# Transthoracic Rebiopsy for Mutation Analysis in Lung Adenocarcinoma: Outcomes and Risk Factors for the Acquisition of Nondiagnostic Specimens in 199 Patients

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## Abstract

**We investigated the clinical and procedure-related risk factors for the acquisition of nondiagnostic transthoracic rebiopsy specimens. The adequacy rate for tissue samples for mutation analysis was 90%. An internal low-attenuation area in the target lesion on computed tomography was an independent predictor for the acquisition of nondiagnostic specimens for mutation analysis during rebiopsy.**

**Purpose:** To determine the outcomes of transthoracic rebiopsy for epidermal growth factor receptor (*EGFR*) mutation in patients with lung adenocarcinoma and to explore the clinical and procedure-related risk factors for the acquisition of nondiagnostic rebiopsy specimens. **Patients and Methods:** We retrospectively reviewed 367 patients with lung adenocarcinoma who underwent transthoracic core needle biopsy for mutation analysis from September 2011 to October 2016. Of these, 199 patients underwent rebiopsy. Patient characteristics, treatment history, target lesion characteristics, and procedure-related factors were evaluated. The adequacy rate of specimens for mutation analysis was evaluated. Univariable and multivariable analyses were performed to determine the independent predictors of nondiagnostic specimens. **Results:** Ninety percent of specimens (179 of 199) were adequate for mutation analysis. The *EGFR* mutation (exon 18-21) was 65% (117 of 179) and the *EGFR* T790M mutation 33% (59 of 179) of specimens. In univariable analysis, an internal low-attenuation area in the target lesion ($P = .001$) and pleural contact ($P = .004$) on computed tomography were significant risk factors for nondiagnostic specimens. In multivariable analysis, an internal low-attenuation area in the target lesion (odds ratio = 7.333; 95% confidence interval, 1.755-30.633; $P = .006$) was an independent predictor for acquisition of nondiagnostic specimens. **Conclusion:** Image-guided transthoracic rebiopsy to obtain specimens for mutation analysis in lung adenocarcinoma provides high diagnostic accuracy, with a low rate of nondiagnostic specimens. The presence of internal low-attenuation area in the target lesion on computed tomography was an independent predictor for acquiring nondiagnostic specimens.

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## Introduction

Non–small-cell lung cancer (NSCLC) is a heterogeneous collection of diseases with diverse molecular characteristics.[1,2] Optimal management of advanced NSCLC is based on matching drug profiles with specific mutations found in the patient's primary tumor. A tyrosine kinase inhibitor (TKI) is the recommended first-line therapy for patients whose tumor has the epidermal growth factor receptor (*EGFR*) mutation.[3-5] Despite an initial dramatic response to *EGFR*-TKI therapy, most patients eventually experience a progression of disease owing to the development of resistance.[3,6,7] The *EGFR* T790M mutation accounts for over half of the resistance

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to first- or second-generation *EGFR*-TKIs[8-10] and the recent development of the third-generation *EGFR*-TKIs that effectively target T790M emphasizes an urgent need for the development of effective methods that identify this mutation.[11,12] Accordingly, rebiopsy is essential to determine the mechanisms underlying resistance to TKIs and is now recommended in the National Comprehensive Cancer Network guidelines to guide the selection of second-line therapies.

To date, several studies have reported the feasibility and outcomes of rebiopsy for mutation analysis in patients with NSCLC who were treated with *EGFR*-TKIs.[13-17] The success rate of rebiopsy after *EGFR*-TKI failure has been reported to be approximately 70% to 90%.[13,16,17] However, previous studies have limitations due to relatively small sample sizes, and, in addition, little is known about the factors that determine appropriate specimen acquisition for mutation analysis. Therefore, the purposes of this study were to determine the outcomes of transthoracic rebiopsy for assessing *EGFR* mutations, including the T790M mutation, in patients with lung adenocarcinoma and to explore the clinical features, target-lesion characteristics, and procedure-related factors associated with the acquisition of nondiagnostic rebiopsy specimens.

