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Scientific Research

Characteristics of Non-Small Cell Lung Cancer Located in the Right Middle Lobe According to a Retrospective Study of Recurrence and Prognosis*

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ABSTRACT

Background: Some studies have suggested that among all cases of lung cancer, the outcome of lung cancer located in the right middle lobe (RML) is the worst. However, with the advances in the diagnosis and treatment methods of lung cancer over the last couple of decades, we investigated whether the prognosis of primary lung cancer located in the RML still remains inferior to that of lung cancer arising from other lobes. **Methods:** Between July 2003 and December 2011, 505 consecutive patients with non-small cell lung cancer (NSCLC) underwent surgical resection at our institution. Of these, 32 patients (6.3%) had tumors arising from the RML. **Results:** The rate of incomplete resection was higher for cancer located in the RML than that for cancer arising from other lobes. Significant associations were noted between cancer located in the RML and the rate of lymph node metastasis and initial locoregional recurrence. Multivariate analysis identified lymph node metastasis and location in the RML as independent risk factors influencing the recurrence-free survival ($p = 0.006$), although location in the RML was not extracted as an independent risk factor influencing the overall survival ($p = 0.060$). **Conclusion:** Despite the recent advances in the treatment of lung cancer, evaluation of complete resection revealed that the outcome of cancer located in the RML is still the worst among cancer of all the lobes. Further early diagnosis and adjuvant therapy are needed for improving the prognosis of cancer located in the RML.

Keywords: Right Middle Lobe; Non-Small Cell Lung Cancer; Prognosis

1. Introduction

The right middle lobe (RML) is the smallest lobe of the lungs. Primary lung cancer originating from the RML is much less common than that arising from the other lobes, and is estimated to account for 3.8% - 6.7% of all lung cancers [1-3]. Some studies have reported that among all lung cancers, cancers arising in the RML have the worst outcomes [1-3]. On the other hand, a recent report demonstrated that the outcome of cancer located in the RML was no worse than that of cancers arising from other lobes of the lung [4]. However, all of the earlier reports were published between 15 and 35 years ago. Over the decades since, marked advances have been made in the diagnosis and treatment of lung cancer, and we investigated whether primary lung cancer located in the RML may still be associated with a poor prognosis.

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2. Patients and Methods

2.1. Study Population

We conducted this retrospective study in a total of 505 patients with non-small cell lung cancer (NSCLC) who underwent resection at the Kawasaki Medical School Hospital between July 2003 and December 2011. Of these, 32 patients (6.3%) had tumors arising from the RML. Among the 505 patients, 31 cases (6.1%) were incomplete resection including exploratory thoracotomy. We excluded these 31 patients, and enrolled the remaining 474 patients in whom complete resection could be accomplished in this study. The histological diagnosis of the tumors was based on the criteria of the World Health Organization, and the TNM stage was determined according to the criteria updated in 2009. This study was conducted with the approval of the institutional Ethics Committee of Kawasaki Medical School.

2.2. Statistical Analysis

Statistical analysis was performed for examining the significances of differences among the groups and the possible correlations between cancer of the RML and other lobes and the clinicopathological features using the Fisher exact test or the χ^2 test, as appropriate. An unpaired t-test was used for comparison of the continuous data. Multivariate analyses were performed using a logistic regression analysis. To explore the association between recurrence-free survival (RFS)/overall survival (OS) and cancer of the RML or other lobes, a Kaplan-Meier survival analysis was performed by stratifying significant predictor variables identified using the Cox proportional hazards model. All the statistical analyses were conducted using the SPSS software (Version 17.0; SPSS Incorporation, Chicago, IL). All statistical tests were two-sided, and probability values of <0.05 were regarded as denoting statistical significance.

3. Results

3.1. Clinical Characteristics

The median follow-up period was 32.6 months (range 1 - 99 months). The rate of incomplete resection was higher for cancer located in the RML than for that arising from the other lobes (**Table 1**). The characteristics of the 474 patients enrolled in this study are summarized in **Table 2**. The patients ranged in age from 34 to 90 years (mean, 69.5), and there were 297 men and 177 women.

3.2. Relation between Location of Cancer and the Clinicopathological Characteristics

There were 28 (5.9%) patients with NSCLC located in the RML, consisting of 18 men and 10 women, with a mean age of 68.7 years. The tumors included 20 adenocarcinomas (71.4%), seven squamous cell carcinomas (25.0%), and one large cell carcinoma. Ten patients (35.7%) had lymph node metastasis. Significant associations of the cancer located in the RML were observed with the rate of male (64.3% vs. 62.6%, p = 0.034) and the rate of lymph node metastasis (36.0% vs. 17.7%, p = 0.018), but not with the mean of age (p = 0.703), histological type (p = 0.751), or the rate of pleural invasion (21.4% vs. 11.9%, p = 0.138) (**Table 3**).

Table 1. The rate of incomplete resection cases (n = 505).

	Resection		
Location	Incomplete (A)	Complete (B)	A/A+B (%)
RML	4	28	12.50
RUL	8	162	4.71
RLL	6	100	5.66
LUL	9	110	7.56
LLL	4	74	5.13

RML vs. other lobes p = 0.124 (p-value was calculated by Fisher exact test.) RML: right middle lobe; RUL: right upper lobe; RLL: right lower lobe; LUL: left upper lobe; LLL: left lower lobe.

Table 2. Patient characteristics enrolled in this study (n = 474).

	Number	%
Sex		
Male	297	62.7
Female	177	37.3
Age, mean ± SD	69.5 ± 9.9	
Histology		
Adenocarcinoma	326	68.7
Squamous cell	107	22.6
Large cell	24	5.1
Adenosquamous	9	1.9
Pleomorphic	8	1.7
Pathological stage		
IA	216	45.6
IB	125	26.4
II (A + B)	73	15.4
III (A + B)	57	12.0
IV	3	0.6

3.3. Relation between the Location of Cancer and the Risk of Recurrence

One hundred twenty-one (25.5%) patients developed cancer recurrence after resection. Significant association of cancer located in the RML was observed with the rate of recurrence (46.4% vs. 24.2%, p = 0.009). The initial recurrence sites are presented in **Table 4**. The initial recurrence site was analyzed: of 474 patients, 53 patients (43.8%) had locoregional relapse, 76 patients (62.8%) had distant relapse, and 8 (6.6%) had both. Significant association of cancer located in the RML was observed with the rate of locoregional recurrence (25.0% vs.10.3%, p = 0.012), but not with that of distant-site recurrence (25.0% vs. 15.5%, p = 0.183).

3.4. Prognostic Analysis

The RFS of patients with cancer located in the RML was significantly worse than that of the patients with cancer arising from the other lobes (p = 0.018, according to the

Table 3. Clinicopathological feature according to the lobe (n = 474).

Location	RML	RUL	RLL	LUL	LLL	p-value
						(RML vs. Others)
Number	28	162	100	110	74	
Sex (%)						
Male	18 (64)	110 (68)	55 (55)	68 (62)	46 (62)	0.034
Female	10 (36)	52 (32)	45 (45)	42 (38)	28 (38)	
Age, mean	68.7	69.8	68.4	69.9	69.8	0.703
Histology (%)						0.751
Squamous cell	7 (25)	31 (19)	24 (24)	26 (24)	19 (26)	
Non-Squamous cell	21 (75)	131 (81)	76 (76)	84 (76)	55 (76)	
Adenocarcinoma	20	117	70	74	45	
Large cell	1	11	3	4	5	
Adenosquamous	0	1	2	4	2	
Pleomorphic	0	2	1	2	3	
Pathological lymph node status (%)						0.018
pN0	18 (64)	141 (87)	79 (79)	92 (84)	55 (74)	
pN1 - 2	10 (36)	21 (13)	21 (21)	18 (16)	19 (26)	
pN1	3	8	77	10	11	
pN2	7	13	14	8	8	
Pathological pleural invasion status (%)						0.138
pl 0 - 1	22 (79)	138(85)	96 (96)	94 (85)	65 (88)	
pl 2 - 3	6 (21)	24 (15)	4 (4)	16 (15)	9 (12)	

p-value was calculated by χ^2 test. RML: right middle lobe; RUL: right upper lobe; RLL: right lower lobe; LUL: left upper lobe; LLL: left lower lobe.

log-rank test; **Figure 1(a)**). The OS of the patients with cancer located in the RML tended to be worse than that of the patients with cancer arising from the other lobes, although this association was not statistically significant ($p = 0.109$ according to the log-rank test; **Figure 1(b)**). Multivariate analysis identified lymph node metastasis and location in the RML as independent risk factors influencing the RFS ($p = 0.006$, **Table 5(a)**). However, multivariate analysis identified only lymph node metastasis as an independent risk factor influencing the OS; location in the RML tended to be independent risk factor, however, the association was not statistically significant ($p = 0.060$, **Table 5(b)**).

