Identifying factors causing failure of nodal staging by endobronchial ultrasound-guided transbronchial needle aspiration in non-small cell lung cancer

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Background: Endobronchial ultrasound-guided transbronchial needle aspiration (EBUS-TBNA) is recommended for nodal staging in non-small cell lung cancer (NSCLC). Although this method may rarely fail, reports on the causes are few. We therefore retrospectively investigated the factors causing failure of nodal staging by EBUS-TBNA.

Methods: Consecutive patients who underwent EBUS-TBNA at National Cancer Center Hospital between January 2017 and December 2020 for systematic nodal staging in NSCLC were extracted. The nodal stages at diagnosis including EBUS-TBNA and at treatment were investigated separately, and unmatched cases were defined as failures. Factors associated with them were explored while dividing the cases into punctured and not-punctured groups.

Results: Of the 264 patients, 21 (8.0%) failed the nodal staging: 10 (3.8%) in the punctured group and 11 (4.2%) in the not-punctured group. The latter was subdivided into the following three categories: (I) difficult-to-reach; (II) omission due to false-positive rapid on-site cytologic evaluation (ROSE) results; and (III) non-significant EBUS findings. The nodal staging failure rate was significantly higher in cases with driver oncogenes positive than in those negative (16.1% *vs.* 3.3%, P=0.026) for adenocarcinomas. Note that all cases categorized as non-significant EBUS findings involved various driver oncogenes.

Conclusions: The present study demonstrated the risk of false positives with ROSE and the involvement of driver oncogenes as factors associated with nodal staging failure in NSCLC by EBUS-TBNA, in addition to limitations of the procedure itself, including sampling performance and reachability. Especially in adenocarcinoma patients with driver oncogenes, their nodal staging results should be interpreted cautiously.

Keywords: Endobronchial ultrasound-guided transbronchial needle aspiration (EBUS-TBNA); nodal staging; staging failure; non-small cell lung cancer (NSCLC); bronchoscopy

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Introduction

Lung cancer is the leading cause of cancer-related deaths, with an estimated 2.2 million new cases and 1.8 million deaths worldwide in 2020, among which 85% of the deaths were due to non-small cell lung cancer (NSCLC) (1).

Accurate nodal staging is crucial for the prognosis and treatment of NSCLC (2-5) without distant metastases. However, nodal staging by contrast-enhanced computed tomography (CT) and ^{18}F-fluorodeoxyglucose-positron emission tomography (FDG-PET) is insufficient (2,3). Therefore, invasive nodal staging involving the collection of pathologic specimens from lymph nodes (LNs) is recommended for patients suspicious of mediastinal LN metastasis (N2 or higher) and is also considered in the presence of the following: primary lesion >3 cm, primary lesion centrally located, or N1 suspected on imaging (6-8).

Endobronchial ultrasound-guided transbronchial needle aspiration (EBUS-TBNA) is a minimally invasive and standardized technique for sampling of lesions contacting the central airway, especially hilar and mediastinal LNs. It has a high diagnostic value in nodal staging, with sensitivities and specificities of 80–96% and 100%,

respectively (2,3,9-11). Thus, when utilized as the initial examination, it is safer, more cost-effective, and equally as efficient as surgical nodal staging (11-17).

Nevertheless, the occasional failure of nodal staging by EBUS-TBNA can lead to delayed diagnosis and inappropriate treatment strategies. Notably, clinical stage III treatment plans vary based on nodal staging results. When EBUS-TBNA reveals mediastinal involvement, further confirmatory tests are generally unnecessary due to the exceedingly low likelihood of false-positive results (18). The primary concern arises from false-negative EBUS-TBNA outcomes, where patients are potentially downstaged to a clinical stage N2, resulting in incorrect surgical indications. While current guidelines recommend that cases of negative EBUS-TBNA with strong suspicion of LN metastases should undergo additional surgical staging, such as cervical mediastinoscopy (8,19), a recent randomized controlled trial has reported the validity of omitting it (20).

Furthermore, the landscape of early-NSCLC treatment is evolving with the introduction of neoadjuvant therapies, including immune checkpoint inhibitor combinations for non-oncogene-driven NSCLC and molecular targeted therapies for oncogene-driven NSCLC, as indicated by several ongoing phase III trials (21). In the future, there is anticipated to be an increase in the number of cases that are currently deemed inoperable but have the potential to be downstaged through preoperative treatments and thus to become eligible for surgical resection.

