Endoscopic ultrasound-guided fine needle aspiration and endobronchial ultrasound-guided transbronchial needle aspiration: Are two better than one in mediastinal staging of non–small cell lung cancer?

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Objective: The role of combined endobronchial ultrasound-guided transbronchial needle aspiration (EBUS-TBNA) and endoscopic ultrasound-guided fine needle aspiration (EUS-FNA) with a single bronchoscope is poorly understood. The purpose of the present study was to elucidate the roles of EBUS-TBNA and EUS-FNA with a single bronchoscope in the preoperative hilar and mediastinal staging of non–small cell lung cancer (NSCLC).

Methods: A total of 150 patients with potentially resectable known or suspected NSCLC were enrolled in our prospective study. EBUS-TBNA was performed, followed by EUS-FNA, with an EBUS bronchoscope for N2 and N3 nodes ≥5 mm in the shortest diameter on ultrasound images, in a single session.

Results: EBUS-TBNA was performed for 257 lymph nodes and EUS-FNA for 176 lymph nodes. Of the 150 patients, 146 had a final diagnosis of NSCLC. Of these 146 patients, 33 (23%) had N2 and/or N3 nodal metastases. The sensitivity of EBUS-TBNA, EUS-FNA, and the combined approach per patient was 52%, 45%, and 73%, respectively (EBUS-TBNA vs the combined approach, $P = .016$, McNemar's test). The corresponding negative predictive value was 88%, 86%, and 93%. Two patients (1%) developed severe cough from EBUS-TBNA.

Conclusions: The combined endoscopic approach with EBUS-TBNA and EUS-FNA is a safe and accurate method for preoperative hilar and mediastinal staging of NSCLC, with better results than with each technique by itself. (J Thorac Cardiovasc Surg 2014;148:1169-77)

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Supplemental material is available online.

Endoscopic ultrasound (EUS)-guided needle techniques, including endobronchial ultrasound-guided (EBUS) transbronchial needle aspiration (EBUS-TBNA) and EUS-guided fine needle aspiration (EUS-FNA), have been recommended as the test of choice for mediastinal staging of non–small cell lung cancer (NSCLC).[1] Although either EBUS-TBNA[2,3] or EUS-FNA[4,5] alone has been found to be an effective method, the combination of EBUS-TBNA and EUS-FNA has been reported to be more accurate than either method alone,[6-10] because EBUS-TBNA and EUS-FNA have complementary roles for mediastinal exploration.[11] However, the combination method has had some issues regarding the availability of expensive equipment and expertise. To overcome these problems, the utility of EUS-FNA with an EBUS bronchoscope in place of an EUS endoscope has been advocated.[12-14] Although the procedure requires some experience and skill, it can be performed by a bronchoscopist with an EBUS bronchoscope and thus enable a simple combined transbronchial and transesophageal endoscopic approach. To date, a few investigators[12,13] have suggested the efficacy of combined EBUS-TBNA and EUS-FNA with an EBUS bronchoscope in the mediastinal staging of NSCLC. However, because no prospective study has clearly demonstrated that the diagnostic value of the combined method is superior to that of each method alone, the roles remain unknown. The purpose of the present study was to elucidate the role of combined EBUS-TBNA and EUS-FNA with a single bronchoscope in preoperative hilar and mediastinal staging of NSCLC. The primary endpoint of the present study was to compare the diagnostic value of the combined method to that of each method by itself. The secondary endpoints were safety and the procedure duration.

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Abbreviations and Acronyms

CT = computed tomography
EBUS = endobronchial ultrasound
EBUS-TBNA = endobronchial ultrasound-guided transbronchial needle aspiration
EUS = endoscopic ultrasound
EUS-FNA = endoscopic ultrasound-guided fine needle aspiration
NSCLC = non–small cell lung cancer
TBNA = transbronchial needle aspiration

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METHODS

Patients

We performed a prospective study that had been approved by the institutional review board of Nagoya Medical Center (identifier, 2009-251) and registered with the University Hospital Medical Information Network-Clinical Trials Registry (identifier, UMIN000002882). From December 2009 to August 2012, 150 patients with potentially operable, pathologically proven or clinical or radiologically suspected, NSCLC were enrolled in the present study. The operability was decided from the radiologic findings, including chest computed tomography (CT), positron emission tomography-CT, and brain magnetic resonance imaging, and the patients' condition. Patients with stage T4 or M1 disease according to the International Association for the Study of Lung Cancer staging system[15,16] were excluded. Patients with bulky N2 or N3 disease were also excluded. In our institution, we usually perform bronchoscopy for diagnosis and mediastinal staging in a separate setting; however, we sometimes perform EBUS-TBNA for highly suspicious mediastinal lymph nodes as an initial diagnostic test. Such patients with pathologically proven N2 or N3 disease were not included in the present study. All patients provided written informed consent. The baseline characteristics of the 150 patients are listed in Table 1.

TABLE 1. Patient and lesion characteristics

Characteristic	Value
Patients (n)	150
Gender	
Male	103 (69)
Female	47 (31)
Age (y)	
Mean ± standard deviation	68.3 ± 8.6
Range	33-83
Smoking history	
Never	30 (20)
Former	52 (35)
Current	68 (45)
Primary lesion location by bronchopulmonary segment	
Right upper lobe	51 (34)
Right middle lobe	3 (2)
Right lower lobe	24 (16)
Left upper lobe	34 (23)
Lingula	7 (5)
Left lower lobe	31 (21)
Final histopathologic classification	
Non–small cell lung cancer	
Adenocarcinoma	89 (59)
Squamous cell carcinoma	48 (32)
Large cell carcinoma	3 (2)
Adenocarcinoma + squamous cell carcinoma	2 (1)
Adenocarcinoma + large cell carcinoma	1 (1)
Squamous cell carcinoma + small cell carcinoma	1 (1)
Adenocarcinoma + small cell carcinoma	1 (1)
Sarcomatoid carcinoma	1 (1)
Other	
Small cell carcinoma	2 (1)
Tuberculosis	1 (1)
Organizing pneumonia	1 (1)
Preprocedural diagnosis for non–small cell lung cancer	
Diagnosed	137 (91)
Undiagnosed, but suspected	13 (9)

Data presented as n (%), unless otherwise noted.

Procedures

For EBUS-TBNA and EUS-FNA, a convex probe ultrasound bronchoscope (BF-UC260F-OL8 or BF-UC260FW; Olympus, Tokyo, Japan) and 22-gauge needles (NA-201SX-4022; Olympus) were used. The endoscopic procedures were performed with the patient under local anesthesia with lidocaine and conscious sedation with intravenous midazolam by staff pulmonologists or supervised pulmonary residents. EBUS-TBNA was performed first, followed by EUS-FNA, in a single session.

EBUS-TBNA was performed in the manner similar to the one we have previously described.[17] The procedure was performed with the patient in the supine position. After anesthetizing the upper airway with lidocaine, an EBUS bronchoscope was inserted into the trachea through the mouth, and lidocaine was administered into the trachea and bronchus through the working channel. Next, a balloon attached to the transducer was inflated with saline solution. It was then brought into contact with the airway wall and moved in all directions to identify the lesions for sampling. Once the target lesion had been visualized by ultrasound, a dedicated needle was passed through the working channel of the EBUS bronchoscope and advanced through the tracheobronchial wall into the lesion under real-time ultrasound visualization. After the central stylet had been removed, suction was applied using a syringe while manipulating the needle back and forth within the lesion. After sampling, the suction was released slowly, and the needle was retracted. The specimen collected in the lumen of the needle was first pushed out with the central stylet and then blown by air with a syringe onto a glass slide. The visible tissue fragment on the glass slide was then collected and transferred into numbered separate containers filled with formalin for histologic examination. The remaining specimen on the glass slide was smeared with another glass slide and fixed in 95% alcohol for cytologic examination. To clarify the role and diagnostic ability of each needle aspiration procedure, rapid on-site cytologic examination was not used. EBUS-TBNA was performed for N3 nodes, followed by the N2 nodes that were ≥5 mm in the shortest diameter on the ultrasound images. N1 nodes were examined after the N2 nodes if the attending physician or examiner considered it necessary. Two punctures were made for each lymph node, as previously reported by Herth and colleagues.[18] The lymph node location examined and the duration of the procedure from insertion to removal of an EBUS bronchoscope were recorded.

