thin-section computed tomography (CT) [11]. A contrast-enhanced CT-scan was performed to evaluate the entire lung for preoperative staging. In addition, the main tumor was evaluated preoperatively to estimate the extent of ground-glass opacity (GGO) with thin-section helical CT-scan with 1–3 mm collimation. Images were reconstructed with a field of view of 15–20 cm. The lung was photographed with a window level of −500 to −700 H and a window width of 1000–2000 H as a 'lung window,' and with a window level of 30–60 H and a window width of 350–600 H as a 'mediastinal window.' The consolidation component was defined as an area of increased opacification that completely obscured the underlying vascular markings. GGO was defined as an area of a slight, homogenous increase in density that did not obscure the underlying vascular markings. Minimally-invasive lung cancer was tentatively defined as a tumor with a maximum diameter of consolidation of the maximum tumor diameter (consolidation/tumor ratio, C/T ratio) <0.5, indicating a tumor with a wide GGO area. All patients underwent posterolateral thoracotomy or anterior thoracotomy with the incision ranging from 8 to 12 cm. Eighteen (36.7%) were males and 31 (63.3%) were females (Table 1). Malignant tumors were found in 40 (81.2%). No patient underwent blood transfusion. The mean operative time was 167.3 min, with a range of 65–315 min. Resected regions were the following; right S1a+S3b in one patient, S2 in one, S3 in two, S6 in six, S8 in four, left upper division in 20, lingular division in five and S6 in six patients (Table 2).

When performing segmentectomy, the inter-segmental plane was developed with mechanical stapling and/or electrocautery. The inter-segmental plane was divided with only mechanical staplers in 18 patients, and with electrocautery in the other 31 patients. Among these 31 patients electrocarutery and staplers were used in 28 patients, i.e. the combination method. In the combination method, staplers were used mainly for the hilar side of the inter-segmental plane. We believe this technique results in decreased postoperative alveolar air leakage. When using electrocautery, the output was 60 joules for developing an inter-segmental plane.

The following factors were analyzed to investigate the relationship between these methods and the clinicopathological features; age, gender, histological diagnoses, the length of postoperative thoracic drainage, operative time, intraoperative blood loss, tumor size, postoperative pleurodesis, preoperative FEV_1, and preserved FEV_1. Preserved FEV_1 is calculated as preoperative FEV_1 divided by postoperative FEV_1 (%). Postoperative complications were investigated by the procedures. Statistical analysis was performed with uni- and multivariate analysis using logistic regression analysis. A *P*-value <0.05 is considered to be significant.

Table 1. Overall patient characteristics

Clinicopathological features	Number of patients
Overall	49
Gender	
Male/female	18/31
Age	
Range (mean)	24–81 (65.5)
Disease	
Primary lung cancer	33 (67.3%)
Metastatic tumor	6 (12.2%)
Benign tumor	3 (6.1%)
Others[a]	7 (14.3%)
Resected segments	
Right S1a+S3	1 (2.0%)
Right S2	1 (2.0%)
Right S3	2 (4.1%)
Right S6	9 (18.4%)
Right S8	4 (8.2%)
Left upper division	20 (40.8%)
Left lingular division	5 (10.2%)
Left S6	7 (14.3%)
Operative time (min)	
Range (mean)	65–315 (167.3)
Intraoperative blood loss (ml)	
Range (mean)	3–330 (46.6)
Methods of making an inter-segmental plain	
Stapling	18 (36.7%)
Electrocautery[b]	31 (63.3%)

[a]Others include inflammation, mucosa-associated lymphoid tissue lymphoma, giant bulla, sarcoidosis. [b]This category includes not only electrocautery but also both procedures (see text in detail).

3. Results

Stapler method and the above electrocautery method were used in 18 and 31 patients, respectively. There were no significant relationships between clinicopathological features and both procedures, except gender, operative time, and pleurodesis (Table 3). Women tended to undergo segmentectomy using electrocautery. As to operative time, segmentectomy using stapler needed more time than when using electrocautery. There were no patients who needed postoperative pleurodesis in the stapler group. However, preoperative FEV_1 was independent of the procedures, which meant that both procedures were used equally for patients having COPD. Preserved FEV_1 was not affected by the procedures.

Postoperative complications were found in 12 (29.3%) patients. The following complications occured: air leak resulting from treatments in four patients (8.2%), residual pulmonary torsion in one (2.0%), atelectasis in two (4.1%), hypoxemia in one (2.0%), atrial fibrillation in one (2.0%), liver dysfunction in one (2.0%) and infected wound in two patients (4.1%). Three of four patients with an air leak underwent chemical pleurodesis performed by the use of OK-432. Moreover, one of those three patients with chemical pleurodesis had surgical treatment to close the air leak. These three cases were as postoperative early air

Table 2. Relationship between resected segments and procedures

Segments	Stapling	Electrocautery
Overall	18	31
Right S1a+S3	0	1
Right S2	0	1
Right S3	1	1
Right S6	3	6
Right S8	2	2
Left upper division	9	11
Left lingular division	2	3
Left S6	1	6

Table 3. Relationship between clinicopathological features and procedures used for dividing inter-segmental planes

Variables	Stapling	Electrocautery[a]	*P*-value
Age	67.8 (56–81)	64.3 (24–84)	0.27
Gender (male/female)	12/6	9/22	0.013
Disease (primary lung cancer/others)	11/7	22/9	0.185
Thoracic drainage (days)	2.7	4.7	0.185
Operative time (min)	187.2	155.8	0.018
Intraoperative blood loss (ml)	66.7	34.9	0.12
Tumor size (mm)	20.6	15.6	0.092
Pleurodesis (+/−)	0/18	3/28	0.005
Preoperative FEV_1 (ml)	2.36	2.17	0.289
Preserved FEV_1[b]	90.0%	87.7%	0.652

[a]This category includes not only electrocautery but also both procedures (see text in detail). [b]Preserved FEV_1 is calculated as preoperative FEV_1 divided by postoperative FEV_1 (%).
FEV_1, forced expiratory volume in one second.

