																Same/Different
31	P	F	71	0	1.2	S		8	70	0	MPLC	Ex21: L858R		A		
	S1						Same	3	100						E	
	S2						Different	4	100			Ex21: L858R		D	E	
32	P	F	74	20	4.7	S		16	60	0	MPLC	Ex21: L858R				Different
	S						Different	15	80			Ex19: L747-T751del		B	E	
33	P	M	72	138	1.8	S		16	0	1	MPLC			E		ND
	S						Same	12	0						E	
34	P	F	48	1.8	1.8	S		30	0	0	MPLC	codon12 GAT		E		Different
	S						Same	4	100						A	
35	P	M	60	40	1.2	S		16	95	0	MPLC	Ex19: E746-A750del				Same/Same
	S1						Different	9	90			codon12 TGT		A	D	
	S2						Different	7	80			codon12 TGT		A	D	
36	P	F	67	0	2	S		17	90	0	MPLC					ND
	S						Different	7	100					E	E	

P, primary tumor; S, secondary tumor; M, male; F, female; CEA, carcinoembryonic antigen; M/S, metachronous/synchronous; BAC, bronchioloalveolar carcinoma; pN, pathological nodal status; MPLC, multiple primary lung cancer; PM, pulmonary metastasis; *EGFR*, epidermal growth factor receptor; ND, not determined.

[a] Location of secondary tumors in comparison to the primary tumor (the same lobe or a different lobe).

[b] Proportion of the BAC component in a tumor.

[c] Differential diagnosis of MPLC or PM based on Martini and Melamed's criteria.

[d] Clonality status based on the presence of *EGFR* and *K-ras* mutations.

[e] Sequence of deleted region in exon 19 could not be determined.

and genetically heterogenous [11], we considered that a clone with the L858R mutation metastasized to three secondary tumors from a primary tumor with genetic heterogeneity.

Twenty-three (50%) secondary tumors were categorized into pattern A, B or C and were regarded as exhibiting a different clonality. Eight (17%) secondary tumors were regarded as exhibiting the same clonality (pattern D). The remaining 15 (33%) were categorized into either pattern E or F, and their clonality status could therefore not be determined. An independent analysis of the *EGFR* mutation status enabled a clonality assessment of multifocal lung adenocarcinomas in 21(58%) of the 36 patients.

EGFR mutations were detected in at least one tumor in 22 patients, and no *EGFR* mutations were detected in any of the tumors in 14 patients. The *EGFR* mutations had occurred randomly in 20 (91%) of the 22 patients. Concordant activating *EGFR* mutations in all the multifocal tumors were detected in the remaining two patients. Different activating *EGFR* mutations in all the multifocal tumors were detected in six of the 20 patients with tumors exhibiting random *EGFR* mutations. In total, some type of activating *EGFR* mutation was found in all the multifocal tumors in eight (22%) of the 36 patients.

3.3. Clonality assessment based on K-ras mutation status (Table 2)

K-ras mutations were detected in 19 (23%) of the 82 tumors in total, 9 (25%) of the 36 primary tumors and 10 (22%) of the 46 secondary tumors. A point mutation in codon 12 was found in 17 tumors, and a point mutation in codon 13 was detected in two tumors. The coexistence of *EGFR* and *K-ras* mutations was observed in 2 (2%) of the 82 lung adenocarcinomas, each from a different patient.

Fourteen (30%) secondary tumors were categorized into pattern A, B or C and were regarded as exhibiting a different clonality. Two (4%) secondary tumors were regarded as exhibiting the same clonality (pattern D). The remaining 30 (65%) were categorized into either pattern E or F. An independent analysis of the *K-ras* mutation status enabled a clonality assessment of multifocal lung adenocarcinomas in 15(42%) of the 36 patients.

K-ras mutations were detected in at least one tumor in 15 patients, and no *K-ras* mutations were detected in any of the tumors in 21 patients. The *K-ras* mutations had occurred randomly in 14 (93%) of the 15 patients.

3.4. Clonality assessment based on combined EGFR and K-ras mutation status (Table 2)

Combining the results for *EGFR* and *K-ras* mutation patterns, 23 (64%) patients were regarded as having tumors with a different clonality, four (11%) patients were regarded as having tumors with the same clonality, and three patients (Nos. 8, 10, and 31) were regarded as having both a tumor with the same clonality and one with a different clonality. Therefore, the clonality status of multifocal adenocarcinomas was determined in 30 (83%) of the 36 patients.

Based on Martini and Melamed's criteria [8], 31 (86%) of the 36 patients were diagnosed as having MPLC and the remaining five (14%) patients were diagnosed as having PM. The results of the clonality assessment based on the combined *EGFR* and *K-ras* mutation status was consistent with the differential diagnosis of MPLC or PM according to Martini and Melamed's criteria in 21 (70%) of the 30 patients whose tumor clonality status could be determined (Table 3).

Table 3
Comparison of differential diagnosis of MPLC or PM based on Martini and Melamed's criteria and a clonality assessment based on *EGFR* and *K-ras* mutation status.

	MPLC[a]	PM[a]
Tumors with a different clonality[b]	21	2
Tumors with the same clonality[b]	4	0
Tumors with a different/the same clonality[b]	3[c]	0
Not determined	3	3

MPLC, multiple primary lung cancer; PM, pulmonary metastasis.
[a] Differential diagnosis of MPLC or PM based on Martini and Melamed's criteria.
[b] Clonality assessment of multifocal adenocarcinomas based on *EGFR* and *K-ras* mutation status.
[c] Three patients (Nos. 8, 10, and 31) had both a secondary tumor with the same clonality and one with a different clonality.

3.5. Relationships between EGFR/K-ras mutation status and clinicopathological features

The relationships between the *EGFR/K-ras* mutation status and the clinicopathological features, such as gender, age, smoking status, preoperative serum carcinoembryonic antigen (CEA) level, pathological nodal status, tumor size, or the proportion of BAC component, were evaluated. Among them, gender and smoking status were associated with the *EGFR* mutation status. The frequency of *EGFR* mutations was significantly higher among women than among men ($P=0.041$) and among never smokers than among current or former smokers ($P=0.014$).

3.6. Survival analyses

The median follow-up period for the 31 patients with synchronous multifocal adenocarcinomas was 40 months (ranging from 6 to 118 months). The overall 3-year and 5-year survival rates were 82.1% and 77.3%, respectively (Fig. 1A). The actuarial survival was significantly higher in female patients than in male patients and in patients with MPLC diagnosed according to Martini and Melamed's criteria than in those with PM (Fig. 1B).

However, no statistically significant differences in actuarial survival were observed among patient subgroups stratified according to age, smoking status, preoperative serum CEA level, pathological nodal status, tumor size, proportion of BAC component, clonality status based on *EGFR* mutations, or clonality status based on *EGFR* and *K-ras* mutations (Fig. 1C) (Table 4).

In a multivariate analysis, the differential diagnosis of MPLC or PM according to Martini and Melamed's criteria was the only significant prognostic factor ($P=0.001$).

4. Discussion

With the recent advance of molecular biology, a number of investigators have performed clonality assessments of multifocal lung cancers using markers such as *p53* mutation [12–15], *K-ras* mutation [16–18], *EGFR* mutation [14,17,18], X-chromosome inactivation [2,13], or loss of heterozygosity analyses of various microsatellite markers [13]. We considered both *EGFR* and *K-ras* to be suitable for investigating the clonal origin of lung adenocarcinomas for the following reasons. First, both *EGFR* [19,20] and *K-ras* mutations [19–21] have been found in atypical adenomatous hyperplasia (AAH), which is considered to be a precursor to lung adenocarcinoma. Moreover, a close relationship between *EGFR* or *K-ras* mutation and lung adenocarcinoma pathogenesis has been demonstrated in transgenic mice [22–24]. Therefore, both *EGFR* and *K-ras* mutations are thought to be early events in lung adenocarcinoma pathogenesis. Second, both *EGFR* and *K-ras* mutations are known to be frequent genetic alterations in lung adenocarcinoma, and these mutations are observed in a mutually exclusive

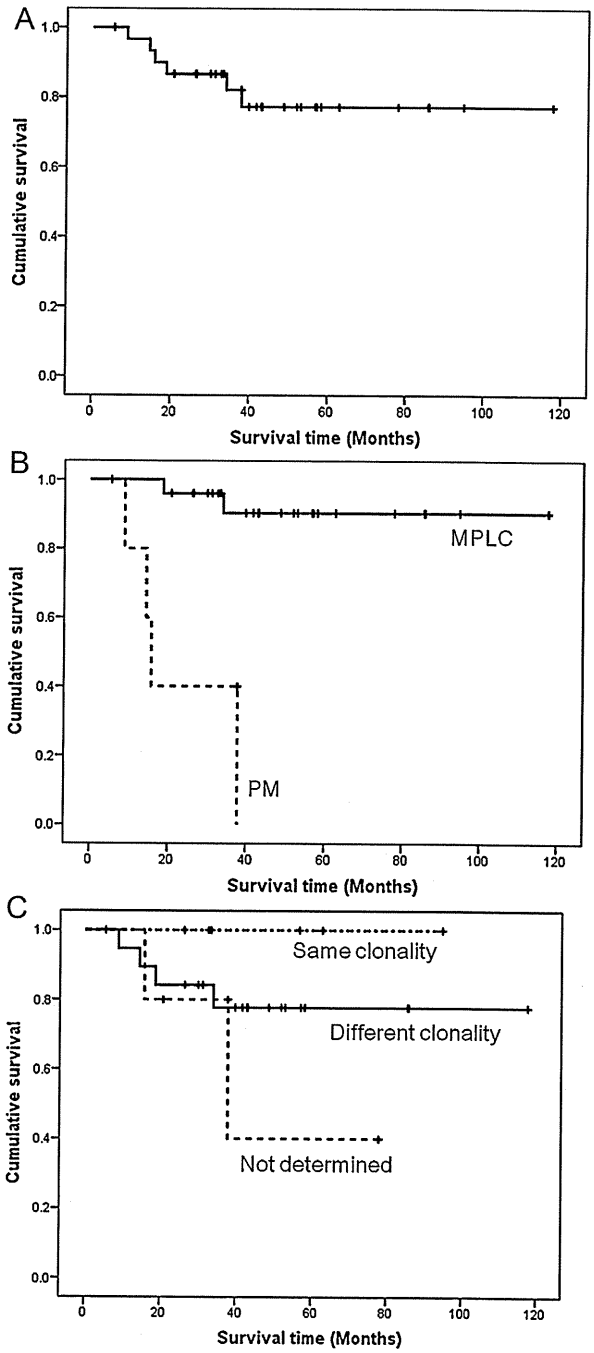

Fig. 1. Survival curves for patients with synchronous multifocal lung adenocarcinomas. (A) Overall patients. (B) Comparison between the outcomes of patients with MPLC and patients with PM diagnosed according to Martini and Melamed's criteria (log-rank test, $P<0.001$). (C) Comparison between the outcomes of patients with tumors exhibiting the same clonality and patients with tumors exhibiting a different clonality based on the presence of *EGFR* and *K-ras* mutations (log-rank test, $P=0.267$).

manner [25]. Therefore, a large portion of multifocal lung adenocarcinomas could be assessed for the clonality status using a combined mutation pattern analysis of *EGFR* and *K-ras*. In the present study, the independent analysis of *EGFR* or *K-ras* mutation status enabled a clonality assessment of multifocal lung adenocarcinomas in 21 (58%) and 15 (42%) of the 36 patients, respectively. However, a clonality assessment was possible in 30 (83%) of the 36

Table 4
Univariate survival analysis of synchronous multifocal lung adenocarcinomas.