4. Comment

There have been several reports focusing on the cancer located in the RML, especially its prognosis. However, all of the earlier reports were published between 15 and 35 years ago. This is the first report focusing on the prognosis of the cancer located in the RML since 2000. In the last couple of decades, much progress has been made in the diagnosis and treatment of lung cancer, such as early diagnosis, less invasive surgery, adjuvant chemotherapy, and molecular-targeted therapy. Therefore, we investigated once again whether primary lung cancer located in the RML may still be associated with a poor prognosis.

In general, the characteristics of lung cancer arising from the middle lobe have been described as follows: 1) high frequency of extralobar invasion or pleural dissemination, and 2) numerous lymphatic drainage sites extending to both the superior and inferior mediastinal zones [4-6]. First, we demonstrated that the rate of incomplete resection was higher for cancers located in the RML than for those located in the other lobes. In our study, incomplete resection was performed in two patients with pleural dissemination, one with mediastinal lymph node involvement, and one residual disease in bronchial

Table 4. Initial recurrence sites according to the lobe (n = 474).

Location	RML	RUL	RLL	LUL	LLL	p-value
						(RML vs. Others)
Number	28	162	100	110	74	
Recurrence cases (%)	13 (46.4)	35 (21.6)	27 (27.0)	29 (26.4)	17 (23.0)	0.009
Initial recurrence sites						
Locoregional (%)	7 (25.0)	12 (7.4)	13 (13.0)	12 (10.9)	9 (12.2)	0.012
Local	0	4	2	1	3	
Lymph node	5*	6*	6*	7*	3*	
Plural dissemination	2	2	5	4	3	
Distant (%)	7 (25.0)	26 (16.0)	16 (16.0)	18 (16.4)	9 (12.2)	0.183
Lung	3	9	6*	5	4	
Brain	2	6	5	3	1	
Bone	1*	5*	2*	5	0	
Liver	0	5	3	4*	4*	
Adrenal	0	1	0	1	0	
Others#	1	0	0	0	0	
Simultaneous*	1*	3*	2*	1*	1*	
Local and distant						

p-value was calculated by χ^2 test. *gastrointestinal metastasis; RML: right middle lobe; RUL: right upper lobe; RLL: right lower lobe; LUL: left upper lobe; LLL: left lower lobe; #gastrointestinal metastasis.

Table 5. Prognostic analysis.

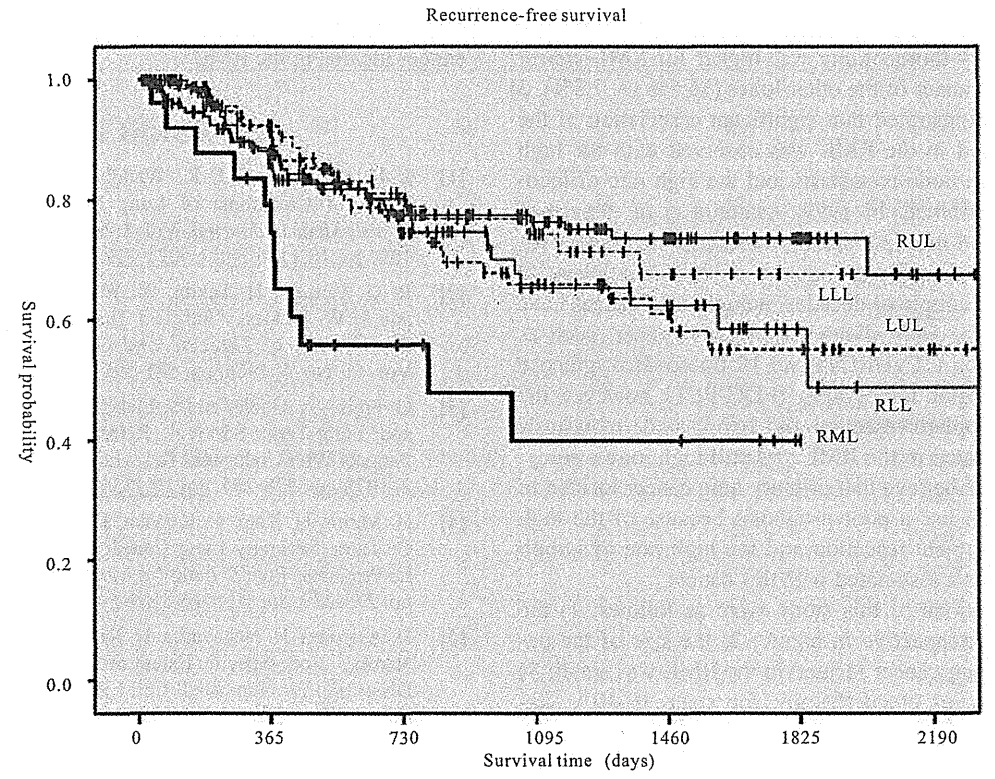
(a) Multivariate analysis of factors predicting recurrence-free survival (n = 474).

Variable		HR	95%CI	p-value
LN metastasis	negative	1.00		
	positive	5.15	3.54 - 7.49	<0.001
Location	RUL	1.00		
	RML	2.53	1.30 - 4.93	0.006
	RLL	1.13	0.67 - 1.91	0.639
	LUL	1.22	0.74 - 1.99	0.439
	LLL	0.83	0.46 - 1.51	0.539

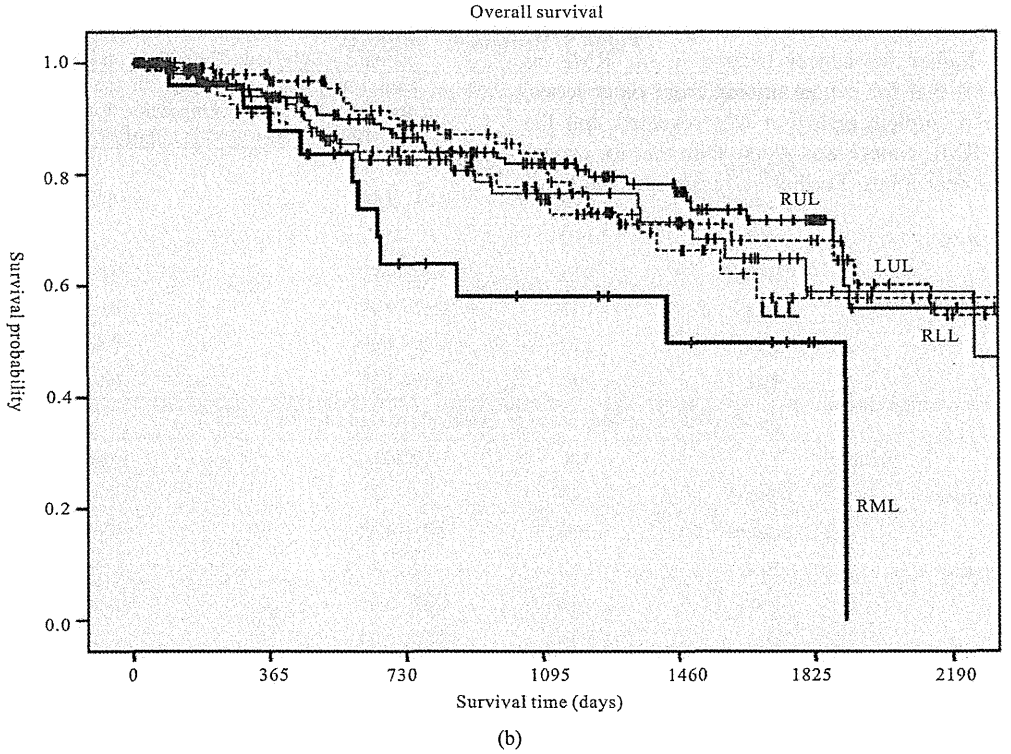
(b) Multivariate analysis of factors predicting overall survival (n = 474).

Variable		HR	95%CI	p-value
LN metastasis	negative	1.00		
	positive	2.98	2.01 - 4.42	<0.001
Location	RUL	1.00		
	RML	1.94	0.97 - 3.87	0.060
	RLL	1.19	0.69 - 2.03	0.536
	LUL	0.99	0.59 - 1.68	0.979
	LLL	1.18	0.67 - 2.08	0.561

HR: hazard ratio; 95%CI: 95% confidence interval; p-value was calculated by log-rank test; LN: lymph node; RML: right middle lobe; RUL: right upper lobe; RLL: right lower lobe; LUL: left upper lobe; LLL: left lower lobe.



(a)



(b)

Figure 1. (a) Kaplan-Meier curve for recurrence-free survival according to the location, log-rank p = 0.018; (b) Kaplan-Meier curve for overall survival according to the location, log-rank p = 0.109.

stump. In 1996, Miura *et al.* also described that the rate of exploratory thoracotomy was higher for RML cancer than for the cancer of the other lobes (16.5% vs. 7.3%). 3) We also demonstrated that significant association of the cancer located in the RML was observed with the high rate of lymph node metastasis and the high rate of loco-regional recurrence. In 1999, Asamura *et al.* described that the rate of multiple-station metastasis was higher for RML cancer than for the cancer of the other lobes. 4) Over the last couple of decades, numerous advances have been made for early diagnosis of lung cancer, such as high-resolution CT (HRCT) and 18-fluoro-deoxyglucose positron emission tomography (FDG-PET), however, the rate of incomplete resection and lymph node metastasis of cancer located in the RML was still high in this study. Therefore, we believe that primary lung cancer located in the RML still has a poor prognosis because of the high risk of incomplete resection and the high rate of lymph node metastasis associated with this cancer.