Table 4. Relationship between postoperative complications and procedures used for dividing inter-segmental planes

Variables	Stapling	Electrocautery[a]	*P*-value
Complications (+/−)	4/14	9/22	0.603

[a]This category includes not only electrocautery but also both procedures (see text in detail).

leak. Meanwhile one of four patients with an air leak experienced empyema and underwent fenestration. This case was a postoperative air leak which occurred seven months after a left upper division segmentectomy. Residual pulmonary torsion occurred in the residual left upper division after segmentectomy of the left lingular division. This case required surgical treatment. Atrial fibrillation and hypoxemia were found in the left upper division segmentectomy. The patient with postoperative hypoxemia required temporary home oxygen therapy. However, home oxygen therapy was discontinued two months after the operation.

Postoperative complications were independent of the method of dividing inter-segmental plane (Table 4). However, patients who underwent left upper division segmentectomy had significantly more complications (Table 5). Intraoperative blood loss was found to be a significant predictor for complications. On multivariate analysis, the resected segment and intraoperative blood loss were found to be significant predictors for postoperative complications (Table 6).

4. Discussion

One of the merits of segmentectomy of the lung is the preservation of postoperative pulmonary function [12]. However, segmentectomy could be associated with more postoperative complications. Segmentectomy of the lung is recognized as a difficult procedure for surgeons compared with lobectomy of the lung, as the division of inter-segmental planes is frequently troublesome. It is facile and convenient for thoracic surgeons to divide inter-segmental planes with mechanical staplers. Some surgeons prefer to use electrocautery for the division. Division with a stapler could lead to less postoperative complications and division with electrocautery can result in better postoperative lung

Table 5. Univariate analysis for the predictive factors for postoperative complications

Variables	Hazard ratio	95% CI	*P*-value[a]
Age	1.065	0.977–1.161	0.151
Gender, female	0.781	0.214–2.857	0.709
Disease, primary lung cancer	0.449	0.121–1.666	0.231
Side, left	3.929	0.757–20.375	0.103
Procedure, left upper division segmentectomy	8.667	1.968–38.157	0.004
Procedure, right/left S6 segmentectomy	0.117	0.014–0.997	0.05
Tumor size[b] (mm)	0.960	0.895–1.013	0.882
Intraoperative blood loss[b] (ml)	1.014	1.001–1.026	0.027
Operative time[b] (min)	1.432	0.369–5.552	0.603
Methods of making an inter-segmental plain (stapler vs. electrocautery[c])	1.432	0.369–5.552	0.603
Preoperative FEV_1	0.504	0.167–1.527	0.226

[a]*P*-value in logistic regression analysis. [b]Continuous valuable. [c]This category includes not only electrocautery but also both procedures (see text in detail).
CI, confidence interval; FEV_1, forced expiratory volume in one second.

Table 6. Multivariate analysis for the predictors for postoperative complications

Variables	Hazard ratio	95% CI	*P*-value[a]
Procedure, left upper division segmentectomy	9.783	1.834–52.178	0.008
Intraoperative blood loss	1.014	1.001–1.028	0.036

[a]*P*-value in logistic regression analysis.
CI, confidence interval.

function. However, there have been few reports on the relationship between the methods of dividing inter-segmental planes and postoperative complications and/or lung function.

When performing segmentectomy we prefer to use electrocautery because of the following reasons; 1) better postoperative lung function can be obtained; 2) better local control can be expected. This investigation is focused on the former point of view. In this study the decision as to which procedures were used depended on surgeons preference. However, preoperative clinical parameters were not associated with the procedures. Thus, both procedures were used equally for patients with severe complications, such as angina pectoris, diabetes mellitus, and/or COPD. Female patients tended to undergo segmentectomy using electrocautery. This observation was associated with the fact that earlier lung cancers showing GGO were found in women, though this should be investigated in the near future. Pleurodesis were not performed at all in patients who underwent segmentectomy using a stapler. This may mean segmentectomy using electrocautery resulted in more prolonged air leakage.

Patients who underwent left upper division segmentectomy had significantly more postoperative complications. Left upper division segmentectomy is considered to be equivalent to right upper lobectomy in terms of resected lung volume. This could mean resected lung volume was associated with the frequency of postoperative complications. Another significant predictor for complications was intraoperative blood loss. Prolonged air leakage may be observed in patients having pleural adhesion and this may be the reason for it. The limitation of this study may be the small number of patients investigated. Further investigations are warranted.

In conclusion, our limited investigation fails to show the significant relationship between the methods of making inter-segmental planes and postoperative complications and/or lung functions. As to the efficacy of segmental resection of the lung, a final decision should be made based on the results of the phase III trials conducted by JCOG [11].