Hence, while EBUS-TBNA offers high reliability in nodal staging, it may not be infallible, prompting the need for an in-depth investigation of factors contributing to the diagnostic failures. In the quest for enhanced diagnostic precision and the ever-evolving landscape of precision medicine, this study aimed to identify factors that may contribute to nodal staging failures via EBUS-TBNA in NSCLC. We present this article in accordance with the STROBE reporting checklist (available at https://tlcr.amegroups.com/article/view/10.21037/tlcr-23-264/rc).

Highlight box

Key findings
- Despite the addition of endobronchial ultrasound-guided transbronchial needle aspiration (EBUS-TBNA), nodal staging in non-small cell lung cancer failed in 21 of 264 patients (8.0%).
- Failures categorized as punctured and not-punctured, with the latter further divided into: (I) difficult-to-reach; (II) omission due to false-positive rapid on-site cytologic evaluation (ROSE) results; and (III) non-significant EBUS findings.
- Adenocarcinoma patients with driver oncogenes had a significantly higher failure rate (16.1% *vs.* 3.3%, P=0.026).

What is known and what is new?
- EBUS-TBNA is recommended for nodal staging, but poorly investigated regarding failure causes.
- Three main causes for "not-punctured group": (I) difficult-to-reach; (II) omission due to false-positive ROSE results; and (III) non-significant EBUS findings.
- Novel finding: significantly higher failure rate in adenocarcinoma with driver oncogenes.

What is the implication, and what should change now?
- Identifying failure causes and improvements enhance diagnostic accuracy in nodal staging.
- Exercise caution in interpreting nodal staging results in adenocarcinoma patients with driver oncogenes.

Methods

Patients

Consecutive patients who underwent EBUS-TBNA at the National Cancer Center Hospital between January 2017 and December 2020 were reviewed. Of them, cases wherein the purpose was systematic nodal staging were included. Note that we perform systematic nodal staging

based on imaging, usually for cases of suspected mediastinal LN metastasis, and often also for cases of centrally located primary tumors and/or N1. We do not cover cases in which extensive mediastinal LN metastases are evident, nor do we establish criteria based on primary tumor size. Cases for (I) re-staging purposes after any treatment; (II) those found to be stage IV; and (III) those diagnosed with other than NSCLC were excluded; and the remaining cases were eligible for analyses.

This study was performed in accordance with the Declaration of Helsinki (as revised in 2013). This study was approved by the National Cancer Center Institutional Review Board (No. 2018-090). The requirement for informed consent was waived due to the retrospective nature of this study. However, the information used in this study was entered into a database independent of the electronic medical record, and patient identification was controlled by a separately assigned study number so that third parties cannot identify them.

Equipment and procedures

Either of the two linear ultrasound bronchoscopes (UC260FW or UC290F; Olympus, Tokyo, Japan) was used, and almost all cases involved the latter after its introduction. Various needle types, including 22-gauge needles (NA-201SX-4022/NA-U401SX-4022, Olympus; Expect™ Pulmonary, Boston Scientific, Natick, MA, USA; EchoTip Ultra®/ProCore®, Cook Medical, Bloomington, IN, USA) or 25-gauge needles (NA-U401SX-4025N, Olympus; Expect™ Pulmonary, Boston Scientific; EchoTip Ultra®/ProCore®, Cook Medical), were used, depending on the timing of the examination and the discretion of the bronchoscopist.

Moderate-to-deep sedation with a combination of intravenous narcotics (fentanyl or pethidine) and sedatives (propofol or midazolam) was administered. All procedures were performed under the supervision of bronchoscopy experts with more than 10 years of experience. The bronchoscope was inserted orally after topical application of aerosolized 8% lidocaine; intratracheal local anesthesia was administered using 2% lidocaine injection.

Visual LN survey through EBUS was performed in the order of the primary lesion (if observable), N1, N2, and N3 stations. The possibility of metastasis to each LN was systematically assessed with a combination of ultrasound modes. First, the following sonographic features on conventional B-mode were observed according to the previous report: short-axis size, shape, margin, echogenicity, and presences of central hilar structure and coagulation necrosis sign (22). Second, the vascularity was evaluated using color/power Doppler mode (23). Third, elastography mode was used when available to assess the relative stiffness of tissues (24). These findings were identified and recorded during real-time evaluation.