Prognostic factors	No.	Survival rates (%)		*P* value[a]
		3-Year	5-Year	
Total	31	82.1	77.3	
Age (years)				
≤65	12	80.2	80.2	0.746
>65	19	83.3	75.8	
Gender				
Male	14	68.4	58.6	0.038
Female	17	94.1	94.1	
Smoking status				
Smoker	18	74.9	66.5	0.158
Nonsmoker	13	92.3	92.3	
Serum CEA level				
Normal	18	79.4	79.4	0.736
Elevated	13	84.6	74.0	
Pathological nodal status				
N0	24	87.5	81.3	0.447
N1 or N2	7	66.7	66.7	
Tumor size				
<30 mm	18	88.2	88.2	0.245
≥30 mm	13	75.2	65.8	
Proportion of BAC component				
<50%	16	65.2	65.2	0.068
≥50%	15	90.0	90.0	
Martini and Melamed's criteria				
MPLC	26	90.4	90.4	<0.001
PM	5	40.0	0.00	
EGFR clonality status				
Same	5	100	100	0.317
Different	16	81.3	81.3	
ND	10	74.1	59.3	
EGFR and *K-ras* clonality status				
Same	6	100	100	0.267
Different	19	77.7	77.7	
vND	6	80.0	40.0	

CEA, carcinoembryonic antigen; BAC, bronchioloalveolar carcinoma; MPLC, multiple primary lung cancer; PM, pulmonary metastasis; *EGFR*, epidermal growth factor receptor; ND: not determined.

[a] Log-rank test.

patients when the results of *EGFR* and *K-ras* mutation analyses were combined.

We showed that both *EGFR* and *K-ras* mutations frequently occurred randomly. Although the numbers of cases were small, several investigators have also reported that *EGFR* and *K-ras* mutations occurred randomly in the same patients with multifocal lung cancers and/or AAHs [18,20,21,26,27]. Multiple primary lung cancers are potentially curable by surgical resection, especially in patients without nodal involvement [28,29]. In this series, no statistical differences in survival were observed between the patients with synchronous multifocal adenocarcinomas exhibiting the same clonality and patients with those exhibiting a different clonality. Therefore, whenever possible, all multifocal adenocarcinomas should be resected in operable patients, regardless of the clonality status.

Recently, in two randomized phase 3 trials, first-line gefitinib monotherapy was shown to improve progression-free survival, compared with standard chemotherapy, in patients with advanced non-small-cell lung cancer harboring *EGFR* mutations [6,7]. EGFR-TKIs could be useful as an alternative treatment for inoperable patients with multifocal adenocarcinomas with activating *EGFR* mutations. In the present series, some type of activating *EGFR* mutation was found in all the multifocal tumors in eight (22%) of the 36 patients. If a surrogate marker for *EGFR* mutations becomes available in the future, these patients may be managed successfully using EGFR-TKIs, since the sampling of all tumors is often impossible practically.

The results of the *EGFR*/*K-ras* clonality assessment were not completely consistent with the differential diagnosis of MPLC or PM according to Martini and Melamed's criteria. In general, differences in genetic alteration patterns are considered to be a good marker for determining tumors of the same (PM) or different origin (MPLC). As shown in the present study, the prognosis of patients with PM according to Martini and Melamed's criteria was worse than that of those with MPLC. However, surprisingly, the clonality status based on *EGFR* and *K-ras* mutations was not prognostic. Although no significant difference was observed, the prognosis of the patients with tumors showing the same clonality was somewhat better than that of those showing a different clonality. All but one patient with synchronous multifocal adenocarcinomas exhibiting the same clonality had *EGFR*-mutated tumors. Therefore, the same *EGFR* mutation might occur simultaneously in a subgroup of multifocal adenocarcinomas through some mechanism other than metastasis. One possibility is the "field effect phenomenon" proposed by Tang et al. [30]. They reported that *EGFR* mutations identical to the tumors were detected in the normal respiratory epithelium in 9 of 21 (43%) patients with *EGFR*-mutated adenocarcinomas but none in patients without mutation in the tumors. A widespread field effect phenomenon caused by some mutagen other than tobacco carcinogen may affect the pathogenesis of multifocal lung adenocarcinoma. Further studies to identify mutagens of *EGFR* are needed to confirm the involvement of the field effect phenomenon in the development of multifocal lung adenocarcinoma.

In summary, *EGFR* and *K-ras* mutations frequently occur randomly in multifocal lung adenocarcinomas. Combined mutation pattern analyses of *EGFR* and *K-ras* may be useful for making decisions regarding treatment strategies for patients with multifocal lung adenocarcinomas. Further well-designed prospective studies with larger numbers of patients are needed to establish guidelines for selecting treatment options, such as surgery or the use of EGFR-TKIs or chemotherapy, based on the *EGFR* and *K-ras* mutation status for patients with multifocal lung adenocarcinomas.

Conflict of interest statement

None declared.

Acknowledgments

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Prognostic Effect of Perioperative Change of Serum Carcinoembryonic Antigen Level: A Useful Tool for Detection of Systemic Recurrence in Rectal Cancer

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Background: The prognosis of patients even with the same stage of rectal cancer varies widely. We analyzed the capability of perioperative change of serum carcinoembryonic antigen (CEA) level for predicting recurrence and survival in rectal cancer patients.

Methods: We reviewed 631 patients who underwent potentially curative resection for stage II or III rectal cancer. Patients were categorized into three groups according to their serum CEA concentrations on the seventh day before and on the seventh day after surgery: group A, normal CEA level (≤5 ng/mL) in both periods; group B, increased preoperative and normal postoperative CEA; and group C, continuously increased CEA in both periods. The prognostic relevance of the CEA group was investigated by analyses of recurrence patterns and survival.

Results: Stage III patients showed higher systemic recurrence ($P = .001$) and worse 5-year survival rates ($P < .0001$) for group C than for groups A and B. On multivariate analysis, the CEA group was a significant predictor for recurrence ($P < .001$; relative risk, 2.740; 95% confidence interval, 1.677–4.476) and survival ($P = .001$; relative risk, 2.174; 95% confidence interval, 1.556–3.308).

Conclusions: The perioperative serum CEA change was a useful prognostic indicator to predict for systemic recurrence and survival in stage III rectal cancer patients.

Key Words: Rectal cancer—Perioperative serum CEA change—Recurrence—Prognosis.

Although the pathologic tumor-node-metastasis stage provides the best prognostic information in rectal cancer patients, the prognosis of patients with the same stage of tumor varies widely, especially in those with stage II and III tumors.[1–5] To identify a subset of patients at high risk for recurrence, several prognostic factors, including molecular and biochemical markers, have been investigated.[6–9] However, the validity of those markers remains controversial, and their clinical application is limited because of their complexity, the difficulties of standardization, and the high cost of measurement.

Carcinoembryonic antigen (CEA) is the most widely used and readily available tumor marker for the management of colorectal cancer. Increased preoperative serum CEA levels are associated with an increased risk of recurrence and poor prognosis,[10–12] and the prognostic effect of serum CEA levels is independent of the tumor-node-metastasis stage.[13–15] However, less work has been performed to evaluate the prognostic significance of early postoperative serum CEA levels after curative resection in combination with preoperative measurements, which reflects the patient status after tumor removal. The purpose of this study was to analyze the capability of perioperative changes in the serum CEA level mea-

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sured in the preoperative and early postoperative period for predicting recurrence and survival in stage II and III rectal cancer patients.

PATIENTS AND METHODS

A total of 715 patients with stage II and III rectal cancer who had undergone potentially curative resection in the Department of Surgery, Yonsei University College of Medicine, from January 1990 to December 1999 were analyzed retrospectively. Rectal cancer was defined as histologically proven adenocarcinoma within 15 cm from the anal verge and was staged according to the 6th edition of the American Joint Committee on Cancer staging system.[16] Excluded from this study were 52 patients who underwent preoperative chemotherapy or radiotherapy, because their preoperative serum CEA levels may have been influenced by preoperative treatment, and 32 patients for whom either the preoperative or postoperative serum CEA data were not available. Thus, 631 patients who underwent curative resection without any preoperative treatment for stage II and III adenocarcinoma of the rectum were included in this study.

Serum CEA levels were measured in the preoperative period and on the seventh postoperative day. All assays were performed in one laboratory by use of the CobasCore immunoassay (Boehringer-Mannheim, Mannheim, Germany) from 1990 to 1994 and, thereafter, by the Elecsys 2010 electrochemiluminescence immunoassay (Roche Diagnostics GmbH, Mannheim, Germany) in which the reference range was ≤5 ng/mL. Patients were categorized into three groups according to their serum CEA concentrations on the preoperative and postoperative seventh day: in group A, the value of the preoperative and postoperative CEA was ≤5 ng/mL; in group B, the value of CEA was >5 ng/mL before surgery and ≤5 ng/mL after surgery; and in group C, both the preoperative and postoperative CEA levels were >5 ng/mL.