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Clonality status of multifocal lung adenocarcinomas based on the mutation patterns of *EGFR* and *K-ras*

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ABSTRACT

Purpose: The purpose of this study is to clarify the clonality status of multifocal lung adenocarcinomas based on the mutation patterns of *epidermal growth factor receptor* (*EGFR*) and *K-ras*.
Methods: We analyzed 82 multifocal lung adenocarcinomas from 36 patients who underwent surgical resection. Genomic DNA was extracted from formalin-fixed, paraffin-embedded tissue and analyzed for *EGFR* and *K-ras* mutations. We determined the clonality status of multifocal lung adenocarcinomas based on the mutation patterns of *EGFR* and *K-ras*. The actuarial survival time was estimated and the prognostic factors were evaluated for 31 patients with synchronous multifocal lung adenocarcinomas.
Results: *EGFR* and *K-ras* mutations were detected in 36 (44%) and 19 (23%) of the 82 tumors, respectively. *EGFR* mutations had occurred randomly in 20 (91%) of the 22 patients with at least one *EGFR* mutated tumor. *K-ras* mutations had occurred randomly in 14 (93%) of the 15 patients with at least one *K-ras* mutated tumor. Combining the results for the *EGFR* and *K-ras* mutation patterns, the clonality status of multifocal lung adenocarcinomas could be determined in 30 (83%) of the 36 patients. No statistically significant difference in the actuarial survival of the patient subgroups stratified according to the clonality status, which was based on the presence of *EGFR* and *K-ras* mutations, was observed.
Conclusions: Both *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.

1. Introduction

Adenocarcinoma is now the most common histological type of lung cancer, followed by squamous cell carcinoma and small cell carcinoma. Bronchioloalveolar carcinoma (BAC) is a specific subtype of adenocarcinoma that disproportionately affects women, Asians, and non-smokers [1]. Adenocarcinomas, including BACs, frequently develop as synchronous and/or metachronous multifocal disease [2]. Although surgical resection is considered to be the best means of obtaining a definitive diagnosis and curative treatment, resecting all the lesions completely is sometimes difficult in patients with a poor cardiopulmonary function or those with numerous pulmonary lesions.

Epidermal growth factor receptor (*EGFR*) mutation is the most important predictor of the efficacy of EGFR tyrosine kinase inhibitors (TKIs) such as gefitinib and erlotinib [3–7]. In contrast, *K-ras* mutations are a useful biomarker of resistance to EGFR-TKIs [5]. Therefore, if multifocal lung adenocarcinomas simultaneously harbor *EGFR* mutations, they can likely be managed successfully using EGFR-TKIs. But, if they simultaneously harbor *K-ras* mutations, the use of EGFR-TKIs is not preferred. If *EGFR* and *K-ras* mutations are random events in multifocal lung adenocarcinomas, the efficacy of EGFR-TKIs would be limited to only the tumors carrying *EGFR* mutations, and not to those carrying *K-ras* mutations.

The purpose of this study was to clarify the clonality status of multifocal lung adenocarcinomas. The present study, to our knowledge, is the largest investigation of the clonality status of multifocal lung adenocarcinomas based on the mutation patterns of *EGFR* and *K-ras*.

2. Materials and methods

This retrospective review was performed under a waiver of authorization approved by the institutional review board of Juntendo University School of Medicine.

2.1. Patients

Between September 1996 and December 2008, 1047 patients with primary lung cancers underwent pulmonary resection. Among

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them, 57 patients had synchronous or metachronous multifocal lung cancers. Patients with pneumonic-type mucinous BAC were excluded. Patients whose tumor tissues were not available for molecular analyses were also excluded. Therefore, we conducted a retrospective review of a total of 82 multifocal lung adenocarcinomas in 36 patients.

2.2. Histological examination

The differential diagnosis of multiple primary lung cancer (MPLC) or pulmonary metastasis (PM) was clinicopathologically performed according to the criteria proposed by Martini and Melamed [8]. The proportion of the BAC component was evaluated microscopically on all the slides, including the largest cut surface of the tumor, using hematoxylin and eosin staining and elastica van Gieson staining. The BAC component was defined as the component of lepidic growth patterns of tumor cells.

2.3. Molecular analyses

DNA extraction and mutation analyses for *EGFR* and *K-ras* were conducted at Mitsubishi Chemical Medience Corporation (Tokyo, Japan). Genomic DNA was extracted from formalin-fixed, paraffin-embedded tissue. Serial slices at 5 μm were made from each block for tumor cell dissection. After deparaffinization with xylene, the tissue sections were stained with hematoxylin and eosin, and the target tumor lesions were macroscopically dissected to minimize contamination with normal tissue. The peptide nucleic acid-locked nucleic acid (PNA-LNA) polymerase chain reaction (PCR) clamp method [9] was used for *EGFR* mutation analysis, while the peptide nucleic acid (PNA)-mediated PCR clamping method [10] was used for the *K-ras* mutation analysis.

2.4. Clonality assessment

In synchronous multifocal tumors, the largest tumor was defined as the "primary tumor" and the remaining tumors were defined as "secondary tumors". In metachronous multifocal tumors, the first tumor was defined as the "primary tumor" and the tumors that developed after the surgical resection of the first tumor were defined as "secondary tumors".

First, we separately compared *EGFR* and *K-ras* mutation statuses between each primary and secondary tumor and classified the results as belonging to one of six different patterns of multifocal tumors (Table 1): pattern A, mutation in only the primary tumor; pattern B, different mutations in the primary and secondary tumors; pattern C, mutation in only the secondary tumor; pattern D, identical mutations in the primary and secondary tumors; pattern E, no mutations both in the primary and secondary tumors; and pattern F, undetermined mutation status in either the primary or secondary tumor regardless of the mutation status in the other tumor. Patterns A, B and C were regarded as indicating different clonal origins, whereas pattern D was regarded as indicating the same clonal origin. Patterns E and F were regarded as indicating an undetermined clonality status.

Table 1
Patterns of *EGFR* and *K-ras* mutations.

Clonality status	Pattern	Primary tumor	Secondary tumor
Different clonality	A	●	○
	B	●	■
	C	○	●
Same clonality	D	●	●
Not determined	E	○	○
	F	Any/?	?/any

EGFR, epidermal growth factor receptor; ●/■, mutation positive; ○, mutation negative; ?, clonality status could not be determined.