Subsequently, targeted lesions with sonographic findings suggestive of malignancy (22,25) or at locations critical to determine the treatment protocols were punctured in the order of the N3, N2, N1 stations, and primary lesion (if approachable) to avoid contamination from higher LNs. EBUS-TBNA was performed using a negative pressure syringe (10 or 20 mL) with around 30–50 agitations per puncture. If there was considerable blood backflow, lower negative pressure or a slow-pull method was applied to the subsequent passes. Whenever possible, the puncture was repeated at least twice to ensure the sample adequacy, while referring to the results of rapid on-site cytologic evaluation (ROSE). Although not performed routinely, transesophageal endoscopic ultrasound with bronchoscope-guided fine needle aspiration (EUS-B-FNA) was also aggressively conducted for cases with LNs with difficult transbronchial access, such as #5 and #8.

Definition of nodal stage

Nodal stages were investigated separately at diagnosis and treatment. The former was categorized as "unconfirmed", relying on the results of EBUS-TBNA in addition to imaging modalities such as CT and FDG-PET. In contrast, the "confirmed" nodal stages were determined based on the pathological stages of patients who underwent surgery. For patients who did not undergo surgery, the confirmed stages were based on the consensus results of the multidisciplinary team.

Since the prognoses have been reported to differ between patients with single- and multiple-station N2 (N2a and N2b, respectively) (26), and the difference has a significant impact on the indication for surgery, they were investigated separately.

Variables

We defined cases where the unconfirmed and confirmed stages matched as "nodal staging successes", while those that did not match were defined as "failures". In this study, we focused on the failure cases and explored factors

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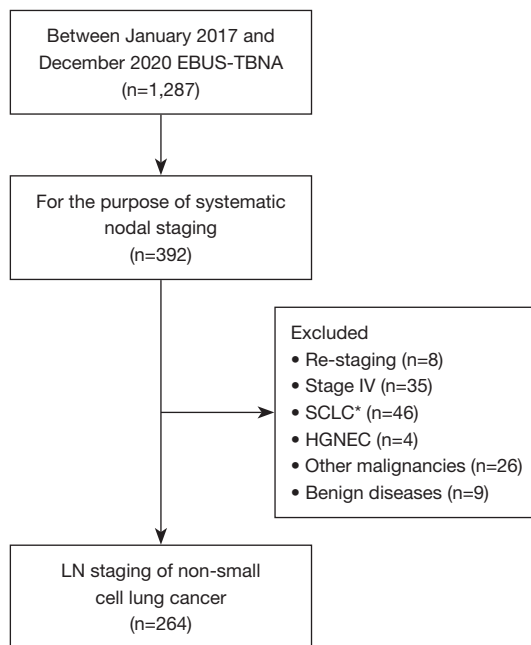So et al. Staging failure causes in NSCLC

Figure 1 Flow diagram of the analyzed patients. *, SCLC includes combined SCLC. EBUS-TBNA, endobronchial ultrasound-guided transbronchial needle aspiration; SCLC, small cell lung carcinoma; HGNEC, high-grade neuroendocrine carcinoma; LN, lymph node.

associated with them. Specifically, since there is an essential difference between (I) cases that were punctured but resulted in failures and (II) cases that were not punctured and resulted in failures, these two groups, "punctured" and "not-punctured" were investigated separately.

The following patient data were collected: age, sex, smoking history, side and location of the primary lesion, lesion size, nodal stage by CT/FDG-PET, and histological type. The smoking history was classified as "never/light" and "heavy", with the Brinkman index bordering 400. The location was determined by dividing the distance from the pulmonary hilum into three equal parts referring to the previous report: the central two-thirds was defined as "central" and the rest was defined as "peripheral" (27). The lesion size was measured as the short diameter on axial CT images. The histological type was divided into adenocarcinoma and non-adenocarcinoma.

If biomarker tests for driver oncogenes had been performed, the results were also extracted. Since the types and methods of those tests varied from time to time, cases in which at least epidermal growth factor receptor (*EGFR*)

and anaplastic lymphoma kinase (*ALK*) were examined were included.

Statistical analyses

Descriptive statistics are presented as numerical values and frequencies (percentages) or medians (ranges). Univariable analyses were performed using Fisher's exact test. All statistical analyses were performed using EZR version 1.55 (Saitama Medical Center, Jichi Medical University, Saitama, Japan), a graphical user interface for R (The R Foundation for Statistical Computing, Vienna, Austria) (28). All P values were two-sided, and those less than 0.05 were considered statistically significant.

Results

During the study period, we performed EBUS-TBNA for 1,287 patients, and 392 cases were intended for the systematic nodal staging. As per the inclusion criteria, 264 cases were included in the analyses (*Figure 1*).