The limitations of this study were as follows: 1) the study was retrospective in nature; 2) the size of the patients with lung cancer located in the RML was small; 3) routine adjuvant chemotherapy for stage IB-IIIA was started in 2006; 4) routine FDG-PET for staging was started in 2007. Therefore, in this study, the role of adjuvant chemotherapy or FDG-PET remains uncertain.

In conclusion, despite recent advances in the diagnosis and treatment of lung cancer, the rate of incomplete resection was higher for cancer located in the RML as compared with that for cancer arising from other lobes, evaluation of complete resection was revealed that the outcome for RML cancer was worse than that for cancer arising from other lobes. Further early diagnosis and adjuvant therapy are needed for improving the prognosis of cancer located in the RML.

REFERENCES

[1] J. H. Gifford and J. K. Waddington, "Review of 464 Cases of Carcinoma of Lung Treated by Resection," *British Medical Journal*, Vol. 1, No. 5021, 1957, pp. 723-730. doi:10.1136/bmj.1.5021.723

[2] R. G. Vincent, H. Takita, W. W. Lane, A. C. Gutierrez and J. W. Pickren, "Surgical Therapy of Lung Cancer," *The Journal of Thoracic and Cardiovascular Surgery*, Vol. 71, No. 4, 1976, pp. 581-591.

[3] G. Freise, A. Gabler and S. Liebig, "Bronchial Carcinoma and Long-Term Survival. Retrospective Study of 433 Patients Who Underwent Resection," *Thorax*, Vol. 33, No. 2, 1978, pp. 228-234. doi:10.1136/thx.33.2.228

[4] H. Miura, H. Kato, C. Konaka, J. Usuda, O. Uchida and O. Taira, "Primary Lung Cancer of the Middle Lobe. Is Its Prognosis Poor?" *Lung Cancer*, Vol. 14, No. 2-3, 1996, pp. 273-279. doi:10.1016/0169-5002(96)00553-3

[5] H. Asamura, H. Nakayama, H. Kondo, R. Tsuchiya and T. Naruke, "Lobe-Specific Extent of Systematic Lymph Node Dissection for Non-Small Cell Lung Carcinomas According to a Retrospective Study of Metastasis and Prognosis," *The Journal of Thoracic and Cardiovascular Surgery*, Vol. 117, No. 6, 1999, pp. 1102-1111. doi:10.1016/S0022-5223(99)70246-1

[6] Y. Sakao, S. Okumura, M. Mingyon, H. Uehara, Y. Ishikawa and K. Nakagawa, "The Impact of Superior Mediastinal Lymph Node Metastases on Prognosis in Non-Small Cell Lung Cancer Located in the Right Middle Lobe," *Journal of Thoracic Oncology*, Vol. 6, No. 3, 2011, pp. 494-499. doi:10.1097/JTO.0b013e31820b8891

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Scientific Research

Current status of Radiologic Diagnosis for Mediastinal Lymph Node Metastases of Non-Small-Cell Lung Cancer: Retrospective Study of pN2 Cases

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ABSTRACT

Objective: Advances in diagnostic imaging techniques, such as ^{18}F-fluorodeoxyglucose positron emission tomography (FDG-PET), have led to greater accuracy in preoperative mediastinal staging for patients with non-small-cell lung cancer (NSCLC), but surgical staging remains the "gold standard" for diagnosis. A proper understanding of the current accuracy of diagnostic imaging is needed for further improvements. **Methods:** Forty-three patients who underwent resection for NSCLC involving mediastinal lymph node (MLN) metastasis at our hospital between June 2003 and May 2011 were enrolled in this study. We conducted a retrospective study of the radiological and pathological findings for 53 metastatic MLNs in the 43 patients. **Results:** The preoperative imaging modality was computed tomography (CT) alone for 18 patients (22 MLNs) and CT and FDG-PET for 25 patients (31 MLNs). The sensitivities of CT and FDG-PET were 41.5% and 58.0%, respectively. The sensitivity of CT did not differ according to any clinicopathological factors, but the sensitivity of FDG-PET tended to be higher for primary tumors with high SUV_{max} values and for non-adenocarcinomas. In the lymph nodes, all micrometastatic foci ≤ 2 mm were PET-negative, but 4 lymph nodes with metastatic foci larger than 10 mm were also PET-negative. **Conclusions:** For the diagnostic imaging of MLN, FDG-PET has a greater sensitivity than contrast-enhanced CT based on "size criteria", but it is still not sufficiently sensitive and is influenced by various factors. At present, histological confirmation of MLNs is necessary when making decisions regarding treatment plans and the type of surgical procedure that should be performed.

Keywords: Non-Small-Cell Lung Cancer; Mediastinal Lymph Node Metastasis; Positron Emission Tomography; Computed Tomography

1. Introduction

Mediastinal lymph node (MLN) metastasis is the most important factor in determining both the treatment strategy and the prognosis of patients with non-small-cell lung cancer (NSCLC) without distant metastasis [1,2]. Several non-invasive and invasive diagnostic procedures have been used for mediastinal staging before surgery with curative intent, including computed tomography (CT), ^{18}F-fluorodeoxyglucose positron emission tomography (FDG-PET), and endobronchial ultrasound with transbronchial needle aspiration (EBUS-TBNA), mediastinoscopy, and video-assisted thoracoscopy (VATS). Non-invasive radiographic staging with contrast-enhanced CT and FDG-PET remain the standard procedures. However, neither CT nor FDG-PET is sufficiently sensitive or specific for the diagnosis of MLN metastasis [3-6]. The American College of Chest Physicians (ACCP) guidelines and the European Society of Thoracic Surgeons (ESTS) guidelines state: "in patients with discrete lymph node enlargement, staging by CT or FDG-PET is not sufficiently accurate, and invasive surgical procedures are recommended." [7-9].

In the current study, we investigated the diagnostic significance of CT and FDG-PET for identifying MLN metastases and compared the findings obtained using these imaging modalities with the histological results.

2. Patients and Methods

2.1. Study Population

Of the 53 patients who underwent surgery and were diagnosed as having pN2 NSCLC at the Kawasaki Medical School Hospital, Kurashiki, Japan, between June 2003 and May 2011, we included 43 patients who had under-

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gone contrast-enhanced CT before surgery in the current study. Ten patients who underwent only plain CT preoperatively were excluded.

A total of 53 metastatic MLNs were evaluated in the 43 patients. The histological type was adenocarcinoma in 24 patients, squamous cell carcinoma in 12, large cell carcinoma in 3, adenosquamous carcinoma in 2, and pleomorphic carcinoma in 2. Localization of the primary tumor was peripheral-type in 39 patients and central-type in 4. The pathological N2 status was single station in 34 patients and multiple stations in 9 (**Table 1**). The lymph node station was determined according to the lymph node map proposed by the International Association for the Study of Lung Cancer (IASLC). Written informed consent was obtained from each patient, and the study was approved by the institutional review board of Kawasaki Medical School and Hospital (IRB No. 967).

2.2. CT Imaging

The CT scans were performed using a 16-detector-row device (LightSpeed 16; GE Healthcare, Milwaukee, WI), and the scanning parameters were as follows: exposure settings, 323 to 432 mA, 0.5 s/rotation at 120 kVp; collimation, 16 × 0.625 or 1.25 mm; beam pitch, 1.75:1. A 10-mm-thick contiguous collimation was used to evaluate the entire lung for the preoperative evaluations. The size of the tumors and MLNs were determined digitally based on the findings of a thin-section CT scan.

For each metastatic MLN station, the short-axis diameter of the largest lymph node was measured using CT. The "size criteria" of the CT definition for MLN metastasis was a short-axis diameter of 10 mm or larger.

2.3. FDG-PET Imaging

FDG-PET was performed using a dedicated PET/CT scanner (Discovery ST Elite; GE Healthcare, Kyoto, Japan). The axes of the multidetector CT and PET systems were mechanically aligned so that the patient could be moved from the CT to the PET scanner gantry by simply changing the position of the examination table. The resultant PET and CT scans were coregistered with hardware. PET/CT scanning was performed at 115 minutes after the intravenous injection of 150 to 220 MBq of 18-fluorodeoxyglucose (FDG scan; Universal Giken, Nihon Medi-Physics, Tokyo, Japan). The regions of interest were placed three-dimensionally over the lung cancer nodules. A semiquantitative analysis of the images was performed by measuring the maximal standardized uptake value (SUV_{max}) of the lesions. The SUV was calculated using the following equation: tumor activity concentration/(injected dose/body weight).

The FDG-PET criteria for MLN metastasis were defined as FDG uptake coinciding with MLNs irrespective of the SUV_{max} and exhibiting a left-right asymmetry.