Next, we determined the clonality status based on combining the results of the mutation patterns for *EGFR* and *K-ras* genes. A secondary tumor was classified as exhibiting a different clonality if either *EGFR* or *K-ras* mutation belonged to pattern A, B or C but both *EGFR* and *K-ras* mutations did not belong to pattern D. A secondary tumor was classified as exhibiting the same clonality whenever either the *EGFR* or *K-ras* mutation belonged to pattern D. The clonality status was regarded as undetermined for secondary tumors in which both *EGFR* and *K-ras* mutations belonged to either pattern E or F.

2.5. Statistical analysis

The relationships between *EGFR*/*K-ras* mutation status and the clinicopathological features were statistically evaluated using a chi-square test or a Fisher's exact test.

Survival analyses were performed only for the patients with synchronous multifocal adenocarcinomas, since most of the patients (31/36) had synchronous tumors. The length of survival was defined as the interval in days between the day of surgical intervention and the date of either death or the last follow-up. The survival rates were calculated using the Kaplan–Meier method, and the curve differences were tested using the log-rank test. Multivariate analyses of independent prognostic factors were performed using Cox's proportional hazards model. A *P*-value of less than 0.05 was considered statistically significant. All statistical analyses were performed using the SPSS statistical software package (version 17.0, SPSS Inc., Chicago, IL).

3. Results

3.1. Clinicopathological characteristics of patients (Table 2)

The patients comprised 18 men and 18 women. The median age at the time of the first operation was 67 years (range 44–79 years). Twenty-two patients (61%) had a smoking history (either current or ex-smoker). Synchronous multifocal adenocarcinomas were noted in 31 patients (86%) and metachronous ones were noted in 5 patients (16%). The median size of the tumors was 16 mm (range 1–105 mm). Twenty secondary tumors (43%) were located in the same lobe as the primary tumor, and 26 (57%) were located in a different lobe. The number of patients according to the pathological nodal status was 29 with N0, 3 with N1, and 4 with N2, respectively. Therefore, the majority of patients in the present study did not have lymph node involvement.

3.2. Clonality assessment based on EGFR mutation status (Table 2)

EGFR mutations were detected in 36 (44%) of the 82 tumors in total: 20 (56%) of the 36 primary tumors, and 16 (35%) of the 46 secondary tumors. The point mutation L858R in exon 21 and a deletion in exon 19 were detected in 18 and 17 tumors, respectively. T790M in exon 20, which has been recognized as a mutation that confers resistance to EGFR-TKIs, was detected in one tumor.

Simultaneously, two tumors in one patient (No. 16) harbored double *EGFR* mutations and one tumor in one patient (No. 27) had three different types of *EGFR* mutations.

Patient No. 27 had a primary tumor with three different types of *EGFR* mutations and three secondary tumors with one *EGFR* mutation (L858R in exon 21) identical to one in the primary tumor. We classified this patient as having tumors exhibiting the same clonality (pattern D), which corresponds to PM according to the *EGFR* mutation status. Because lung adenocarcinoma is morphologically

Table 2
Clinicopathological characteristics and molecular findings of 82 multifocal lung adenocarcinomas from 36 patients.

Case	P/S	Sex	Age	Pack-year	CEA	M/S	Location of secondary tumors[a]	Tumor size	BAC (%)[b]	pN	PM or MPLC[c]	*EGFR* mutation	*EGFR* mutation pattern	*K-ras* mutation	*K-ras* mutation pattern	Clonality[d]
1	P	F	57	0	4.3	S		32	30	0	MPLC	Ex21: L858R				Same
	S						Same	32	80			Ex21: L858R	D	codon12 AGT	C	
2	P	F	67	0	1.4	S		10	100	0	MPLC			codon12 GCT		Different
	S						Same	2	100				F		A	
3	P	F	44	24	2	S		18	0	0	MPLC					ND
	S						Same	2	70				F		E	
4	P	M	75	55	10.3	S		32	30	0	MPLC	Ex19: L747-T751del				Different
	S						Same	6	60				A		E	
5	P	M	61	10	2.7	S		45	20	2	MPLC			codon12 GAT		Different
	S						Same	5	40				E		A	
6	P	F	78	0	2.9	S		25	40	0	MPLC	Ex19: E746-A750del				Different
	S						Same	1	100				A		E	
7	P	F	68	0	4.3	S		33	40	0	MPLC	Ex21: L858R				Same
	S						Different	16	60			Ex21: L858R	D		E	
8	P	F	60	0.2	3.8	S		50	60	1	MPLC	Ex21: L858R				Same/Different
	S1						Different	3	60				A	codon13 AGC	C	
	S2						Same	5	100			Ex21: L858R	D		E	
9	P	M	58	0	1.2	S		23	40	0	MPLC	Ex19: E746-A750del				Different
	S						Different	5	100			Ex19: del[e]	B		E	
10	P	F	66	20	1.8	S		25	60	0	MPLC	Ex19: L747-E749del Ex19: A750P				Same/Different
	S1						Same	10	60			Ex19: L747-E749del Ex19: A750P	D		E	
	S2						Same	13	60			Ex18: G719S	B		E	
11	P	M	68	86	5.2	M		42	0	0	MPLC			codon12 TGT		Different
	S						Different	18	60				E		A	
12	P	F	74	54	14.1	S		52	80	0	MPLC	Ex19: E746-A750del				Different
	S						Same	6	100				A	codon12 GCT	C	
13	P	F	73	0	3	S		15	50	0	MPLC	Ex21: L858R				Different/Different
	S1						Same	8	100				A		E	
	S2						Different	6	100				A		E	
14	P	M	63	40	3.2	M		20	0	0	MPLC			codon12 GCT		Different
	S						Different	12	10				E		A	
15	P	F	78	0	6.5	S		40	80	0	MPLC			codon12 GAT		Different
	S						Same	7	80			Ex21: L858R	C		A	