Patients were followed up every 3 months for the first 3 years after surgery, every 6 months for the next 2 years, and yearly thereafter. Each visit included a medical history, a physical examination, including a rectal examination, and measurement of the serum CEA concentration. Routine radiological examinations consisting of chest radiography, abdominopelvic computed tomography or ultrasonography, whole-body bone scintigraphy, and colonoscopy or double-contrast barium enema were performed 6 months after surgery and annually thereafter, as well as on suspicion of recurrence. The main patterns of recurrence were recorded as the first site of detectable failure at the time of diagnosis. Determination of recurrence was made by clinical and radiological examinations or by histological confirmation. Recurrences were classified into locoregional (disease within the pelvis), systemic (disease outside the pelvis), or combined. The patients were followed up until death or the cutoff date (December 31, 2003). Overall, 10 patients (1.6%) were lost to follow-up. There were two operative mortalities within 30 days of surgery. The median duration of follow-up at the cutoff date was 74.7 months (range, 10.6–167.8 months).

Data analyses were performed by using SPSS version 10.0 for Windows (SPSS, Inc., Chicago, IL). The intergroup comparisons of clinicopathologic variables were performed by using the analysis of variance test for continuous variables and the two-tailed χ^2 test for discrete variables. The lost cases and operative mortality cases were treated as censored data for the analysis of survival rates. The overall survival rate was estimated and compared according to the Kaplan-Meier method and log-rank test, respectively. Multivariate analyses using logistic regression analysis and Cox's proportional hazard model were used to identify the independent risk factors that influenced recurrence and survival, respectively. A P value $< .05$ was considered statistically significant.

RESULTS

Comparison of Clinicopathologic Features

The clinicopathologic features of the three CEA groups categorized by preoperative and early postoperative serum CEA concentrations are summarized in Table 1. The patient distribution in the groups was 381 patients in group A, 166 in group B, and 84 in group C. There were no significant differences among the groups with regard to age, sex, or location of the tumor, whereas tumor size, perirectal fat invasion, and the number of metastatic lymph nodes showed significant differences. Tumor size and perirectal fat invasion were stratified according to preoperative CEA levels. Tumor size was significantly larger for groups B (5.61 cm) and C (5.63 cm) than for group A (5.04 cm; $P = .001$). Perirectal fat invasion was more common in groups B (94.7%) and C (94.1%) than in group A (90.2%; $P < .001$). However, the mean number of metastatic lymph nodes was stratified

TABLE 1. *Clinicopathologic features in patients with stage II and III rectal cancer according to CEA group*[a]

Clinicopathologic features	Group A (n = 381)	Group B (n= 166)	Group C (n = 84)	*P* value
Age (y)	56.0 ± 12.5	57.6 ± 12.0	57.6 ± 11.8	.281
Sex				.061
Male	219 (57.5)	92 (55.4)	59 (70.2)	
Female	162 (42.5)	74 (44.6)	25 (29.8)	
Location				.910
Upper	74 (19.4)	28 (16.9)	18 (21.4)	
Middle	133 (34.9)	60 (36.1)	27 (32.1)	
Lower	174 (45.7)	78 (47.0)	39 (46.4)	
Histological grade[b]				.046
Low	324 (85.0)	149 (89.8)	79 (94.0)	
High	57 (15.0)	17 (10.2)	5 (6.0)	
Tumor size (cm)	5.04 ± 1.73	5.61 ± 2.01	5.63 ± 1.78	.001
Perirectal fat invasion				< .001
Negative	37 (9.8)	8 (5.3)	5 (5.9)	
Positive	344 (90.2)	158 (94.7)	79 (94.1)	
LN metastasis				.037
Negative	167 (43.8)	76 (45.8)	25 (29.0)	
Positive	214 (56.2)	90 (54.2)	59 (70.2)	
No. of positive LNs	2.46 ± 4.95	2.51 ± 4.70	5.45 ± 8.92	< .001
No. of retrieved LNs	23.77 ± 14.94	24.81 ± 17.57	24.98 ± 16.53	.698
Adjuvant treatment				.592
Yes	325 (85.3)	147 (88.6)	72 (85.7)	
No	56 (14.7)	19 (11.4)	12 (14.3)	
Recurrence				< .001
No	267 (70.1)	108 (65.1)	39 (46.4)	
Yes	114 (29.9)	58 (34.9)	45 (53.6)	

Data are n (%) or mean ± SD.

[a] CEA, carcinoembryonic antigen; LN, lymph node Group A, normal (≤ 5ng/mL) preoperative CEA/normal postoperative CEA; group B, increased preoperative CEA/normal postoperative CEA; group C, increased preoperative CEA/increased postoperative CEA.

[b] Based on 6th edition American Joint Committee on Cancer classification.

according to postoperative CEA levels, which were higher for group C (5.45) than for groups A (2.46) and B (2.51; $P < .001$). There was no significant difference in the number of retrieved lymph nodes and adjuvant treatment among the three groups.

Recurrence Patterns According to CEA Group and Risk Factors for Recurrence

Of 268 stage II and 363 stage III rectal cancer patients, 65 (24.3%) and 152 (41.9%) patients developed recurrence, respectively. According to CEA group, 114 (29.9%) group A, 58 (34.9%) group B, and 45 (53.6%) group C patients developed recurrent disease ($P < .001$; Table 1). For stage III patients, systemic recurrence was significantly higher in group C (47.5%) than in groups A (24.4%) and B (22.2%; $P = .001$), whereas, for stage II patients, no significant difference was observed among the three groups (group A, 13.7%; group B, 13.3%; group C, 28.0%; $P = .077$). In terms of locoregional recurrence, there was no significant difference among the three groups in stage II ($P = .939$) and III ($P = .420$) patients (Figs. 1 and 2). Logistic regression analysis revealed that perirectal fat invasion, lymph node metastasis, and CEA group were correlated independently with postoperative recurrence (Table 2).

Survival Rate According to CEA group and Predictors for Survival

For stage III patients, group C (35.4%) had a lower 5-year survival rate than groups A (64.1%) and B (54.1%; $P < .0001$), whereas there was no significant difference among the three groups for stage II patients ($P = .552$; Figs. 3 and 4). Cox's proportional hazard model analysis showed that age, histological grade, perirectal fat invasion, lymph node metastasis, and CEA group were the independent prognostic factors (Table 3).

DISCUSSION

The major findings of this study were that rectal cancer patients who had increased preoperative serum CEA levels could be divided into two groups according to their early postoperative serum CEA level. The patients who had continuously increased serum CEA levels both in the preoperative and early

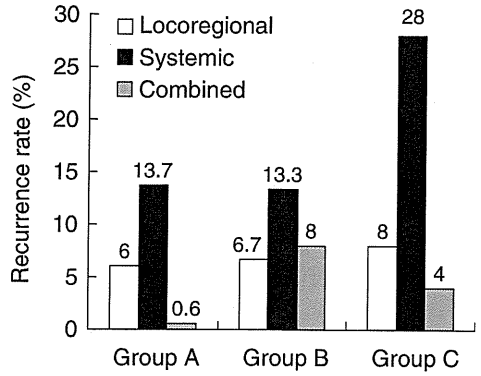

FIG. 1. Recurrence patterns in patients with stage II rectal cancer according to carcinoembryonic antigen group. There were no significant differences in locoregional (P = .939) and systemic (P = .077) recurrence rates among the three groups.

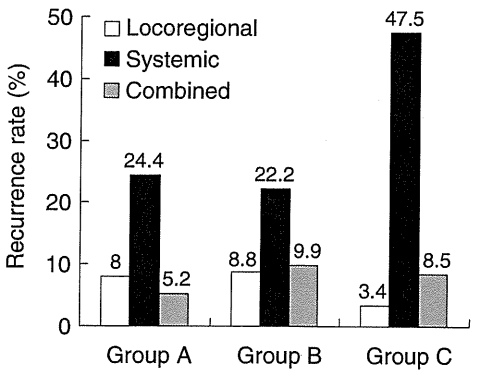

FIG. 2. Recurrence patterns in patients with stage III rectal cancer according to carcinoembryonic antigen group. There were no significant differences in locoregional recurrence rates among the three groups (P = .420). However, the systemic recurrence rate was significantly higher in group C than in groups A and B (P = .001).

TABLE 2. *Logistic regression analysis of risk factors for recurrence*

Covariate	RR	95% CI	P value
Perirectal fat invasion (absence vs. presence)	2.219	1.094–4.502	.027
Lymph node metastasis (absence vs. presence)	2.041	1.430–2.912	< .001
CEA groups[a]			< .001
A	1		
B	1.398	.942–2.074	
C	2.740	1.677–4.476	

RR, relative risk; CI, confidence interval; CEA, carcinoembryonic antigen.

[a] CEA group A, normal (≤ 5ng/mL) preoperative CEA/normal postoperative CEA; B, increased preoperative CEA/normal postoperative CEA; C, increased preoperative CEA/increased postoperative CEA.

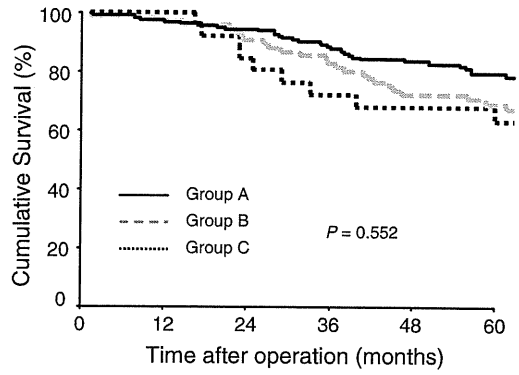

FIG. 3. Survival curves in patients with stage II rectal cancer according to carcinoembryonic antigen group. There were no significant differences in 5-year survival rates among the three groups (group A, 79.1%; group B, 71.0%; group C, 66.0%, P = .552).

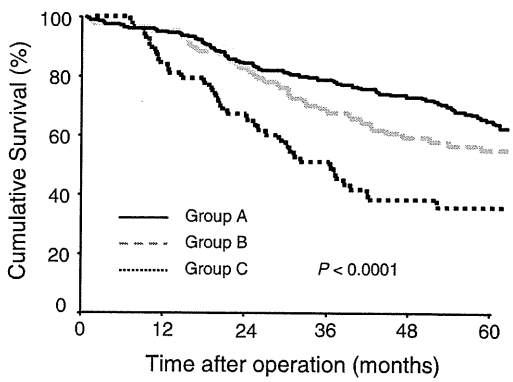

FIG. 4. Survival curves in patients with stage III rectal cancer according to carcinoembryonic antigen group. The 5-year survival rate was significantly lower in group C (35.4%) than in groups A (64.1%) and B (54.1%; P < .0001).

postoperative period showed more frequent systemic recurrence and worse survival rates than those who had increased preoperative but normal early postoperative serum CEA levels in stage III rectal cancer.