After EBUS-TBNA, EUS-FNA was performed at the left lateral position, as previously described.[19] An EBUS bronchoscope was inserted and advanced through the esophagus while examining the structure around the esophagus by ultrasound. Once the target lesion had been identified, it was punctured through the esophagus with another needle to avoid contamination from the EBUS-TBNA samples under real-time ultrasound guidance. Next, the needle was manipulated back and forth within the lesion

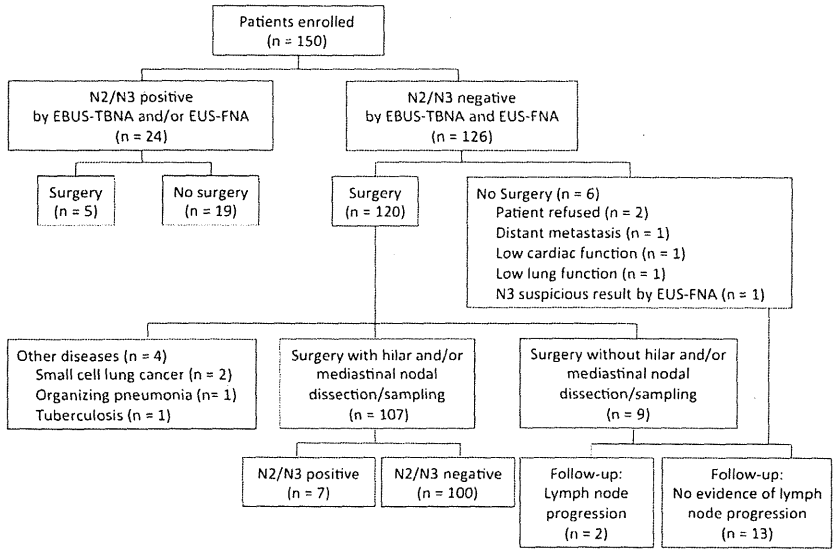

FIGURE 1. Clinical course of patients enrolled in the study. *EBUS-TBNA*, Endobronchial ultrasound-guided transbronchial needle aspiration; *EUS-FNA*, endoscopic ultrasound with bronchoscope-guided fine needle aspiration.

while applying suction under ultrasound guidance and then retracted to collect the aspirated specimen. The handling of the sampled specimens, the size criteria and order in each lymph node for needle aspiration, and the puncture number was the same as for the EBUS-TBNA procedure. To clarify the role and diagnostic ability of each procedure, EUS-FNA was performed even for lymph nodes that had been evaluated using EBUS-TBNA.

Surgical resection with lymph node dissection and/or examination was performed for patients with no evidence of N2 or N3 metastasis and for patients whose attending physician considered it appropriate. An experienced thoracic surgeon decided the operative procedure.

Final Diagnosis

The final diagnosis of lymph node metastases was established by the results of the surgical procedure, EBUS-TBNA and/or EUS-FNA, or radiologic evidence of lymph node progression. If no lymph node progression was seen on CT ≥6 months after EBUS-TBNA and/or EUS-FNA, the lymph nodes were regarded as benign.[20] Suspicious findings from the needle aspiration procedure were regarded as negative in our analysis. The positive findings from the needle aspiration procedure were regarded as true-positive results in our analysis, because the occurrence of false-positive results has been reported to be extremely rare.[1]

TABLE 2. Locations of lymph nodes evaluated by EBUS-TBNA and EUS-FNA

Lymph node location	EBUS-TBNA (n)	EUS-FNA (n)
2R	12	0
2L	0	4
3p	1	3
4R	65	1
4L	56	66
5	0	2
7	77	79
8	0	5
10L	10	16†
11R	20*	0
11L	16	0
Total	257	176

EBUS-TBNA, Endobronchial ultrasound-guided transbronchial needle aspiration; *EUS-FNA*, endoscopic ultrasound-guided fine needle aspiration. *Included 2 N1 lymph nodes. †Included 3 N1 lymph nodes.

Statistical Analysis

The sensitivity of EBUS-TBNA, EUS-FNA, and combined EBUS-TBNA and EUS-FNA approach had been assumed to be 70%, 70%, and 93%, respectively, according to the findings from a previous study.[8] From this information, we planned to accrue ≥129 patients with malignancy to help us detect any superiority in the diagnostic sensitivity of the combined EBUS-TBNA and EUS-FNA procedure compared with a single procedure (EBUS-TBNA or EUS-FNA) at a significance level of 0.05, with 80% statistical power. The homogeneity in the performance of the 2 diagnostic procedures was assessed using the exact McNemar test. Diagnostic sensitivity and the negative predictive value were calculated using the standard definitions, and the 95% confidence intervals were calculated based on the binomial distribution. The mean values and percentages are presented, as appropriate. Continuous variables were analyzed using the Mann-Whitney *U* test. The *P* value was 2-tailed. Statistical analyses were performed using a statistical software program (PASW Statistics, version 18; SPSS, Inc, Chicago, Ill).

RESULTS

Patients

Figure 1 and Figure E1 show the clinical course of the 150 patients enrolled in the present study. A total of 146 patients had a final diagnosis of NSCLC. Of these 146 patients, 121, including 5 with N2- and/or N3-positive results by EBUS-TBNA and/or EUS-FNA, underwent surgery. The surgical procedures were pneumonectomy with nodal dissection or sampling in 3, lobectomy with or without nodal dissection or sampling in 102, segmentectomy with or without nodal dissection or sampling in 6,

TABLE 3. Diagnostic values of EBUS-TBNA and EUS-FNA stratified by patient

	EBUS-TBNA		EUS-FNA		EBUS-TBNA + EUS-FNA	
Diagnostic value	n/Total (%)	95% CI	n/Total (%)	95% CI	n/Total (%)	95% CI
Sensitivity*	17/33 (52)	34-69	15/33 (45)	28-64	24/33 (73)	54-87
Specificity	113/113 (100)	97-100	113/113 (100)	97-100	113/113 (100)	97-100
Positive predictive value	17/17 (100)	81-100	15/15 (100)	78-100	24/24 (100)	85-100
Negative predictive value	113/129 (88)	81-93	113/131 (86)	79-92	113/122 (93)	86-97
Accuracy	130/146 (89)	83-94	128/146 (88)	81-93	137/146 (94)	89-97

EBUS-TBNA, Endobronchial ultrasound-guided transbronchial needle aspiration; *EUS-FNA*, endoscopic ultrasound-guided fine needle aspiration; *CI*, confidence interval. *EBUS-TBNA versus combined approach, $P = .016$; EUS-FNA versus combined approach, $P = .004$; McNemar's test.

wedge resection with nodal dissection or sampling in 8, and thoracotomy with mediastinal exploration in 2.

EBUS-TBNA and EUS-FNA

Two patients (1%) in whom severe cough had developed during the EBUS-TBNA procedure could not undergo additional EBUS or EUS evaluation. No other complications, including hemorrhage, mediastinitis, or pneumothorax, were observed. The median dose of midazolam used was 4 mg (range, 2-8). EBUS-TBNA was performed for 257 lymph nodes (median, 8.2 mm in the shortest diameter on CT; range, 3.4-17.1) in 121 patients. EUS-FNA was performed for 176 lymph nodes (median, 7.8 mm; range, 4.1-17) in 107 patients. The lymph node locations sampled by the procedures are listed in Table 2.

In the 146 patients with NSCLC, 33 (23%) were diagnosed with N2 or N3 disease. The final tumor and nodal stage and TNM classification determined from the final staging procedures (surgery, endoscopic needle aspiration, or radiologic findings) were as follows: T1 in 71, T2 in 55, T3 in 19, and T4 in 1; N0 in 103, N1 in 10, N2 in 30, and N3 in 3; stage IA in 57, IB in 26, IIA in 16, IIB in 10, IIIA in 29, IIIB in 4, and IV in 4.

The diagnostic values of the procedures per patient are summarized in Table 3. The diagnostic sensitivity of the combined approach was significantly greater than that of each procedure alone (EBUS-TBNA vs combined approach; $P = .016$, EUS-FNA vs combined approach; $P = .004$). The details of the patients with mediastinal metastases diagnosed only by EUS-FNA and EBUS-TBNA are listed in Tables 4 and 5, respectively. Representative patients diagnosed with mediastinal metastasis only by EUS-FNA are shown in Figures 2 and 3. Surgery alone revealed mediastinal metastases in an additional 7 patients (only micrometastases in 2). The lymph node locations were as follows: stations 2R in 1, 4R and 7 in 1, 7 in 1, 5 in 3, and 6 in 1. The positive yield of EBUS-TBNA and EUS-FNA according to lymph node size is presented in Table 6. The sensitivity of EBUS-TBNA and EUS-FNA stratified by lesion is provided in Table 7.

Of the 24 patients with N2 or N3 disease confirmed by EBUS-TBNA and/or EUS-FNA, 19 did not undergo surgical resection but received chemotherapy (n = 7) or chemoradiotherapy (n = 12). The remaining 5 patients underwent surgical resection, followed by chemotherapy (n = 3) or chemoradiotherapy (n = 2).

The duration of the procedures is listed in Table 8. When we examined ≤ 2 lymph nodes, the duration of EUS-FNA was significantly shorter than that of EBUS-TBNA.

