence of bone metastases and the ability to detect driver oncogene mutations in serum using ddPCR. No other significant differences in metastatic sites were observed between the two groups.

Discussion

Key findings

This prospective study evaluated the diagnostic performance of ddPCR analysis of BWs for detecting actionable driver oncogene mutations in NSCLC patients. Our findings demonstrate the potential utility of liquid biopsy techniques, particularly ddPCR analysis of BW samples, in the molecular profiling of NSCLC.

The key findings demonstrate a high concordance between genotyping results from BW samples analyzed by ddPCR and tumor tissue analyzed by standard methods. Overall, ddPCR analysis of BW samples detected EGFR mutations in 31.5% of cases, which was identical to the mutation rate observed in tumor tissue. For the most

Table 5 Factors related to the detection of driver oncogene by liquid biopsy

Factor	Positive (n=6)	Negative (n=17)	P value
Age, years	69 [46–81]	73 [44–86]	0.62
Sex (male/female)	2/4	7/10	>0.99
Smoking habit			
Non-smoker/smoker	3/3	8/9	>0.99
Pack-year	27.3±12.4	32.5±15.8	0.54
Mutations			
L858R	2 (33.3)	9 (52.9)	0.64
Ex19del	2 (33.3)	3 (17.6)	0.58
G719X	0	1 (5.9)	>0.99
KRAS G12/13	2 (33.3)	3 (17.6)	0.58
BRAF V600	0	1 (5.9)	>0.99
T stage			>0.99
1–2	4 (66.7)	11 (64.7)	
3–4	2 (33.3)	6 (35.3)	
N stage			0.62
0–1	1 (16.7)	2 (11.8)	
2–3	5 (83.3)	15 (88.2)	
M stage			0.34
0–1a	1 (16.7)	7 (41.2)	
1b–1c	5 (83.3)	10 (58.8)	
Metastatic lesion			
Lung	3 (50.0)	8 (47.1)	>0.99
Bone	5 (83.3)	5 (29.4)	0.05
Pleural	2 (33.3)	5 (29.4)	>0.99
Liver	1 (16.7)	1 (5.9)	0.46
Spleen/adrenal	1 (16.7)	0	0.26
Brain	0	5 (29.4)	>0.99

Data are presented as median [range], number, mean ± standard deviation, or n (%).

common EGFR mutations (L858R and exon 19 deletions), the sensitivity of BW genotyping was 100% and specificity of BW genotyping were 98.0% compared to tissue genotyping.

Notably, we observed a significantly shorter TAT for BW samples analyzed by ddPCR compared to tumor tissue samples analyzed by standard methods (4.4±1.8 *vs.* 20.4±7.7 days, P<0.001).

Strengths and limitations

These findings suggest that BW samples can serve as a reliable alternative to tumor biopsy for molecular profiling in NSCLC. The high concordance between BW and tissue genotyping is likely attributable to the ability to detect carcinoma cells in the majority (79%) of BW samples evaluated by cytology.

A limitation of this study is the lack of evaluation of other molecular alterations beyond the common EGFR, KRAS and BRAF mutations. Comprehensive genomic profiling is increasingly employed to identify additional targetable drivers and mechanisms of resistance. Additionally, while this study focused on the diagnostic utility of BW, the clinical impact of BW genotyping on treatment outcomes and patient survival warrants further investigation.

Comparison with similar researches

While similar techniques have been explored, the specific combination of BW and ddPCR has not been previously reported to our knowledge. Several studies have previously investigated molecular testing of BW samples. Lee *et al.* demonstrated the utility of BW fluid for EGFR mutation detection (14), while Ryu *et al.* and others have explored various molecular testing approaches (15-18). Our study builds upon this foundation by implementing a simplified workflow that achieves rapid TAT while maintaining high diagnostic accuracy. Asaka *et al.* conducted a comparable study using bronchoalveolar lavage fluid (BALF) instead of BW for EGFR mutation analysis (19). BALF is a method of administering about 150 mL of saline solution, injecting and collecting lavage fluid to the alveolar level, and obtaining samples of cells and pathogens. It is highly invasive and involves a high risk of complications such as dyspnea and hypoxemia. On the other hand, BW is a method of administering about 10–20 mL of saline solution and are used to evaluate cells and pathogens in the airways. It is minimally invasive but has the disadvantage of obtaining few samples. Their findings demonstrated that BALF samples could detect EGFR mutations at rates equivalent to tissue samples. Notably, they reported cases where EGFR mutations were detected even in samples where lung cancer cells were not cytologically identified. In a related study focusing on pleural effusions, researchers reported a sensitivity of 81.8% and specificity of 80.0% for EGFR mutation detection (20). These results, while promising, suggest that our BW-based approach may offer superior performance, particularly in terms of specificity. When