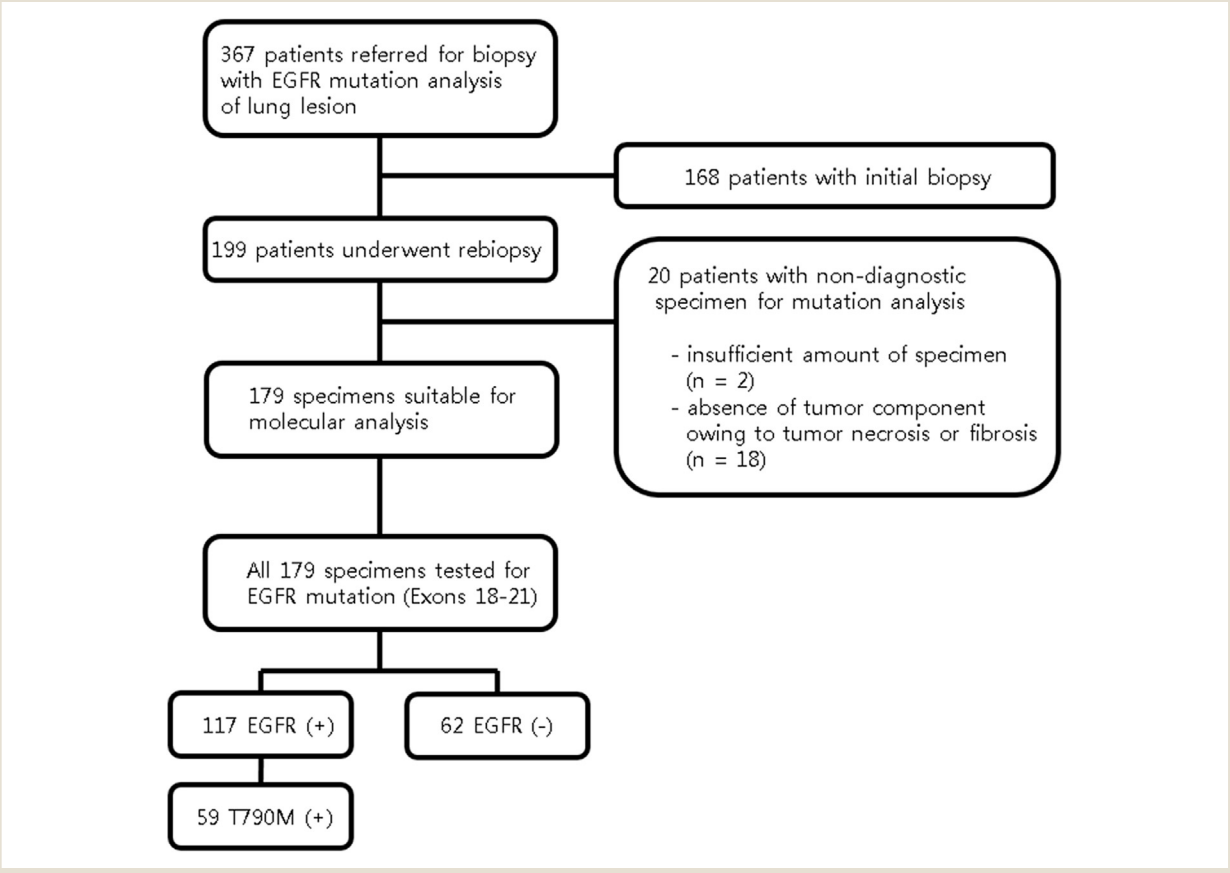
## Patients and Methods

Institutional review board approval was obtained, and the requirement for informed patient consent was waived owing to the retrospective study design.