Table 1. Patients' characteristics.

		n = 43
Age (years)	Median	71
	Range	45 - 83
Sex	Male	27
	Female	16
Smoking Index (pack-years)	0	17
	1 - 20	7
	>20	19
Tumor Marker (CEA)	≤5.0 ng/mL	19
	>5.0 ng/mL	23
	unknown	1
Tumor Location	Central	4
	Peripheral	39
Tumor Size (cm)	Average	3.2
	Range	1.2 - 6.6
Histology	Adenocarcinoma	24
	Squamous cell carcinoma	12
	Large cell carcinoma	3
	Adenosquamous carcinoma	2
	Pleomorphic carcinoma	2
pN1 Status	pN1(−)	11
	pN1(+)	32
pN2 Status	N2 single station	34
	N2 multiple station	9

CEA: carcinoembryonic antigen.

2.4. Microscopic Measurement of the Size of the Lymph Nodes and Metastatic Foci

The dissected lymph nodes were histologically examined using 10% formalin-fixed and paraffin-embedded sections with hematoxylin and eosin staining. In each lymph node containing metastases, the lymph node with the largest metastatic foci was selected, and the long-axis and short-axis diameters of the metastatic foci in the lymph nodes were microscopically measured.

2.5. Statistical Analysis

The clinical and pathological parameters were compared using a chi-square test. Univariate analyses were performed

using the log-rank test. Differences were considered significant if $P \leq 0.05$. The statistical analyses were performed using a statistical software package (SPSS).

3. Results

3.1. CT and FDG-PET Findings

The characteristics and radiologic findings of 53 metastatic MLNs are shown in **Table 2**. Of 53 metastatic MLNs, 49 lymph nodes were visible on preoperative CT and the remaining 4 were not. The lymph node short-axis diameters were 4.7 - 25.1 mm (median, 9.6 mm) and were ≥ 10 mm in 22 lymph nodes. The sensitivity of CT was 41.5%.

FDG-PET was performed in 26 of 43 patients, with a total of 31 MLNs imaged. Eighteen lymph nodes showed FDG uptake (13 nodes with $SUV_{max} \geq 2.5$; 5 nodes with $SUV_{max} < 2.5$), and the remaining 13 nodes showed no FDG uptake. The sensitivity of FDG-PET was 58.0%.

When defining either CT or FDG-PET findings as positive for lymph node metastasis (cN2), diagnostic imaging combining CT and FDG-PET had a diagnostic sensitivity of 67.7%.

3.2. Correlation between CT/FDG-PET Findings and Clinicopathological Factors

We investigated the clinicopathological factors influencing the sensitivity of CT and FDG-PET (**Table 3**). We did not find any factors influencing the sensitivity of CT for the diagnosis of MLN metastasis. In contrast, the sensitivity of FDG-PET for the diagnosis of MLN metastasis was significantly higher ($P = 0.001$) when the primary tumor SUV_{max} was > 10.0. Also, in terms of histology, the sensitivity tended to be higher for non-adenocarcinoma than for adenocarcinoma ($P = 0.075$).

3.3. Size of Metastatic Foci in Lymph Nodes

The size of the metastatic foci could be measured pathologically in 44 metastatic MLNs. These foci were

Table 2. Characteristics and radiologic findings of 53 metastatic mediastinal lymph nodes.

		N	Detail		
pN2 Station	Upper Zone	26	#2R (n = 1), #3a (n = 2), #4L (n = 3), #4R (n = 20)		
	AP Zone	7	#5 (n = 6), #6 (n = 1)		
	Subcarinal Zone	17	#7 (n = 17)		
	Lower Zone	3	#8L (n = 1), #8R (n = 1), #9L (n = 1)		
Histology	Adenocarcinoma	33			
	Non-Adenocarcinoma	20			
CT Findings	Detected	49	Short-Axis (mm)	Average	10.0 mm
				Range	4.7 - 25.1 mm
				>10 mm	n = 22
				≤10 mm	n = 27
	Not Detected	4			
PET Findings	FDG Uptake (+)	18	SUV_{max}	Average	5.2
				Range	1.0 - 12.1
				>2.5	n = 13
				≤2.5	n = 5
	FDG Uptake (−)	13			
CT and PET	CT (+)*/PET (+)**	7			
	CT (+)/PET (−)	3			
	CT (−)/PET (+)	11			
	CT (−)/PET (−)	10			

CT: computed tomography; PET: positron emission tomography; FDG: 18F-fluorodeoxyglucose; SUV_{max}: the maximal standardized uptake value; *CT (+): short-axis diameter of mediastinal lymph node ≥ 10 mm; **PET (+): presence of asymmetrical FDG uptake coincided with mediastinal lymph node.

Table 3. Correlations with clinicopathological factors in 53 metastatic mediastinal lymph nodes.

		CT Findings		PET Findings	
		Sensitivity (%)	*P*	Sensitivity (%)	*P*
Age	≥70 yr	47	0.275	73	0.055
	<70 yr	71		47	
Sex	Male	60	0.882	45	0.178
	Female	57		89	
Smoking Status	Smoker	58	0.933	50	0.162
	Non-Smoker	60		77	
CEA	>5.0 ng / mL	60	0.949	52	0.340
	≤5.0 ng / mL	48		66	
Histology	Adenocarcinoma	64	0.293	44	0.075
	Non-Adenocarcioma	50		76	
Location	Central	75	0.509	100	0.222
	Peripheral	58		55	
Primary Tumor					
Size	>3.0 cm	55	0.514	68	0.221
	≤3.0 cm	64		46	
SUV_{max}	>10.0	62	0.526	87	0.001
	≤10.0	73		26	
pN1 Status	pN1 (+)	59	0.965	57	0.882
	pN1 (-)	58		60	
pN2 Status	Superior	63	0.415	59	0.790
	Aortic	71		67	
	Inferior	50		50	

CT: computed tomography; PET: positron emission tomography; CEA: carcinoembryonic antigen; SUV_{max}: the maximal standardized uptake value.

significantly larger (P = 0.001) in CT-positive lymph nodes with a diameter of 10 mm or more. In contrast, no statistical correlation was observed between the size of the metastatic foci and the FDG uptake (**Figures 1** and **2**).

3.4. False-Negative Lymph Nodes with FDG-PET

Thirteen lymph nodes from 9 patients showed no FDG uptake, as summarized in **Table 4**. In 10 of these 13 nodes, the histology was adenocarcinoma. The metastatic foci were micrometastatic lesions ≤ 2 mm in 7 of 13 nodes. On the other hand, in 4 of the lymph nodes, the metastatic focus showed no FDG uptake, despite having a long axis greater than 10 mm.

4. Discussion

MLN metastasis is the most important factor when determining the treatment plan for resectable NSCLC. Recent advances in diagnostic imaging technology, particularly the introduction of FDG-PET, have enabled more accurate reoperative diagnoses. In meta-analyses of reports describing the diagnosis of lymph node metastasis during the years 2000-2009, FDG-PET demonstrated a better diagnostic rate than CT, with a sensitivity of 70% - 85% and a specificity of 90% - 95%, compared to a sensitivity of 60% and a specificity of 75% - 80% for CT. [3-6] However, the accuracy of FDG-PET differed according to various factors, such as histology[10], lymph node station [11], size of lymph nodes [12,13], size of metastatic foci inside lymph nodes [13,14], and the localization of primary tumor [12]. Moreover, SUV_{max} was affected by various factors including the equipment that was used, the imaging conditions, image reconstruction, and the patient's condition; thus, comparisons among

Table 4. Pathological findings of 13 lymph nodes with false-negative of FDG-PET results.

Case	Histology	Primary Tumor			pN2 Nodes		
		Location	Size (mm)	SUV_{max}	Station	Size (mm)	Microscopic Size of Metastatic Foci (mm)
1	Ad	RU	31	10.8	#4R	(−)	<1 × 1
2	Ad	RL	45	7	#4R	7.6	<1 × 1
					#7	10.0	<1 × 1
3	Ad	LU	30	4.1	#6	6.8	<1 × 1
4	Ad	LU	25	5.3	#4L	8.2	<1 × 1
					#5	(−)	<1 × 1
5	AdSq	RL	22	5.2	#7	8.5	6 × 3
6	Ad	RU	18	4.5	#2R	9.6	2 × 1
					#4R	12.0	10 × 8
7	Sq	RL	25	8.8	#3a	9.9	12 × 11
8	Sq	LL	25	10.6	#7	4.8	12 × 11
9	Ad	RM	31	7.6	#4R	10.5	28 × 14
					#7	8.2	5 × 4

Ad: adenocarcinoma, AdSq: adenosquamous carcinoma. Sq: squzmous cell carcinoma, RU: rught upper lobe, RM: right middle lobe, RL: right lower lobe, LU: left upper lobe, LL: left lower lobe.