The locoregional extent of tumor and the regional lymph node status, assessed pathologically, are the standards for staging and are the most useful criteria to plan treatment, project prognosis, and measure outcomes in colorectal cancer.[1,17] However, some patients with the same stage of cancer would show different prognostic outcomes and form a heterogeneous group, as with stage II and III rectal cancer.[2–5] For this reason, a large number of potential prognostic factors, including molecular and biochemical markers such as p53, K-*ras*, microsatellite instability, and thymidylate synthase, have been proposed to aid the traditional

TABLE 3. *Cox's proportional hazard model analysis of prognostic factors*

Covariate	RR	95% CI	*P* value
Age (<55 vs. ≥55 y)	1.560	1.201–2.188	.001
Histological grade (low vs. high)[a]	1.442	.009–2.060	.044
Perirectal fat invasion (absence vs. presence)	1.779	1.078–2.937	.024
Lymph node metastasis (absence vs. presence)	2.029	1.543–2.667	<.001
CEA group[b]			<.001
A	1		
B	1.315	.982–1.759	
C	2.174	1.556–3.308	

RR, relative risk; CI, confidence interval: CEA, carcinoembryonic antigen.

[a] Based on the 6th edition American Joint Committee on Cancer classification.

[b] CEA, group A, normal (≤ 5ng/mL) preoperative CEA/normal postoperative CEA; B, increased preoperative CEA/normal postoperative CEA; C, increased preoperative CEA/increased postoperative CEA.

staging system.[7–9,18] However, the clinical application of these markers is not widely available because the methods of detection are complicated, expensive, and not automated and because the reference ranges are not consistent among the study groups.

CEA is the most widely accepted and frequently used tumor marker worldwide in colorectal cancer, and the method of measurement is standardized, readily available, and not costly. Most studies on CEA in colorectal cancer have focused on the prognostic effect of preoperative CEA levels[10–15] and on the usefulness of postoperative CEA monitoring for early detection of recurrence after curative surgery and for assessment of response to chemotherapy in metastatic colorectal cancer.[19–22]

Previous studies reported that an increased preoperative CEA level was correlated with a high rate of recurrence and that the degree of elevation was also associated with the outcome of patients with Dukes' B/C colorectal cancer.[10–15] In the College of American Pathologists Consensus Statement in 1999, the prognostic factors in colorectal cancer were categorized according to the strength and reliability of the published evidence in the literature. Preoperative CEA elevation is classified into category I, which includes factors definitely proven to be of prognostic import on the basis of multiple statistically robust published trials and generally used in patient management, together with tumor extent, regional lymph node metastasis, blood or lymphatic vessel invasion, and residual tumor after surgery.[23] However, the prognostic significance of early postoperative CEA, which reflects the response to surgical treatment, has not been investigated extensively.

Although a few studies evaluated the relationship between the perioperative serum CEA change and prognosis in colorectal cancer patients, those involved only a small number of patients or included both colon and rectal cancer patients.[24,25] Moreover, the time of postoperative blood sampling for CEA measurement was not consistent, ranging from 1 week to 4 weeks after surgery,[24,25] a variation that may affect the value of postoperative serum CEA.

In this study, we investigated the prognostic value of the perioperative serum CEA change by assessing preoperative and early postoperative concentrations in patients with stage II and III rectal cancer. This study included only rectal cancer patients and involved a relatively large number of patients. Moreover, all the early postoperative serum CEA concentrations were measured on the postoperative seventh day: a time point that took into account the half-life of CEA[26] and reduced the possible effects of adjuvant treatment on the postoperative serum CEA levels. We analyzed the pattern of recurrence based on the CEA group, which was not evaluated in the previous studies. According to our results, the patients with increased preoperative serum CEA levels could be divided into two groups: one with normal serum CEA levels in the early postoperative period (group B) and the other with continuously increased serum CEA levels (group C). For stage III rectal cancer patients, those who had normal postoperative serum CEA levels showed a prognosis similar to that of patients with normal preoperative serum CEA levels, whereas patients with increased preoperative and early postoperative serum CEA levels had more frequent systemic failure and worse survival rates. Although the statistical difference was not significant ($P = .077$), the patients with stage II disease showed a tendency for systemic failure similar to that of stage III patients. Patients with increased preoperative serum CEA that failed to normalize in the early postoperative period showed frequent systemic recurrence compared with those who had normal preoperative or normal early postoperative serum CEA. The marginal statistical difference might come from the small number of recurrences observed in stage II patients, which could be insufficient to differentiate the high-risk group from the low-risk group for recurrence and death.

In summary, perioperative serum CEA change may be a useful tool for prediction of systemic failure after curative resection in stage III rectal cancer. Our findings suggest that a more accurate prediction of prog-

nosis and systemic recurrence can be obtained from early postoperative serum CEA levels, which reflect the status of curative resection, together with preoperative serum CEA values. During postoperative follow-up, careful attention should be given to stage II and III patients with a high probability of systemic failure based on their perioperative CEA levels. In addition, perioperative serum CEA change can be an aid to sort patients into a more homogeneous group for the application of new treatment strategies.

In conclusion, perioperative serum CEA changes in the preoperative and early postoperative period are predictive of systemic recurrence and prognosis in stage III rectal cancer patients. Early postoperative serum CEA combined with the preoperative value could serve as a useful prognostic indicator.

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Risk factors for morbidity after pulmonary resection for lung cancer in younger and elderly patients

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Abstract

The aim of this study was to evaluate the perioperative morbidity, mortality, and risk factors for morbidity after lung cancer resection in younger and elderly patients. This study retrospectively reviewed 1073 patients with non-small cell lung cancers (NSCLC) who underwent pulmonary resection. The risk factors for morbidity were analyzed independently in groups of 664 younger (<70 years) patients and 409 elderly (≥70 years) patients. Co-morbidities, such as hypertension, ischemic heart disease, and renal insufficiency were more frequently observed in the elderly group in comparison to the younger group. However, there were no statistical differences in the rates of overall morbidity and 30-day mortality between the younger and elderly groups (36% vs. 42% and 0.3% vs. 0.5%, respectively). Multivariate analyses revealed the risk factors for morbidity to be % forced expiratory volume in 1 s (FEV_1), the extent of pulmonary resection and tumor histology in the younger group, and smoking, hypertension, renal insufficiency and % diffusing capacity of the lung to carbon monoxide (DLCO) in the elderly group, respectively. In conclusion, the rate of morbidity and mortality in elderly patients were similar to those observed in younger patients. However, perioperative management should be cautiously performed while taking into account the risk factors for morbidity especially in elderly patients because they frequently have various co-morbidities.

Keywords: Lung cancer; Elderly patients; Risk factors

1. Introduction

Non-small cell lung cancer (NSCLC) is the leading cause of cancer-related death in Western countries as well as in Japan. The incidence of NSCLC diagnosed in the elderly population is rising due to the increasing life expectancy. The data of the cancer registry of Japan in 2003 showed that as many as 63.2% of lung cancers were diagnosed in patients older than 70 years of age and 24.6% in those older than 80 years of age [1]. Although surgery offers the best chance of cure for early-stage NSCLC, surgical intervention in the elderly patients has been performed with a great deal of hesitation because of the high incidence of surgical mortality and morbidity [2, 3]. The naturally shortened life expectancy and possible sequelae leading to an impaired quality of life are also additional reasons for hesitation. However, recent advances in anesthetic management, surgical techniques and perioperative management now allow for the surgical resection of NSCLC in elderly patients. In fact, many investigators reported that surgical intervention for NSCLC in the elderly is justified in terms of morbidity, mortality and residual quality of life [4–8].

Aging is associated with a significant prevalence of co-morbidities [3]. Moreover, decreased reserves in various vital organs are observed in the elderly in comparison to younger patients. Therefore, perioperative complications occurring in elderly patients, even if they are not severe, sometimes result in fatal conditions. More cautious perioperative management should therefore be conducted in elderly patients with the above distinctive characteristics in the elderly in mind. However, there have been few reports analyzing the risk factors for morbidity associated with NSCLC resection independently in younger and elderly patients [5]. The aim of this study was to evaluate the perioperative morbidity, mortality, and risk factors for morbidity after lung cancer resection in younger and elderly patients.

2. Patients and methods

This study retrospectively reviewed 1073 patients with NSCLCs who underwent pulmonary resection between September 1996 and October 2009 at our institute. Our surgical policy in lung cancer patients without severe co-morbidities favored lobectomy with mediastinal lymph node dissection (MLD) over limited resections, and pneumonectomy is avoided, whenever possible. Induction chemotherapy and/or radiotherapy were not routinely performed if complete resection was possible based on the radiological findings. No patient in this series received induction therapy. There were 677 males and 396 females, ranging in age from 23

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to 86 years with a median age of 65 years. Fifty-two (4.8%) patients were octogenarians.

Routine preoperative assessments included medical history, physical examination, basic blood tests, electrocardiogram (ECG) with exercise stress test, pulmonary function test and blood gas examination. All patients with cardiovascular disease, suspicious symptoms, or ECG abnormalities in an exercise stress test consulted a cardiologist and had additional tests, such as echocardiography and coronary angiography were performed. The diabetic status was strictly controlled under the support by diabetes specialists during the perioperative period.

Clinical staging was based on a computed tomography (CT) of the chest and upper abdomen, brain CT or magnetic resonance imaging, radionuclide bone scan, and/or positron emission tomography with fluorine-18 fluorodeoxyglucose. The mediastinal and hilar lymph node status was defined as positive if the chest CT showed that the shorter axis of any node was larger than 1 cm. Mediastinoscopy and endobronchial ultrasound were not routinely performed.

Morbidity was defined as any postoperative events, such as bacterial pneumonia, interstitial pneumonia, arrhythmia, prolonged air leak requiring >7 days of postoperative chest tube drainage, delirium/confusion, atelectasis, bleeding, chylothorax, empyema, bronchopleural fistula, or respiratory failure.

The following risk factors for morbidity were evaluated independently in groups of 664 younger (<70 years) patients and 409 elderly (≥70 years) patients: gender, body mass index, smoking status (pack-year), the presence of preoperative co-morbidities [diabetes mellitus, arrhythmia, hypertension, ischemic heart disease (IHD), and liver dysfunction], serum creatinine (Cr) level, forced expiratory volume in 1 s (FEV_1)%, %vital capacity (VC), %diffusing capacity of the lung to carbon monoxide (DLCO), past history of cancers, the extent of pulmonary resection and MLD, clinical and pathological stage, and histological cell type.