Table 2 (*Continued*)

Case	P/S	Sex	Age	Pack-year	CEA	M/S	Location of secondary tumors[a]	Tumor size	BAC (%)[b]	pN	PM or MPLC[c]	*EGFR* mutation	*EGFR* mutation pattern	*K-ras* mutation	*K-ras* mutation pattern	Clonality[d]
16	P	F	60	0	3.1	S		32	5	2	MPLC	Ex21: L858R Ex19: del[e]				Different
	S						Different	6	30			Ex18: G719S Ex19: del[e]	B		E	
17	P	M	78	32.5	1.2	S		29	10	0	MPLC			codon12 GCT		Different
	S						Same	15	100				E	codon12 GTT	B	
18	P	M	72	52	15.2	S		36	0	2	MPLC			codon12 GCT		Different
	S						Different	13	0				E	codon12 TGT	B	
19	P	M	58	0	1.8	S		27	60	0	MPLC	Ex21: L858R				Different/Different
	S1						Different	8	90			Ex19: E746-A750del	B		E	
	S2						Different	22	10				A	codon13 GAC	C	
20	P	M	70	50	0.5	S		20	100	0	MPLC	Ex21: L858R				Different
	S						Different	5	100			Ex19: del[e]	B		E	
21	P	M	66	45	1.8	S		18	20	2	PM					ND
	S						Same	6	10				F		E	
22	P	M	74	90	23.1	S		35	0	0	PM	Ex19: E746-A750del				Different/Different
	S1						Different	25	0				A		E	
	S2						Different	25	0				A		E	
23	P	M	61	21	6.2	S		25	0	1	PM					Different/ND
	S1						Different	7	0				E		E	
	S2						Same	12	0			Ex19: E746-A750del	C		E	
24	P	M	56	19	7.1	S		105	0	0	PM	Ex18: G719S				Different
	S						Different	22	0				A		E	
25	P	M	79	90	3.6	S		70	80	0	PM					ND
	S						Same	7	100				E		E	
26	P	M	49	60	6	M		27	0	0	MPLC					Different
	S						Different	57	0				E	codon12 GAT	C	
27	P	F	68	0	4.2	M		35	40	0	MPLC	Ex19: L747-S752del Ex19: E746V Ex21: L858R Ex20: T790M				Same/Same/Same
	S1						Different	19	70			Ex21: L858R	D		E	
	S2						Different	12	100			Ex21: L858R	D		E	
	S3						Different	12	100			Ex21: L858R	D		E	
28	P	F	78	0	2.4	S		22	60	0	MPLC	Ex18: G719S				Different
	S						Same	20	90				A		E	
29	P	M	63	60	21.3	M		31	70	0	MPLC					Different
	S						Different	15	0				E	codon12 AGT	C	
30	P	F	63	0	1.4	S		21	70	0	MPLC	Ex19: E746-A750del				Different
	S						Different	15	100				A		E	

																Same/Different
31	P	F	71	0	1.2	S		8	70	0	MPLC	Ex21: L858R	A		E	
	S1						Same	3	100							
	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		E	ND
	S						Same	12	0							
34	P	F	48	1.8	1.8	S		30	0	0	MPLC	codon12 GAT			A	Different
	S						Same	4	100				E			
35	P	M	60	40	1.2	S		16	95	0	MPLC	Ex19: E746-A750del				Same/Same
	S1						Different	9	90				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