The baseline patients' characteristics and details of the nodal staging are summarized in *Table 1*. The median [range] age was 70 [31–90] years, and 185 patients (70.1%) were male. The primary lesions were located mainly on the right side [175 cases (66.3%)] and at the peripheral location [209 cases (79.2%)]. Of the 724 lesions punctured in total, the most frequent site was #4R [145 lesions (20.0%)] followed by #7 [122 lesions (16.9%)] and #4L [104 lesions (14.4%)]. The histologic type was mostly adenocarcinoma in 154 (58.3%), followed by squamous cell carcinoma in 77 (29.2%), NSCLC, not otherwise specified (NOS) in 13 (4.9%), and others in 20 (7.6%).

All but 11 cases were evaluated with FDG-PET in conjunction with CT. However, 189 cases (71.6%) were correctly staged without EBUS-TBNA, whereas with the addition of EBUS-TBNA, 243 cases (92.0%) were correctly staged (Table S1). There were 21 cases (7.9%) that were upstaged and 33 cases (12.5%) that were downstaged. In other words, despite the addition of EBUS-TBNA, 21 cases (8.0%) failed the nodal staging. Although we analyzed the association between the failure cases and the clinical factors, there were no significant differences (*Table 2*).

Next, all 21 patients who failed the nodal staging are summarized (*Table 3*): 10 were in the punctured group and 11 were in the not-punctured group. The latter, when reviewed in detail down to the causes that prevented

Table 1 Baseline patients' characteristics and details of nodal staging by EBUS-TBNA (n=264)

Characteristic	Value
Age (years), median [range]	70 [31–90]
Sex, n (%)	
Male	185 (70.1)
Female	79 (29.9)
Smoking history, n (%)	
Never/light	192 (72.7)
Heavy	72 (27.3)
Primary lesion side, n (%)	
Right	175 (66.3)
Left	89 (33.7)
Primary lesion location, n (%)	
Peripheral	209 (79.2)
Central	55 (20.8)
Punctured lesion (n=724), n (%)	
#1R	6 (0.8)
#2L[†]	7/1 (1.1)
#2R	66 (9.1)
#3p	16 (2.2)
#4L[†]	97/7 (14.4)
#4R	145 (20.0)
#5[†]	4/11 (2.1)
#7	122 (16.9)
#8[†]	11/6 (2.3)
#10L	8 (1.1)
#10R	15 (2.1)
#11L	43 (5.9)
#11R	83 (11.5)
#12L	12 (1.7)
#12R	7 (1.0)
#13L	1 (0.1)
#14R	1 (0.1)
Primary lesion[†]	50/5 (7.6)
Histological type, n (%)	
Adenocarcinoma	154 (58.3)
Squamous cell carcinoma	77 (29.2)
NSCLC, NOS	13 (4.9)
Others	20 (7.6)

Table 1 (*continued*)

Table 1 (*continued*)

Characteristic	Value
Driver oncogene, n (%)	
Positive	65 (24.6)
Negative	105 (39.8)
Not evaluated	94 (35.6)

[†], marked lesions include those in which EUS-B-FNA was performed. The left side of the slash indicates the number of cases sampled by EBUS-TBNA and the right side indicates that by EUS-B-FNA, respectively. EBUS-TBNA, endobronchial ultrasound-guided transbronchial needle aspiration; NSCLC, non-small cell lung cancer; NOS, not otherwise specified; EUS-B-FNA, transesophageal endoscopic ultrasound with bronchoscope-guided fine needle aspiration.

Table 2 Univariable analysis of factors associated with staging failure (n=264)

Variable	Success (n=243)	Failure (n=21)	P value
Age (years), n (%)			>0.99
≤70	127 (92.0)	11 (8.0)	
>70	116 (92.0)	10 (8.0)	
Sex, n (%)			>0.99
Male	170 (91.9)	15 (8.1)	
Female	73 (92.4)	6 (7.6)	
Smoking history, n (%)			0.610
Never/light	178 (92.7)	14 (7.3)	
Heavy	65 (90.3)	7 (9.7)	
Primary lesion side, n (%)			0.639
Right	162 (92.6)	13 (7.4)	
Left	81 (91.0)	8 (9.0)	
Primary lesion location, n (%)			0.264
Peripheral	190 (90.9)	19 (9.1)	
Central	53 (96.4)	2 (3.6)	
Primary lesion size (mm), n (%)			0.646
≤30	146 (91.2)	14 (8.8)	
>30	97 (93.3)	7 (6.7)	
Suspected nodal stage on imaging, n (%)			>0.99
≥1	216 (91.9)	19 (8.1)	
0	27 (93.1)	2 (6.9)	
Histological type, n (%)			0.820
Adenocarcinoma	141 (91.6)	13 (8.4)	
Non-adenocarcinoma	102 (92.7)	8 (7.3)	

