Compared to plasma, bronchial washing fluid shows higher diagnostic yields for detecting EGFR-TKI sensitizing mutations by ddPCR in lung cancer

Sang Hoon Lee
Yonsei University College of Medicine
Eun Young Kim
Yonsei University College of Medicine
Taehee Kim
Yonsei University College of Medicine
Yoon Soo Chang (yschang@yuhs.ac)
Yonsei University College of Medicine

Research

Keywords: BWF, plasma, ddPCR, mEGFR, lung cancer

Posted Date: February 15th, 2020

DOI: https://doi.org/10.21203/rs.2.23312/v1

Version of Record: A version of this preprint was published at Respiratory Research on June 9th, 2020.
See the published version at https://doi.org/10.1186/s12931-020-01408-x.

Abstract

Background: The diagnostic rate of advanced lung adenocarcinoma must be improved. In this study, we compared the EGFR-tyrosine kinase-sensitizing mutation (mEGFR) detection rate of bronchial washing fluid (BWF) to that of blood in lung adenocarcinoma using the tissue genotype as a standard reference.

Methods: Paired blood-bronchial washing specimens were collected from 73 patients with lung cancer. The tumor EGFR mutation status was determined by tumor tissue genotyping of plasma and BWFs by digital droplet PCR.

Results: Thirty-five of 73 cases had wild-type EGFR, and 19 had L858R and E19d mutations. The study cohort included 26, 10, 10, and 27 patients with stage I, II, III, and IV disease. The area under the curve (AUC) of the BWF was 0.895 (95% CI: 0.822–0.969) and that of the plasma was 0.686 (95% CI: 0.592–0.780). The AUC obtained from BWF was significantly higher than that from plasma ($p < 0.001$). The fractional abundance (Fa) was higher in the BWF of mEGFR-positive cases ($p = 0.004$), facilitating easy threshold setting and discrimination between mEGFR-positive and -negative cases. When the genotyping results of blood and BWF were compared in early lung cancer (I–IIIA), the diagnostic yields were significantly higher in BWF and showed the same tendency in the advanced stage, suggesting that it may reflect the genotype status in early-stage patients.

Conclusions: The mEGFR genotype results obtained from BWF showed higher diagnostic efficacy than those from the blood. BWF may be a useful substitute for plasma-based genotyping in lung cancer.

1. Background

In 2018, approximately two million people were newly diagnosed with lung cancer worldwide and 1.76 million people died of this devastating disease, accounting for 18.4% of cancer-related deaths.[1, 2] Because the associated symptoms are not initially detectable and are similar to those of other respiratory diseases, lung cancer is typically diagnosed in advanced stages, when the disease cannot be cured by surgical resection. Lung cancer, which is difficult to cure by surgical resection, is treated with chemotherapy, targeted agents, and immune checkpoint inhibitors based on biomarkers along with radiotherapy.

Because of advancements in translational research, the outcomes and quality of life of patients with lung cancer have greatly improved through the use of target therapy, including agents targeting EGFR, ALK, and ROS1 compared to conventional treatments such as chemotherapy[3]. In a landmark placebo-controlled study of advanced non-small cell lung cancer (NSCLC) refractory to previous chemotherapy, evaluated in the Iressa Survival Evaluation in Lung Cancer (ISEL) trial, a EGFR-tyrosine kinase inhibitor (TKI) showed efficacy in a population subset, suggesting that biomarkers are needed to predict therapeutic responses.[4] The IPASS trial, associated with the ISEL study which identified an EGFR activating mutation,[5] showed that EGFR-TKI is more effective in patients with lung adenocarcinoma and EGFR-TKI-sensitizing mutation (mEGFR).[6] Further phase III trials comparing first- or second-line EGFR-

TKI treatment with platinum doublets confirmed the benefits on progression-free survival and the response rate of patients with lung adenocarcinoma and mEGFR.[7] Therefore, it is important to identify target genes and accurately manage lung cancer; mEGFR is considered a strong biomarker for predicting the response to EGFR-TKI.

Tissue biopsy specimens are used as a part of the standardized protocol to detect EGFR target mutations, but the process of obtaining tissue biopsy samples is invasive or impossible depending on the patient's condition, tumor location, and size. Based on flexible bronchoscopy biopsy, sensitivity of lung cancer diagnosis was 34% in peripheral tumors of diameter <2 cm and 63% in peripheral lesions of diameter >2 cm;[8] these suboptimal diagnostic yields make the identification of molecular biomarkers difficult. In contrast, 82.9% of specimens obtained by percutaneous core needle biopsies are appropriate for molecular marker analysis, whereas 15.3% of examinees experienced pneumothorax and 9.4% showed chest tube insertion as a complication of the procedure.[9]