Univariate analyses were performed by the χ^2-test. All of the variables that were found to be significant in the univariate analyses were entered into the multivariate analyses using a forward step-wise logistic regression model (0.05 for entry and 0.10 for removal probability). A P-value of <0.05 was considered to be statistically significant. All statistical analyses were performed using the SPSS statistical software package (Version 17.0, SPSS Inc, Chicago, IL, USA).

3. Results

Several clinical and pathological characteristics of patients were significantly different between the elderly and younger group (Table 1). Co-morbidities, such as hypertension ($P<0.001$), IHD ($P=0.002$), and renal insufficiency ($P=0.001$) were more frequently observed in the elderly group in comparison to the younger group. Patients with lower FEV_1%, %VC and %DLCO were more common in the elderly group than in the younger group ($P<0.001$, $P=0.005$, and $P<0.001$, respectively). The frequency of previous cancers other than lung cancer were higher in the elderly group than in the younger group ($P=0.001$). Standard pulmonary resection (lobectomy or pneumonectomy, $P=0.005$) and MLD ($P<0.001$) were more frequently performed in the younger group in comparison to the elderly group. The frequency of squamous cell carcinoma in the elderly patients was higher than in the younger patients ($P=0.006$).

At least one morbidity occurred in 241 (36%) of 664 younger patients and 171 (42%) of 409 elderly patients, respectively ($P=0.071$). The frequency of arrhythmia (8% vs. 14%, $P<0.001$), prolonged air leak (6% vs. 11%, $P=0.005$), and delirium/confusion (0.8% vs. 6%, $P<0.001$) after surgery were higher in the elderly group in comparison with younger group. Two patients (0.3%) in the younger group and two patients (0.5%) in the elderly group died within 30 days after surgery ($P=0.624$). The causes of death were aspiration pneumonia and acute exacerbation of interstitial pneumonia in the younger group, and cerebral infarction and hemoptysis in the elderly group. The postoperative events were summarized in Table 2.

Univariate analyses showed that gender, smoking status, the presence of hypertension, FEV_1%, %VC, the extent of pulmonary resection and MLD, clinical stage, and histological cell type were significantly associated with morbidity in the younger group. Gender, smoking status, the presence of diabetes mellitus and hypertension, serum Cr level, FEV_1%, %DLCO, and histological cell type were all found to be significant risk factors for morbidity in the elderly group (Table 3).

A multivariate analysis showed that FEV_1%, the extent of pulmonary resection and histological cell type remained significant risk factors for morbidity in the younger group (Table 4). Smoking status, the presence of hypertension, serum Cr level and %DLCO were significant in the elderly group (Table 5).

Subgroup analyses based on the number of significant risk factors identified by the multivariate analyses were performed independently in the younger and elderly groups. The risk grade was defined as follows: low-risk: patients without any risk factors; moderate risk: patients with 1 or 2 risk factors in the younger group and patients with 1–3 risk factors in the elderly group; high-risk: patients with all risk factors. The rates of morbidity in the younger group were 7.7% (4/52) in the low-risk, 36.4% (211/579) in the moderate risk, 78.8% (26/33) in the high-risk subgroup, respectively. The rates of morbidity in the elderly group were 2.0% (1/48) in the low-risk, 46.5% (165/355) in the moderate risk, 83.3% (5/6) in the high-risk subgroup, respectively.

4. Discussion

The present study retrospectively evaluated the risk factors for morbidity after lung cancer resection independently in younger (<70 years) and elderly (≥70 years) patients. Although the definition of an elderly patient is arbitrary, a cut-off of 70 years of age was selected because the incidence of age-related physiological changes begins to increase after 70 years [9]. In addition, the incidence of overall co-morbidities in the elderly group was significantly higher than that in the younger group. Among them, hypertension, IHD, renal insufficiency, and low preoperative

Table 1. Patient characteristics

Variable	Subset	<70 years (n=664)	≥70 years (n=409)	P-value
Gender	Male	418 (63%)	259 (63%)	0.90
	Female	246 (37%)	150 (37%)	
Body mass index	<25	500 (75%)	295 (72%)	0.34
	≥25	127 (19%)	87 (21%)	
Smoking (pack-year)	≤40	391 (59%)	224 (55%)	0.23
	>40	224 (34%)	151 (37%)	
At least one co-morbidity	(−)	200 (30%)	78 (19%)	<0.001
	(+)	464 (70%)	331 (81%)	
Diabetes mellitus	(−)	582 (88%)	355 (87%)	0.68
	(+)	82 (12%)	54 (13%)	
Arrhythmia	(−)	654 (98%)	396 (97%)	0.066
	(+)	10 (2%)	13 (3%)	
Hypertension	(−)	512 (77%)	265 (65%)	<0.001
	(+)	152 (23%)	144 (35%)	
IHD	(−)	633 (95%)	370 (90%)	0.002
	(+)	31 (5%)	39 (10%)	
Liver disease	(−)	636 (96%)	396 (97%)	0.40
	(+)	28 (4%)	13 (3%)	
Cr (mg/dl)	≤1.0	525 (79%)	298 (73%)	0.001
	>1.0	36 (5%)	43 (11%)	
FEV_1%	<70%	117 (18%)	137 (33%)	<0.001
	≥70%	523 (78%)	255 (62%)	
%VC	<80%	60 (9%)	59 (14%)	0.005
	≥80%	579 (87%)	332 (81%)	
%DLCO	<60%	177 (27%)	205 (50%)	<0.001
	≥60%	415 (63%)	151 (40%)	
Past history of lung cancer	(−)	642 (97%)	395 (97%)	0.92
	(+)	22 (3%)	14 (3%)	
Past history of other cancers	(−)	595 (90%)	338 (83%)	0.001
	(+)	69 (10%)	71 (17%)	
Mode of surgery	Pneumonectomy	26 (4%)	4 (1%)	0.005
	Lobectomy	563 (85%)	329 (80%)	0.065
	Segmentectomy	31 (5%)	35 (9%)	0.010
	Wedge resection	44 (7%)	41 (10%)	0.045
MLD	(−)	95 (14%)	117 (29%)	<0.001
	(+)	569 (86%)	292 (71%)	
Clinical stage	I	437 (66%)	280 (68%)	0.075
	II, III, IV	137 (21%)	65 (16%)	
Pathological stage	I	419 (63%)	267 (65%)	0.47
	II, III, IV	245 (37%)	142 (35%)	
Histology	Adenocarcinoma	504 (76%)	274 (67%)	0.006
	Squamous cell carcinoma	119 (18%)	103 (25%)	
	Others	41 (6%)	32 (8%)	

IHD, ischemic heart disease; Cr, serum creatinine level; FEV_1, forced expiratory volume in 1 s; VC, vital capacity; DLCO, diffusing capacity of the lung to carbon monoxide; MLD, mediastinal lymph node dissection.

pulmonary functions (lower FEV_1%, %VC and %DLCO) were more frequently observed in the elderly group in comparison to the younger group. However, there were no significant differences in terms of the rates of 30-day mortality and overall morbidities between these two groups. Kilic et al. reported segmentectomy to be associated with a decreased morbidity and mortality in elderly patients with early-stage NSCLC in comparison to lobectomy [10]. MLD was shown to be an independent risk factor for postoperative complications in octogenarians with clinical stage I NSCLC based on a review of nationwide data collected by the Japanese Joint Committee of Lung Cancer Registry [11]. Despite the higher morbidity rates of standard operative procedures, long-term survival is comparable between lobectomy and limited resection [12] and between MLD and non-MLD [13] in elderly patients with NSCLC. Limited pulmonary resection (wedge resection or segmentectomy) and omission of MLD were more frequently performed for the elderly group than the younger group in the present series. Appropriate decision-making for the surgical procedure might be one of the reasons for the equal safety and feasibility of surgical intervention in the elderly group in comparison to the younger group.

Although the prevalence of both clinical and pathological early stage disease was not significantly different between

Table 2. Postoperative events

Event	All (n=1073)	<70 years (n=664)	≥70 years (n=409)	P-value
Thirty-day mortality	4 (0.4%)	2 (0.3%)	2 (0.5%)	0.62
Overall morbidities	412 (38%)	241 (36%)	171 (42%)	0.071
Bacterial pneumonia	11 (1%)	5 (0.8%)	6 (1%)	0.26
Interstitial pneumonia	3 (0.3%)	2 (0.3%)	1 (0.2%)	0.86
Arrhythmia	110 (10%)	51 (8%)	59 (14%)	<0.001
Prolonged air leak	84 (8%)	40 (6%)	44 (11%)	0.005
Delirium/confusion	29 (3%)	5 (0.8%)	24 (6%)	<0.001