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Table 3 Summary of patients who failed nodal staging (n=21)

Group	Age (years)	Sex	Primary lesion site	Failed lesion	Histological type	Driver oncogene	Failure cause
P	68	Male	Left hilum	#4R	Sq	Negative	False negative
P	81	Female	RUL	#7	Sq	Negative	False negative
P	65	Male	RLL	#4R	AdSq	Negative	False negative
P	64	Male	RUL	#2R, #4R	Sq	Not evaluated	False negative
P	75	Female	LLL	#4L	Sq	Negative	False negative
P	65	Male	RLL	#7	Pleomorphic	Not evaluated	False negative
P	74	Male	RLL	#4R	Ad	*EGFR*	False negative
P	67	Male	LUL	#5	Ad	Negative	False negative
P	65	Male	LUL	#5	Ad	Not evaluated	False negative
P	57	Male	ML	#1R	Ad	*RET*	False negative
NP	82	Male	RLL	#14R	Sq	Not evaluated	Difficult-to-reach
NP	66	Male	LLL	#10L	Sq	Negative	Difficult-to-reach
NP	54	Male	LLL	#5	Ad	Not evaluated	Omission due to false-positive ROSE results
NP	75	Male	LUL	#10L, #12L, #13L	Ad	Negative	Omission due to false-positive ROSE results
NP	79	Female	LLL	#4L, #5, #7, #8, #11L	Ad	*HER2*	Non-significant EBUS findings
NP	53	Female	RLL	#7, #11R, #12R	Ad	*ALK*	Non-significant EBUS findings
NP	77	Male	RUL	#2R	Ad	*MET*	Non-significant EBUS findings
NP	71	Female	RLL	#7	Ad	*EGFR*	Non-significant EBUS findings
NP	69	Female	RUL	#2R	Ad	*ALK*	Non-significant EBUS findings
NP	73	Male	RLL	#4R	Ad	*EGFR*	Non-significant EBUS findings
NP	85	Male	RUL	#2R	Ad	*EGFR*	Non-significant EBUS findings

P, punctured; Sq, squamous cell carcinoma; RUL, right upper lobe; RLL, right lower lobe; AdSq, adenosquamous carcinoma; LLL, left lower lobe; Ad, adenocarcinoma; *EGFR*, epidermal growth factor receptor; LUL, left upper lobe; ML, middle lobe; *RET*, rearranged during transfection; NP, not-punctured; ROSE, rapid on-site cytologic evaluation; *HER2*, human epidermal growth factor receptor 2; EBUS, endobronchial ultrasound; *ALK*, anaplastic lymphoma kinase; *MET*, mesenchymal-epithelial transition.

punctures, were subdivided into the following three categories: (I) two of difficult-to-reach LNs; (II) two of omission at lower level LNs due to false-positive ROSE results at the higher level; and (III) seven of LNs with non-significant EBUS findings (i.e., <5 mm in the short diameter, not corresponding to the puncture target).

Last, focusing on biomarker tests, 65 of the 170 patients who were checked for driver oncogenes were positive. Stratified analysis was performed separately for adenocarcinoma and non-adenocarcinoma, considering the difference in driver oncogene positive rates; the association between histology type and nodal staging failure is expressed in *Table 4*. In cases of adenocarcinoma, the nodal staging failure rate was significantly higher in cases positive for driver oncogenes than in negative cases [9/56 (16.1%) *vs.* 2/60 (3.3%), P=0.026]. Note that all cases categorized as non-significant EBUS findings in the not-punctured group involved various driver oncogenes (*Table 3*).