Liquid biopsy using plasma is a simple, repeatable, and less invasive method and may overcome the disadvantages and limitations of tissue biopsy. However, this method exhibits some disadvantages, with different assay platforms associated with different sensitivities, specificities, and analytical approaches.[10] Furthermore, circulating tumor DNA (ctDNA) is present in only 0.1–1.0% of cell-free DNA (cfDNA) in the plasma and its half-life is approximately 90 min. Thus, the results obtained from plasma are less accurate than those obtained by conventional tissue biopsy. By-products from the flexible bronchoscopy procedure, such as bronchial washing fluid (BWF) and/or bronchoalveolar lavage fluid (BALF), may be a useful alternative to biopsy specimens. In patients with suspected lung cancer, bronchoscopy is routinely performed, and the BALF is a specific material that can reflect the characteristics of the lung compartment.[11] Carvalho et al. performed mass spectrometry using the BALF from 90 patients suspected of having lung cancer. They identified significantly different biomarkers between the lung cancer and non-lung cancer groups.[12] These studies suggest that compared to plasma, BALF may be more effective for diagnosing lung cancer.

Here, we compared the EGFR detection performance of using plasma, which is the standard for liquid biopsy, to that using BWF obtained during routine bronchoscopy by using the droplet digital PCR (ddPCR) platform. We confirmed that the BWF is more useful for detecting mEGFR in lung adenocarcinoma.

2. Materials And Methods

2.1 Clinical specimens. Paired blood-bronchial washing specimens from 73 patients with NSCLC were collected from June 2016 to May 2019. Detailed inclusion criteria for the enrolled cases were as follows: (1) pathologically proven NSCLC, (2) tumor EGFR mutation status identified by genotyping of tumor tissue obtained when plasma and BWF were obtained, and (3) informed consent for the collection and use of BWF and blood samples. Patients with rare EGFR TKI-sensitizing mutations were excluded from the study.

BWF and blood samples were obtained at the time of the initial visit for pathologic examination of lung tumor and the interval of securing paired specimens was less than 24 h. BWF was collected from the residue after sending the specimen for routine cytologic examination and microbial study. If the obtained specimen was less than 5 mL, an additional specimen was obtained by bronchial washing once or twice and processed within 3 h from collection by centrifuging at 1,800 ×*g* for 10 min at 4°C. The supernatant was stored at -80°C until analysis. Seven milliliters of blood were obtained using a Streck tube® (Streck, La Vista, NE, USA), transferred to the laboratory within 8 h of collection, centrifuged at 1,800 ×*g* for 10 min at 4°C, and stored at -80°C. DNA was extracted from the plasma using a QIAamp circulating nucleic acid kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. This study was performed in accordance with the amended Declaration of Helsinki and was approved by the independent hospital institutional review board (approval no. 3-2016-0225 and 3-2017-0321).

2.2 ddPCR. ddPCR was performed according to the manufacturer's instructions (Bio-Rad, Hercules, CA, USA). mEGFR was detected using probes for Exon19del (BR12002392, Bio-Rad) and EGFR L858R (BR186dHsaCP2000021; Bio-Rad). As a positive control, SNU1330 cells harboring an EGFR exon 19 deletion mutation (E19del) (homozygote) and H1975 cells containing the L858R and T790M mutations were used in each experiment. First, droplets were generated using a QX100 Droplet Generator (Bio-Rad), and then PCR amplification was performed using a thermal cycler (Bio-Rad). After PCR, droplets were streamed in single flow on a QX200 Droplet Reader (Bio-Rad) to count the fluorescence-positive and fluorescence-negative droplets. Data were processed using QuantaSoft software (Bio-Rad). The thresholds of the ddPCR results were determined using QuantaSoft software and then manually inspected for further validation. The ddPCR results passed quality control when the number of droplets was more than 9,000 and the wild-type gene sequence was present at more than 100 copies/mL.[12] Only data passing initial quality control were further analyzed. Positivity was defined when the fractional abundance (Fa) was ≥0.044% (sensitivity 42.1%, specificity 91.4%) for blood samples and ≥0.015% in the bronchial washing samples.

2.3 Statistical analysis. Categorical and continuous parameters were evaluated by Chi-square test or an independent sample *t*-test, respectively. The Spearman correlation test was used to evaluate relationships between two variables. The area under the curve (AUC) and sensitivity/specificity between plasma and BWF were generated and compared using pROC and gmodels R package, respectively. A p-value less than 0.05 was considered as significant. Statistical analyses were performed using SPSS version 25.0 (SPSS Inc., Chicago, IL, USA) or R Statistical Package (Institute for Statistics and Mathematics, Vienna, Austria, ver. 3.5.3, www.R-project.org).