Table 3. Univariate analysis of the risk factors for morbidity

Variable	Younger patients (<70 years)		Elderly patients (≥70 years)	
	Odds ratio (95% CI)	*P*-value	Odds ratio (95% CI)	*P*-value
Gender		<0.001		<0.001
Female	1.00 (reference)		1.00 (reference)	
Male	1.96 (1.39–2.77)		2.21 (1.44–3.38)	
Body mass index		0.97		0.53
<25	1.00 (reference)		1.00 (reference)	
≥25	1.01 (0.67–1.51)		0.85 (0.53–1.39)	
Smoking (pack-year)		<0.001		<0.001
≤40	1.00 (reference)		1.00 (reference)	
>40	1.89 (1.35–2.65)		2.18 (1.43–3.33)	
Diabetes mellitus		0.076		0.028
(−)	1.00 (reference)		1.00 (reference)	
(+)	1.52 (0.96–2.43)		1.90 (1.07–3.38)	
Arrhythmia		0.81		0.14
(−)	1.00 (reference)		1.00 (reference)	
(+)	1.17 (0.33–4.20)		2.29 (0.74–7.12)	
Hypertension		0.014		0.002
(−)	1.00 (reference)		1.00 (reference)	
(+)	1.59 (1.10–2.29)		1.91 (1.27–2.89)	
IHD		0.29		0.56
(−)	1.00 (reference)		1.00 (reference)	
(+)	1.47 (0.71–3.04)		1.22 (0.63–2.36)	
Liver dysfunction		0.95		0.38
(−)	1.00 (reference)		1.00 (reference)	
(+)	0.97 (0.44–2.15)		1.65 (0.55–5.00)	
Cr (mg/dl)		0.27		0.004
≤1.0	1.00 (reference)		1.00 (reference)	
>1.0	1.47 (0.74–2.93)		2.61 (1.35–5.05)	
FEV_1%		<0.001		<0.001
≥70%	1.00 (reference)		1.00 (reference)	
<70%	2.46 (1.64–3.70)		2.05 (1.34–3.12)	
%VC		0.38		0.61
≥80%	1.00 (reference)		1.00 (reference)	
<80%	0.77 (0.44–1.37)		1.16 (0.66–2.02)	
%DLCO		0.022		0.003
≥60%	1.00 (reference)		1.00 (reference)	
<60%	1.52 (1.06–2.17)		1.94 (1.26–3.00)	
Past history of lung cancer		0.083		0.64
(−)	1.00 (reference)		1.00 (reference)	
(+)	0.38 (0.13–1.14)		0.77 (0.52–2.33)	
Past history of other cancers		0.11		0.25
(−)	1.00 (reference)		1.00 (reference)	
(+)	0.64 (0.37–1.11)		1.35 (0.81–2.25)	
Extent of pulmonary resection		<0.001		0.47
Less than lobectomy	1.00 (reference)		1.00 (reference)	
Lobectomy or more	4.77 (2.33–9.75)		1.21 (0.72–2.01)	
MLD		<0.001		0.19
(−)	1.00 (reference)		1.00 (reference)	
(+)	3.51 (1.98–6.25)		1.34 (0.86–2.09)	
Clinical stage		0.006		0.083
II–IV	1.00 (reference)		1.00 (reference)	
I	0.58 (0.39–0.86)		0.62 (0.36–1.07)	
Histology		<0.001		0.022
Non-sq	1.00 (reference)		1.00 (reference)	
Sq	2.05 (1.37–3.05)		1.69 (1.08–2.65)	

IHD, ischemic heart disease; Cr, serum creatinine level; FEV_1, forced expiratory volume in 1 s; VC, vital capacity; DLCO, diffusing capacity of the lung to carbon monoxide; MLD, mediastinal lymph node dissection; Sq, squamous cell carcinoma; CI, confidence interval.

the younger and elderly group, squamous cell carcinoma histology was more frequent in the elderly group than the younger group. The reasons for the difference in the histological tumor type according to the chronological age remain unclear. However, several researchers previously reported the same findings [8, 12]. Interestingly, a squamous cell carcinoma histology was a significant risk factor for morbidity according to the univariate analysis both in the younger and elderly group, and in the younger group, it was also significant in the multivariate analysis.

Several studies have found that risk factors for morbidity after pulmonary resection in elderly patients are increased age [14], co-morbidities [4], the extent of pulmonary resection (pneumonectomy) [15], surgical approach (thoracotomy) [14], and neoadjuvant therapy [5]. The current multivariate analysis found that heavy smoking history, the pre-

Table 4. Multivariate analysis of the risk factors for morbidity in younger patients (<70 years)

Variable	Odds ratio	95% CI	*P*-value
FEV_1%			<0.001
≥70%	1.00 (reference)		
<70%	2.34	1.46–3.76	
Extent of pulmonary resection			<0.001
Less than lobectomy	1.00 (reference)		
Lobectomy or more	6.29	2.59–15.3	
Histology			0.009
Non-sq	1.00 (reference)		
Sq	1.88	1.17–3.01	

FEV_1, forced expiratory volume in 1 s; Sq, squamous cell carcinoma.

Table 5. Multivariate analysis of the risk factors for morbidity in elderly patients (≥70 years)

Variable	Odds ratio	95% CI	*P*-value
Smoking (pack-year)			<0.001
≤40	1.00 (reference)		
>40	2.78	1.66–4.65	
Hypertension			0.005
(−)	1.00 (reference)		
(+)	2.09	1.24–3.52	
Cr (mg/dl)			0.008
≤1.0	1.00 (reference)		
>1.0	3.06	1.35–6.93	
%DLCO			0.001
≥60%	1.00 (reference)		
<60%	2.46	1.47–4.11	

Cr, serum creatinine level; DLCO, diffusing capacity of the lung to carbon monoxide.

sence of hypertension, renal insufficiency and low %DLCO were significant risk factors for the elderly patients. The risk grade for morbidity based on the number of risk factors clearly stratified the patients according to the incidence of morbidity. The risk grade could be used to assist physicians and patients make decisions regarding surgery. Although chronological age is no longer a firm limitation to surgery with regard to postoperative morbidity and mortality, postoperative morbidity is well known to have a significant impact on the patients' quality of life. Therefore, more cautious patient selection and more careful perioperative management based on the age-appropriate risk grading should be performed to make surgery an optimal intervention for NSCLC patients.

The main limitation of the present study is its retrospective nature. Although our surgical policy in lung cancer patients without severe co-morbidities is lobectomy with MLD, regardless of chronological age, the final decision to perform surgical procedures may be biased based on the judgment of each primary surgeon or the wishes of the patients or their family. Although the risk factors identified in this study do not necessarily imply the true risk factors in elderly patients, we believe that such risk factors may possibly even be good candidates for use as true risk factors in these patients.

In summary, the rate of perioperative morbidity and mortality after NSCLC resection in elderly patients were similar to those in younger patients. However, perioperative management should be cautiously performed while taking into account the risk factors for morbidity, especially in elderly patients because they frequently have various co-morbidities.

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The usefulness of mutation-specific antibodies in detecting epidermal growth factor receptor mutations and in predicting response to tyrosine kinase inhibitor therapy in lung adenocarcinoma

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ABSTRACT

Introduction: Among the mutations of epidermal growth factor receptor (*EGFR*), deletions in exon 19 (DEL), and point mutations in exon 21 (L858R) predict the response to EGFR-tyrosine kinase inhibitors (TKIs) in primary lung adenocarcinoma. The ability to detecting such mutations using immunohistochemistry (IHC) would be advantageous.
Methods: The molecular-based and IHC-based *EGFR* mutations were analyzed in 577 lung adenocarcinomas using high resolution melting analysis (HRMA) and 2 mutation-specific antibodies, respectively.
Results: In the molecular-based analyses, DEL was detected in 135 cases (23%), and L858R was detected in 172 cases (30%). In the IHC-based analyses, a positive reaction was detected in 59 cases (10%) for the DEL-specific antibody, and in 139 cases (24%) for the L858R-specific antibody. With the molecular-based results set as the gold standard, the sensitivity and specificity of the DEL-specific antibody were 42.2% and 99.5%, respectively, while the sensitivity and specificity of the L858R-specific antibody were 75.6% and 97.8%, respectively. The antibody specificities improved when the threshold for the mutation-positive reactions was set as >50% of immunopositive tumor cells. The significant predictors of the clinical response to EGFR-TKI were molecular-based *EGFR* mutations ($p < 0.001$) and IHC-based *EGFR* mutations ($p = 0.001$). However, a multivariate analysis revealed that only molecular-based *EGFR* mutations were significantly correlated with the clinical response ($p < 0.001$).
Conclusions: Mutation-specific antibodies demonstrated extremely high specificities, but their sensitivities were not higher than those of molecular-based analyses. However, IHC should be performed before a molecular-based analysis, because it is more cost-effective and can effectively select candidates for EGFR-TKI therapy.

1. Introduction

Many human receptor tyrosine kinases mediate signals that promote the proliferation and survival of cancer cells. Activation of tyrosine kinases appears to be the causal event in many human malignancies [1]. The importance of this finding is reflected in the development of new anticancer drugs that specifically target these activated proteins. The clinical success of tyrosine kinase inhibitors (TKIs), such as imatinib for the treatment of chronic myeloid leukemia and gastrointestinal stromal tumors, has prompted intensive efforts to identify and target additional oncogene kinases as a broad therapeutic strategy for selected patient populations [2,3].

A subset of non-small cell lung cancer (NSCLC), particularly adenocarcinomas, has activating mutations in the *epidermal growth factor receptor* (*EGFR*) gene [4,5]. The most prevalent *EGFR* mutations are deletions in exon 19 (DEL) and a point mutation at codon 858 in exon 21 (L858R); together, these account for more than 90% of all *EGFR* mutations. These 2 types of *EGFR* mutations cause sustained activation of *EGFR*, followed by the selective activation of Akt and signal transduction, and the activation of

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transcription signaling pathways: altogether, these promote cell survival [4,6].

EGFR-TKIs are competitive inhibitors of the adenosine triphosphate-binding clefts within the tyrosine kinase domain of *EGFR* [7]; they effectively inhibit the critical antiapoptotic signals transduced by the mutant receptors [6]. The clinicopathologic parameters of female gender, East Asian ethnicity, adenocarcinoma histology, and nonsmoking status are strong predictors of the response to EGFR-TKIs [4,5,8,9]. Moreover, the DEL and L858R mutations were also revealed to be strong predictors [10–14]. Therefore, the detection of such mutations provides both patients and physicians with important information regarding the optimal choice for therapy.

Direct sequencing is the gold standard method to detect *EGFR* mutations. However, to obtain precise data, high-quality DNA extracted from an adequate amount of pure tumor cells is required, and this is expensive and time-consuming. Recently, other indirect methods were developed to detect *EGFR* mutations, including Scorpion ARMS, the peptide nucleic acid-locked nucleic acid PCR clamp, mutant-enriched PCR, the smart amplification process, and high-resolution melting analysis (HRMA) [15,16]. These methods have high sensitivities, and can be applied to specimens in which cancer cell content is low. However, they invariably require technical labor and sophisticated instruments, and are therefore, not applied in most pathology laboratories.

Compared to molecular techniques, immunohistochemistry (IHC) is a fast and cost-effective method that can be performed in most pathology laboratories on not only fresh, but also archival, formalin-fixed tissue samples. Recently, some authors revealed the correlation between EGFR mutations and EGFR phosphorylation detected by IHC [17,18]. Additionally, EGFR phosphorylation antibodies exhibited a correlation with response to EGFR-TKIs [18]. However, these antibodies recognize EGFR phosphorylation regardless of mutational status. More recently, highly sensitive and specific rabbit monoclonal antibodies against the 2 most common mutations were developed for detecting *EGFR* mutations [19–24].

The main purpose of the present study was to explore the use of the 2 mutation-specific antibodies for DEL and L858R for detecting *EGFR* mutations. Additionally, we compared the molecular-based and the IHC-based *EGFR* mutational status to the response to EGFR-TKI.