considering the performance of ddPCR specifically, a study using tissue samples demonstrated 87.5% sensitivity and 100% specificity in detecting primary EGFR mutations in treatment-naïve patients, with overall positive and negative predictive values of 100% and 80%, respectively (21). Our findings of 100% sensitivity and 98.0% specificity for common EGFR mutations using BW samples are comparable to, and in some aspects surpass, these tissue-based results.

Regarding blood-based samples, we previously reported a sensitivity of 63.3% for EGFR mutation detection using qPCR on plasma samples (22). Reports from other research groups have shown a wide range of sensitivities, varying from 53% to 84.6% (23-25). This variability likely stems from differences in detection methods, the lower shedding of ctDNA, and the extent of metastatic disease in the studied populations. While blood-based liquid biopsies offer a non-invasive approach, it's important to note that their current sensitivity is generally lower than tissue-based methods. This limitation should be carefully considered when interpreting results or choosing diagnostic strategies for NSCLC patients (14,26,27).

The growing importance of non-invasive diagnostic techniques in lung cancer management cannot be understated. Liquid biopsy approaches, including the detection of circulating tumor cells (CTCs) and cfDNA, have emerged as promising alternatives to traditional tissue sampling (28-30). While our study focused on BWs, the broader field of liquid biopsy encompasses various biological materials that can be sampled non-invasively, offering potential for both initial diagnosis and disease monitoring. Recent advances in CTC detection technologies have demonstrated particular utility in early cancer detection and treatment response monitoring. The detection of driver mutations through liquid biopsy has shown concordance rates of 60–80% with tissue testing, particularly in advanced disease stages. These approaches are especially valuable in cases where tissue biopsy is challenging or contraindicated, or when serial sampling is needed to monitor treatment response or resistance development (31).

Explanations of findings

Our study demonstrates that ddPCR analysis of precipitated cells from BW provides rapid and accurate molecular profiling results. The high concordance between BW and tissue genotyping (98.6%) and significantly shorter TAT (4.4 *vs.* 20.4 days, P<0.001) highlight the practical advantages of this approach in clinical settings. While recent studies have shown the potential of cell-free supernatant for comprehensive molecular testing, our method focusing on precipitated cells offers a simplified and efficient workflow for detecting common driver mutations that influence immediate treatment decisions.

Our serum-based ddPCR analysis showed limited sensitivity for detecting driver oncogene mutations compared to tissue genotyping (7.8% *vs.* 33.3% for EGFR mutations). This lower detection rate can be attributed to two main factors: first, the use of serum rather than plasma for cfDNA analysis, as serum contains higher levels of non-cancer cfDNA that can dilute tumor-derived DNA signals (32). Second, the short half-life of cfDNA (several minutes to 1–2 hours) may have impacted detection rates due to processing time constraints. Notably, mutation detection in serum was associated with the presence of bone metastases (83.3% *vs.* 29.4%, P=0.05), suggesting that tumor burden influences cfDNA detection rates (33).

Implications and actions needed

While serum-based liquid biopsy had limited utility in this study, it may play a complementary role to tumor genotyping in monitoring treatment response and clonal evolution.

Future studies should assess the performance of ddPCR-based genotyping from BW samples across a broader panel of biomarkers.

The implementation of BW ddPCR in clinical settings offers several potential benefits, including reduced invasiveness compared to tissue biopsy, faster TAT, and the ability to perform repeated testing for monitoring purposes. However, challenges such as standardization of collection and processing protocols, as well as integration into existing diagnostic workflows, need to be addressed.

Conclusions

This study demonstrates the high diagnostic accuracy and rapid TAT of ddPCR-based genotyping using BWs for detecting common actionable driver mutations in NSCLC. BWs represent a viable liquid biopsy alternative to tumor tissue for comprehensive molecular profiling, potentially improving the speed and efficiency of personalized treatment selection in lung cancer patients. Future research should focus on expanding the panel of detectable mutations and assessing the long-term clinical impact of this approach on patient outcomes.

Acknowledgments

None.