3. Results

3.1. Demographic characteristic of study population. Table 1 describes the characteristics of enrolled cases. The mean age of the study population was 65.3 ± 9.8 years, and 38 (52.1%) patients were male and 35 (47.9%) were female. Twenty-eight (38.4%) patients had a history of smoking and the mean smoking level of all-life was 12.6 ± 21.4 pack-year. Nearly all enrolled patients had adenocarcinoma

(89.0%), and one (1.4%) had pulmonary sarcomatoid carcinoma. The average of the longest diameter of tumors was 3.5 ± 2.0 cm. Twenty-five patients were in stage I, 10 in stage II, 10 in stage III, and 27 in stage IV. Of the 73 patients, 35 (47.9%) showed wild-type EGFR and 38 showed mutations in the EGFR-tyrosine kinase domain; 19 patients had L858R substitution and 19 showed E19del, respectively. Baseline characteristics did not significantly differ between early (stages I–IIIA) and advanced (stages IIIB–IV) stages of lung cancer, except for stage.

3.2 AUC predicts tissue EGFR mutation. First, we compared the diagnostic yields of blood and BWF in all cases. The AUC was 0.717 (95% confidence interval (CI): 0.592–0.842) when L858R was detected in a blood sample, whereas the value was 0.961 (95% CI: 0.901–1.000) for BWF (Table 2, Figure 1A). Testing with BWF more accurately predicted the presence of L858R in tumor tissue than in blood, and the difference in AUC was significant (p-value < 0.001, DeLong's test for two correlated ROC curves). Predicting the presence of E19del in tumor tissue showed similar results to the findings of the L858R test, showing that the AUC in the E19del test using blood was only 0.632 (95% CI: 0.519–0.745), while that from BWF was 0.858 (95% CI: 0.746–0.969) (Table 2, Figure 1B). Similar to L858R, BWF was more useful for predicting E19del compared to blood, and the difference in the AUC was significant (p<0.001, DeLong's test for two correlated ROC curves).

By combining the results of L858R and E19del, variables were simplified and the usefulness of blood and BWF were compared for predicting the tissue EGFR mutation status. The AUC for detecting tumor EGFR mutations using blood samples was 0.686 (95% CI: 0.592–0.780), whereas that using BWF was 0.895 (95% CI: 0.822–0.969), showing a significant difference between specimens (p-value < 0.0001, DeLong's test for two correlated ROC curves). Compared to the Fa value obtained from the BWF with that from the plasma in each patient, the Fa was significantly higher in BWF than in plasma in mEGFR-positive cases (p-value = 0.004, Wilcoxon signed rank test), indicating that BWF can be used to easily distinguish the positivity or negativity of ddPCR compared to plasma (Figure 2).

3.3 EGFR mutation status prediction sensitivity. When cut-off values were set based on the above results obtained from each sample group, the cut-off Fa value from the blood sample was 0.044% and that from BWF was 0.015%. When these cut-off values were applied, the sensitivity for predicting tissue E19del mutation using blood samples was 31.6%, whereas that using BWF was 68.4% (Figure 3), demonstrating that BWF is superior for detecting E19del mutation in tumor tissue (p-value = 0.005, McNemar's Chi-squared test). Similar results were obtained when predicting tumor tissue L858R substitution; the sensitivity using the blood sample was 47.4%, while that using BWF was 89.5% (p-value = 0.005, McNemar's Chi-squared test). The same findings were obtained when comparing the usefulness of blood and BWF in predicting the tissue EGFR mutation status by combining the L858R and E19Del values as one variable (p-value < 0.0001, McNemar's Chi-squared test). The results obtained using both specimens showed high specificity, and no significant difference was observed (data not shown).

3.4 BWF showed good diagnostic yields. The detection rate of mEGFR from blood samples was dependent on the disease stage. To confirm whether these findings can be applied to BWF, we

investigated the mEGFR detection yields in each sample type by dividing the stage of lung cancer into early stages and advanced stages and then compared the detection rate between each type of sample in each stage group (Table 3 and Figure 3). In the early stage group (stage I–IIIA; n = 38), the AUC value of blood samples for predicting tissue mEGFR was 0.504, whereas that of BWF was 0.768, showing a significant difference between sample types (p-value = 0.008). Although the specificity was higher in both sample types, the sensitivity of the results from blood samples was only 15.0%, while that from BWF was 65.0%. In the advanced stage group (stage IIIB–IV, n = 35), the AUC value obtained from BWF was higher than that from the blood; AUC from BWS was 1.000 (95% CI: 0.899–1.000), and 0.879 (95% CI: 0.724–0.964) from blood. This shows that the results from BWF predicted the tumor tissue mEGFR status more accurately than that by using plasma (p-value = 0.043, DeLong's test for two correlated ROC curves).

The results from the blood and BWF data were highly specific, but the sensitivity from BWF-derived values was significantly higher than that from the blood. The values from BWF also accurately reflected the tumor mEGFR status in early and advanced-stage tumors.