2. Materials and methods

2.1. Case selection

After obtaining institutional review board approval, the specimens used in the present study were obtained from 577 Japanese patients who underwent a surgical resection for primary lung adenocarcinoma at the National Cancer Center Hospital, Tokyo, Japan, between 1993 and 2009. Histological diagnosis was based on the latest World Health Organization classification of lung tumors [25].

2.2. Analysis of EGFR mutational status by molecular technique

The materials analyzed for the molecular-based mutational status were as follows: fresh frozen (in liquid nitrogen), surgically resected tissue specimens from 505 patients (88%); methanol-fixed, paraffin-embedded, surgically resected tissue specimens from 36 patients (6%); and ethanol-fixed, imprint cytologic smears obtained from the fresh-cut surface of resected tumor specimens from 36 patients (6%). We used HRMA for detecting the DEL and L858R mutations, routinely performed at our institution. HRMA is well validated, and has been previously shown to accurately reflect *EGFR* mutational status [15].

2.3. Tissue microarray construction

The representative tumor regions to be sampled for the tissue microarray (TMA), were carefully selected and marked on a hematoxylin-eosin-stained slide. The TMAs were assembled using a manual tissue-arraying instrument (Azumaya, Tokyo, Japan). Considering tumor heterogeneity, 2 replicate 2-mm cores were routinely sampled from different regions of each tumor.

2.4. Immunohistochemistry

For the immunohistochemical staining, the 4-μm-thick TMA sections were deparaffinized. A heat-induced epitope retrieval with Target Retrieval Solution (Dako, Carpinteria, CA, USA) was performed. The primary antibody used were a rabbit monoclonal antibody against human *EGFR* with the DEL (E746-A750del) mutation (1:100, clone 6B6, Cell Signaling Technology, Danvers, MA, USA) and a rabbit monoclonal antibody against human *EGFR* with the L858R mutation (1:200, clone 43B2, Cell Signaling Technology). The antibodies were diluted in SignalStain (Cell Signaling Technology), and slides were incubated with each primary antibody for 1 h, at room temperature. The immunoreactions were detected using the EnVision Plus system (Dako) and 3,3′-diaminobenzidine, followed by counterstaining with hematoxylin. We used positive and negative controls for the IHC that previously confirmed the mutational status by using molecular analyses.

2.5. Immunohistochemical scoring system for mutation-specific antibodies

The immunoreactivity for each mutation-specific antibody was evaluated by using light microscopy at magnifications of 4 and 10× with objective lenses. Immunoreactivity was classified on the basis of cytoplasmic intensity. The following scoring system was used: negative intensity, 0 (defined as no immunoreactivity with any intensity); weak intensity, 1 (defined as the immunoreactivity only observed in 10× objective lenses); moderate intensity, 2 (defined as the immunoreactivity easily detected in 4× objective lenses, but less intense than the positive control); and strong intensity, 3 (defined as immunoreactivity equal to or stronger than the positive control; Fig. 1A and B). We also evaluated the extent of each intensity as a percentage (0–100%). Next, an expression score was obtained by multiplying the intensity by the percentage values (range, 0–300) for each core. Finally, the staining scores obtained in 2 cores were averaged, and the result was used as the representative score for each case. In the case of loss of tumor cells in 1 of the 2 cores during IHC, the staining score for the other core was used. We set the threshold at a staining score of 10; therefore, a staining score <10 was categorized as negative and a score ≥10 was categorized as positive. Additionally, we set another threshold for positive cases, defined as >50% of immunopositive tumor cells with any intensity.

2.6. Evaluation of the response to EGFR-TKI

Of the 577 patients, 116 received systemic therapy with EGFR-TKI gefitinib (250 mg daily) after tumor relapse. The therapeutic effect of gefitinib was complete response (CR) in 3, partial response (PR) in 61, stable disease (SD) in 13, and progressive disease (PD) in 37. Two patients were not evaluable for the clinical response due to the withdrawal of gefitinib caused by drug-induced liver dysfunction. The clinical response to gefitinib was determined using standard bidimensional measurements [26]. Responders were defined as patients with CR or PR, and non-responders were defined as patients with SD or PD.

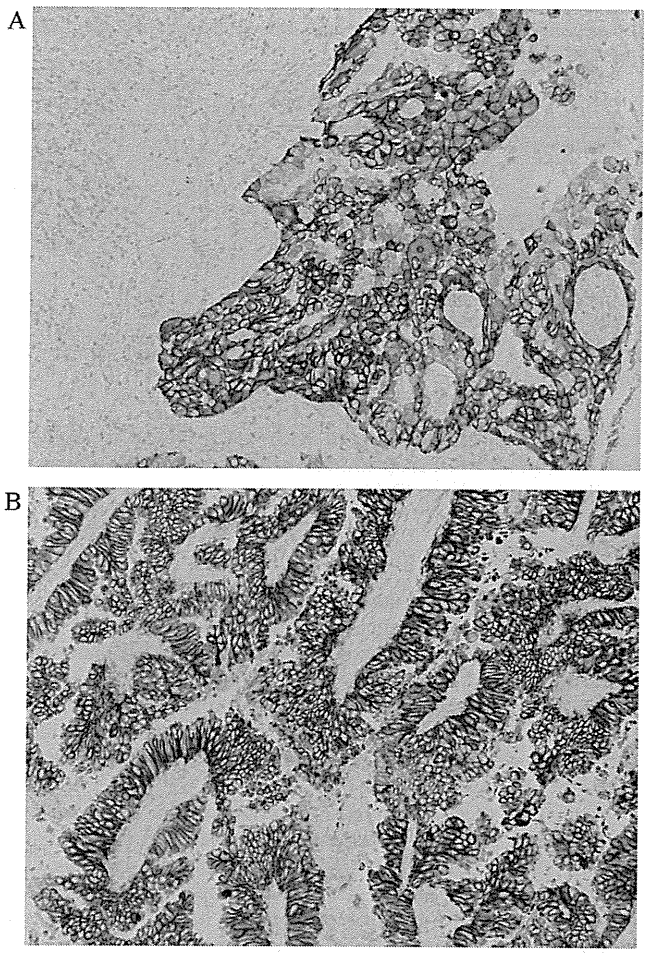

Fig. 1. A representative immunohistochemistry staining of intensity 3 for the DEL-specific antibody (1A, top) and the L858R-specific antibody (1B, bottom). The case 1A/1B harbored the molecular based DEL/L858R status.

2.7. Statistical analyses

Statistical analyses were performed using SPSS 12.0 for Windows (SPSS, Chicago, IL, USA). Chi-square tests for categorical variables were used and $p < 0.05$ was regarded as statistically significant.

3. Results

3.1. Clinicopathologic parameters

There were 319 males and 258 females with median age at surgery being 60 years (range, 30–82). A total of 343 patients had never/light smoking status with Brinkman index of <400, and 234 patients had smoking status with Brinkman index of ≥400. The pathological tumor stage (p-stage) was I in 331, II in 74, III in 164, and IV in 8 cases.

3.2. Molecular-based EGFR mutational status

After analyzing the *EGFR* mutational status by HRMA, DEL (m-DEL) was detected in 135 cases (23%), and L858R (m-L858R) was detected in 172 cases (30%). The remaining 270 cases (47%) were regarded as wild-type (m-WT), because neither the DEL nor the L858R mutation was detected.

Table 1A
Usefulness of DEL-specific antibody in detecting *EGFR* mutation of DEL under the threshold for the mutation-positive defined as staining score ≥10 and >50% of immunopositive tumor cells.

IHC-based *EGFR* mutation of DEL	Molecular-based *EGFR* mutation of DEL	
Staining score ≥10	(+)	(−)
(+)	57	2
(−)	78	440
Sensitivity = 42.2%; specificity = 99.5%		
>50% of immunopositive tumor cells		
	(+)	(−)
(+)	28	0
(−)	107	442
Sensitivity = 20.7%; specificity = 100.0%		

EGFR, epidermal growth factor receptor; DEL, deletions in exon 19; IHC, immunohistochemistry.

3.3. IHC-based EGFR mutational status

Although the tumor tissues of 52 of the 2308 cores (2.3%) were lost during the IHC procedure, at least 1 of the 2 cores contained tumor tissue in all cases. A positive immunoreactivity for the DEL-specific antibody was observed in 59 cases (10%). A positive immunoreactivity for the L858R-specific antibody was observed in 139 cases (24%). The remaining 379 cases were regarded as negative because neither the DEL- nor the L858R-specific antibody was positive. The immunohistochemical expression using DEL- and L858R-specific antibodies was mutually exclusive.

3.4. Correlation between the molecular-based and the IHC-based EGFR mutational status

We compared the molecular-based and IHC-based mutational status using molecular-based mutational status as the gold standard. The 59 cases that were positive for the DEL-specific antibody consisted of 57 cases with m-DEL, and 2 cases with m-WT. The sensitivity and specificity for the DEL-specific antibody was 42.2% and 99.5%, respectively (Table 1A). The 139 cases that were positive for the L858R-specific antibody consisted of 130 cases with m-L858R, and 9 cases with m-WT. The sensitivity and specificity for the L858R-specific antibody was 75.6% and 97.8%, respectively (Table 1B). Combining the results using these 2 antibodies, the overall sensitivity and specificity were 60.9% and 98.7%, respectively.

Table 1B
Usefulness of L858R-specific antibody in detecting *EGFR* mutation of L858R under the threshold for the mutation-positive defined as staining score ≥10 and >50% of immunopositive tumor cells.

IHC-based *EGFR* mutation of L858R	Molecular-based *EGFR* mutation of L858R	
Staining score ≥10	(+)	(−)
(+)	130	9
(−)	42	396
Sensitivity = 75.6%; specificity = 97.8%		
>50% of immunopositive tumor cells		
	(+)	(−)
(+)	83	5
(−)	89	400
Sensitivity = 48.3%; specificity = 98.8%		

EGFR, epidermal growth factor receptor; L858R, L858R mutation in exon 21; IHC, immunohistochemistry.

Table 2
Correlation between the clinicopathologic parameters of 577 patients and the response to EGFR-TKI.

	Responder (CR+PR, $n=64$)	Non-responder (SD+PD, $n=50$)	p-Value
Age			
≥65	20	18	0.690
<65	44	32	
Gender			
Male	35	27	1.000
Female	29	23	
Smoking status			
Brinkman index <400	45	33	0.687
Brinkman index ≥400	19	17	
p-Stage			
IA–IIB	26	19	0.848
IIIA–IV	38	31	

EGFR, epidermal growth factor receptor; TKI, tyrosine kinase inhibitor.