### *Patients*

We retrospectively reviewed 367 consecutive transthoracic core needle biopsy cases for *EGFR* mutational analysis of lung adenocarcinoma was requested by the Oncologic Division of the Department of Internal Medicine between September 2011 to October 2016. The procedure was an initial biopsy in 168 patients who were therefore excluded from analysis. The remaining 199 patients who underwent rebiopsy for evaluation of *EGFR*-TKI sensitivity and newly developed mutations, including the T790M mutation, were included in the final study population: these patients had progressive disease on conventional chemotherapy (n = 187) or *EGFR*-TKI treatment (n = 156) (Figure 1). Progressive disease was determined by medical oncologists at our institution, based on the published consensus guidelines.[18,19] Rebiopsy was defined as a biopsy performed after the tumor progressed despite adequate initial chemotherapy or *EGFR*-TKI treatment.[20]

**Figure 1** Patient Flow Diagram. Flowchart Showing Patient Inclusion and Exclusion Criteria




Abbreviation: *EGFR* = epidermal growth factor receptor.

### Procedure

All patients underwent a diagnostic chest computed tomography (CT) scan for planning the biopsy procedure. A coagulation screening test was performed before all procedures. All rebiopsy procedures were performed percutaneously under C-arm cone-beam CT (n = 183), CT (n = 15), or ultrasonography (n = 1) guidance. The C-arm cone-beam CT system was equipped with a single-plane C-arm angiography unit that had a flat-panel detector (Allura Xper FD20; Philips Healthcare, Best, Netherlands) adopting an indirect digital radiographic method. All procedures were performed by (n = 54) or under the supervision of (n = 145) attending thoracic radiologists with more than 10 years of experience in transthoracic needle biopsy. The patient's position was chosen depending on the location of the target lesion and the easiest access route was determined to avoid large vessels, the heart, severe emphysema, and interlobular fissures. Biopsies were performed with 18 gauge (n = 167) or 20 gauge (n = 27) core needles (TSK Acecut [n = 191] or Stericut [n = 7]; TSK Laboratory, Japan) with an 11 or 22 mm throw. In some cases, needle size (n = 5) and needle type (n = 1) were not available on electrical medical record review. The choice of needle excursion was based on the lesion size and location. Details of our cone-beam CT-guided needle biopsy procedures are described elsewhere.[21]

### Pathologic and Molecular Analyses

One experienced pathologist (Y.L.C., with 8 years of experience in genomic study) evaluated all tissue specimens. DNA extracted from the tumor samples was analyzed for the *EGFR* mutation by direct sequencing of exons 18 to 21 or with more sensitive polymerase chain reaction-based assays as previously published.[22-24] Negative results were correlated with concurrent cytologic or histologic slides to confirm adequate tumor content relative to associated nonneoplastic tissue to prevent false negative results. Samples with a confirmed *EGFR* mutation in exons 18 to 21 were tested for the *EGFR* T790M mutation by fragment analysis.[24]

### Data Collection

Demographic and clinical features of the study population were collected from the electronic medical records. Patient age, sex, smoking status (current, ex-smoker, or nonsmoker), initial diagnosis (pathology and staging), prior local or systemic treatment, and type of chemotherapy (conventional chemotherapy or *EGFR*-TKI treatment) were recorded.

CT characteristics of the target lesions, such as tumor size (longest diameter), tumor morphology including air-bronchogram and internal low-attenuation area, tumor solidity, pleural contact, peribronchovascular location, and zonal location (upper, middle, lower) were independently reviewed by 2 thoracic radiologists (B.D.N. and T.J.K., with 5 and 18 years of experience in thoracic CT interpretation, respectively). If disagreement occurred in the analysis, a consensus reading between these 2 observers was performed.

Biopsy-related factors were also evaluated by review of intraprocedural images and radiology reports. The number of pleural punctures, core biopsy specimen length, patient position, guiding methods, core needle size (18- or 20 gauge), needle types (coaxial or noncoaxial), and experience of the operator (attending radiologists or clinical fellows) were documented.

### Statistical Analysis

Statistical analyses were conducted by SPSS Statistics 18.0 software (IBM, Armonk, NY). Patient characteristics and the variables associated with success or failure of rebiopsy were summarized using descriptive statistics, including number, mean, median, standard deviation, minimum and maximum values for continuous variables, and frequency for categorical variables. Clinical, target-lesion characteristics, and biopsy-related factors between rebiopsy success and failure were compared by the Mann-Whitney *U* test. Multivariable logistic regression analysis was conducted to identify independent predictors for the acquisition of nondiagnostic rebiopsy specimens. In all statistical analyses, $P < .05$ was considered statistically significant.