DISCUSSION

In the present study, we investigated the efficacy of combined EBUS-TBNA and EUS-FNA with a single bronchoscope in preoperative hilar and mediastinal staging of NSCLC. We demonstrated greater sensitivity with the combined approach than with either alone. In addition, the feasibility and safety were high. We were able to

TABLE 4. Details of 7 patients with mediastinal metastases diagnosed by EUS-FNA but not EBUS-TBNA

Pt. no.	Age (y)	Gender	Histopathologic type	Lymph node location with positive EUS-FNA results (shortest diameter on CT, mm)	EBUS-TBNA
1	76	Male	Squamous cell carcinoma	5 (8.7), 7 (7.5)	Not performed
2	64	Male	Squamous cell carcinoma	5 (16.5)	Not performed
3	65	Female	Squamous cell carcinoma	7 (17.0)	Negative
4	72	Male	Squamous cell carcinoma	7 (9.6)	Negative
5	60	Male	Squamous cell carcinoma	2L (9.5)	Not performed
6	79	Male	Squamous cell carcinoma	4L (7.4)	Negative
7	34	Female	Adenocarcinoma	7 (7.8)	Negative

EUS-FNA, Endoscopic ultrasound-guided fine needle aspiration; *EBUS-TBNA*, endobronchial ultrasound-guided transbronchial needle aspiration; *Pt. no.*, patient number; *CT*, computed tomography.

TABLE 5. Details of 9 patients with mediastinal metastases diagnosed by EBUS-TBNA but not EUS-FNA

Pt. no.	Age (y)	Gender	Histopathologic type	Lymph node location with positive EBUS-TBNA results (shortest diameter on CT, mm)	EUS-FNA
1	70	Male	Adenocarcinoma	4R (12.9)	Not performed
2	54	Male	Non–small cell carcinoma	4R (10.3), 7 (9.5)	Not performed for 4R, suspicious result for 7
3	66	Male	Non–small cell carcinoma	4R (10.3)	Not performed
4	69	Female	Adenocarcinoma	4R (14.2)	Not performed
5	61	Male	Adenocarcinoma	4R (9.9)	Not performed
6	70	Male	Adenocarcinoma	4R (6.0)	Not performed
7	69	Female	Adenocarcinoma	4R (6.0)	Not performed
8	63	Male	Squamous cell carcinoma	4R (10.9)	Not performed
9	76	Male	Squamous cell carcinoma	4R (13.8), 2R (9.7)	Not performed

EBUS-TBNA, Endobronchial ultrasound-guided transbronchial needle aspiration; *EUS-FNA*, endoscopic ultrasound-guided fine needle aspiration; *Pt. no.*, patient number; *CT*, computed tomography.

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complete the procedures in all but 2 patients, who had developed a bad cough during EBUS-TBNA.

The development of EUS endoscopes and EBUS bronchoscopes has dramatically changed the approach to mediastinal staging of NSCLC. Although controversial,[21] several investigators have reported that the diagnostic sensitivity of EBUS-TBNA[3,4] or EUS-FNA[4,5] was similar or greater than that of mediastinoscopy, which has been considered the reference standard for mediastinal staging of lung cancer. Thus, EBUS-TBNA or EUS-FNA has become increasingly accepted as a staging procedure before surgical biopsy.[1,22] A recent review study reported that the diagnostic sensitivity of EBUS-TBNA and EUS-FNA was 89% (range, 46%-97%) and 89% (range, 45%-100%), respectively.[1] Either procedure alone seems sufficiently sensitive as a single method; however, the sensitivity is likely to be affected by the prevalence of the malignancy or suspected nodal locations accessible by each method.[1] Thus, EBUS-TBNA, which can access the paratracheal, subcarinal, and hilar regions, and EUS-FNA, which can access the subcarinal, aortopulmonary window, and lower mediastinal regions, are complementary in the mediastinal staging of lung cancer.[11] EBUS-TBNA and EUS-FNA combined can access nearly all mediastinal lymph nodes. Several investigators[6-10,23] have reported the usefulness of combined EBUS-TBNA and EUS-FNA. Wallace and colleagues[8] compared the diagnostic accuracy of conventional TBNA, EBUS-TBNA, and EUS-FNA for mediastinal staging of lung cancer. These procedures were performed sequentially at the same session in 138 patients.[8] The sensitivity of conventional TBNA, EBUS-TBNA alone, EUS-FNA alone, and combined EBUS-TBNA and EUS-FNA was 36%, 69%, 69%, and 93%, respectively.[8] Szlubowski and colleagues[9] investigated the diagnostic value of EBUS-TBNA and EUS-FNA in 120 patients with NSCLC with normal-size mediastinal nodes. The sensitivity of EBUS-TBNA, EUS-FNA, and combined EBUS-TBNA and EUS-FNA was 46%, 50%, and 68%, respectively (EBUS-TBNA alone vs combined EBUS-TBNA and EUS-FNA, $P = .04$). Annema and colleagues[23] conducted a randomized trial of 241 patients to compare surgical staging alone and combined EBUS-TBNA and EUS-FNA followed by surgical staging. The sensitivity of combined EBUS-TBNA and EUS-FNA followed by surgical staging was significantly greater than surgical staging alone (94% vs 79%, $P = .02$). As these positive

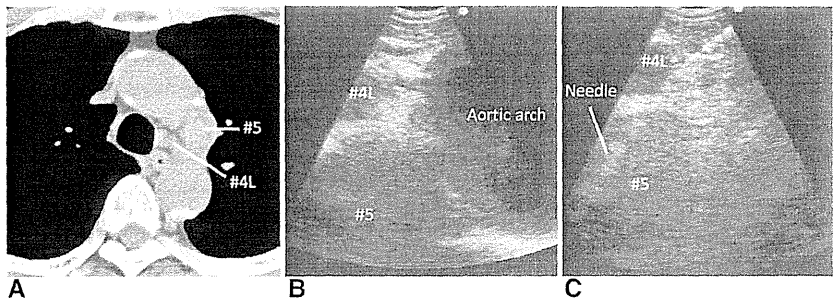

FIGURE 2. Transesophageal endoscopic ultrasound-guided fine needle aspiration for the subaortic lymph node (no. 5), which provided the only evidence of N2 disease (squamous cell carcinoma). The results of endobronchial ultrasound-guided transbronchial needle aspiration and transesophageal endoscopic ultrasound-guided fine needle aspiration for the left lower paratracheal lymph node (no. 4L) were both negative. A, Computed tomography image. B and C, Transesophageal endoscopic ultrasound images.

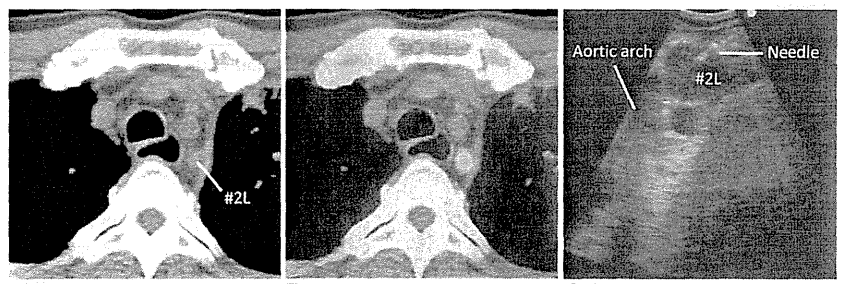

FIGURE 3. Transesophageal endoscopic ultrasound-guided fine needle aspiration for the left upper paratracheal lymph node (no. 2L), which provided the only diagnosis of N2 disease (squamous cell carcinoma). A, Computed tomography image. B, Positron emission tomography-computed tomography image. C, Transesophageal endoscopic ultrasound image.

results have emerged, the combined approach with EBUS-TBNA and EUS-FNA has been described as the best mediastinal staging procedure by endoscopy in recent review studies.[22,24]