Discussion

This study demonstrated the nodal staging failure cases

Table 4 Stratified analysis of driver oncogene involvement considering histological type (n=170)

Histological type	Driver oncogene	Nodal staging			P value
		Success	Failure	Total	
Adenocarcinoma	Positive	47	9	56	0.026
	Negative	58	2	60	
	Total	105	11	116	
Non-adenocarcinoma	Positive	9	0	9	0.576
	Negative	40	5	45	
	Total	49	5	54	

by EBUS-TBNA in NSCLC and its associated factors. There were 21 cases (8.0%) of nodal staging failure: 10 (3.8%) in the punctured group and 11 (4.2%) in the not-punctured group. The not-punctured group was subdivided into the following three categories: (I) difficult-to-reach; (II) omission due to false-positive ROSE results; and (III) non-significant EBUS findings. Focusing on biomarker tests, the nodal staging failure rate was significantly higher in adenocarcinoma cases that were positive for driver oncogenes than in negative cases [9/56 (16.1%) *vs.* 2/60 (3.3%), P=0.026]. Note that all cases categorized as having non-significant EBUS findings in the not-punctured group involved various driver oncogenes. To the best of our knowledge, few studies have investigated the factors leading to the failure of nodal staging by EBUS-TBNA and evaluated their relationship to driver oncogenes.

LNs with a short diameter of ≥1 cm on chest CT are generally diagnosed as positive for metastasis, with the sensitivity and specificity of 59% and 78%, respectively (9). Meanwhile, FDG-PET has the sensitivities and specificities of 80–90% and 85–95%, respectively (2,3). The sensitivities of the systematic nodal staging by EBUS-TBNA have been reported to be 86–92% (2,3,9-11). In the present study, the sensitivities before and after EBUS-TBNA were 71.6% and 92.0%, respectively. While FDG-PET was utilized with CT in almost all cases, nodal staging through EBUS-TBNA produced more valuable results and mostly aligned with those reported in the previous literatures.

The punctured failures could have been influenced by a variety of factors, such as the number of times each LN was punctured, needle size used, and bronchoscopist's experience. However, a systematic review and meta-analysis showed similarly high diagnostic sensitivities of EBUS-TBNA for diagnosing NSCLC regardless of the needle

size used (29). Furthermore, it has been reported that the diagnostic yield did not increase after three or more punctures (9), nor did experienced physicians perform the punctures (30). Thus, false negatives can be considered a limitation in the diagnostic performance of EBUS-TBNA itself.

On the other hand, in the not-punctured group, one of the causes was difficult-to-reach LNs, including #10L behind the left pulmonary artery trunk and #14R. Approaches to these LNs require overcoming the challenges of limited mobility due to the combination of ultrasound bronchoscopes and needles. Some LNs were difficult to approach with the conventional ultrasound bronchoscope, but since the advent of the newer bronchoscope, the miniaturization of the ultrasound tip and the modified puncture angle have improved this situation considerably (31,32). Nevertheless, although it is currently almost impossible to approach the intrapulmonary LNs, a prototype ultrasound bronchoscope with an even thinner tip has been reported (33), which should make the approach feasible in the future. In addition, although we have not actively performed it for staging purposes, the utility of EBUS-guided transvascular sampling for lesions located behind major vessels has been reported (34) and may be considered.

Another cause was that lower-level LNs, necessary for the true staging, were not punctured due to the false-positive ROSE results at the higher level. ROSE during EBUS-TBNA has been reported to contribute to improved diagnostic outcomes, reduced puncture attempts, and decreased complications (35). Although only a few articles have described the concordance rate of ROSE in EBUS-TBNA as compared to the final cytology, the accuracy has been reported to range from 89% to 98% (35-37). It is generally rare to encounter false-positive results with

EBUS-TBNA, but it is possible with ROSE (35,38). In particular, reactive bronchial epithelial cells have the potential to mimic neoplastic cells and may pose a challenge for less experienced cytopathologists when interpreting the results. It should be noted that false-positive results with ROSE have been reported not only in cases of lung cancer, but also in cases of granulomatous diseases (39). Therefore, it is crucial to be aware of these potential challenges and maintain accuracy control when using ROSE in conjunction with nodal staging.

The remaining cause was that the EBUS findings were deemed non-significant and thus were not considered for punctures. The LNs to be sampled for staging are mainly three mediastinal LNs, including #4R, #4L, and #7, usually with the short diameter of 5 mm or greater (40). However, the LNs in this category were visualized as tiny lesions, did not meet this size criterion, and thus resulted in false negatives. Interestingly, all these patients had adenocarcinoma with various driver oncogenes. In an additional stratified analysis, adenocarcinomas with positive driver oncogenes were significantly more likely to fail the staging than those without. According to Marulli *et al.*, LN metastasis was more common in patients with adenocarcinoma when the clinical stage was the same, and the relative risk of unexpected postoperative LN metastasis was about seven times higher than that in patients with squamous cell carcinoma (41). Furthermore, Seto *et al.* reported an increased incidence of subclinical LN metastasis in *EGFR* mutation-positive and *ALK* translocation-positive adenocarcinomas (42). Based on the above, adenocarcinoma patients with driver oncogenes are considered to be at high risk for LN metastasis, even if the image findings are obscure, which would have contributed to the staging failures. We present a representative case of nodal staging failure in a 53-year-old female with *ALK* translocation-positive adenocarcinoma (*Figure 2*).