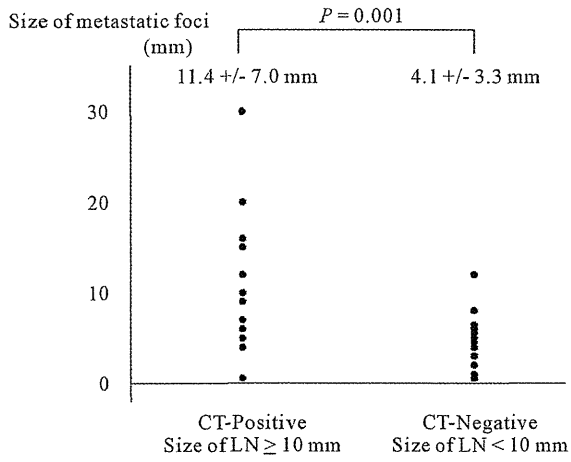

Figure 1. Distribution of sizes of metastatic foci in CT-positive (short-axis ≥ 10 mm) and CT-negative (short-axis < 10 mm) lymph nodes as observed using CT.

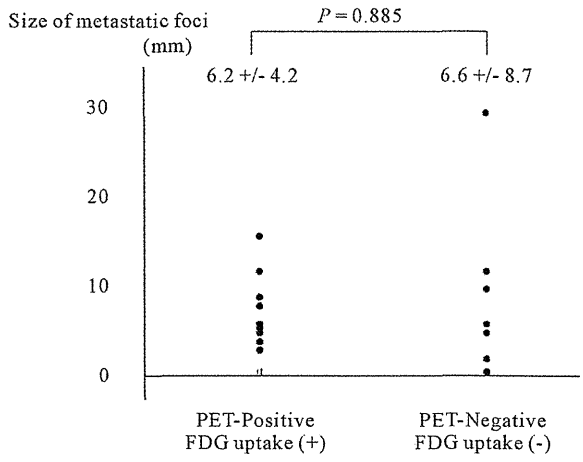

Figure 2. Distribution of sizes of metastatic foci in PET-positive and PET-negative lymph nodes as observed using FDG-PET.

multiple institutions are problematic. For these reasons, no clear diagnostic "criteria" have been established for FDG-PET diagnosis of MLN metastasis. In our study, when we adopted 1) a clear FDG uptake compared with the area around the mediastinal tissue and 2) a left-right asymmetry as the criteria for positive metastasis, the sensitivity was 58%, which was similar to the results of previous reports. We also observed differences in FDG-PET sensitivity according to histology (44% for adenocarcinoma versus 77% for non-adenocarcinoma, $P = 0.075$) and SUV_{max} of primary tumor (26% for tumors with $SUV_{max} \leq 10$ versus 87% for tumors with $SUV_{max} > 10$, $P = 0.001$); however, the sensitivity did not differ according to the localization of primary tumors (central-type or peripheral-type), the metastatic lymph node station, or the presence of hilar lymph node metastases. These re-

sults demonstrate that when judging the presence of MLN metastasis using FDG-PET diagnostic images, various pathological factors must be taken into account in addition to the lymph node size and SUV_{max}. Thus, we believe that it may be difficult to establish "cN2 criteria" for FDG-PET imaging.

Using FDG-PET, the underestimation of the SUV_{max} in small pulmonary lesions has been reported, and this issue is an important problem in the diagnosis of lymph node metastasis. The sizes of the metastatic foci in lymph nodes influence the diagnostic ability, and FDG-PET is relatively poor at identifying metastatic foci of 4 - 10 mm in size [13,14]. In our study, all the lymph node metastatic foci with FDG uptake were ≥3 mm in size, and the absence of FDG uptake occurred in micrometastatic lesions ≤2 mm in size. This low ability to detect small metastatic foci is a limitation of FDG-PET. On the other hand, we unexpectedly observed 4 lymph nodes that were PET-negative despite being at least 10 mm in size and having metastatic lesions distributed throughout the whole lymph node. Such lymph nodes would have been assessed as "CT-positive, PET-negative" in clinical practice and would likely have been judged as reactive lymph node swelling (cN0).

EBUS-TNBA is a new, minimally invasive method for histologically confirming metastatic MLNs. However, biopsies are only performed for lymph nodes with suspected metastases based on CT or FDG-PET findings. In addition, not all MLNs are approachable using EBUS-TNBA or a mediastinoscopy. Thus, diagnostic imaging still plays a major role in preoperative staging, yet the diagnostic accuracy of these techniques has not improved. In recent years, induction therapy followed by surgery has become more common for the treatment of resectable cN2 NSCLC. We are also seeing a growing diversity of therapeutic and surgical procedures at different clinical stages, e.g., a simplified lymphadenectomy, such as a lobe-selective lymph node dissection, and limited resections for small-sized NSCLC. Preoperative diagnostic imaging is therefore becoming even more important. A careful approach conducted with an understanding of the current state of diagnostic imaging and its uncertainties and with the application of EBUS-TBNA or mediastinoscopy, when necessary, in addition to pathological examinations, such as intraoperative biopsy, is required.

Our study has some limitations. First, the numbers of patients and lymph nodes that were examined were relatively small, and only 60% of all the lymph nodes were imaged using FDG-PET. Second, we only studied lymph nodes that were confirmed as being histologically metastatic. A study of the CT and FDG-PET findings for a larger number of patients and lymph nodes, including non-metastatic lymph nodes, is needed.

5. Conclusion

The sensitivity of diagnostic imaging for MLN metastasis was 41.5% for contrast-enhanced CT and 58.0% for FDG-PET. These sensitivities equate to a false-negative rate of over 30%, even when CT and FDG-PET are used in combination. The accuracy of diagnostic imaging differs according to various factors, so histological confirmation is essential for choosing a treatment strategy and surgical procedures.

REFERENCES

[1] C. F. Mountain, "Revisions in the International System for Staging Lung Cancer," *Chest*, Vol. 111, No. 6, 1997, pp. 1710-1717. doi:10.1378/chest.111.6.1710

[2] J. LoCicero, "Surgical Treatment of Non-Small Cell Lung Cancer. In: T. W. Shields, J. LoCicero, B. P. Ronald and V. W. Rusch, Eds., *General Thoracic Surgery*, 7th Edition, Lippincott Williams & Wilkins, Philadelphia, 2009, pp. 1388-1425.

[3] B. A. Dwamena, S. S. Sonnad, J. O. Angobaldo and R. L. Wahl, "Metastases from Non-Small Cell Lung Cancer: Mediastinal Staging in the 1990s—Meta-Analytic Comparison of PET and CT," *Radiology*, Vol. 213, No. 2, 1999, pp. 530-536.

[4] M. K. Gould, W. G. Kuschner, C. E. Rydzak, C. C. Maclean, A. N. Demas, H. Shigemitsu, *et al.*, "Test Performance of Positron Emission Tomography and Computed Tomography for Mediastinal Staging in Patients with Non-Small-Cell Lung Cancer," *Annals of Internal Medicine*, Vol. 139, No. 11, 2003, pp. 879-892.

[5] O. Birim, A. P. Kappetein, T. Stijnen and A. J. Bogers, "Meta-Analysis of Positron Emission Tomographic and Computed Tomographic Imaging in Detecting Mediastinal Lymph Node Metastases in Non-Small Cell Lung Cancer," *The Annals of Thoracic Surgery*, Vol. 79, No. 1, 2005, pp. 375-381. doi:10.1016/j.athoracsur.2004.06.041

[6] Y. L. Lv, D. M. Yuan, K. Wang, X. H. Miao, Q. Qian, S. Z. Wei, *et al.*, "Diagnostic Performance of Integrated Positron Emission Tomography/Computed Tomography for Mediastinal Lymph Node Staging in Non-Small Cell Lung Cancer: A Bivariate Systematic Review and Meta-Analysis," *Journal of Thoracic Oncology*, Vol. 6, No. 8, 2011, pp. 1350-1358. doi:10.1097/JTO.0b013e31821d4384

[7] G. A. Silvestri, M. K. Gould, M. L. Margolis, L. T. Tanoue, D. McCrory, E. Toloza, *et al.*, "American College of Chest Physicians. Noninvasive Staging of Non-Small Cell Lung Cancer: ACCP Evidenced-Based Clinical Practice Guidelines (2nd Edition)," *Chest*, Vol. 132, Suppl. 3, 2007, pp. 178S-201S.

[8] F. C. Detterbeck, M. A. Jantz, M. Wallace, J. Vansteenkiste, G. A. Silvestri and American College of Chest Physicians, "Invasive Mediastinal Staging of Lung Cancer: ACCP Evidence-Based Clinical Practice Guidelines (2nd Edition)," *Chest*, Vol. 132, Suppl. 3, 2007, pp. 202S-220S.