Although the accuracy seems to be optimal, the combined EBUS-TBNA and EUS-FNA approach has a serious drawback: the necessity for both an EBUS bronchoscope and an EUS endoscope. Furthermore, most bronchoscopists might not be familiar with the handling of an EUS scope, adding the requirement for an additional experienced endoscopist to perform the combined procedure. The equipment and expertise would not be available in most institutions. In the technique described by Ohnishi and colleagues,[10] each procedure can be performed separately at different specialized centers; however, that could lead to high costs and be time-consuming. To date, several investigators have demonstrated the feasibility, safety, and effectiveness of EUS-FNA with an EBUS bronchoscope for diagnosing benign[19,25] and malignant[12-14,26-28] disease. In the combined transbronchial and transesophageal approach, the use of the EBUS bronchoscope in place of the EUS endoscope for the transesophageal approach is more practical, because all procedures can be performed by a bronchoscopist using an EBUS bronchoscope. The combined approach using a single bronchoscope seems much simpler, more cost effective,[29] and less-time consuming than the approach using both an EBUS bronchoscope and an EUS endoscope. To date, a few studies have reported on its usefulness for preoperative mediastinal staging of lung cancer. Hwangbo and colleagues[13] reported the effectiveness of adding EUS-FNA with an EBUS bronchoscope to EBUS-TBNA in the mediastinal staging of NSCLC. In 150 patients with potentially operable lung cancer, EBUS-TBNA was performed, followed by EUS-FNA for the patients in whom the mediastinal lymph nodes were inaccessible or difficult to access using EBUS. The sensitivity, negative predictive value, and accuracy increased from 84% to 91%, 93% to 96% and 95% to 97% by adding EUS-FNA to EBUS-TBNA, respectively. No complication associated with EUS-FNA was observed in their study.[13] Herth and colleagues[12] investigated the feasibility and efficacy of EBUS-TBNA and EUS-FNA with a single bronchoscope for 150 patients with proven or suspected lung cancer with enlarged mediastinal lymph nodes. They also demonstrated that the combination of EBUS-TBNA and EUS-FNA increased the diagnostic sensitivity compared with each method alone (EBUS-TBNA, 92%; EUS-FNA, 89%; combined approach, 96%) without any complications. Although the sensitivity of the combined approach was greater than that of EBUS-TBNA alone in the studies by Hwangbo and colleagues[13] and Herth and colleagues,[12] the effect of adding EUS-FNA did not seem as large because of the high sensitivity of EBUS-TBNA alone. Our study has shown more clearly the greater effectiveness of adding EUS-FNA to EBUS-TBNA compared with previous studies.

The sensitivity of EBUS-TBNA and EUS-FNA in our study seemed to be lower than that in previous studies. The reasons might have been the low prevalence of

TABLE 6. Yield of EBUS-TBNA and EUS-FNA stratified by mediastinal nodal size on CT

Mediastinal lymph nodes in shortest diameter on CT (mm)	Patients (n)	Total with N2-N3 disease (n)	Patients with positive results (n)		
			EBUS-TBNA	EUS-FNA	EBUS-TBNA + EUS-FNA
<10	107	12	3 (25)	4 (33)	7 (58)
≥10	39	21	14 (67)	11 (52)	17 (81)
Total	146	33	17 (52)	15 (45)	24 (73)

Data in parentheses are percentages. *EBUS-TBNA*, Endobronchial ultrasound-guided transbronchial needle aspiration; *EUS-FNA*, endoscopic ultrasound-guided fine needle aspiration; *CT*, computed tomography.

TABLE 7. Sensitivity of EBUS-TBNA and EUS-FNA per lesion

Lymph node location	Total pathologically proven malignant lesions by surgery or needle aspiration (n)	Positive results (n)*		
		EBUS-TBNA	EUS-FNA	EBUS-TBNA + EUS-FNA
2R	5	4 (80)	0 (0)	4 (80)
2L	1	0 (0)	1 (100)	1 (100)
4R	13	12 (92)	0 (0)	12 (92)
4L	4	1 (25)	4 (100)	4 (100)
5	5	0 (0)	2 (40)	2 (40)
6	1	0 (0)	0 (0)	0 (0)
7	14	7 (50)	10 (71)	12 (86)
Total	43	24 (56)	17 (40)	35 (81)

Data in parentheses are percentages. *EBUS-TBNA*, Endobronchial ultrasound-guided transbronchial needle aspiration; *EUS-FNA*, endoscopic ultrasound-guided fine needle aspiration. *Results of lesions without punctures during EBUS or EUS were regarded as negative.

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malignancy (23%), which has been reported to affect the sensitivity.[1] In addition, our study included consecutive patients with or without enlarged mediastinal lymph nodes, regardless of the lymph node location. In fact, 4 of 7 patients, who had been diagnosed with N2 or N3 disease only by surgery, had single station 5 or 6 lymph node metastases. Other possible reasons include procedural or technical issues, such as the number of aspirations (2 aspirations per lesion in our study) or the level of the examiner's skill. Previous studies of EBUS-TBNA for mediastinal staging of NSCLC have recommended ≥2 needle aspirations per lymph node station[30] and >2 lymph node stations.[31] However, optimal results were obtained by 3 needle aspirations[30] and 4 lymph node stations[31] in those studies.

EUS endoscopes have some distinct diagnostic advantages over EBUS bronchoscopes, including the availability of larger and longer needles, better visibility with the endoscope and ultrasound, with a wider ultrasound scanning range, and adjustability of the protruding needle angle using the elevator. These factors are why conventional EUS-FNA surpasses EUS-FNA with an EBUS bronchoscope in diagnostic ability. Although a few studies,[13,26] ours among them, have included a few cases with successful EUS-FNA for the station 5 lymph node, it cannot be assessed by EUS-FNA using an EBUS bronchoscope in most cases. The accessibility of conventional EUS-FNA for station 5 lymph nodes would be much better than that of EUS-FNA with an EBUS bronchoscope. In addition, the adrenal glands[32] or even station 6 lymph nodes[33] can be potentially evaluated using conventional EUS-FNA. Although conventional EUS-FNA was not performed for any patients in our study, it might provide additional diagnostic information in certain cases. Nevertheless, the simplicity of EUS-FNA with an EBUS bronchoscope seems much more practical. One nonrandomized study that included 214 patients with lung cancer suggested that combined EBUS-TBNA and EUS-FNA with a single bronchoscope was equally effective and less time-consuming than combined EBUS-TBNA and conventional EUS-FNA.[14] To resolve the issue regarding whether EUS-FNA with an EBUS bronchoscope can be substituted for that with an EUS endoscope, additional randomized studies are required.

The reason for adding EUS-FNA to EBUS-TBNA is to provide results for the lymph node stations that cannot be assessed using EBUS-TBNA. EUS-FNA can access station 8 or 9 or, occasionally, 5 lymph nodes, which are usually inaccessible using EBUS-TBNA. In addition, it could also

TABLE 8. Duration of procedures stratified by the number of lymph node stations sampled

Lymph node stations sampled (n)	EBUS			EUS			EBUS + EUS*			
		Procedure time (min)			Procedure time (min)			Procedure time (min)		
	Patients (n)	Median	Range	Patients (n)	Median	Range	Patient (n)	Median	Range	*P* value†
0‡	29 (19)	6.5	4.0-23.0	41 (28)	3.5	1.3-14.0	23 (15)	14.8	7.5-34.5	<.01
1	40 (27)	13.9	9.0-36.5	47 (32)	8.8	4.3-21.0	37 (25)	22.5	12.8-39.5	<.01
2	43 (29)	18.8	12.0-43.8	52 (35)	12.5	8.3-28.0	31 (21)	30.8	19.3-56.8	<.01
3	26 (17)	20.8	14.8-41.0	7 (5)	17	15.0-23.0	35 (23)	34	21.8-63.8	.09
4	9 (6)	32.3	23.0-42.0	1 (1)	24	24.0	20 (13)	40.9	35.8-66.3	NA
5	1 (1)	36.8	36.8	0 (0)	—	—	1 (1)	44.3	44.3	NA
6	2 (1)	32	29.5-34.5	0 (0)	—	—	3 (2)	50.3	51-64.8	NA
Total	150 (100)	16.5	4.0-43.8	148 (100)	10.1	1.3-28.0	150 (100)	29	7.5-66.3	<.01

EBUS, Endobronchial ultrasound; *EUS*, endoscopic ultrasound; *NA*, not available. *Duration from EBUS bronchoscope insertion into the trachea until removal from the esophagus. †EBUS versus EUS. ‡Examined by ultrasonography but not sampled because no target lesions were >5 mm.

play an important role for some cases with other lymph nodes difficult to access using EBUS-TBNA, including station 2L, 4L,[13,27] and 7 lymph nodes.[6] The accessibility to station 4L will be much better with EUS-FNA than with EBUS-TBNA in most cases. Even in station 7 lymph nodes, which have been reported as the most frequent location for EBUS-TBNA, the visibility of EUS has been superior to that of EBUS in some cases. Thus, additional EUS-FNA can be recommended, especially for patients with mediastinal lymph nodes that are inaccessible, difficult to access, or not clearly visualized from the airway.

To date, several investigators have suggested that EBUS-TBNA, which provides high sensitivity, can be an alternative to mediastinoscopy.[3,34] Although our study was not designed to compare combined EBUS-TBNA and EUS-FNA with mediastinoscopy, the combined procedure seems to be reasonable as a first pathologic mediastinal staging test, because it can reduce the need for additional invasive surgical staging procedures. However, a substantial number of patients (n = 9) had false-negative EBUS-TBNA and EUS-FNA results in our study; thus, its role seems to be complementary, rather than an alternative, to surgical staging procedures.