4. Discussion

This study showed that in ddPCR of liquid biopsy samples, BWF samples are more reliable than blood samples for reflecting the tumor mEGFR status. Additionally, BWF can be used to analyze both L858R and E19del and is more sensitive than plasma in detecting every stage of lung cancer.

Although tissue biopsy is the gold standard method for molecular genotyping in lung cancer, liquid biopsy may play an important role as a complementary method for detecting targeted genes and predicting the clinical course or outcome[13, 14] and to detect lung cancer in an early stage.[15] Liquid biopsy is relatively non-invasive, safe, and simple procedure. Therefore, it can be performed in patients with poor medical condition or when the location or size of lung cancer is difficult to biopsy.

Many studies have shown the feasibility of liquid biopsy in lung cancer, but some limitations remain.[16, 17] Blood samples are mainly used for liquid biopsy, including ctDNA, circulating tumor cells, platelets, exosomes, and microRNAs, which provide specific genetic information of the tumor. Although the proportion of ctDNA can vary depending on the tumor burden, stage, vascularization, and biologic feature, this value is generally only 0.1–1.0%. Furthermore, ctDNA has a relatively short half-life, from approximately 16 to 150 min.[18] Thus, if sample processing is delayed, ctDNA would not reflect the precise experimental results. To overcome this limitation, we froze the BWF and blood samples within 3 and 8 h, respectively, and we used Streck tube®, which maintained the stability of cfDNA for up to 14 days and CTC for up to 7 days. Recently, Krug el al.[19] demonstrated that compared to ctDNA, combined isolation of exosomal RNA (exoRNA) and cfDNA detected EGFR mutation and T790M with improved sensitivity (96% in activating EGFR mutation and 90% for T790M). They showed the largest improvement in sensitivity (26% to 74%), particularly in patients with intrathoracic metastatic diseases by using combined exoRNA and ctDNA. Therefore, applying exoRNA and ctDNA in BWF specimens may greatly

improve the detection of EGFR mutations, even in the early stage of lung cancer, but further studies are needed to evaluate this.

BAL plays a supporting role in the diagnosis of lung cancer.[20] Since the 1980s, various studies have shown the usefulness of BAL in diagnosing lung malignancy.[21] BAL showed a diagnostic yield of 33–90% in diffuse malignant pulmonary infiltrates, although the value differed according to the cancer type. Particularly, in NSCLC such as squamous cell carcinoma and adenocarcinoma, the diagnostic yield was 50% and 77%, respectively.[21] Park et al.[22] suggested that BAL fluid was effective for detecting the EGFR mutation status. Although their study involved a small number of subjects (n = 20), a high concordance rate (91.7%) was observed between the BALF and tissue for detecting EGFR mutations by using PNA-mediated PCR clamping and PANAMutyper with fluorescence melting curve analysis. However, their study included only three patients with early stage lung cancer, and they did not report the difference in the detection rate between the BALF and plasma. Our study included 38 patients with an early stage of lung cancer, and the sensitivity was 65% with BWF, while it was only 15.0% with plasma. There was a greater improvement in the sensitivity and diagnostic yield with BWF compared to that with blood in an early stage of lung cancer. Therefore, we suggest that, rather than plasma, BWF can be used to detect mutational variations, regardless of the lung cancer stage.

Our study showed BWF could be substituted for biopsy to confirm EGFR mutation and may shorten the time from diagnosis to treatment by avoiding the delay for biopsy and confirmation of biopsy results. BWF specimens are simple to collect, and obtaining BWF is safer than lung biopsy because only simple bronchoscopy needs to be performed, for which hospitalization is not required.

Additionally, we investigated the relationship between tumor size and mEGFR detection rate. There may be a significant agreement of the mEGFR status between liquid biopsy and tissue biopsy for larger-sized tumors, but no significant association was noted between tumor size and concordance of mEGFR for liquid biopsy and tissue biopsy (data not shown). This suggests that the diagnostic yield of liquid biopsy with BWF depends mainly on staging rather than on tumor size. However, as our study sample size was small, further studies using large sample sizes are needed.

5. Conclusion

Compared to plasma, liquid biopsy using BWF is more effective for identifying mEGFR. This may be useful for avoiding invasive tissue biopsy and complications such as pneumothorax or bleeding following tissue biopsy. Furthermore, BWF may be an alternative method for re-biopsy to detect the presence of the T790M mutation via ddPCR.

Abbreviations

ctDNA = circulating tumor DNA; mEGFR = EGFR-tyrosine kinase sensitizing mutation; ddPCR = digital droplet PCR; AUC = area under the curve; TKI = tyrosine kinase inhibitor; cfDNA = cell free DNA; BWF =

bronchial washing fluid; BALF = bronchoalveolar lavage fluid.