[9] P. De Leyn, D. Lardinois, P. E. Van Schil, R. Rami-Porta,

B. Passlick, M. Zielinski, *et al.*, "ESTS Guidelines for Preoperative Lymph Node Staging for Non-Small Cell Lung Cancer," *European Journal Cardio-Thoracic Surgery*, Vol. 32, No. 1, 2007, pp. 1-8. doi:10.1016/j.ejcts.2007.01.075

[10] A. Billé, E. Pelosi, A. Skanjeti, V. Arena, L. Errico, P. Borasio, *et al.*, "Preoperative Intrathoracic Lymph Node Staging in Patients with Non-Small-Cell Lung Cancer: Accuracy of Integrated Positron Emission Tomography and Computed Tomography," *European Journal Cardio-Thoracic Surgery*, Vol. 36, No. 3, 2009, pp. 440-445. doi:10.1016/j.ejcts.2009.04.003

[11] R. J. Cerfolio, B. Ojha, A. S. Bryant, C. S. Bass, A. A. Bartalucci and J. M. Mountz, "The Role of FDG-PET Scan in Staging Patients with Non-Small Cell Carcinoma," *The Annals of Thoracic Surgery*, Vol. 76, No. 3, 2003, pp. 861-866. doi:10.1016/S0003-4975(03)00888-9

[12] N. Al-Sarraf, R. Aziz, K. Gately, J. Lucey, L. Wilson, E. McGovern, *et al.*, "Pattern and Predictors of Occult Mediastinal Lymph Node Involvement in Non-Small Cell Lung Cancer Patients with Negative Mediastinal Uptake on Positron Emission Tomography," *European Journal Cardio-Thoracic Surgery*, Vol. 33, No. 1, 2008, pp. 104-109. doi:10.1016/j.ejcts.2007.09.026

[13] W. Yang, Z. Fu, J. Yu, S. Yuan, B. Zhang, D. Li, *et al.*, "Value of PET/CT versus Enhanced CT for Locoregional Lymph Nodes in Non-Small Cell Lung Cancer," *Lung Cancer*, Vol. 61, No. 1, 2008, pp. 35-43. doi:10.1016/j.lungcan.2007.11.007

[14] H. Nomori, K. Watanabe, T. Ohtsuka, T. Naruke, K. Suemasu and K. Uno, "The Size of Metastatic Foci and Lymph Nodes Yielding False-Negative and False-Positive Lymph Node Staging with Positron Emission Tomography in Patients with Lung Cancer," *The Journal of Thoracic and Cardiovascular Surgery*, Vol. 128, No. 3, 2004, pp. 396-401. doi:10.1016/j.jtcvs.2004.03.020

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ORIGINAL RESEARCH

Heterogeneity of the *EGFR* mutation status between the primary tumor and metastatic lymph node and the sensitivity to *EGFR* tyrosine kinase inhibitor in non-small cell lung cancer

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Abstract The purpose of this study was to clarify the distribution of epidermal growth factor receptor (*EGFR*) mutations between primary tumors (PT) and metastatic lymph node (MLN) in patients with resected non-small cell lung cancer (NSCLC) and to identify a better predictive marker of the response to *EGFR* tyrosine kinase inhibitor (EGFR-TKI). We conducted a retrospective review of the data of 70 lung cancer patients with lymph node metastasis who underwent surgical resection. Analysis to detect *EGFR* mutations was performed by a peptide nucleic acid–locked nucleic acid polymerase chain reaction clamp method. *EGFR* mutations were detected in 15.7 % of both the PT and MLN and in 14.3 % of the PT only. The response rate to EGFR-TKI tended to be higher in patients with *EGFR* mutations in the MLN, as all patients with *EGFR* mutations in the MLN showed disease control to treatment with EGFR-TKI. Our results demonstrated that the *EGFR* mutation status of MLN is a predictive marker of the response to EGFR-TKI therapy in patients with recurrent NSCLC after surgical resection.

Keywords Epidermal growth factor receptor mutation · Non-small cell lung cancer · Metastatic lymph node · *EGFR* tyrosine kinase inhibitor

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Introduction

Lung cancer is a leading cause of cancer-related death worldwide. The most effective treatment of early stage (IA–IIIA) non-small cell lung cancer (NSCLC) is surgical resection. In addition, adjuvant chemotherapy after the resection of stage II–IIIA NSCLC is now the "standard of care" based on the results of three large-scale phase III trials and individual patient meta-analyses [1–4]. However, up to 60 % of patients with NSCLC with lymph node metastasis show relapse after surgery [5, 6].

The prognosis of patients with NSCLC is critically dependent on the extent of metastatic spread of the tumor cells at the time of surgery. Tumor cells acquire the metastatic phenotype through the process of clonal evolution occurring during the multistep process of tumor progression [7]. Significant progress in the understanding of the biology and molecular mechanisms of lung cancer has allowed some new molecular targeted therapies to be developed. The most well-known molecular target is mutation of the epidermal growth factor receptor (EGFR). *EGFR* tyrosine kinase inhibitors (EGFR-TKIs) have been shown to have a dramatic clinical effect in a significant proportion of patients with NSCLC [8]. In 2004, such response to EGFR-TKIs was identified to be related to the presence of some type of gene mutation in the tyrosine kinase domain of *EGFR* [9, 10]. *EGFR* mutations associated with clinical sensitivity to EGFR-TKIs have been shown to occur more frequently in lung cancer patients who are females, nonsmokers, Asians, and have adenocarcinoma [11, 12].

Several studies in Asia have shown that patients with *EGFR*-mutated lung cancers show objective response rates of 70–80 % to treatment with EGFR-TKIs, while 20–30 %

of these patients show no response to EGFR-TKI therapy [13–15]. In most studies, the *EGFR* mutation status has been determined based on analysis of the primary tumors (PT). In general, the EGFR-TKIs resistance in a proportion of patients with *EGFR* mutations is explained by the T790M mutation [16]. In addition, it has been speculated that the absence of response to EGFR-TKIs might be due to the discordance of the *EGFR* mutation status between the PT and the metastatic lesions.

Thus, to investigate the heterogeneity of the *EGFR* mutation status between the PT and metastatic lesions, we examined the *EGFR* mutation patterns in PT and the corresponding local metastatic lymph nodes (MLN) using resected samples. The purpose of this study was to clarify the existence of any discordant *EGFR* mutation patterns between the PT and MLN and the clinical usefulness of *EGFR*-targeted therapies in the treatment of patients with NSCLC.

Patients and methods

Study population

Seventy patients of NSCLC with lymph node metastasis who were treated by surgical resection with systematic lymph node dissection at the Kawasaki Medical School Hospital between 2004 and 2010 were enrolled in this study. None of the patients had received radiation therapy prior to the surgery. The histological diagnosis of the tumors was based on the criteria of the World Health Organization, and the TNM stage was determined according to the criteria in 2009. Informed consent was obtained from each patient for the study of tissue samples from the resected surgical specimens. This study was conducted with the approval of the institutional ethics committee of the Kawasaki Medical School.

EGFR mutation analysis

Analysis to detect *EGFR* mutations was performed in the resected, paraffin-embedded lung cancer tissues (the PT and one of their corresponding local MLN) by the peptide nucleic acid–locked nucleic acid polymerase chain reaction (PNA-LNA PCR) clamp method [17]. In this study, the PNA-LNA PCR clamp assay was performed at the Mitsubishi Kagaku Bio-Clinical Laboratories, Inc.

Drug administration

Sixteen patients received oral gefitinib treatment for disease recurrence, at the dose of 250 mg once daily. These patients were continued on gefitinib therapy until the detection of disease progression or the development of intolerable toxicity.

Assessment of response Tumor recurrences were diagnosed based on the findings on imaging, including thoracic computed tomography, fluorodeoxyglucose positron emission tomography, and brain magnetic resonance imaging, and not necessarily confirmed by histopathology. Objective tumor response and its duration were assessed according to the RECIST criteria, and the response of all the responders was still confirmed to be valid more than 4 weeks after the initial assessment of the response [18].

Statistical analysis

`Statistical analysis was performed for examining the significances of the differences among the groups and any possible correlations between presence/absence of *EGFR* gene mutations and the clinicopathological features using Fisher's exact test or chi-square test, as appropriate. An unpaired *t* test was used for the comparison of continuous data. All the statistical analyses were conducted using the SPSS software (Version 17.0; SPSS Incorporation, Chicago, IL, USA). All statistical

Table 1 The patient characteristics

Characteristics	Number of patients	Percent
Age (years)		
<70	30	42.9
≥70	40	57.1
Sex		
Male	46	65.7
Female	24	34.3
Smoking status		
Smoker	48	68.6
Never smoker	22	31.4
Histology		
Adenocarcinoma	35	50.0
Squamous cell carcinoma	24	32.9
Adenosquamous carcinoma	5	6.8
Large cell carcinoma	4	5.5
Pleomorphic carcinoma	2	2.7
Pathological lymph node status		
pN1	29	39.7
pN2	41	60.3
Neoadjuvant chemotherapy		
Yes	7	10.0
No	63	90.0
Adjuvant chemotherapy		
Yes	41	58.6
No	29	41.4

Table 2 *EGFR* mutation status of the PT and MLN

PT	Lymph node	
	Mutant	Wild type
Mutant	11	10
Wild type	0	49

tests were two-sided, and probability values of <0.05 were regarded as denoting statistical significance.