A sequential EBUS and EUS examination in a single session seemed to be a well-tolerated and safe procedure. We could complete both procedures in all but 2 patients, who had developed a severe cough during EBUS-TBNA. No other complications were observed except for the minor usual ones associated with endoscopy, such as a minimal amount of blood from the puncture site, a mild cough, or pharyngeal discomfort at EBUS bronchoscope insertion. The present study was performed in the outpatient setting; thus, some minor self-healing complications that occurred after the procedures, including a low-grade fever, might have been underestimated. However, no patients required a specific treatment, including antibiotics for prophylaxis or treatment, in our study.

This was a single-center, nonrandomized study, which was a potential limitation. In a consecutive examination using a single bronchoscope, the order of the transbronchial approach followed by the transesophageal approach seemed reasonable to minimize the risk of infection; however, it could have affected the accuracy and safety of each procedure. Furthermore, it is well-known that the yield of endoscopic procedures largely depends on the examiner's experience and skill. Our results might not be readily duplicated by less experienced examiners, and better results might be achieved by more skilled examiners. Another limitation was the reliability of the final diagnosis. In the present study, 34 patients had no surgical confirmation of N2 and N3 disease. Thus, the risk exists of inaccuracy if the reference standard is used for referent values. In addition, not all hilar or mediastinal lymph nodes were explored during surgery, which could have led to an overestimation of the endoscopic diagnostic value. However, it would have affected the diagnostic value of each procedure equally; thus, our conclusions regarding the significant superiority of the combined method are well founded.

We, therefore, consider that the combined endoscopic approach with EBUS-TBNA and EUS-FNA with a single bronchoscope is an accurate and safe method for preoperative hilar and mediastinal staging of NSCLC and better than each technique alone.

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EDITORIAL COMMENTARY

Pathologic staging of the mediastinum: When and how?

Jacob A. Klapper, MD, and Chadrick E. Denlinger, MD

Pathologic staging of mediastinal lymph nodes before surgical resection is the accepted standard for patients with non–small cell lung cancer (NSCLC).[1] Mediastinoscopy, endobronchial ultrasound (EBUS), and endoscopic ultrasound are all acceptable means of obtaining tissue. Two prospective studies compared the sensitivity of EBUS with mediastinoscopy among patients with NSCLC and concluded that the 2 modalities are equivalent.[2,3] In the first study patients were randomized to either mediastinoscopy or EBUS followed by mediastinoscopy if EBUS was negative. The sensitivity of mediastinoscopy alone was 79% compared with 85% for EBUS. The addition of mediastinoscopy in patients previously evaluated by EBUS increased the sensitivity to 94%.[2] In the second study, by Yasufuku and colleagues,[3] each patient was evaluated by both EBUS and mediastinoscopy and the sensitivities of the 2 were 81% and 79%, respectively.

The sensitivity of 73% for combined EBUS and endoscopic ultrasound presented by Oki and colleagues[4] in this issue is congruent with prior surgical series where patients went on to resection if the mediastinum was

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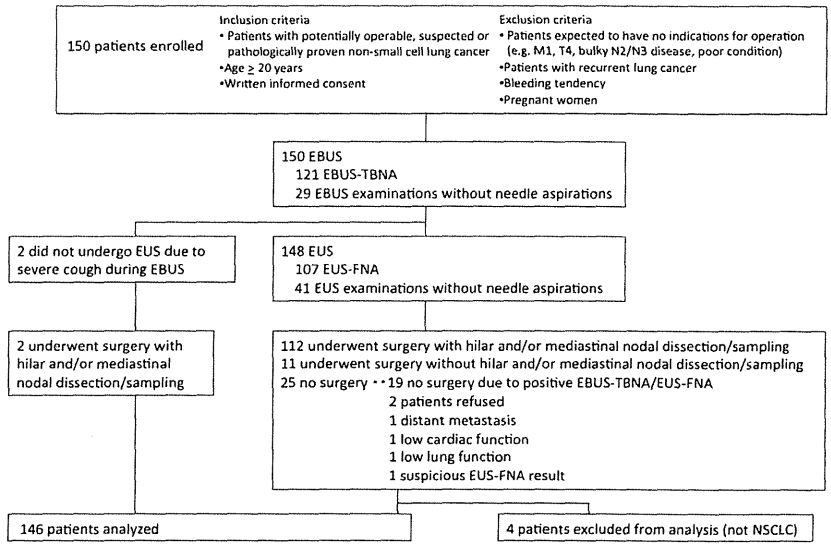

FIGURE E1. CONSORT diagram showing the clinical course of patients enrolled in the study.

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ORIGINAL ARTICLE

First-line gefitinib therapy for elderly patients with non-small cell lung cancer harboring EGFR mutation: Central Japan Lung Study Group 0901

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Abstract
Background The population of elderly patients with lung cancer is increasing worldwide. Although first-line gefitinib is one of the standard treatments for advanced non-small cell lung cancer (NSCLC) harboring epidermal growth factor receptor (EGFR) mutation, few data have been reported regarding gefitinib and elderly patients.
Patients and methods Chemotherapy-naïve patients aged 70 years or older with stage IIIB or IV NSCLC harboring EGFR-activating mutation were enrolled and treated with 250 mg of gefitinib daily until disease progression. The primary end point was response rate, and secondary end points were survival, safety, and quality of life.
Results Twenty patients were enrolled, and the median age was 79.5 years (range 72–90). Overall response rate was 70 % (95 % CI 45.7–88.1 %), and the disease control rate was 90 % (95 % CI 68.3–98.7 %). The median progression-free survival and overall survival time were 10.0 and 26.4 months, respectively. The Functional Assessment of Cancer Therapy-Lung Cancer Subscale (FACT-LCS) scores improved significantly 4 weeks after the initiation of gefitinib ($P = 0.037$) and maintained favorably over a 12-week assessment period. Among the seven items of FACT-LCS, shortness of breath and cough improved

This trial is registered at UMIN-CTR, Number UMIN000001863.

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significantly after 4 weeks of treatment ($P = 0.046$ and $P = 0.008$, respectively). The most common adverse events were rash and liver dysfunction. Although Grade 1 pneumonitis developed in one patient, no treatment-related death was observed.

Conclusion First-line gefitinib therapy is effective and feasible for elderly patients harboring EGFR mutation, and improves disease-related symptoms, especially pulmonary symptoms like shortness of breath and cough.

Keywords Non-small cell lung cancer · EGFR mutation · Elderly · Gefitinib · Quality of life · First-line treatment

Introduction

Lung cancer is the leading cause of cancer mortality. Non-small cell lung cancer (NSCLC) accounts for 85 % of lung cancer cases, with at least 40 % of the patients at an advanced stage. The population of elderly patients with lung cancer is increasing worldwide. Two-thirds of the lung cancer cases are diagnosed in patients over the age of 65, and the median age at diagnosis is 70 years [1, 2].

Aging is associated with physiologic changes in organ function and altered drug pharmacokinetics. Furthermore, the presence of comorbidities and polypharmacy is frequent in elderly populations. Elderly patients are more likely to experience severe hematologic and non-hematologic toxicity from conventional chemotherapy than their younger counterparts [3]. Before the discovery of driver mutations including epidermal growth factor receptor (EGFR) mutation, single-agent chemotherapy was considered to be a standard of care for elderly patients with advanced NSCLC [4–6]. Although carboplatin and weekly paclitaxel doublet chemotherapy improved overall survival compared with vinorelbine or gemcitabine monotherapy in the IFCT-0501 trial, accompanying toxicity such as Grade 3 or Grade 4 neutropenia, febrile neutropenia, and asthenia was more frequent in the doublet chemotherapy arm [7]. Therefore, investigations of effective treatments with less toxicity are needed for this population.

Gefitinib is an orally administered EGFR tyrosine kinase inhibitor (TKI) that blocks signal transduction pathways implicated in the proliferation and survival of cancer cells. Since EGFR somatic mutation was reported to be strongly related to the response of EGFR-TKI therapy, several studies have demonstrated the efficacy of gefitinib for NSCLC harboring EGFR-activating mutation [8–11]. Two phase III studies comparing gefitinib with platinum doublet chemotherapy as a first-line treatment for NSCLC patients with EGFR mutation showed that the gefitinib group had a higher response and longer progression-free survival than a standard chemotherapy group [12, 13]. However, these studies targeted patients aged 75 years or younger, and few data were available on the efficacy and feasibility of first-line gefitinib therapy for elderly NSCLC patients with EGFR mutation. Therefore, we started our current study of this population. The present study included the assessment of quality of life (QOL) besides the efficacy and feasibility of treatment.