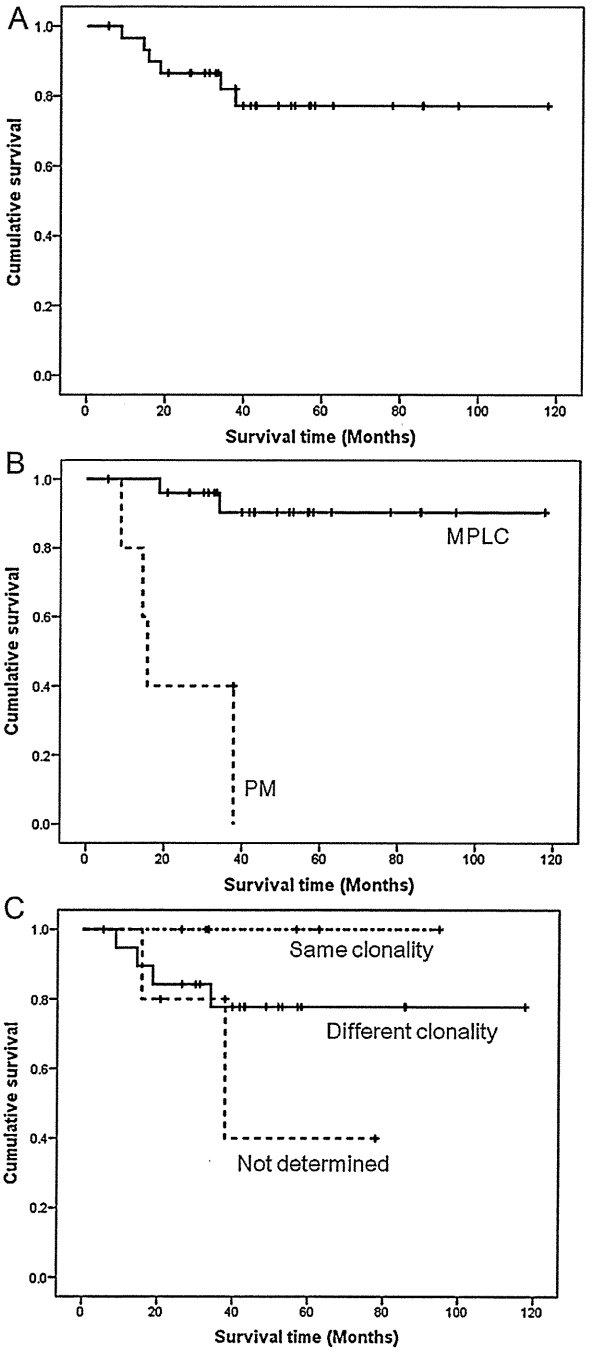

Fig. 1. Survival curves for patients with synchronous multifocal lung adenocarcinomas. (A) Overall patients. (B) Comparison between the outcomes of patients with MPLC and patients with PM diagnosed according to Martini and Melamed's criteria (log-rank test, $P < 0.001$). (C) Comparison between the outcomes 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

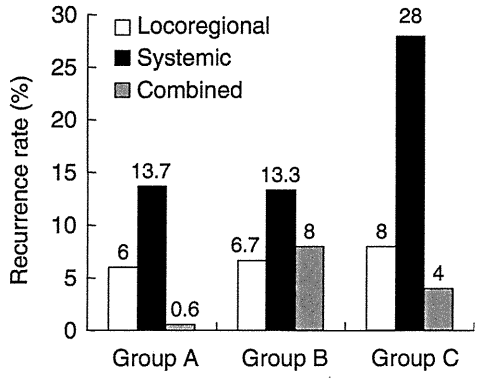

FIG. 1. Recurrence patterns in patients with stage II rectal cancer according to carcinoembryonic antigen group. There were no significant differences in locoregional (P = .939) and systemic (P = .077) recurrence rates among the three groups.

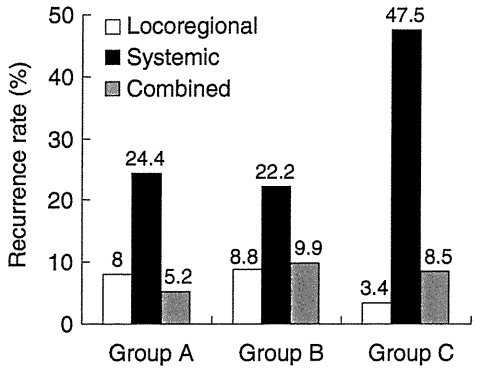

FIG. 2. Recurrence patterns in patients with stage III rectal cancer according to carcinoembryonic antigen group. There were no significant differences in locoregional recurrence rates among the three groups (P = .420). However, the systemic recurrence rate was significantly higher in group C than in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

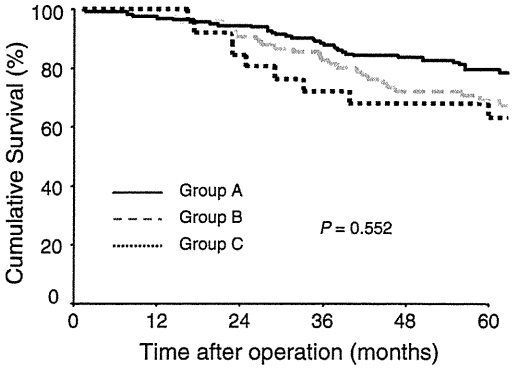

FIG. 3. Survival curves in patients with stage II rectal cancer according to carcinoembryonic antigen group. There were no significant differences in 5-year survival rates among the three groups (group A, 79.1%; group B, 71.0%; group C, 66.0%, P = .552).

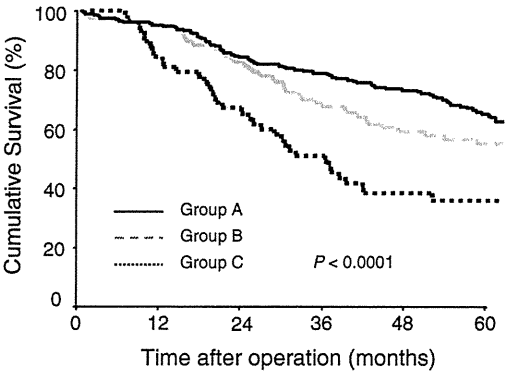

FIG. 4. Survival curves in patients with stage III rectal cancer according to carcinoembryonic antigen group. The 5-year survival rate was significantly lower in group C (35.4%) than in groups A (64.1%) and B (54.1%; P < .0001).

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.

ACKNOWLEDGMENT

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Hybrid Video-Assisted Thoracic Surgery Basilar (S9-10) Segmentectomy

Yoshihiro Miyata, MD, PhD, and Morihito Okada, MD, PhD

We perform segmentectomy for patients with cT1N0 non-small cell lung cancer (NSCLC) of 2 cm or less, even in good-risk patients. Hilar dissection and intersegmental dissection are performed by using mainly direct visualization through the access thoracotomy, which is called hybrid video-assisted thoracic surgery (VATS). Identification of the intersegmental plane is performed by selective jet ventilation under bronchofiberscopy. With this method, the segment to be removed can be inflated, while the segments to be preserved are kept deflated. When the intersegmental plane is being divided by electrocautery, direct visualization during the hybrid VATS approach is extremely important, because a 3-dimensional understanding of the pulmonary anatomy is crucial to avoid ambiguous procedures.