Results

Clinical characteristics

The characteristics of the patients are summarized in Table 1. The patients ranged in age from 37 to 83 years (mean, 69.1 years), and there were 46 men and 24 women. The majority of patients (35, 50.0 %) had adenocarcinoma, while 24 (32.9 %) had squamous cell carcinoma, 5 (6.8 %) had adenosquamous carcinoma, 4 (5.5 %) had large cell carcinoma, and 2 (2.7 %) had pleomorphic carcinoma. Pathological N1 disease was confirmed in 29 (39.7 %) patients and N2 disease in 41 (60.3 %) patients.

EGFR mutation status of the PT and the lymph node metastases

EGFR gene mutations were found in the PT of 21 (31.5 %) patients and MLN of 11 (15.1 %) patients. All patients with *EGFR* mutations in the MLN also had *EGFR* mutations in the PT. Of 21 patients with *EGFR* mutations in the PT, 10 showed no *EGFR* mutations in the MLN. Of the 49 patients who were *EGFR* mutation-negative in the PT, none showed mutations in the MLN either (Table 2).

Relation between the mutation status of *EGFR* and the clinicopathological characteristics

We categorized the 70 patients into three groups according to the presence/absence of *EGFR* mutations in the PT and MLN, as follows: group 1 (n=11): *EGFR* mutation-positive in both the PT and MLN; group 2 (n=10): *EGFR* mutation-positive only in the PT; group 3 (n=49): *EGFR* mutation-negative in both the PT and MLN. Group 3 had a higher proportion of men ($p<0.001$), current smokers ($p<0.001$), and patients with squamous cell carcinoma ($p<0.001$) than group 1+2, while no significant association of the *EGFR* mutation-

Table 3 Association of *EGFR* mutation and clinicopathological variables

Characteristics	*EGFR* mutation status (PT/MLN)				
	Group1	Group2		Group3	
	Mut/Mut	Mut/WT	p value (G1 vs. G2)	WT/WT	p value (G3 vs. G1+2)
Patients, number	11	10		49	
Age (mean), years	72.2	66.9	0.142	68.9	0.745
Sex			0.149		<0.001
Male	1	4		41	
Female	10	6		8	
Smoking status			0.311		<0.001
Smoker	1	3		44	
Never smoker	10	7		5	
Histology			0.366		<0.001
Adenocarcinoma	10	9		16	
Squamous cell	0	1		23	
Adenosquamous	1	0		4	
Large cell	0	0		4	
Pleomorphic	0	0		2	
Tumor size (mean), mm	34.7	37.3	0.734	37.8	0.657
Pathological nodal status			0.387		0.540
pN1	6	3		20	
pN2	5	7		29	

Mut mutant, *WT* wild type

Table 4 Response to EGFR-TKI: patients

EGFR mutation status					
Case	Age/sex	Histology	PT	Lymph node	Response
1	70/F	AS	Exon 19 del	Exon 19 del	SD
2	60/F	AD	L858R	L858R	PR
3	69/M	AD	L858R	L858R	SD
4	65/F	AD	L858R	L858R	CR
5	73/F	AD	L858R	L858R	PR
6	76/F	AD	L858R	L858R	PR
7	81/F	AD	Exon 19 del	Exon 19 del	PR
8	78/F	AD	Exon 19 del	Exon 19 del	PR
9	59/F	AD	L858R	L858R	SD
10	57/F	AD	Exon 19 del	WT	PD
11	66/F	AD	Exon 19 del	WT	PR
12	75/M	AD	Exon 19 del	WT	CR
13	53/M	AD	Exon 19 del	WT	CR
14	61/F	AD	L858R	WT	PR
15	74/F	AD	L858R	WT	PD
16	77/F	AD	L858R	WT	PD

AD adenocarcinoma, *AS* adenosquamous carcinoma

negative status was observed with age (p=0.745), tumor size (p=0.657), or pathological lymph node status (N1 or N2) (p=0.540) (Table 3). No significant associations with any clinicopathological characteristics were observed in group 1 and group 2 (Table 3).

Response to EGFR-TKI

Of the 21 patients in group 1 and group 2, 16 patients were treated with gefitinib. All the nine patients in group 1 showed disease control (complete response+partial response+stable disease). On the other hand, of the seven patients in group 2, 4 (57.1 %) showed disease control and the remaining three showed progressive disease. The disease control rate tended to be higher in group 1 than in group 2 (p=0.062) (Tables 4 and 5).

Table 5 Response to EGFR-TKI: association of *EGFR* mutation and response to EGFR-TKI

EGFR mutation (PT/MLN)			
Response	Mut/Mut	Mut/WT	p value
CR+PR+SD	9	4	0.062
PD	0	3	

Mut mutant, *WT* wild type

Discussion

This study demonstrated the existence of discordance of the *EGFR* mutation status between the PT and MLN in patients with NSCLC. This is the first report of the investigation of the *EGFR* mutation gene status using the PNA-LNA PCR clamp assay and of the sensitivity of NSCLC patients with postoperative recurrence to treatment with EGFR-TKI in relation to the *EGFR* mutation status.

To date, the heterogeneity of the distribution of *EGFR* mutations is still controversial. Several reports have described the discordance of *EGFR* mutation status in NSCLC patients between the PT and metastatic tumors [19, 20], or even among parts of the PT [21–23]. On the other hand, a few reports have also suggested that such heterogeneous distribution of *EGFR* mutations is rare [24]. Therefore, the issue of discordance of the *EGFR* mutation status between the PT and metastatic sites, including the MLN, in NSCLC patients remains under debate. The reason for the discordant expression of *EGFR* mutations between the primary and metastatic lesions also remain controversial, although recent research suggests the following: (1) presence of intratumoral heterogeneity of *EGFR* mutations, (2) occurrence of changes in *EGFR* mutations during the course of disease progression, and (3) technical limitations in the methods used for the assessment of *EGFR* mutations. As for intratumoral heterogeneity, Yatabe et al. clearly demonstrated, using the Cycleave PCR™ method, that intratumoral heterogeneous distribution of the *EGFR* mutations is extremely rare [24]. As for the occurrence of changes in *EGFR*

mutations during the course of disease progression, Ji et al. demonstrated that *EGFR* mutations represent an early event in the pathogenesis of lung adenocarcinoma [25]. Thus, the discrepancy of the *EGFR* mutation status between the PT and metastatic lesions in NSCLC patients would also seem to depend on the method used for the assessment of *EGFR* mutations.

Over the past 3 years, several methods have been proposed for the detection of *EGFR* mutations. Previously, the direct sequencing method was usually used; however, the sensitivity of direct sequencing was suboptimal for clinical tumor samples and mutation detection by direct sequencing was associated with a higher frequency of false-negative results. In the literature, the PNA-LNA clamp method, Scorpion amplification refractory mutation system method, mutant-enriched PCR method, and Cycleave PCR™ method have been reported to be more sensitive than the direct sequencing method. To date, most reports describing discordance in the *EGFR* mutation status between the PT and metastatic tumors have used the direct sequencing method. For example, Park et al. demonstrated, by using heteroduplex analysis, that a considerable proportion of NSCLC patients showed discrepancy in the *EGFR* mutation status between the PT and MLN [19]. In their series, ten cases were *EGFR* mutation-negative in the MLN while being mutation-positive in the PT and seven cases were *EGFR* mutation-positive in the MLN while being mutation-negative in the PT. However, our results demonstrated that all patients with *EGFR* mutations in the MLN had *EGFR* mutations in the PT. This difference was considered to be a consequence of the method of assessment of the *EGFR* mutation. We believed that our results were highly reliable for the assessment of *EGFR* mutations, as the PNA-LNA clamp assay has been shown as a high-sensitivity detection method.

Our results demonstrated a tendency towards the existence of associations between the *EGFR* mutation status in the PT/MLN and clinical response to treatment with EGFR-TKI. Especially, the response rate to EGFR-TKI tend to be higher in patients with *EGFR* mutations in the MLN, and all patients with *EGFR* mutations in the MLN showed disease control to EGFR-TKI therapy. On the other hand, only half of patients with *EGFR* mutations in the PT but not in the MLN showed disease control to EGFR-TKI therapy. Thus, heterogeneity in the expression of *EGFR* mutations in the PT and MLN would appear to be correlated with the initial response to gefitinib. This is the first report of the investigation of the associations between heterogeneity of the *EGFR* mutation status in the PT/MLN and the clinical response to EGFR-TKIs.

Conclusion

In conclusion, the *EGFR* mutation status is often discordant between the PT and MLN in patients with NSCLC, and this heterogeneity may explain the variable clinical responses of these patients to EGFR-TKI therapy. Our results demonstrated that the *EGFR* mutation status in the MLN may be a better predictive marker of the response to EGFR-TKI therapy in patients with recurrent NSCLC after surgical resection. Further study will be required to characterize the heterogeneity of the *EGFR* mutation status between the PT and metastatic lesions and its correlation with the clinical responses to EGFR-TKI therapy in patients with NSCLC.

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Conflict of interest The authors have no conflicts of interests to declare.