Footnote

Reporting Checklist: The authors have completed the STARD reporting checklist. Available at https://tlcr.amegroups.com/article/view/10.21037/tlcr-24-772/rc

Data Sharing Statement: Available at https://tlcr.amegroups.com/article/view/10.21037/tlcr-24-772/dss

Peer Review File: Available at https://tlcr.amegroups.com/article/view/10.21037/tlcr-24-772/prf

Funding: This work was supported by the Advanced Medical Research Grant from the City of Yokohama (No. 202).

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://tlcr.amegroups.com/article/view/10.21037/tlcr-24-772/coif). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by Yokohama City University Hospital (No. F220900066) and informed consent was obtained from all individual participants. Yokohama City University Medical Center was informed and agreed with this study.

References

1. Bray F, Ferlay J, Soerjomataram I, et al. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 2018;68:394-424.
2. Bade BC, Dela Cruz CS. Lung Cancer 2020: Epidemiology, Etiology, and Prevention. Clin Chest Med 2020;41:1-24.
3. Thandra KC, Barsouk A, Saginala K, et al. Epidemiology of lung cancer. Contemp Oncol (Pozn) 2021;25:45-52.
4. Maemondo M, Inoue A, Kobayashi K, et al. Gefitinib or chemotherapy for non-small-cell lung cancer with mutated EGFR. N Engl J Med 2010;362:2380-8.
5. Rosell R, Carcereny E, Gervais R, et al. Erlotinib versus standard chemotherapy as first-line treatment for European patients with advanced EGFR mutation-positive non-small-cell lung cancer (EURTAC): a multicentre, open-label, randomised phase 3 trial. Lancet Oncol 2012;13:239-46.
6. Kobayashi N, Miura K, Kaneko A, et al. Tailoring Therapeutic Strategies in Non-Small-Cell Lung Cancer: The Role of Genetic Mutations and Programmed Death Ligand-1 Expression in Survival Outcomes. Cancers (Basel) 2023;15:5248.
7. Lindeman NI, Cagle PT, Aisner DL, et al. Updated Molecular Testing Guideline for the Selection of Lung Cancer Patients for Treatment With Targeted Tyrosine Kinase Inhibitors: Guideline From the College of American Pathologists, the International Association for the Study of Lung Cancer, and the Association for Molecular Pathology. J Thorac Oncol 2018;13:323-58.
8. Heerink WJ, de Bock GH, de Jonge GJ, et al. Complication rates of CT-guided transthoracic lung biopsy: meta-analysis. Eur Radiol 2017;27:138-48.
9. Siravegna G, Marsoni S, Siena S, et al. Integrating liquid biopsies into the management of cancer. Nat Rev Clin Oncol 2017;14:531-48.
10. Reckamp KL, Melnikova VO, Karlovich C, et al. A Highly Sensitive and Quantitative Test Platform for Detection of NSCLC EGFR Mutations in Urine and Plasma. J Thorac Oncol 2016;11:1690-700.
11. dMIQE Group, Huggett JF. The Digital MIQE Guidelines Update: Minimum Information for Publication of Quantitative Digital PCR Experiments for 2020. Clin Chem 2020;66:1012-29.
12. de Kock R, Deiman B, Kraaijvanger R, et al. Optimized (Pre) Analytical Conditions and Workflow for Droplet Digital PCR Analysis of Cell-Free DNA from Patients with Suspected Lung Carcinoma. J Mol Diagn 2019;21:895-902.
13. Kanda Y. Investigation of the freely available easy-to-