Recently, neoadjuvant therapies have attracted attention as an alternative to conventional adjuvant therapies for perioperative treatment. Based on the results of the CheckMate 816 trial, neoadjuvant nivolumab plus chemotherapy has already been introduced into clinical practice for NSCLC (43). However, *EGFR* mutation- or *ALK* translocation-positive patients were excluded in the trial. The KEYNOTE-671 trial also reported the efficacy of neoadjuvant pembrolizumab plus chemotherapy followed by surgery and adjuvant pembrolizumab for NSCLC (44). However, it is argued that very few patients

with *EGFR*-mutation or *ALK*-translocation were enrolled in this trial, limiting insights into the efficacy of this combination therapy for these subgroups. On the other hand, following the ADAURA trial of adjuvant osimertinib for *EGFR* mutation-positive NSCLC, which showed longer overall survival in patients who received osimertinib (45), another trial of osimertinib in the neoadjuvant setting is underway (ClinicalTrials.gov identifier: NCT04351555, JapicCTI-205325). Moreover, an umbrella trial investigating neoadjuvant therapies for various driver oncogenes is in progress, and it is becoming increasingly important to examine these genetic statuses preoperatively even in resectable cases (46). Thus, our results suggest the need for caution when interpreting nodal staging in patients who are preoperatively determined to be driver oncogene-positive.

Our study has several limitations. First, this was a retrospective, single-center study, and the type of the ultrasound bronchoscope and needles used depended on the timing of the examination and/or the discretion of each bronchoscopist. Second, in confirming the final nodal stages, not all cases were determined by their postoperative pathological stages. For non-surgical cases, the confirmed stages were determined based on the consensus results of the multidisciplinary team. Although the confirmed stages may be somewhat inaccurate, this study included patients who were upstaged after EBUS-TBNA, thus avoiding unnecessary surgeries. The presence of these cases is one of the important indicators of the efficacy of nodal staging as well, and their inclusion would be reasonable to reflect actual clinical practice. Third, not all patients underwent biomarker tests for driver oncogenes, and the types and methods used were subject to the time period. Nevertheless, the fact that nodal staging was prone to failure in adenocarcinomas that were positive for driver oncogenes raises significant attention.