References

1. Arriagada R, Bergman B, Dunant A, Le Chevalier T, Pignon JP, Vansteenkiste J, International Adjuvant Lung Cancer Trial Collaborative Group (2004) Cisplatin-based adjuvant chemotherapy in patients with completely resected non-small-cell lung cancer. N Engl J Med 350:351–360
2. Winton T, Livingston R, Johnson D, Rigas J, Johnston M, Butts C et al (2005) Vinorelbine plus cisplatin vs. observation in resected non-small-cell lung cancer. N Engl J Med 352:2589–2597
3. Douillard JY, Rosell R, De Lena M, Carpagnano F, Ramlau R, Gonzáles-Larriba JL et al (2006) Adjuvant vinorelbine plus cisplatin versus observation in patients with completely resected stage IB–IIIA non-small-cell lung cancer (Adjuvant Navelbine International Trialist Association [ANITA]): a randomised controlled trial. Lancet Oncol 7:719–727
4. Pignon JP, Tribodet H, Scagliotti GV, Douillard JY, Shepherd FA, Stephens RJD et al (2008) Lung adjuvant cisplatin evaluation: a pooled analysis by the LACE Collaborative Group. J Clin Oncol 26:3552–3559
5. Asamura H, Goya T, Koshiishi Y, Sohara Y, Eguchi K, Mori M et al (2008) Prognosis of 13,010 resected lung cancers. J Thorac Oncol 3:46–52
6. Asamura H, Nakayama H, Kondo H, Tsuchiya R, Naruke T (1999) Lobe-specific extent of systematic lymph node dissection for non-small cell lung carcinomas according to a retrospective study of metastasis and prognosis. J Thorac Cardiovasc Surg 117:1102–1111
7. Fidler IJ, Hart IR (1982) Biological diversity in metastatic neoplasms: origins and implications. Science 217:998–1003
8. Fujiwara K, Kiura K, Ueoka H, Tabata M, Hamasaki S, Tanimoto M (2003) Dramatic effect of ZD1839 ("Iressa") in a patient with advanced non-small lung cancer and poor performance status. Lung Cancer 40:73–76
9. Paez JG, Janne PA, Lee JC, Tracy S, Greulich H, Gabriel S et al (2004) EGFR mutations in lung cancer: correlation with clinical response to gefitinib therapy. Science 304:1497–1500
10. Lynch TJ, Bell DW, Sordella R, Gurubhagavatula S, Okimoto RA, Brannigan BW et al (2004) Activating mutations in the epidermal growth factor receptor underlying responsiveness of non-small-cell lung cancer to gefitinib. N Engl J Med 350:2129–2139
11. Mitsudomi T, Kosaka T, Endoh H, Horio Y, Hida T, Mori S et al (2005) Mutations of the epidermal growth factor receptor gene predict prolonged survival after gefitinib treatment in patients with

non-small-cell lung cancer with postoperative recurrence. J Clin Oncol 23:2513–2520
12. Mok TS, Wu YL, Thongprasert S, Yang CH, Chu DT, Saijo NS et al (2009) Gefitinib or carboplatin-paclitaxel in pulmonary adenocarcinoma. N Engl J Med 361:947–957
13. Maemondo M, Inoue A, Kobayashi K, Sugawara S, Oizumi S, Isobe H et al (2010) Gefitinib or chemotherapy for non-small-cell lung cancer with mutated EGFR. N Engl J Med 362:2380–2388
14. Mitsudomi T, Morita S, Yatabe Y, Negoro S, Okamoto I, Tsurutani JS et al (2005) Gefitinib versus cisplatin plus docetaxel in patients with non-small-cell lung cancer harbouring mutations of the epidermal growth factor receptor (WJTOG3405). Lancet Oncol 11:121–128
15. Zhou C, Wu YL, Chen G, Feng J, Liu XQ, Wang C et al (2011) Erlotinib versus chemotherapy as first-line treatment for patients with advanced EGFR mutation-positive non-small-cell lung cancer (OPTIMAL, CTONG-0802). Lancet Oncol 12:735–742
16. Kobayashi S, Boggon TJ, Dayaram T, Jänne PA, Kocher O, Meyerson M et al (2005) EGFR mutation and resistance of non-small-cell lung cancer to gefitinib. N Engl J Med 24:786–792
17. Nagai Y, Miyazawa H, Huqun, Tanaka T, Udagawa K, Kato M, Fu et al (2005) Genetic heterogeneity of the epidermal growth factor receptor in non-small cell lung cancer cell lines revealed by a rapid and sensitive detection system, the peptide nucleic acid–locked nucleic acid PCR clamp. Cancer Res 65:7276–7282
18. Eisenhauer EA, Therasse P, Bogaerts J, Schwartz LH, Sargent D, Ford R et al (2009) New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1). Eur J Cancer 45:228–247
19. Park S, Holmes-Tisch AJ, Cho EY, Shim YM, Kim J, Kim HS (2009) Discordance of molecular biomarkers associated with epidermal growth factor receptor pathway between primary tumors and lymph node metastasis in non-small cell lung cancer. J Thorac Oncol 4:809–815
20. Schmid K, Oehl N, Wrba F, Pirker R, Pirker C, Filipits M (2009) EGFR/KRAS/BRAF mutations in primary lung adenocarcinomas and corresponding locoregional lymph node metastases. Clin Cancer Res 15:4554–4560
21. Taniguchi K, Okami J, Kodama K, Higashiyama M, Kato K (2008) Intratumor heterogeneity of epidermal growth factor receptor mutations in lung cancer and its correlation to the response to gefitinib. Cancer Sci 99:929–935
22. Nakano H, Soda H, Takasu M, Tomonaga N, Yamaguchi H, Nakatomi K et al (2008) Heterogeneity of epidermal growth factor receptor mutations within a mixed adenocarcinoma lung nodule. Lung Cancer 60:136–140
23. Sakurada A, Lara-Guerra H, Liu N, Shepherd FA, Tsao MS (2008) Tissue heterogeneity of EGFR mutation in lung adenocarcinoma. J Thorac Oncol 3:527–529
24. Yatabe Y, Matsuo K, Mitsudomi T (2011) Heterogeneous distribution of EGFR mutations is extremely rare in lung adenocarcinoma. J Clin Oncol 29:2972–2977
25. Ji H, Li D, Chen L, Shimamura T, Kobayashi S, McNamara K et al (2005) The impact of human EGFR kinase domain mutations on lung tumorigenesis and in vivo sensitivity to EGFR-targeted therapies. Cancer Cell 9:485–495

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Membrane-bound estrogen receptor-α expression and epidermal growth factor receptor mutation are associated with a poor prognosis in lung adenocarcinoma patients

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Abstract

Background: The purpose of this study is to clarify the correlations between the expression of membrane-bound estrogen receptor-α (mERα) and epidermal growth factor receptor (EGFR) mutation and clinicopathological factors, especially in relation to the prognosis, in patients with lung adenocarcinoma.

Methods: We conducted a retrospective review of the data of 51 lung adenocarcinoma patients with tumors measuring less than 3 cm in diameter. Immunohistochemical staining for mERα expression and detection of the *EGFR* mutation status were performed.

Results: Among the 51 patients, the tumors in 15 showed both mERα expression and *EGFR* mutation. ("double positive") Significant associations between "double positive" and vascular invasion, vascular endothelial growth factor expression, and Ki-67 expression were observed. A multivariate analysis revealed that only "double positive" was an independent risk factor influencing the recurrence-free survival.

Conclusions: Presence of mERα expression together with *EGFR* mutation was found to be an independent prognostic factor for survival in patients with lung adenocarcinoma, suggesting cross-talk between mERα and *EGFR* mutation.

Keywords: Membrane-bound estrogen receptor-α, Epidermal growth factor receptor mutation, Lung adenocarcinoma

Background

Lung cancer is a leading cause of cancer-related death worldwide. The recent increase in interest in lung cancer appears to be attributable to the marked increase in the global prevalence of adenocarcinoma. Especially, adenocarcinoma appears to have a predilection for women, and the association of adenocarcinoma with a smoking habit may be less than that for the other histological subtypes of lung cancer [1,2]. These features of lung adenocarcinoma suggest that some factors peculiar to sex may be involved in the clinicopathology of this cancer, and some preference for female-associated pathways in the development of this form of lung cancer.

Estrogen exerts most of its effects in breast cancer via its receptors expressed in the tumor tissue; estrogen receptor (ER) α and ß. In breast cancer, the expression of ERα is a useful marker that provides information on the patient prognosis and the potential efficacy of hormone therapy [3]. Since ER α and ß are also well known to be expressed in both normal lung epithelial cells and lung cancers, a possible role of estrogen has been proposed in lung carcinogenesis [4]. Known for decades, ERα is a nuclear steroid receptor that is expressed in breast, ovarian, and endometrial tissue, but antibodies used to detect ERα in breast cancer show little or no reactivity in lung cancer tissues. On the other hand, non-nuclear (membrane-bound) ERα was described in 2002. Using this

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