Declarations

Ethics approval and consent to participate

This study was performed in accordance with the amended Declaration of Helsinki and was approved by the independent hospital institutional review board (approval no. 3-2016-0225 and 3-2017-0321).

Consent for publication: All authors provided final approval of the version to be published

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Funding: This work was supported by the National Research Foundation of Korea (grant number NRF-2017R1A2B4009017).

Conflict of interest: none

Author contributor

YSC and SHL conceived and designed the study. All authors contributed to participant recruitment, and data collection/acquisition. EYK and TK analyzed the data and performed the statistical analysis. YSC and SHL wrote the first draft of the manuscript. All authors critically evaluated the data, reviewed the manuscript, and approved the final version.

References

1. Siegel RL, Miller KD, Jemal A: **Cancer statistics, 2018.** *CA Cancer J Clin* 2018, **68:**7-30.
2. de Groot PM, Wu CC, Carter BW, Munden RF: **The epidemiology of lung cancer.** *Transl Lung Cancer Res* 2018, **7:**220-233.
3. Chan BA, Hughes BG: **Targeted therapy for non-small cell lung cancer: current standards and the promise of the future.** *Transl Lung Cancer Res* 2015, **4:**36-54.
4. Thatcher N, Chang A, Parikh P, Rodrigues Pereira J, Ciuleanu T, von Pawel J, Thongprasert S, Tan EH, Pemberton K, Archer V, Carroll K: **Gefitinib plus best supportive care in previously treated patients with refractory advanced non-small-cell lung cancer: results from a randomised, placebo-controlled, multicentre study (Iressa Survival Evaluation in Lung Cancer).** *Lancet* 2005, **366:**1527-1537.
5. Lynch TJ, Bell DW, Sordella R, Gurubhagavatula S, Okimoto RA, Brannigan BW, Harris PL, Haserlat SM, Supko JG, Haluska FG, et al: **Activating mutations in the epidermal growth factor receptor**

underlying responsiveness of non-small-cell lung cancer to gefitinib. *N Engl J Med* 2004, **350:**2129-2139.

6. Mok TS, Wu YL, Thongprasert S, Yang CH, Chu DT, Saijo N, Sunpaweravong P, Han B, Margono B, Ichinose Y, et al: **Gefitinib or carboplatin-paclitaxel in pulmonary adenocarcinoma.** *N Engl J Med* 2009, **361:**947-957.
7. Gonzalez-Larriba JL, Lazaro-Quintela M, Cobo M, Domine M, Majem M, Garcia-Campelo R: **Clinical management of epidermal growth factor receptor mutation-positive non-small cell lung cancer patients after progression on previous epidermal growth factor receptor tyrosine kinase inhibitors: the necessity of repeated molecular analysis.** *Transl Lung Cancer Res* 2017, **6:**S21-S34.
8. Rivera MP, Mehta AC, Wahidi MM: **Establishing the diagnosis of lung cancer: Diagnosis and management of lung cancer, 3rd ed: American College of Chest Physicians evidence-based clinical practice guidelines.** *Chest* 2013, **143:**e142S-e165S.
9. Tam AL, Kim ES, Lee JJ, Ensor JE, Hicks ME, Tang X, Blumenschein GR, Alden CM, Erasmus JJ, Tsao A, et al: **Feasibility of image-guided transthoracic core-needle biopsy in the BATTLE lung trial.** *J Thorac Oncol* 2013, **8:**436-442.
10. Diaz LA, Jr., Bardelli A: **Liquid biopsies: genotyping circulating tumor DNA.** *J Clin Oncol* 2014, **32:**579-586.
11. Oumeraci T, Schmidt B, Wolf T, Zapatka M, Pich A, Brors B, Eils R, Fleischhacker M, Schlegelberger B, von Neuhoff N: **Bronchoalveolar lavage fluid of lung cancer patients: mapping the uncharted waters using proteomics technology.** *Lung Cancer* 2011, **72:**136-138.
12. Carvalho AS, Cuco CM, Lavareda C, Miguel F, Ventura M, Almeida S, Pinto P, de Abreu TT, Rodrigues LV, Seixas S, et al: **Bronchoalveolar Lavage Proteomics in Patients with Suspected Lung Cancer.** *Sci Rep* 2017, **7:**42190.
13. Luo W, Rao M, Qu J, Luo D: **Applications of liquid biopsy in lung cancer-diagnosis, prognosis prediction, and disease monitoring.** *Am J Transl Res* 2018, **10:**3911-3923.
14. Hong MH, Kim HR, Ahn BC, Heo SJ, Kim JH, Cho BC: **Real-World Analysis of the Efficacy of Rebiopsy and EGFR Mutation Test of Tissue and Plasma Samples in Drug-Resistant Non-Small Cell Lung Cancer.** *Yonsei Med J* 2019, **60:**525-534.
15. Liang W, Zhao Y, Huang W, Liang H, Zeng H, He J: **Liquid biopsy for early stage lung cancer.** *J Thorac Dis* 2018, **10:**S876-S881.
16. Lim M, Kim CJ, Sunkara V, Kim MH, Cho YK: **Liquid Biopsy in Lung Cancer: Clinical Applications of Circulating Biomarkers (CTCs and ctDNA).** *Micromachines (Basel)* 2018, **9**.
17. Cabanero M, Tsao MS: **Circulating tumour DNA in EGFR-mutant non-small-cell lung cancer.** *Curr Oncol* 2018, **25:**S38-S44.
18. Saarenheimo J, Eigeliene N, Andersen H, Tiirola M, Jekunen A: **The Value of Liquid Biopsies for Guiding Therapy Decisions in Non-small Cell Lung Cancer.** *Front Oncol* 2019, **9:**129.
19. Krug AK, Enderle D, Karlovich C, Priewasser T, Bentink S, Spiel A, Brinkmann K, Emenegger J, Grimm DG, Castellanos-Rizaldos E, et al: **Improved EGFR mutation detection using combined exosomal RNA**