## Results

### Patients

The demographics and characteristics of the patients who underwent rebiopsy are summarized in Table 1. The study population consisted of 199 patients (mean age, 59.7 years; range, 32-84 years) including 76 male subjects (mean age, 59.9 years; range, 34-80 years) and 123 female subjects (mean age, 59.6 years; range, 32-84 years). All patients underwent prior treatment before rebiopsy with conventional chemotherapy (187/199, 94%), *EGFR*-TKI therapy (156/199, 78%), and/or surgery (43/199, 22%). The mean duration of follow-up after treatment was 31 months (median, 21 months; range, 1-303 months).

### Results of Rebiopsy

Rebiopsy specimens were adequate for mutation analysis in 90% of patients (179 of 199). In 20 patients, the specimens were inadequate for mutation analysis owing to the absence of tumor components in the sample because of tumor necrosis or fibrosis (n = 18) or an insufficient amount of specimen (n = 2). Repeat rebiopsy was performed in 8 of 20 patients. Specimen was adequate for mutation analysis in 7 and was inadequate in 1 (bronchoscopic biopsy). T790M mutation was positive in 1 of 5 transthoracic biopsies and was negative in 6 (4 transthoracic, 1 surgical, and 1 liver biopsy).

There were several significant differences in the target-lesion characteristics between the rebiopsy success and failure groups on univariable analysis (Table 2). An internal low-attenuation area on contrast-enhanced CT was more frequent in the rebiopsy failure group compared with the rebiopsy success group (5/20, 25% vs. 7/179, 4%, $P = .001$). Target-lesions with pleural contact were found more frequently in the rebiopsy failure group than the rebiopsy success group (12/20, 60% vs. 51/179, 28%; $P = .004$). There were no significant procedure-related factors, such as number of pleural punctures, patient position, guiding methods, or needle size and type. Nondiagnostic rebiopsy specimens were more frequently found by the fellow operator (18/20, 90%) than the attending radiologist operator (2/20, 10%), although this difference was not significant ($P = .070$).

**Table 1** Patient Characteristics and Rebiopsy Results

| Characteristic | Value |
|---|---|
| Sex, male | 76 (38) |
| Age at biopsy, years (mean ± SD [range]) | 60 ± 11.2 (32-84) |
| **Smoking History** | |
| Current smoker | 17 (9) |
| Ex-smoker | 50 (25) |
| Nonsmoker | 132 (66) |
| **Initial Histologic Diagnosis** | |
| Adenocarcinoma | 199 (100) |
| **ECOG Performance Status** | |
| 0 | 131 (66) |
| 1 | 48 (24) |
| 2 | 16 (8) |
| 3 | 4 (2) |
| **Initial Biopsy Method**[a] | 155 (100) |
| Transthoracic needle biopsy | 81 (52) |
| Surgery | 45 (29) |
| Bronchoscopy | 20 (13) |
| Other | 9 (6) |
| **Initial Tumor Staging** | |
| IA/IB | 11 (6)/8 (4) |
| IIA/IIB | 8 (4)/2 (1) |
| IIIA/IIIB | 15 (8)/6 (3) |
| IV | 143 (72) |
| Unidentified | 6 (3) |
| **Treatment History** | |
| Chemotherapy | 199 (100) |
| Conventional chemotherapy | 187 (94) |
| First-generation TKI | |
| Gefitinib | 98 (49) |
| Erlotinib | 42 (21) |
| Second-generation TKI—Afatinib | 31 (16) |
| Third-generation TKI | |
| Osimertinib | 40 (20) |
| Olmutinib | 10 (5) |
| Surgery | 43 (22) |
| Therapy duration, months (mean ± SD [range]) | 31 ± 34.1 (0-303) |

Data are presented as n (%) unless otherwise indicated.
Abbreviations: ECOG = Eastern Cooperative Oncology Group; TKI = tyrosine kinase inhibitor.
[a]Available in 155 of 199 patients who underwent rebiopsy.