Conclusions

The present study demonstrated the risk of false positives with ROSE and the involvement of driver oncogenes as factors associated with nodal staging failure in NSCLC by EBUS-TBNA, in addition to limitations of the procedure itself, including sampling performance and reachability. Especially in adenocarcinoma patients with driver oncogenes, the possibility of metastases was significantly high even in LNs with obscure image findings, and their nodal staging results should be interpreted cautiously.
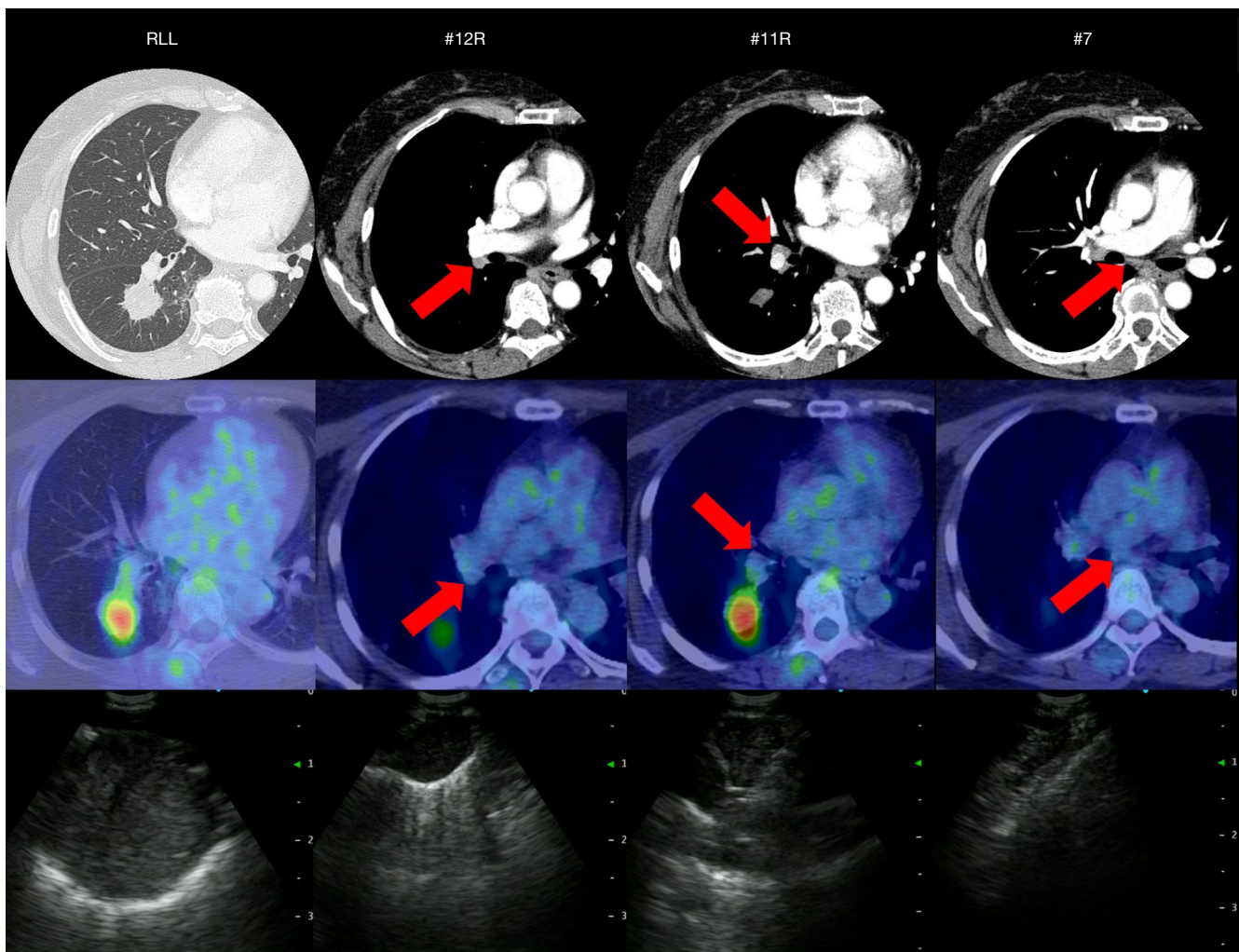
Figure 2 Representative case of nodal staging failure. A 53-year-old female with adenocarcinoma of the lung originating in her RLL; CT and FDG-PET showed no accumulation other than the primary tumor. Through the systematic nodal staging with EBUS-TBNA, #12R and #11R were punctured, while #7 was not punctured due to lack of findings suggestive of malignancy. As a result, #12R was positive for metastasis; however, #11R detected only atypical cells. In the resected surgical specimens, metastases were proven widely in the following LNs: #11R and #7. The patient was later found to be positive for *ALK* translocation and relapsed 2 years after the surgery. Arrows indicate the LNs punctured. RLL, right lower lobe; CT, computed tomography; FDG-PET, ^{18}F-fluorodeoxyglucose-positron emission tomography; EBUS-TBNA, endobronchial ultrasound-guided transbronchial needle aspiration; LN, lymph node; *ALK*, anaplastic lymphoma kinase.

Footnote

Reporting Checklist: The authors have completed the STROBE reporting checklist. Available at https://tlcr.amegroups.com/article/view/10.21037/tlcr-23-264/rc

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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. This study was performed in accordance with the Declaration of Helsinki (as revised in 2013). This study was approved by the National Cancer Center Institutional Review Board (No. 2018-090). The requirement for informed consent was waived due to the retrospective nature of this study.

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Table S1 Shifts of nodal stage with imaging plus EBUS-TBNA ("unconfirmed" nodal stage) to the pathological stage based on surgery or multidisciplinary team consensus ("confirmed" nodal stage)

"Unconfirmed" nodal stage	"Confirmed" nodal stage					
	0	1	2a	2b	3	Total
0	50[†]	3	4	0	0	57
1	0	40[†]	2	1	0	43
2a	0	0	63[†]	9	2	74
2b	0	0	0	52[†]	0	52
3	0	0	0	0	38[†]	38
Total	50	43	69	62	40	264

The sum of the numbers with asterisks ([†]) where "unconfirmed" nodal stages and "confirmed" nodal stages matched were 243. The diagnostic accuracy with imaging plus EBUS-TBNA was 92.0% (243/264). EBUS-TBNA, endobronchial ultrasound-guided transbronchial needle aspiration.