and circulating tumor DNA in NSCLC patient plasma. *Annals of Oncology* 2018, **29:**700-706.

20. Semenzato G, Poletti V: **Bronchoalveolar lavage in lung cancer.** *Respiration* 1992, **59 Suppl 1:**44-46.
21. Poletti V, Poletti G, Murer B, Saragoni L, Chilosi M: **Bronchoalveolar lavage in malignancy.** *Semin Respir Crit Care Med* 2007, **28:**534-545.
22. Park S, Hur JY, Lee KY, Lee JC, Rho JK, Shin SH, Choi CM: **Assessment of EGFR mutation status using cell-free DNA from bronchoalveolar lavage fluid.** *Clinical Chemistry and Laboratory Medicine* 2017, **55:**1489-1495.

Tables

Table 1. Baseline characteristics of the study population

	Total (n= 73)	Early stage of lung cancer (n=38)	Advanced stage of lung cancer (n=35)
Age (year)	65.3 ± 9.8	65.0 ± 8.1	65.7 ± 11.5
Sex (F/M)	35/38 (47.9/52.1)	20/18 (52.6/47.4)	15/20 (42.9/57.1)
Smoking status			
Never smoker	45 (61.6)	25 (65.8)	20 (57.1)
Former smoker	22 (30.1)	10 (26.3)	12 (34.3)
Current smoker	6 (8.2)	3 (7.9)	3 (8.6)
Smoking amount (pack-year)	12.6 ± 21.4	11.3 ± 20.0	14.1 ± 23.2
Tumor type			
Adenocarcinoma	65 (89.0)	32 (84.2)	33 (94.3)
Squamous cell carcinoma	7 (9.6)	5 (13.2)	2 (5.7)
Sarcomatoid carcinoma	1 (1.4)	1 (2.6)	-
Tumor size (cm)	3.5 ± 2.0	3.0 ± 2.0	3.9 ± 1.9
Lung cancer stage			
I/II/III/IV	26/10/10/27	26/10/2/-	-/-/8/27
EGFR genotyping			
Wild type	35 (47.9)	18 (47.4)	17 (48.6)
E19del	19 (26.0)	12 (31.6)	7 (20.0)
L858R	19 (26.0)	8 (21.1)	11 (31.4)

Table 2. Sensitivity, specificity, and concordance rate of ddPCR according to EGFR mutation

	L858R			E19del		
	Plasma	BWF	p-value	Plasma	BWF	p-value
AUC	0.717 (0.592-0.842)	0.961 (0.901-1.0)	<0.001	0.632 (0.519-0.745)	0.858 (0.746-0.969)	<0.001
Sensitivity (%)	47.37	89.47		31.58	68.42	
Specificity (%)	98.15	96.30		94.44	98.15	
Concordance rate	82.2% (60/73)	94.5% (69/73)		76.7% (56/73)	84.9% (62/73)	

Abbreviation: BWF, bronchial washing fluid; AUC, area under curve

Table 3. Sensitivity, specificity, and concordance rate of ddPCR according to lung cancer stage

	Early stage of lung cancer (n=38)			Advanced stage of lung cancer (n=35)		
	Plasma	BWF	p-value	Plasma	BWF	p-value
AUC	0.504 (0.338-0.670)	0.768 (0.603-0.889)	0.008	0.879 (0.724-0.964)	1.0 (0.899-1.000)	0.043
Sensitivity (%)	15.0	65.0		72.2	100.0	
Specificity (%)	100.0	88.9		100.0	100.0	