Multivariable regression analysis indicated that an internal low-attenuation area in the target lesion on contrast-enhanced CT was an independent predictor of nondiagnostic rebiopsy specimens (odds ratio = 8.575; 95% confidence interval, 1.705-43.138; $P$ = .009) (Figure 2 and Table 3).

Regarding the safety of rebiopsy, the overall complication rate was 4% (8 of 199). Pneumothorax was the most common complication (7/199, 3.5%) and 3 patients required chest tube placement. Mild hemoptysis occurred in one patient (0.5%). There were no serious complications, such as massive hemoptysis, air embolism, or death.

### *Results of Rebiopsy for* EGFR *Mutation Analysis*

Of the 179 patients whose specimens were assayed for the *EGFR* mutation at rebiopsy, the *EGFR* mutation was present in those of 117 patients (65%). The detailed results of the *EGFR* mutation analyses are summarized in Table 4. At initial biopsy, the specimens of 84 (54%) of 155 patients were positive for the *EGFR* mutation and that of one patient (0.6%) was positive for the *EGFR* T790M mutation. Of the 117 patients with *EGFR* mutation at rebiopsy, 59 (50%) were positive for the *EGFR* T790M mutation after conventional chemotherapy or *EGFR*-TKI therapy. Of these 59 patients, 50 (85%) were treated with a third-generation TKI, such as osimertinib (n = 40) or olmutinib (n = 10).

## Discussion

Our study suggests that the use of image-guided transthoracic rebiopsy for mutation analysis in lung adenocarcinoma is safe and effective. The adequacy rate for tissue samples for mutation analysis was 90%. The presence of internal low-attenuation areas in the target lesion on contrast-enhanced CT was an independent predictor of nondiagnostic rebiopsy specimen acquisition.

In our study, 90% (179 of 199) of the tissue samples were adequate for mutation analysis, including that for the *EGFR* T790M mutation. Our results were comparable to the published success rates of the rebiopsy procedure, which ranged from 73% to 95%.[13,15,16] The relatively high success rate in our study is attributed to cone-beam CT guidance and the use of core biopsy. Cone-beam CT-guided percutaneous needle biopsy has been shown to be highly accurate and useful for the histologic diagnosis of pulmonary nodules.[17,21] In addition, only core biopsy was performed for mutation analysis in our study, which might have reduced potential problems related to cytologic specimens from fine needle aspiration, such as an insufficient amount of tissue for mutation analysis.[25]

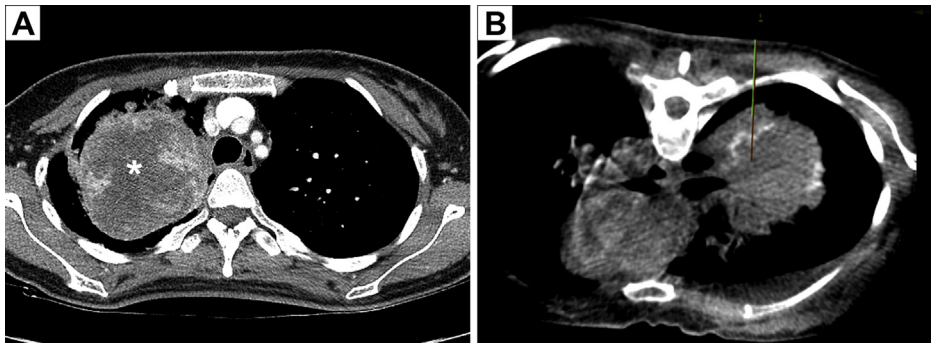
Although several studies have reported the feasibility and outcome of rebiopsy for mutation analysis in advanced NSCLC, little is known about the factors related to the appropriate acquisition of rebiopsy specimens for mutation analysis. It is noteworthy that this was the largest study regarding rebiopsy performed thus far that also analyzed the clinical features and procedure-related factors for the acquisition of nondiagnostic rebiopsy specimens. A recent study evaluated the predictive factors for the acquisition of nondiagnostic samples during rebiopsy for the *EGFR* T790M mutation and no clinical or technical factors were found to be related to the acquisition of nondiagnostic samples.[13] However, this study was limited by a small sample size and the number of nondiagnostic samples was too small to identify predictors for acquiring nondiagnostic biopsy specimens (n = 9). In our study, we found that an internal low-attenuation area in the target lesion on CT was an independent predictor of nondiagnostic specimen acquisition for mutation analysis. An internal low-attenuation area in the target lesion may suggest a necrotic or nonviable tumor. Most of the inadequate specimens in our study were due to total necrosis (90%, 18/20). Judging from these results, total necrosis in the rebiopsy specimen corresponded to an internal low-attenuation area on CT and might have been closely related to nondiagnostic rebiopsy sample acquisition. This result is also comparable to previous studies, which reported that the larger the lesion size, the greater the