Patients and methods

Patient eligibility

Patients aged 70 years or older with a histologically or cytologically proven diagnosis of non-small cell lung cancer were eligible for this study. Other eligibility criteria included the following: EGFR-activating mutation (either exon 19 deletion or L858R in exon 21); measurable disease; stage IIIB/IV or postoperative recurrence; no prior therapy including chemotherapy or radiotherapy of the primary tumor; Eastern Cooperative Oncology Group (ECOG) performance status of 0–2; an adequate organ function defined as leukocyte count $\geq$3,000/mm^3, platelet count $\geq$100,000/mm^3, hemoglobin $\geq$9.0 g/dl, aspartate aminotransferase and alanine aminotransferase $\leq$100 IU/l, total bilirubin $\leq$1.5 mg/dl, serum creatinine $\leq$1.5 mg/dl, and PaO_2 at rest $\geq$60 mmHg. Patients with any of the following criteria were ineligible: superior vena caval syndrome; history of serious drug allergy; massive pleural or pericardial effusion or ascites that required drainage; interstitial lung disease or pulmonary fibrosis detected by conventional computed tomography of the chest; symptomatic brain metastasis; other concurrent active malignancy; pregnancy, lactation, or other concomitant serious medical conditions. All patients gave written informed consent before enrollment. The study protocol was approved by each institutional review board and was carried out in accordance with the Declaration of Helsinki 1964 (as revised 2000).

Study design and treatment

This was a single-arm, prospective, multicenter, phase II trial. Patients were treated with 250 mg of oral gefitinib daily. Therapy was continued unless there was evidence of disease progression, unacceptable toxicity, or withdrawal of consent. If Grade 3 toxicity other than pneumonitis was observed, gefitinib was discontinued for a maximum of 4 weeks. After the toxicity recovered to the level of Grade 2, gefitinib was given every other day. If toxicity further improved, gefitinib was given daily. If Grade $\geq$1 pneumonitis or Grade 4 toxicity other than pneumonitis was observed, the patient was removed from the study.

Evaluation of response and toxicity

The pretreatment baseline evaluation included a complete medical history and physical examination, complete blood cell count, blood chemistry studies, computed tomography scan of the chest and abdomen, computed tomography or magnetic resonance imaging of the brain, bone scintigraphy or positron emission tomography, arterial blood gas analysis, pulmonary function tests, and electrocardiography. Tumor response was assessed every 2 months during the first year after enrollment and every 3 months between 12 and 18 months. Thereafter, the interval was at the physician's discretion.

The Response Evaluation Criteria in Solid Tumors (RECIST) were used for response assessment [14]. Disease control rate (DCR) was defined as the rate of complete response (CR) plus partial response (PR) plus stable disease (SD). An extramural review was conducted to validate staging and response. Toxicity was evaluated according to the National Cancer Institute Common Terminology Criteria (version 3.0).

Quality of life (QOL) was assessed with the Functional Assessment of Cancer Therapy-Lung Cancer Subscale (FACT-LCS) questionnaire version 4. The maximum attainable score on the FACT-LCS was 28, with which the patient was considered to be asymptomatic. Patients were asked to complete the FACT-LCS questionnaire at the time of enrollment and at 4, 8, and 12 weeks after the initiation of treatment.

Mutational analysis of EGFR

Epidermal growth factor receptor (EGFR) genetic testing methods included either direct sequencing, PCR invader, peptide nucleic acid-locked nucleic acid PCR clamp, or the combination of fragment analysis and the Cycleave method.

Statistical analyses

The primary end point of this study was the response rate. We calculated the sample size based on Simon's two-stage design of the phase II study [15]. Assuming that a response rate of 60 % from eligible patients would indicate potential usefulness, and that a rate of 30 % would be the lower limit of interest (with a power of 0.8 at a one-sided significance level of 0.05), accrual of 17 eligible patients was required. Therefore, we planned to accrue a total of 19 patients, assuming there would be a 10 % dropout rate. The duration of survival was measured from the day of enrollment, and the overall survival curve and progression-free survival curve were calculated according to the method of Kaplan and Meier [16]. Repeated-measures analysis of variance was used to assess the differences in the FACT-LCS between baseline and each point during the treatment. Comparisons of the FACT-LCS scores with the baseline scores were adjusted for multiple comparisons using the Dunnett–Hsu test. The software SAS/Proc Mixed version 9.2 (SAS Institute Inc., Cary, NC) was used for statistical analysis. All comparisons were two-sided, and the statistical significance level was set at $P < 0.05$.

Results

Patient characteristics

Between April 2009 and March 2011, 20 patients were enrolled in this study. Sixteen patients (80 %) were aged 75 years or older, and the median age was 79.5 years (range 72–90 years old) (Table 1). All of the 20 patients had adenocarcinoma, 13 (65 %) were female, two (10 %) had an ECOG performance status of 2, and 12 (60 %) had exon 19 deletion mutations.

Tumor responses and survival

Overall response rate was 70 % (95 % CI 45.7–88.1 %), and the disease control rate was 90 % (95 % CI 68.3–98.7 %) (Table 2). Although the response of one patient who developed pneumonitis was not evaluable, progressive disease was observed in only one patient. The median progression-free survival and overall survival time were 10.0 and 26.4 months, respectively (Figs. 1, 2).

Table 1 Patient characteristics

Characteristics	$N = 20$	(%)
Age, years		
Median (range)	79.5 (72–90)	
Sex		
Male	7	35
Female	13	65
Smoking status		
Never smoker	14	70
Former/current smoker	6	30
ECOG performance status		
0	13	65
1	5	25
2	2	10
Stage		
IIIB	4	20
IV	15	75
Postoperative recurrence	1	5
Type of EGFR mutation		
Exon 19 deletion	12	60
L858R	8	40

ECOG Eastern Cooperative Oncology Group

Table 2 Response rate

Response	$N = 20$	% (95% CI)
Partial response	14	70
Stable disease	4	20
Progressive disease	1	5
Inevaluable	1	5
Overall response rate	14	70 % (45.7–88.1)
Disease control rate	18	90 % (68.3–98.7)

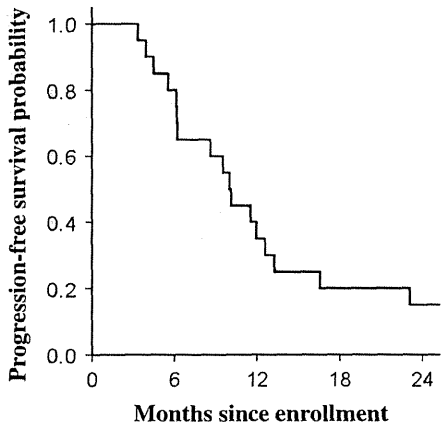

Fig. 1 Kaplan–Meier progression-free survival curve with gefitinib

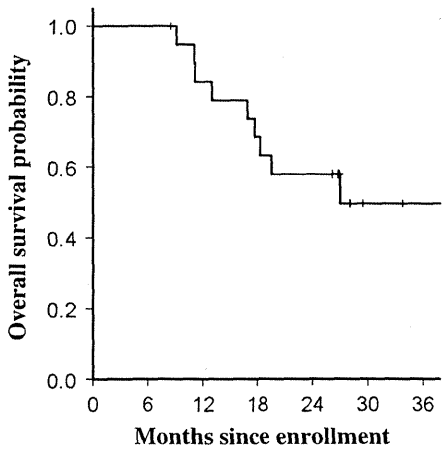

Fig. 2 Kaplan–Meier survival curve with gefitinib

Quality-of-life assessment

All 20 patients completed the FACT-LCS questionnaire at registration and after 4, 8, and 12 weeks of treatment. The adjusted mean FACT-LCS score was 22.8 ± 1.0 at baseline and 25.1 ± 0.7 at 4 weeks. The score improved significantly at 4 weeks ($P = 0.037$) and maintained favorably during the 12-week assessment period (Fig. 3). FACT-LCS consisted of seven items: shortness of breath, cough, chest tightness, ease of breathing, changes in appetite, body weight loss, and disruptions to clear thinking. Among those seven items, shortness of breath and cough improved significantly after 4 weeks of treatment ($P = 0.046$ and $P = 0.008$, respectively).