Abbreviation: BWF, bronchial washing fluid; AUC, area under curve

Figures

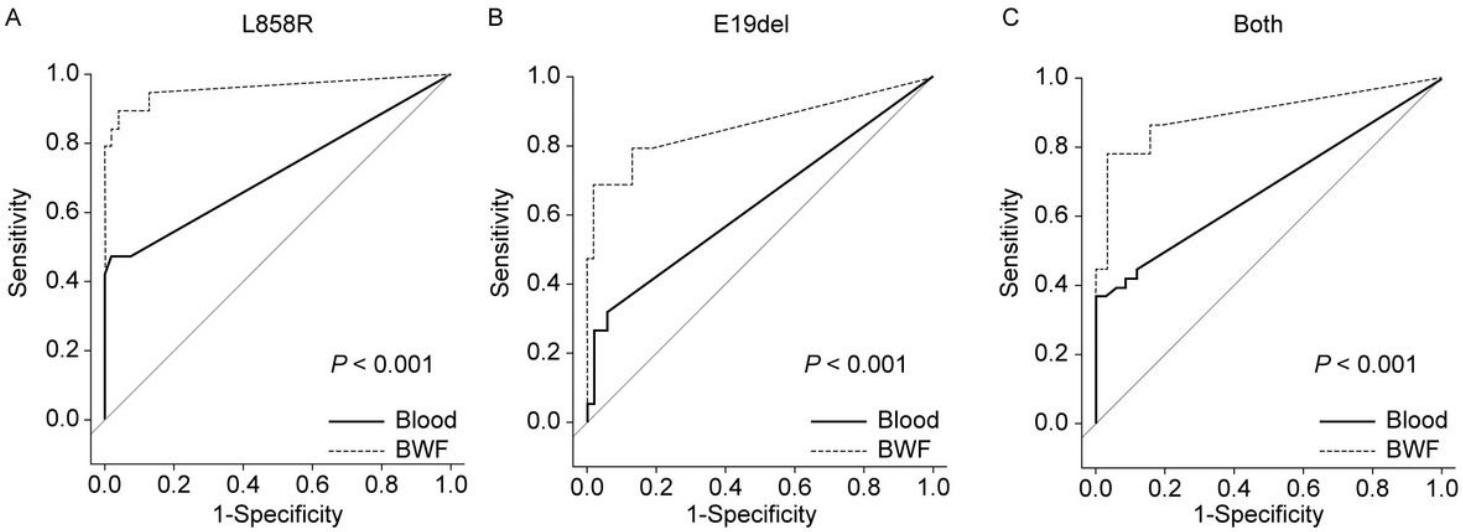

Figure 1

Comparison of receiver operator characteristic (ROC) curves according to sample (plasma or BWF) and EGFR mutational genotype. (A) L858R, (B) E19del, and (C) Both. BWF, bronchial washing fluid

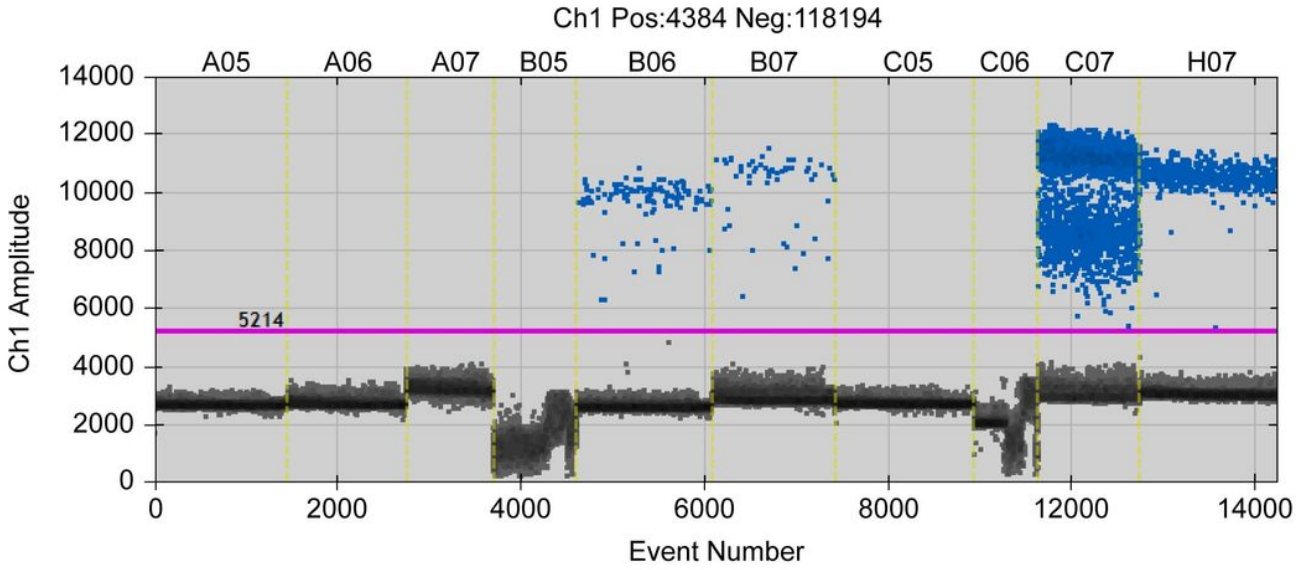

Figure 2

Example of difference of ddPCR results according to samples; Bronchial washing fluid (C07 column) specimens showed clearly distinguishable mutational droplets compared to plasma (B06 and B07 column). H07 column is positive control.