**Table 2** Comparison of Clinical Characteristics, Target Lesion Characteristics, and Biopsy-Related Factors Between Patients With Rebiopsy Success and Failure

| Parameter | Success (N = 179) | Failure (N = 20) | *P* |
|---|---|---|---|
| **Clinical Characteristics** | | | |
| Sex, male | 66 (58) | 10 (50) | .253 |
| Age at biopsy, years (mean ± SD [range]) | 60 ± 11.3 (32-84) | 60 ± 11.7 (34-78) | .923 |
| Smoking History | | | .212 |
| Current smoker | 14 (8) | 3 (15) | |
| Ex-smoker | 43 (24) | 7 (35) | |
| Treatment History | | | |
| Chemotherapy | 179 (100) | 20 (100) | 1.000 |
| Surgery | 38 (21) | 5 (25) | .700 |
| Therapy duration, months (mean ± SD [range]) | 30 ± 28.8 (0-158) | 40 ± 65.4 (6-303) | .867 |
| **Target Lesion Characteristics** | | | |
| Primary lesion | 131 (73) | 13 (65) | .439 |
| Metastasis | 48 (27) | 7 (35) | .424 |
| Size, mm (mean ± SD [range]) | 43 ± 20.9 (11-120) | 46 ± 19.8 (16-85) | .480 |
| Air bronchogram | 20 (11) | 1 (5) | .395 |
| Internal low-attenuation area | 7 (4) | 5 (25) | .001[a] |
| Solidity | | | .560 |
| Solid | 176 (98) | 20 (100) | |
| Subsolid | 3 (2) | 0 | |
| Contact with pleura | 51 (28) | 12 (60) | .004[a] |
| Peribronchovascular | 52 (29) | 8 (40) | .313 |
| Zone Location | | | .206 |
| Upper | 83 (46) | 7 (35) | |
| Middle | 27 (15) | 2 (10) | |
| Lower | 69 (39) | 11 (55) | |
| **Biopsy-Related Factors** | | | |
| No. of pleural puncture (mean ± SD [range]) | 1 ± 0.5 (1-4) | 1 ± 0.4 (2-3) | .562 |
| Specimen length, mm (mean ± SD [range]) | 16 ± 5.8 (5-22) | 16 ± 5.2 (10-22) | .514 |
| Position | | | .818 |
| Supine | 59 (33) | 6 (30) | |
| Prone | 119 (66) | 14 (70) | |
| Decubitus | 1 (1) | 0 | |
| Guidance Methods | | | .164 |
| Cone-beam CT | 163 (91) | 20 (100) | |
| CT | 15 (8) | 0 | |
| US | 1 (1) | 0 | |
| Needle Size | | | .152 |
| 18 gauge | 148 (83) | 19 (95) | |
| 20 gauge | 26 (15) | 1 (5) | |
| Needle Type | | | .336 |
| Noncoaxial | 171 (96) | 20 (100) | |
| Coaxial | 7 (4) | 0 | |
| Operator | | | .070 |
| Attending thoracic radiologist | 52 (29) | 2 (10) | |
| Clinical fellow | 127 (71) | 18 (90) | |

Data are presented as n (%) unless otherwise indicated. Needle size (n = 5) and needle type (n = 1) were not available on electronic medical record review.
Abbreviations: CT = computed tomography; US = ultrasound.
[a]Statistically significant results of difference between success and failure of parameters by Mann-Whitney *U* test ($P < .05$).