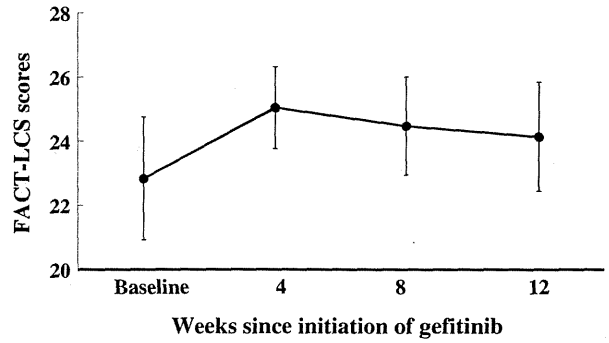

Fig. 3 FACT-LCS scores before treatment and at 4, 8, and 12 weeks after initiation of gefitinib. *Abbreviation FACT-LCS* Functional Assessment of Cancer Therapy-Lung Cancer Subscale

Toxicity

Toxicity data for all 20 patients are listed in Table 3. Non-hematologic toxicity was the principal toxicity from gefitinib treatment and mainly consisted of liver dysfunction, skin rash, anorexia, diarrhea, and fatigue. Grade 3 or Grade 4 liver dysfunction occurred in 3 patients (15 %) but no other Grade 3 or Grade 4 toxicity was occurred. One case of Grade 1 pneumonitis developed in an 87-year-old woman. She had no specific symptoms; however, routine chest X-ray on day 14 showed an increase in density in the bilateral lower lung fields. Since subsequent chest computed tomography revealed bilateral diffuse interstitial opacities and the bronchoalveolar lavage findings were consistent

Table 3 Adverse events ($N = 20$)

	Grade 1	Grade 2	Grade 3	Grade 4	Grades 3–4
AST/ALT	8	4	2	1	3
Rash	8	10	0	0	0
Anorexia	8	2	0	0	0
Diarrhea	6	2	0	0	0
Fatigue	6	2	0	0	0
Mucositis	1	3	0	0	0
Nausea	3	0	0	0	0
Pneumonitis	1	0	0	0	0

AST aspartate aminotransferase, *ALT* alanine aminotransferase

with pneumonitis, gefitinib was discontinued and the treatment with oral prednisolone (0.5 mg/kg/day) was started. Although the pneumonitis was stable, pulmonary and brain metastases gradually progressed and she died of progression of lung cancer 6 months after the occurrence of this adverse event. No treatment-related death was observed.

Discussion

The present study evaluated the efficacy and feasibility of first-line gefitinib treatment for elderly patients harboring EGFR mutation, achieving the response rate of 70 % and disease control rate of 90 %. After we started this phase II study, three groups reported comparable results of response rates from 45.5 to 74 %, and progression-free survival of 9.7–12.9 months for similar populations [17–19]. Efficacy of the present study is also comparable to the results obtained from non-elderly phase III studies. Two prospective studies (WJTOG3405 and NEJ002) and subset analysis of EGFR-mutated patients in the IPASS showed response rates of 62.1–73.7 % and progression-free survival of 9.2–10.8 months [11–13, 20]. From these data, gefitinib treatment for elderly EGFR-mutated patients appears to be as effective as that for the younger population. A randomized trial of EGFR-TKI focusing on efficacy is needed to further improve survival of elderly patients.

We also revealed that disease-related symptoms improved significantly with gefitinib therapy. FACT-LCS score improved more than two points, which is considered a clinically meaningful change [21]. Although superior QOL results were reported with gefitinib versus chemotherapy in the IPASS and NEJ002 studies, the QOL benefit for the elderly population has not been reported [22, 23]. Among the seven items of FACT-LCS, shortness of breath and cough improved significantly. This finding is in accordance with two previous QOL analyses during gefitinib treatment. Cella et al. [24] found that more patients showed an improvement in the pulmonary items of FACT-LCS, such as shortness of breath, cough, or chest tightness than in the non-pulmonary items in the IDEAL2 study, which evaluated two doses of gefitinib for the mutation-unselected population. Oizumi et al. [23] reported that more patients showed an improvement in pain and shortness of breath in the gefitinib arm in the NEJ002 study. With regard to the speed of symptom improvement, our data demonstrated significant improvement at the first follow-up, namely at 4 weeks of treatment. A former analysis reported that the median time to symptom improvement was as immediate as 10 days with gefitinib [24]. In light of its rapid effect, gefitinib could be a good treatment option for patients suffering from pulmonary symptoms like cough or dyspnea.

Toxicity in the present study was generally mild and well tolerated. Grade 3 or Grade 4 adverse events were only in three cases of liver dysfunction. No unpredicted toxicity or treatment-related death was observed. On the other hand, a subgroup analysis of a phase III study of erlotinib treatment indicated that elderly patients experienced significantly more toxicity and tended to discontinue treatment more than their younger counterparts [25]. This difference may be partly explained by the difference in EGFR-TKIs. Gefitinib 250 mg is about one-third of the maximum tolerated dose, and erlotinib 150 mg is just the maximum tolerated dose [26, 27]. Accordingly, gefitinib may have some safety margin, especially for the frail population. In the present study, the oldest patient, aged 90 years, was able to continue gefitinib therapy for about 7 months with side effects no more severe than Grade 2 mucositis and Grade 2 rash.

Pneumonitis is one of the most serious adverse events related to EGFR-TKI therapy. In our previous study evaluating gefitinib in mutation-unselected elderly NSCLC patients, three out of 30 patients (10 %) had pneumonitis, two of them with a Grade $\geq$3 [28]. In the present study, Grade 1 pneumonitis developed in one patient (5 %). Since risk factors of pneumonitis include smoking, preexisting interstitial lung disease, and older age, careful monitoring is desirable for elderly patients [29, 30].

In conclusion, the present study revealed that first-line therapy with gefitinib is effective and feasible for elderly patients harboring EGFR mutation, and improves disease-related symptoms.

Conflict of interest Kosuke Takahashi, Hiroshi Saito, Yoshinori Hasegawa, Yasuteru Sugino, and Joe Shindoh received honoraria from AstraZeneca. Yoshinori Hasegawa received research funding for his institute from AstraZeneca.

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ORIGINAL ARTICLE

Observational Study

Prognostic value of *KRAS* and *BRAF* mutations in curatively resected colorectal cancer

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Abstract

AIM: To investigate the prognostic role of *KRAS* and *BRAF* mutations after adjustment for microsatellite instability (MSI) status in Japanese colorectal cancer (CRC) population.

METHODS: We assessed *KRAS* and *BRAF* mutations and MSI status in 813 Japanese patients with curatively resected, stage Ⅰ-Ⅲ CRC and examined associations of these mutations with disease-free survival (DFS) and overall survival (OS) using uni- and multivariate Cox proportional hazards models.

RESULTS: *KRAS* and *BRAF* mutations were detected in 312 (38%) of 812 and 40 (5%) of 811 tumors, respectively. *KRAS* mutations occurred more frequently in females than in males ($P = 0.02$), while the presence of *BRAF* mutations was significantly associated with the female gender ($P = 0.006$), proximal tumor location ($P < 0.001$), mucinous or poorly differentiated histology ($P < 0.001$), and MSI-high tumors ($P < 0.001$). After adjusting for relevant variables, including MSI status, *KRAS* mutations were associated with poorer DFS (HR = 1.35; 95%CI: 1.03-1.75) and OS (HR = 1.46; 95%CI: 1.09-1.97). *BRAF* mutations were poor prognostic factors for DFS (HR = 2.20; 95%CI: 1.19-4.06) and OS (HR = 2.30; 95%CI: 1.15-4.71). Neither the *BRAF* by MSI interaction test nor the *KRAS* by MSI interaction test yielded statistically significant results for DFS and OS.

CONCLUSION: *KRAS* and *BRAF* mutations are associated with inferior survival, independent of MSI status, in

Japanese patients with curatively resected CRC.

Key words: Colorectal cancer; *KRAS*; *BRAF*; Microsatellite instability; Prognostic factor

Core tip: Although *KRAS* and *BRAF* mutations play a critical role in colorectal cancer development, little is known regarding the prognostic role of these genetic alterations after adjustment for microsatellite instability status in Asian populations. To the authors' knowledge, the current study is the first large-scale study to clarify the impact of *KRAS* and *BRAF* mutations on the survival outcomes of colorectal cancer in Asian populations. We found that *KRAS* and *BRAF* mutations were separately associated with inferior disease-free survival and overall survival, independent of microsatellite instability status, in patients with curatively resected colorectal cancer.

Kadowaki S, Kakuta M, Takahashi S, Takahashi A, Arai Y, Nishimura Y, Yatsuoka T, Ooki A, Yamaguchi K, Matsuo K, Muro K, Akagi K. Prognostic value of *KRAS* and *BRAF* mutations in curatively resected colorectal cancer. *World J Gastroenterol* 2015; 21(4): 1275-1283 Available from: URL: http://www.wjgnet.com/1007-9327/full/v21/i4/1275.htm DOI: http://dx.doi.org/10.3748/wjg.v21.i4.1275

INTRODUCTION

Colorectal cancer (CRC) develops through diverse mechanisms such as chromosomal instability (CIN), microsatellite instability (MSI), and epigenetic DNA promoter methylation [CpG island methylator phenotype (CIMP)][1]. CIMP and MSI-high (MSI-H) phenotypes are closely associated. Most sporadic MSI-H tumors develop through CIMP-associated methylation of *MLH1*, and *BRAF* mutations occur frequently in both phenotypes[2,3]. *KRAS* mutations mainly occur in CIN and are partly associated with intermediate CIMP epigenotype[4]. *KRAS* and *BRAF* mutations are mutually exclusive; both cause RAS/RAF/MAPK signaling pathway upregulation and are crucial in CRC development.