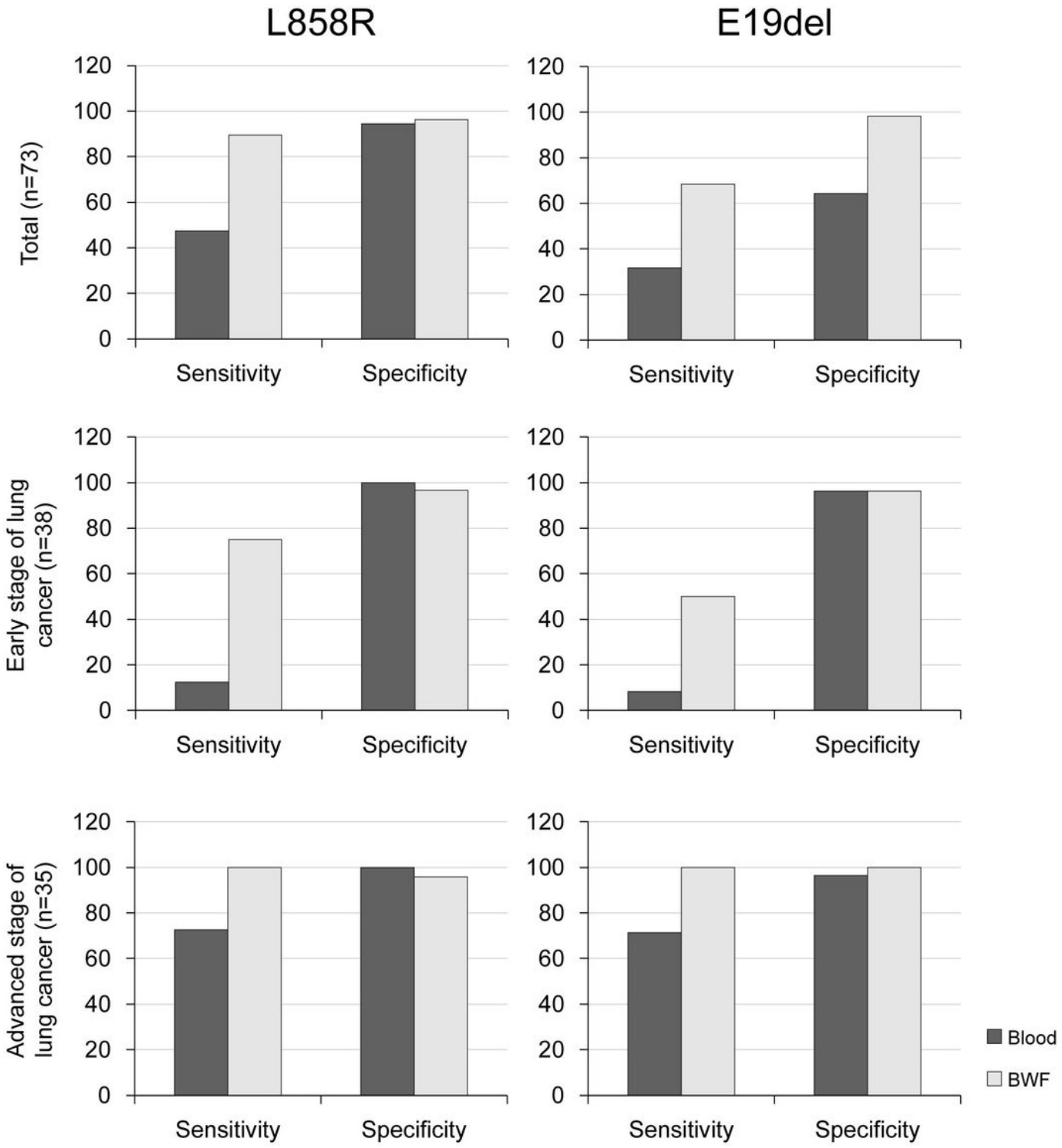

Figure 3

Comparison of sensitivity and specificity according to EGFR mutational genotype and lung cancer stage. (A) Total patients, (B) Early stage of lung cancer (I~IIIA), and (C) Advanced stage of lung cancer (IIIB~IV) BWF, bronchial washing fluid