**Figure 2** Nondiagnostic Specimen Acquisitions in Rebiopsy for *EGFR* T790M Mutation Analysis in a 39-Year-Old Woman With Lung Adenocarcinoma. (A) Axial CT Image Obtained at Level of Aortic Arch Vessels Shows Mass With Large Area of Internal Low-attenuation (Asterisk) in Right Upper Lobe. (B) Cone-beam CT Image Obtained Immediately Before Transthoracic Lung Biopsy Reveals Tumor in Right Upper Lobe. Green and Red Lines Represent Needle Trajectory Planning Using XperGuide Software




Abbreviations: CT = computed tomography; *EGFR* = epidermal growth factor receptor.

incidence of tumor necrosis, leading to sampling error.[26] Therefore, it is important to develop a strategy to increase the yield of rebiopsy by avoiding necrotic or nonviable tumors in rebiopsy planning. Preprocedural CT should be carefully reviewed to plan a trajectory for avoiding necrotic areas in the target lesions. In our study, positron emission tomography (PET)/CT findings were not evaluated because PET/CT scan immediately before rebiopsy was not available in most patients. Previous studies reported that the use of $^{18}$F-flurodeoxyglucose PET/CT scans before transthoracic biopsy may play an important role in appropriate target lesion selection. PET/CT scan is a useful tool for "metabolic biopsy" and a higher diagnostic accuracy could be expected by avoiding metabolic defects on PET/CT during target lesion selection.[17,27,28]

In our study, except for the internal low-attenuation areas in target lesions, no other target lesion characteristics were associated with the acquisition of nondiagnostic specimens. Lesion size is a well-known factor that influences the diagnostic accuracy of transthoracic lung biopsy[29,30]; however, target lesion size was not a significant factor associated with the acquisition of nondiagnostic specimens in our study. This was consistent with the results of previous studies.[13,17] Target lesion selection (between primary and metastatic tumors) or the duration of chemotherapy was also not associated with nondiagnostic specimens in our study, which was consistent with the results of previous studies.[13,17] In addition, no procedure related features, such as biopsy needle size and type, number of procedures, patient position, and type of image guidance, were significantly associated with nondiagnostic specimen acquisition. We performed most rebiopsy procedures under cone-beam CT guidance, and the current evidence suggests that the accuracy of cone-beam CT-guided biopsy is comparable to the results of CT or CT fluoroscopy-guided biopsy.[31,32] These results suggested that rebiopsy in advanced lung cancer might not be different from initial biopsy in terms of technical factors and could be used as the basis for establishing rebiopsy guidelines.

Long-term use of cytotoxic drugs or *EGFR*-TKI may cause lung parenchymal changes, such as traction bronchiectasis or interstitial fibrosis, which potentially increase the risk of complications from rebiopsy.[33,34] Therefore, clinicians who request rebiopsy and

**Table 3** Multivariable Logistic Regression Analysis of Variables Potentially Associated With Failure of Rebiopsy in Patients With Lung Adenocarcinoma

| Variable | *P* | Odds Ratio | 95% Confidence Interval |
|---|---|---|---|
| Air bronchogram | .565 | 0.530 | 0.061, 4.605 |
| Internal low-attenuation area | .006[a] | 7.333 | 1.755, 30.633 |
| Contact with pleura | .052 | 3.140 | 0.992, 9.941 |
| Guided methods | .999 | | |
| Biopsy needle size | .512 | | |
| Operator | .169 | 3.085 | 0.620, 15.364 |

[a]Statistically significant ($P < .05$). Statistical comparison was performed using multivariable logistic regression analysis.