use software 'EZR' for medical statistics. Bone Marrow Transplant 2013;48:452-8.
14. Lee SH, Kim EY, Kim T, et al. Compared to plasma, bronchial washing fluid shows higher diagnostic yields for detecting EGFR-TKI sensitizing mutations by ddPCR in lung cancer. Respir Res 2020;21:142.
15. Ryu WK, Yong SH, Lee SH, et al. Usefulness of bronchial washing fluid for detection of EGFR mutations in non-small cell lung cancer. Lung Cancer 2023;186:107390.
16. Roncarati R, Lupini L, Miotto E, et al. Molecular testing on bronchial washings for the diagnosis and predictive assessment of lung cancer. Mol Oncol 2020;14:2163-75.
17. Murata Y, Nakajima Y, Sato Y, et al. High-efficiency EGFR genotyping using cell-free DNA in bronchial washing fluid. Jpn J Clin Oncol 2024;54:681-8.
18. Park J, Lee C, Eom JS, et al. Detection of EGFR Mutations Using Bronchial Washing-Derived Extracellular Vesicles in Patients with Non-Small-Cell Lung Carcinoma. Cancers (Basel) 2020;12:2822.
19. Asaka S, Yoshizawa A, Matsuda K, et al. A novel, rapid point-of-care test for lung cancer patients to detect epidermal growth factor receptor gene mutations by using real-time droplet-PCR and fresh liquid cytology specimens. Oncol Rep 2017;37:1020-6.
20. Liu X, Lu Y, Zhu G, et al. The diagnostic accuracy of pleural effusion and plasma samples versus tumour tissue for detection of EGFR mutation in patients with advanced non-small cell lung cancer: comparison of methodologies. J Clin Pathol 2013;66:1065-9.
21. Suryavanshi M, Mehta A, Panigrahi MK, et al. The detection of primary and secondary EGFR mutations using droplet digital PCR in patients with nonsmall cell lung cancer. Lung India 2018;35:384-9.
22. Fujii H, Nagakura H, Kobayashi N, et al. Liquid biopsy for detecting epidermal growth factor receptor mutation among patients with non-small cell lung cancer treated with afatinib: a multicenter prospective study. BMC Cancer 2022;22:1035.
23. Xu J, Wu W, Wu C, et al. A large-scale, multicentered trial evaluating the sensitivity and specificity of digital PCR versus ARMS-PCR for detecting ctDNA-based EGFR p.T790M in non-small-cell lung cancer patients. Transl Lung Cancer Res 2021;10:3888-901.
24. Kolesar J, Peh S, Thomas L, et al. Integration of liquid biopsy and pharmacogenomics for precision therapy of EGFR mutant and resistant lung cancers. Mol Cancer 2022;21:61.
25. Williamson DFK, Marris SRN, Rojas-Rudilla V, et al. Detection of EGFR mutations in non-small cell lung cancer by droplet digital PCR. PLoS One 2022;17:e0264201.
26. Zhu YJ, Zhang HB, Liu YH, et al. Association of mutant EGFR L858R and exon 19 concentration in circulating cell-free DNA using droplet digital PCR with response to EGFR-TKIs in NSCLC. Oncol Lett 2017;14:2573-9.
27. Sacher AG, Paweletz C, Dahlberg SE, et al. Prospective Validation of Rapid Plasma Genotyping for the Detection of EGFR and KRAS Mutations in Advanced Lung Cancer. JAMA Oncol 2016;2:1014-22.
28. Jiang H. Latest Research Progress of Liquid Biopsy in Tumor-A Narrative Review. Cancer Manag Res 2024;16:1031-42.
29. Ren F, Fei Q, Qiu K, et al. Liquid biopsy techniques and lung cancer: diagnosis, monitoring and evaluation. J Exp Clin Cancer Res 2024;43:96.
30. Rossi E, Aieta M, Tartarone A, et al. A fully automated assay to detect the expression of pan-cytokeratins and of EML4-ALK fusion protein in circulating tumour cells (CTCs) predicts outcome of non-small cell lung cancer (NSCLC) patients. Transl Lung Cancer Res 2021;10:80-92.
31. Sini C, Tuzi A, Rossi G, et al. Acquired resistance in oncogene-addicted non-small-cell lung cancer. Future Oncol 2018;14:29-40.
32. Pittella-Silva F, Chin YM, Chan HT, et al. Plasma or Serum: Which Is Preferable for Mutation Detection in Liquid Biopsy? Clin Chem 2020;66:946-57.
33. Kustanovich A, Schwartz R, Peretz T, et al. Life and death of circulating cell-free DNA. Cancer Biol Ther 2019;20:1057-67.

Cite this article as: Somekawa K, Kobayashi N, Nagaoka S, Seki K, Kajita Y, Muraoka S, Izawa A, Kaneko A, Otsu Y, Hirata M, Kubo S, Nagasawa R, Murohashi K, Fuji H, Teranishi S, Tashiro K, Watanabe K, Horita N, Hara Y, Kudo M, Kaneko T. Rapid detection of non-small cell lung cancer driver mutations using droplet digital polymerase chain reaction analysis of bronchial washings: a prospective multicenter study. Transl Lung Cancer Res 2025;14(2):353-362. doi: 10.21037/tlcr-24-772