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Keywords: hybrid video-assisted thoracic surgery (VATS), segmentectomy

TECHNIQUE

One 10-mm camera port is placed in the eighth intercostal space over the midaxillary line. An additional transverse skin incision 50-60 mm long for access thoracotomy is made over the auscultatory triangle in the sixth intercostal space for lower lobe segmentectomy (Table 1, Fig. 1). For upper and middle lobe tumors, the access thoracotomy is placed over the midaxillary line in the fourth intercostal space. The access thoracotomy is opened with a silicon rubber wound retractor with no rib spreading. The skin incision can be extended if the operator has difficulty in performing the procedure. Dissection of the intersegmental plane and hilum is performed by using mainly direct visualization through the access thoracotomy, whereas television monitor guidance is invariably used during the procedure when dissecting an area out of direct view, which is called hybrid video-assisted thoracic surgery (VATS). When the intersegmental plane is being completely divided by electrocautery, direct visualization during the hybrid VATS approach is extremely important, because a 3-dimensional understanding of the pulmonary anatomy is crucial to avoid ambiguous procedures.

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ORDER OF DISSECTION OF BRONCHOVASCULAR STRUCTURES

Left Basilar Segmentectomy

Because the pulmonary artery is identified at the interlobar fissure, periarterial dissection is carried out distally to expose the superior segmental branch (A6) and common basal branch (A8 and A9 + 10) to the lower lobe and the lingular branch to the upper lobe (A4 + 5) (Fig. 2). Each branch is exposed and taped, and interlobar, lobar, and segmental lymph nodes (#11, 12, and 13) are removed for intraoperative pathologic analysis. When lymph node metastases are present, the surgical procedure must be converted to a lobectomy. Because the arterial supply to the left basal segment has several variations, exposure of A9 + 10 should be carried out as distally as possible to confirm the identified segmental branch supplying S9 + 10. A9 and A10 are individually ligated and divided. At the proximal site around the hilum, the lung parenchyma, along with A9 + 10, is divided by electrocautery from the proximal to the distal site to separate the targeted S9 + 10 from the preserved S6 and S8. After dividing A9 + 10, B9 + 10 can be seen behind it. Because V9 + 10 runs just behind B9 and B10, it is important not to injure V9 + 10 when B9 + 10 is encircled. Thereafter, the pulmonary ligament is incised, and the inferior pulmonary vein is exposed. V6 and the common basal vein are separately exposed and taped. The lung paren-

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Table 1. Number, Position and Size of the Ports

Port	Size (mm)	Intercostal Space #	Anatomical Lines
1 (camera)	10	8th	MA
2	None	None	None
3	None	None	None
4	None	None	None
Access incision	50-60	6th	Auscultatory triangle

MA, midaxillary.

chyma, along with V6b and V6c, is divided by electrocautery to separate the diseased S9 + 10 from the preserved S6. Then V8 and V9 + 10 are exposed, and the lung parenchyma is divided by electrocautery along V8a and V8b to separate S9 + 10 from S8. This central intersegmental plane must finally be connected to the peripheral inflation-deflation cutting line. After B9 + 10 is isolated and taped, a bronchofiberscope is inserted into B9 + 10, through which high-frequency jet ventilation is conducted. The targeted S9 + 10 is inflated, whereas the preserved S6 and S8 appear collapsed, and a demarcation line is formed between the inflated and deflated lung parenchyma, evidencing the anatomical intersegmental plane (Fig. 3). After jet ventilation fills the targeted S9 + 10, a stapler is applied to cut the targeted B9 + 10 and retain air inside the segment. Alternatively, the distal site of B9 + 10 is ligated first to keep the targeted segment S9 + 10 inflated, and then the site proximal to the ligation is transected and closed with suture. The peripheral stump of B9 + 10 is then lifted, and the anatomical intersegmental plane is used to separate B9 + 10 from the hilum (Fig. 4). Transection of the lung parenchyma tissue is then started from the peripheral site along the inflated and deflated line by electrocautery. This peripheral inflation-deflation cutting line must be connected to the central anatomical intersegmental plane made along the intersegmental vein around the hilum. V9 + 10 running toward the inflated S9 + 10 is identified and finally divided (Fig. 5). Ligation of V9 + 10 is best