**Table 4** Results of *EGFR* Mutation Status

| *EGFR* Status | Initial Biopsy[a] (N = 155) | Rebiopsy (N = 179) |
|---|---|---|
| *EGFR* mutation negative | 71 (46) | 62 (35) |
| *EGFR* mutation positive | 84 (54) | 117 (65) |
| Exon 19 | 43 (28) | 28 (16) |
| Exon 21 (L858R) | 37 (24) | 29 (16) |
| Exon 19 + T790M | 1 (0.6) | 37 (21) |
| Exon 21 + T790M | 0 | 20 (11) |
| Other + T790M | 0 | 2 (1) |
| Other | 3 (2) | 1 (0.5) |

Data are presented as n (%).
Abbreviation: *EGFR* = epidermal growth factor receptor.
[a]Results of *EGFR* status at initial biopsy were available in 155 of 179 patients who successfully underwent rebiopsy.

radiologists who perform the procedure should be familiar with the risks and complications from rebiopsy. In our study, the overall complication rate of rebiopsy was 4% (8/199) and pneumothorax was the most common complication (3.5%, 7/199). The overall incidence and types of complications paralleled those in previous studies.[16,17,35] No serious complication or mortality was reported. The low complication rate in our study might be attributable to our group's experience or rigorous patient selection process. We previously reported our early experience of rebiopsy for advanced lung cancer,[17] and defined the exclusion criteria for rebiopsy. Our results suggested that, if carefully selected, rebiopsy for mutation analysis did not increase the risk of biopsy-related complications, even after long-term chemo- or target therapy.

Our study had several limitations. First, our study was limited by its retrospective nature. The follow-up period and type of treatment varied. Some data were incompletely documented from the outside hospital, which might be related with the increase in the number and percentage of subjects with *EGFR* mutation in the rebiopsy group. Second, our study was performed in a single-center tertiary hospital and may not be representative of the general population. In addition, the use of cone-beam CT may not be routinely available to radiologists in nonacademic centers. Third, the biopsies were performed by several thoracic radiologists during the study period, resulting in variability in experience and inconsistency in technique, but it may serve as a basis for the generalizability of our study. Fourth, the number of patients included in the study, in particular, the number of nondiagnostic specimens, was small, although this study included the largest number of patients who underwent transthoracic rebiopsy reported so far in the literature. Fifth, we did not evaluate the complimentary role of liquid biopsy for mutation analysis, which was unavailable in our routine clinical practice during the study period. Finally, our study did not address other mechanisms of resistance apart from *EGFR* T790M mutation, such as *MET* amplification, *HER2* amplification, or small-cell transformation, which might influence the diagnostic accuracy of rebiopsy.

In spite of the above-mentioned limitations, we can conclude that image-guided transthoracic rebiopsy for mutation analysis in lung adenocarcinoma is a safe and effective method and the success rate of rebiopsy for mutation analysis was 90%. An internal low-attenuation area in the target lesion on CT was the most important independent factor for predicting nondiagnostic specimen acquisition for mutation analysis in rebiopsy.

### *Clinical Practice Points*

- The feasibility and outcomes of rebiopsy for mutation analysis have been reported in patients with lung adenocarcinoma who were treated with *EGFR*-TKIs, whereas little is known about the factors that determine appropriate specimen acquisition for mutation analysis.
- Our study suggests that the use of image-guided transthoracic rebiopsy for mutation analysis in lung adenocarcinoma is safe and effective method for patients with *EGFR* mutations who have progressive disease and need to change chemotherapy drugs.
- The adequacy rate for tissue samples for mutation analysis was 90%.
- The presence of internal low-attenuation areas in the target lesion on contrast-enhanced CT was an independent predictor of nondiagnostic rebiopsy specimen acquisition. Avoiding an internal low-attenuation area in the target lesion on CT will improve the diagnostic accuracy of rebiopsy for mutation analysis.

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## Disclosure

The authors have stated that they have no conflict of interest.

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