KRAS encodes a guanosine triphosphate/guanosine diphosphate binding protein; *KRAS* mutations are observed in approximately 30%-40% CRCs[5-8]. *KRAS* mutations are well known as predictive markers of resistance to epidermal growth factor receptor-targeted antibodies in metastatic CRC, but their prognostic value remains controversial. Some studies have shown that *KRAS* mutations are associated with poorer survival in CRC[8,9], while others found no association[6,7].

BRAF encodes a serine/threonine protein kinase, a downstream effector of the KRAS protein. Activating *BRAF* mutations occur in approximately 4%-20% CRCs[6,10-14], with the vast majority being the V600E hotspot mutation. Although some previous studies have shown that *BRAF* mutations confer poorer prognosis in CRC[10-12], others have not[6,13], probably because of associations with favorable MSI-H CRC prognosis[15-17].

Although genetic background and geographical factors may influence mutation frequency and prognosis, most reports are from Western countries; less data are available regarding the prognostic role of *KRAS* and *BRAF* mutations in Asian populations. Two independent studies from Taiwan and Japan have been published recently. However, both had a small sample size and heterogeneous cohorts including metastatic disease; the study from Taiwan did not examine MSI status[14,18]. Hence, a large homogenous cohort with MSI status is essential for assessing the prognostic value of various clinical or molecular variables in CRC. Here, we clarified associations of *KRAS* and *BRAF* mutations and MSI status with survival outcomes in a larger Japanese cohort of patients with curatively resected CRC.

MATERIALS AND METHODS

Patients and tissue samples

A total of 813 consecutive stage Ⅰ-Ⅲ CRC patients undergoing curative resection at Saitama Cancer Center between July 1999 and May 2006 were included. Written informed consent was obtained from all patients. Patients with the following conditions were excluded: (1) history of radiotherapy or chemotherapy preoperatively; (2) inflammatory bowel disease; or (3) history of familial adenomatous polyposis. Pathological staging was performed according to the tumor, node, and metastasis (TNM) classification system (6th edition)[19]. CRCs were typically divided into 3 types: rectum, distal colon (splenic flexure and descending and sigmoid colon), and proximal colon (cecum and ascending and transverse colon). Adjuvant chemotherapy was administered to 40% (129/322) and 76% (232/307) of stage Ⅱ and Ⅲ CRC patients, respectively. Among 361 patients treated with adjuvant chemotherapy, only 10 patients received combination chemotherapy with 5-fluorouracil, leucovorin, and oxaliplatin, while remaining were treated with single-agent fluoropyrimidines. Patients were followed-up until death or February 2012, whichever came first. We obtained approval from the Ethics Committee of Saitama Cancer Center.

Genomic DNA extraction and KRAS and BRAF mutation analysis

Primary CRCs and paired healthy colorectal mucosa obtained perioperatively were immediately frozen at -80 ℃ until analysis. Genomic DNA was extracted

from fresh frozen specimens using the standard phenol-chloroform extraction method. Exons 2 and 3 of *KRAS* were examined for mutations by denaturing gradient gel electrophoresis, as described previously[20]. The *BRAF* V600E mutation was detected using PCR and restriction enzyme digestion, as described previously[21].

MSI analysis

MSI analysis was performed using fluorescence-based PCR, as described previously[22]. Five Bethesda markers BAT25, BAT26, D5S346, D2S123, and D17S250 were used to classify tumor MSI status. MSI status was graded as MSI-H with 2 or more unstable markers, MSI-low (MSI-L) with only 1 unstable marker, and microsatellite-stable (MSS) with no unstable marker. MSI-positive markers were re-examined at least twice to confirm the result.

Statistical analysis

The aim of this study was to evaluate the impact of *KRAS*/*BRAF* mutations on prognosis in patients with resected CRC. Prognosis was evaluated according to 2 measures: overall survival (OS) and disease-free survival (DFS). OS was defined as the interval from the date of resection until death due to any cause or until the censor date of February 1, 2012. DFS was defined as the time from the date of resection to tumor recurrence, occurrence of a new primary colorectal tumor, or death due to any cause. Survival probability was estimated using the Kaplan-Meier method and compared using the log-rank test. Cox proportional hazards models were used to estimate uni- and multivariate adjusted hazard ratios for DFS and OS according to mutation status. Factors for which the multivariate models were adjusted are age (≥ 65 *vs* < 65), gender (male *vs* female), tumor stage (Ⅲ *vs* Ⅱ *vs* Ⅰ), adjuvant chemotherapy (Yes *vs* No), and status of MSI and *BRAF* or *KRAS* mutations (Yes *vs* No). To further evaluate the potential heterogeneity of the impact of *KRAS* and *BRAF* mutations according to MSI status and other covariates [age (≥ 65 *vs* < 65), gender (male *vs* female), tumor location (distal/rectum *vs* proximal), and stage (Ⅲ *vs* Ⅰ/Ⅱ)], we tested the models that included interaction terms, cross-products of gene mutation status, and another variable of interest in a multivariate Cox model. The likelihood ratio test was performed to determine the significance of the results.

Clinicopathological factor distribution according to gene mutation status was assessed using the χ^2 or Fisher's exact tests for categorical variables, when appropriate, and Student's *t*-test for continuous variables. All statistical analyses were performed using Dr. SPSS Ⅱ software (SPSS Japan Inc., Tokyo, Japan); 2-sided $P < 0.05$ was considered statistically significant.

RESULTS

Clinicopathological characteristics of KRAS and BRAF mutant tumors

Patient characteristics according to *KRAS* or *BRAF* status are summarized in Table 1. MSI status was determined in all cases, whereas mutation status was not determined in 1 case for *KRAS* and 2 for *BRAF*. *KRAS* or *BRAF* mutations were detected in 38% (312/812) and 5% (40/811) of cases, respectively. Only 1 patient harbored *KRAS* and *BRAF* mutations. *KRAS* mutations were more frequent in females than in males (43% *vs* 35%, $P = 0.02$). *BRAF* mutations were significantly more frequent in females than in males (7% *vs* 3%, $P = 0.006$), proximal than in distal or rectal tumors (13% *vs* 1% *vs* 2%, $P < 0.001$), mucinous or poorly differentiated tumors than in moderately or well-differentiated tumors (17% *vs* 4%, $P < 0.001$), and MSI-H tumors than in MSS/MSI-L tumors (36% *vs* 2%, $P < 0.001$).

Survival analysis

The median follow-up time was 87.7 mo (range: 13-148 mo). Based on univariate Cox proportional hazard analysis results (Table 2), greater age (≥ 65), male gender, advanced TNM stage, and presence of *KRAS* mutations were significantly associated with poor prognosis for DFS and OS. For *KRAS* mutant *vs KRAS* wild-type tumors, 5-year DFS was 71% *vs* 77% (log-rank $P = 0.02$; Figure 1A); 5-year OS was 80% *vs* 84%, respectively (log-rank $P = 0.01$; Figure 1B). Presence of *BRAF* mutations was not significantly associated with poorer DFS and OS in the entire cohort. For *BRAF* mutant *vs* wild-type tumors, 5-year DFS was 70% *vs* 75% (log-rank $P = 0.23$; Figure 1C); 5-year OS was 77% *vs* 83% (log-rank $P = 0.11$; Figure 1D), respectively.

In multivariate analysis, adjusting for potential prognostic variables, *KRAS* retained its prognostic impact on DFS (HR = 1.35; 95%CI: 1.03-1.75) and OS (HR = 1.46; 95%CI: 1.09-1.97; Table 3). Presence of *BRAF* mutations was significantly associated with poorer DFS (HR = 2.20; 95%CI: 1.19-4.06) and OS (HR = 2.30; 95%CI: 1.15-4.71) after adjustment (Table 3).

Survival analysis stratified by MSI status

Given the potential prognostic effect of MSI status, we evaluated interactions of *KRAS* or *BRAF* mutations with MSI status (Table 4). The effect of *KRAS* mutations on DFS and OS was limited to patients with MSS/MSI-L tumors (HR = 1.37; 95%CI: 1.05-1.80; HR = 1.49; 95%CI: 1.10-2.02, respectively); however, the *KRAS* by MSI interaction test was not significant ($P = 0.95$ and 0.70, respectively). *BRAF* mutations were significantly associated with reduced OS (HR = 2.74; 95%CI: 1.19-6.30) in MSS/MSI-L, but not MSI-H, tumors.