3.5. Correlation between the molecular-based and IHC-based EGFR mutational status under another threshold

Positive immunoreactive cases for the DEL- or the L858R-specific antibody exhibited lower sensitivities and higher specificities when the threshold for the mutation-positive cases was restricted to >50% of the immunopositive tumor cells with any intensity. The incidence of positive immunoreactive cases for the DEL-specific antibody decreased from 59 to 28 cases—all of which were m-DEL (sensitivity, 20.7%; specificity, 100.0%; Table 1A). The incidence of positive immunoreactive cases for the L858R-specific antibody decreased from 139 to 88 cases, with 83 m-L858R cases and 5 m-WT cases (sensitivity, 48.3%; specificity, 98.8%; Table 1B).

3.6. Comparison of the molecular-based and IHC-based EGFR mutational status and the response to EGFR-TKI

A total of 114 patients were evaluable for the clinical response to EGFR-TKI. They consisted of 38, 39, and 37 patients with tumors harboring m-DEL, m-L858R, and m-WT, respectively; therefore, 68% of patients harbored the molecular-based *EGFR* mutations, and the remaining 32% harbored wild-type *EGFR*. The correlation between the conventional clinicopathologic parameters and the response to EGFR-TKI is shown in Table 2. In the present study, none of these parameters were significantly correlated with the response to EGFR-TKI.

Among the 77 patients harboring the molecular-based *EGFR* mutations, 59 (77%) were responders. In contrast, among the 37 patients without molecular-based *EGFR* mutations, only 5 (14%) were responders. Among the 55 patients with the IHC-based *EGFR* mutations, 40 (73%) were responders. In contrast, among the 59 cases without IHC-based *EGFR* mutations, 24 (41%) were responders (Table 3). Both the molecular- and IHC-based mutational statuses were significantly correlated with the response to EGFR-TKI ($p<0.001$ and $p=0.001$, respectively). We analyzed another threshold of the mutation-specific antibodies, defined as mutation-positive in >50% of the immunopositive tumor cells with any intensity. However, this threshold resulted in a slightly weaker correlation between the IHC-based mutational status and the response to EGFR-TKI ($p=0.012$, Table 3).

3.7. Multivariate analysis of the response to EGFR-TKI

A multivariate analysis of the response to EGFR-TKI with 2 variables (molecular-based mutational status and IHC-based mutational status), which showed a significant correlation by univariate analysis, was performed; only the molecular-based mutational status was significantly correlated with the response to EGFR-TKI ($p<0.001$). The IHC-based mutational status ($p=0.211$) was not significantly correlated (Table 4).

Table 3
Comparison of the molecular-based and IHC-based *EGFR* mutational status and the response to EGFR-TKI.

	Responder (CR+PR, $n=64$)	Non-responder (SD+PD, $n=50$)	p-Value
Molecular-based *EGFR* mutation			
(+)	59	18	<0.001
(−)	5	32	
IHC-based *EGFR* mutation			
Staining score ≥10	40	15	0.001
Staining score <10	24	35	
Immunopositive tumor cells >50%	24	8	0.012
Immunopositive tumor cells ≤50%	40	42	

EGFR, epidermal growth factor receptor; TKI, tyrosine kinase inhibitor; IHC, immunohistochemistry.

4. Discussion

In the present study, we investigated the clinical usefulness of IHC using 2 rabbit monoclonal antibodies against specific mutant *EGFRs* in lung adenocarcinomas. We found that the IHC-based *EGFR* mutational status detected by these antibodies was significantly correlated with the molecular-based *EGFR* mutational status. Furthermore, the IHC-based mutational status showed a significant correlation with the clinical response of tumors in conjunction with EGFR-TKI therapy.

The overall specificity of the 2 mutation-specific antibodies was 99%, and this specificity was consistent with that reported previously [19–24]. There were 11 cases in which the results of IHC examination were positive and those of molecular testing were negative. These false-positive cases might harbor other types of mutations that induce conformational changes in the *EGFR* protein, similar to DEL and L858R [24]. Since none of these 11 patients received EGFR-TKI therapy, the clinical significance of these mutations was not analyzed in the present study.

Despite the significant correlation between clinical response and immunoreactivity, the overall sensitivity of the 2 mutation-specific antibodies was 61%. This sensitivity was the lowest compared to values previously reported by others, which ranged from 78% to 92% [19,21–23]. One possible reason for the lower sensitivity in the present study was the methodological difference in the analysis of the molecular-based *EGFR* mutational status. HRMA, which was used for the molecular *EGFR* mutation analysis in the present study, was more sensitive than direct sequencing. HRMA has been shown to be a highly sensitive method for detecting DEL and L858R in prospective studies, and the detection sensitivity of this assay was reported to be 0.1–10%[15,27,28]. Conversely, the direct sequencing used in previous reports [19,21–23] required the presence of at least 20–25% *EGFR*-mutant cells to detect the DEL and L858R mutations. In the other 2 reports that validated the mutation-specific antibodies by correlating them with the *EGFR* mutational status by using highly sensitive molecular assays (mass spectrometry-based DNA analysis, cycleave PCR, and frag-

Table 4
Multivariate analysis of the response to EGFR-TKI.

	Odds ratio	95% CI	p-Value
Molecular based mutation	40.533	8.691–189.035	<0.001
IHC based mutation	0.421	0.109–1.632	0.211

EGFR, epidermal growth factor receptor; TKI, tyrosine kinase inhibitor; CI, confidence interval; IHC, immunohistochemistry.

ment analysis), the reported sensitivities of IHC-based mutations were lower and partially similar to ours. Brevet et al. have reported that the sensitivity of the DEL-specific antibody was 67%, and that of the L858R-specific antibody was 76%, with the threshold for positive cases defined as moderate staining [20]; Kitamura et al. have reported that the overall sensitivity of these 2 mutation-specific antibodies was 47%, with almost the same threshold for positive cases as our staining score of 10 [24]. Although highly sensitive methods sometimes elicit false positive results, we showed that the response rate to EGFR-TKI in patients with lung tumors harboring HRMA-detected *EGFR* mutations was 77%, and this was consistent with 2 previous reports (82% and 83%) [12,29]. Therefore, HRMA was not likely to have overestimated the *EGFR* mutations.

Most of the extracted DNA in the present study was isolated from fresh frozen tissues or ethanol-fixed imprint cytologic smears, whereas in other reports concerning mutation-specific antibodies, DNA extracted from formalin-fixed, paraffin-embedded tissues was used for molecular *EGFR* mutation analysis [19–22]. Formalin-fixed tissues exhibit non-reproducible sequence alterations more frequently than DNA isolated from frozen tissues. This is because formalin can cross-link cytosine nucleotides on either strand [30]. However, ethanol causes very little chemical change, and therefore preserves nucleic acids better than formalin [30]. Taken together, these data suggest that using a highly sensitive molecular assay and high-quality DNA can reduce false-negative cases. Therefore, the sensitivity of the 2 novel mutation-specific antibodies used in the present study, was decreased.

Another possibility was that the immunopositive tumor cells for the mutation-specific antibodies were not diffusely distributed. When the threshold for mutation-positive was set as >50% of immunopositive tumor cells, the positive cases for the DEL- and L858R-specific antibodies decreased from 59 to 28 cases (47%), and from 139 to 88 cases (63%), respectively. From these decreased rates, the immunopositive tumor cells for DEL were distributed more sparsely and/or focally than those for L858R. These findings, detected by IHC analysis, suggested the presence of heterogeneity in the *EGFR*-mutant cells. Other molecular methods for detecting *EGFR* mutations also revealed the heterogeneous distribution of *EGFR* mutant cells [31–33].

In the present study, the predictors of the EGFR-TKI response were molecular-based (HRMA) *EGFR* mutations ($p < 0.001$), and IHC-based *EGFR* mutations ($p = 0.001$). Two novel mutation-specific antibodies served as the predictors of EGFR-TKI response in the univariate analysis. However, the multivariate analysis revealed that only molecular-based *EGFR* mutations were significantly correlated with the response to EGFR-TKI. Among 6 previous reports on mutation-specific antibodies, 3 analyzed the correlation of IHC-based *EGFR* mutational status with the response to EGFR-TKI, and a significant correlation was found in 2 of these studies [21,24]. The sensitivity and specificity of IHC-based *EGFR* mutations to the EGFR-TKI response calculated in this study were 63% and 70%, respectively. In 2 previous reports, IHC-based *EGFR* mutations showed a sensitivity ranging from 59% to 89% and a specificity ranging from 73% to 96% to the EGFR-TKI response. The last report showed an insignificant correlation between these parameters [22]. The role of IHC in predicting response to EGFR-TKI remains controversial [34]. It is necessary to prospectively study a larger number of cases to determine the usefulness of IHC for the response to EGFR-TKI.

The amount of immunopositive tumor cells did not affect the EGFR-TKI response in the present study. The threshold for mutation-positive, defined as >50% of immunopositive tumor cells, was less significantly correlated with the clinical response to EGFR-TKI than when using judgments by the expression score of 10 ($p = 0.012$). Further discussion regarding whether the percentage of immunopositive tumor cells is correlated with the response to EGFR-TKI, is necessary. The present results showed, for the first time, that the presence of diffusely immunopositive cells does not necessarily predict a response to EGFR-TKI therapy. Therefore, in clinical practice, a threshold for mutation-positive of expression score of 10 should be adopted.

Although the mutation-specific antibodies are not superior to the highly sensitive molecular techniques in detecting *EGFR* mutations, they have some potential advantages. Their excellent specificities [19–24] will serve as the first screening for *EGFR* mutational status, including the human epidermal growth factor 2 status for breast carcinoma [35,36]. In clinical settings, the first screening of IHC enables the omission of molecular *EGFR* mutational analysis in IHC-positive cases. IHC saves time, is cost-effective, and can be performed in most pathology laboratories. Another advantage of IHC over molecular techniques is that it can distinguish between tumor morphology and mutation-bearing cells by light microscopy.

In summary, the mutation-specific antibodies exhibited extremely high specificities, but did not show high sensitivities compared to the highly sensitive molecular method. In clinical practice, IHC using these 2 antibodies is a cost-effective and simple method for detecting *EGFR* mutations in most pathology laboratories, and can quickly evaluate patients for EGFR-TKI therapy.

Conflict of interest statement

All authors have no financial or personal relationship with other people or organization that could inappropriately influence our work.

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