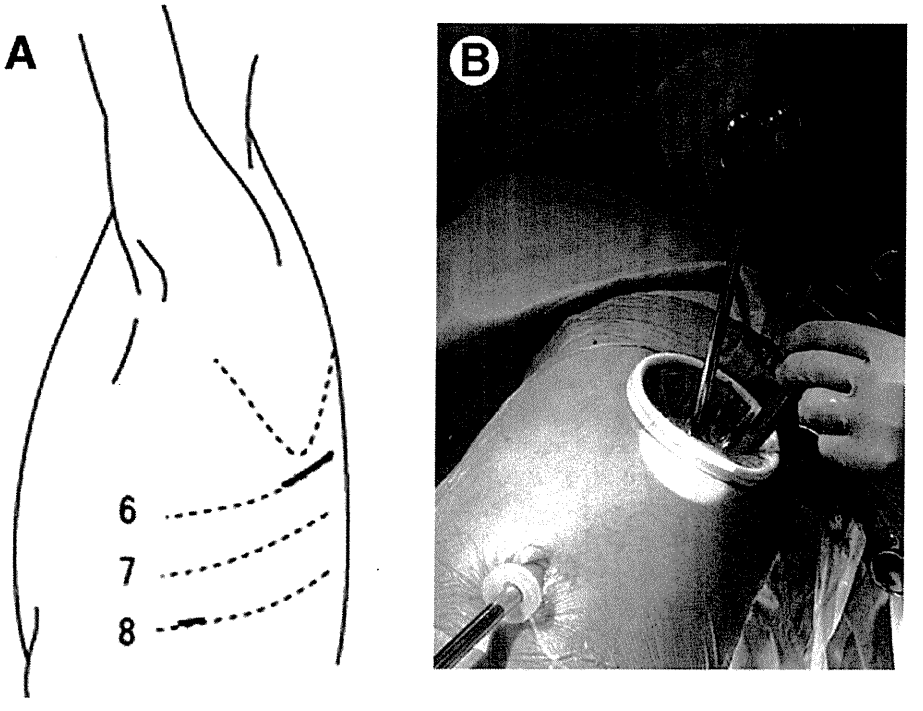

Figure 1. Port placement for the hybrid VATS approach. (A) A camera port (10 mm) is placed in the eighth intercostal space over the midaxillary line, and an access thoracotomy (50-60 mm) is made in the sixth intercostal space over the auscultatory triangle for lower lobe segmentectomy. (B) The access thoracotomy is opened with a silicon rubber wound retractor with no rib spreading. Dissection of the intersegmental plane and hilum is performed by using mainly direct visualization through the access thoracotomy, by using an upside-down grip with 30-cm-long scissors. Most of the procedures are performed with instruments not specialized for endoscopic surgery. A camera port is also used for the introduction of the stapler for pulmonary vessel and bronchi dissection. (Color version of figure is available online at http://www.semthorcardiovascsurg.com.)

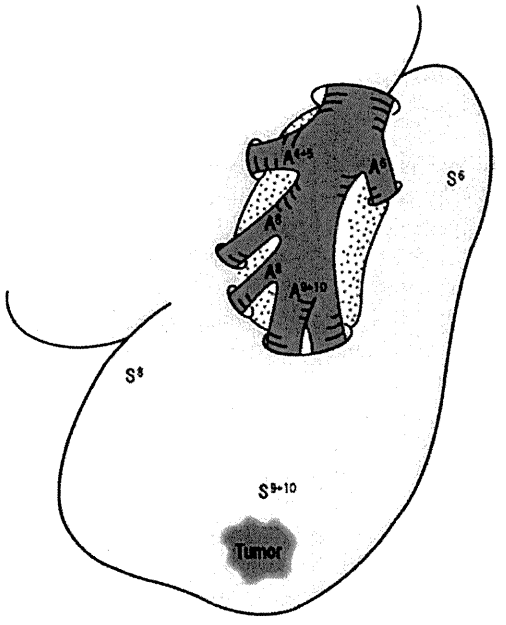

Figure 2. Exposure of the pulmonary artery at the interlobar fissure. Periarterial dissection is carried out distally to expose the superior segmental branch (A6) and common basal branch (A8 and A9 + 10) to the lower lobe and the lingular branch to the upper lobe (A4 + 5). (Color version of figure is available online at http://www.semthorcardiovascsurg.com.)

performed last after the intersegmental plane has been outlined, because venous drainage varies widely. The raw surface of the remaining intersegmental plane after cutting by electrocautery is sealed with polyglycolic acid felt (Neoveil; Kyoto Medical Planning Co, Kyoto, Japan) and fibrin glue (Beriplast; CSL Behring, Tokyo, Japan, or Bolheal; The Chemo-Sero-Therapeutic Research Institute, Kumamoto, Japan) to prevent air leakage. Staples might be used for partially dividing the intersegmental plane only when the lung is apparently emphysematous. In case of an incomplete fissure, the access thoracotomy should be extended without hesitation if the operator has difficulty in performing the procedure.

Right Basilar Segmentectomy

The procedure does not change much with the side on which the tumor is present.

ADJUNCTS TO FACILITATE IDENTIFICATION OF ANATOMICAL STRUCTURES

Preoperative simulation of the procedure with high-resolution computed tomography is important to identify proper anatomical structures including the intersegmental veins that need to be preserved. Intraoperative recognition of the segmental bronchus, which is the most consistent landmark of the segmental anatomy, is confirmed by using a bronchofiberscope. After the segmental bronchus is isolated, the bronchofiberscope is inserted into the targeted segmental bronchus. The tip of the bronchofiberscope is recognized at the surgical field by the operator, because the operator can see the light of the tip and lead it to a suitable place on the targeted bronchus. The anatomical intersegmental plane between the inflated segment to be resected and the deflated area to be preserved is visualized just after selective jet ventilation.

OUTCOMES

Operative time is 90-120 minutes. Blood loss is 10-50 mL. Conversion to thoracotomy is 0%. Conversion to lobectomy is 0%. Air leak >7 days is 0%.

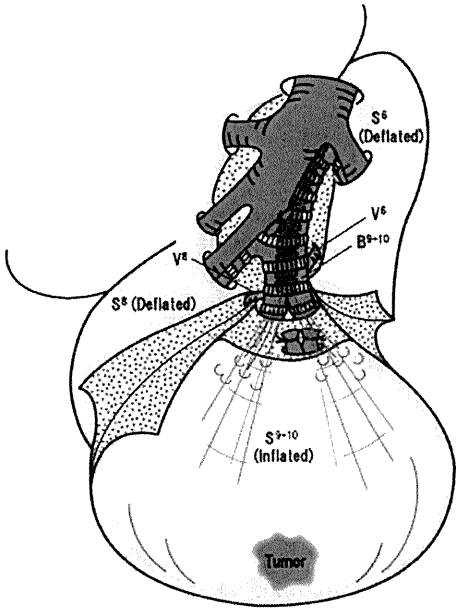

Figure 3. Identification of the intersegmental plane by selective jet ventilation. After B9 + 10 is isolated and taped, a bronchofiberscope is inserted into B9 + 10, through which high-frequency jet ventilation is conducted. The targeted S9 + 10 is inflated, whereas the preserved S6 and S8 appear collapsed, and a demarcation line is formed between the inflated and deflated lung parenchyma, evidencing the anatomical intersegmental plane. (Color version of figure is available online at http://www.semthorcardiovascsurg.com.)