Table 1. Immunohistochemistry and DNA sequence analysis of 60 NSCLC tumor samples

Patient no.	DNA sequencing of EGFR mutations	Immunohistochemistry		
		delEGFR	L858R	WT EGFR
1	E746-A750	2+	0	3+
2	E746-A750	3+	0	3+
3	E746-A750	3+	0	3+
4	E746-A750	1+	0	2+
5	E746-T751>A*	3+	1+	3+
6	E746-A750	2+	0	3+
7	E746-A750	2+	0	3+
8	E746-A750	2+	0	2+
9	E746-A750	2+	0	3+
10	E746-A750	1+	0	3+
11	E746-A750	1+	0	3+
12	E746-A750	3+	0	3+
13	S752-I759*	1+	2+	3+
14	L747-T751>P*	1+	1+	3+
15	L858R	0	3+	3+
16	L858R	1+	3+	3+
17	L858R	0	2+	3+
18	L858R	0	3+	3+
19	L858R	0	2+	3+
20	L858R	0	3+	3+
21	L858R	0	1+	2+
22	L858R	0	3+	3+
23	L858R	0	3+	3+
24	L858R	1+	3+	3+
25	L858R	1+	1+	3+
26	L858R	0	3+	3+
27	L858R	0	1+	3+
28	L858R	0	3+	3+
29	L858R	0	2+	3+
30	L858R	0	2+	2+
31	L858R	0	1+	3+
32	L858R	0	2+	3+
33	L858R	0	3+	3+
34	No mutation	0	0	3+
35	No mutation	0	0	3+
36	No mutation	0	0	3+
37	No mutation	0	0	3+
38	No mutation	0	0	3+
39	No mutation	0	0	3+
40	No mutation	0	0	3+
41	No mutation	0	0	3+
42	No mutation	0	0	3+
43	No mutation	0	0	3+
44	No mutation	0	0	3+
45	No mutation	0	0	3+
46	T751-I759>NKA*	2+	0	3+
47	L747-P753>S*	1+	0	2+
48	L747-T751>Q*	0	0	3+
49	L747-A750>P*	1+	0	3+
50	E746-A750	3+	0	3+

(*Continued on the following page*)

Table 1. Immunohistochemistry and DNA sequence analysis of 60 NSCLC tumor samples (Cont'd)

Patient no.	DNA sequencing of EGFR mutations	Immunohistochemistry		
		delEGFR	L858R	WT EGFR
51	E746-A750	3+	0	3+
52	E746-A750	3+	0	2+
53	L858R	0	2+	3+
54	L858R	0	3+	2+
55	L858R	0	3+	3+
56	L858R	0	3+	3+
57	No mutation	0	0	2+
58	No mutation	0	0	3+
59	No mutation	0	1+	3+
60	No mutation	0	0	2+

*Rare exon 19 deletion mutations.

confirming that these two antibodies were specific for the two mutations and would function in Western blots.

We next asked whether these mutation-specific antibodies were able to recognize mutant EGFRs in cultured lung cancer cells in immunohistochemical tests (Fig. 2B). Apparent expression of EGFR was seen in all four lung cancer cell lines, QG56, PC9, 11-18, and H1975, when labeled with the control anti-WT EGFR antibody. The deletion-specific antibody labeled only PC9 cells carrying the delE746-A750 EGFR mutation, whereas the antibody specific for the EGFR point mutation labeled only 11-18 and H1975 cells carrying the L858R mutation. Therefore, Western blotting and immunohistochemical analysis consistently showed that each mutation-specific antibody was able to identify the appropriate EGFR-activating mutation present in lung cancer cell lines.

Immunohistochemical analysis of activating EGFR mutations in NSCLC patients

We investigated EGFR mutation status in NSCLC adenocarcinomas showing moderate to strong total EGFR expression from 45 patients who were treated at the Kurume University Hospital by direct DNA sequence analysis (Table 1, patients 1-45). Deletion mutations in exon 19, including delE746-A750, delE746-T751>A, delS752-I759, and delL747-T751>P, were present in 14 patients, the L858R point mutation in exon 21 was present in 19 patients, and WT EGFR was present in 12 patients (Table 1). We then labeled paraffin-embedded samples of the NSCLC adenocarcinomas immunohistochemically for the EGFR mutations. Figure 3A shows representative images of two examples in each case of cancers carrying the delE746-A750 mutation, the L858R mutation, and WT EGFR. The two cases carrying the delE746-A750 mutation were stained strongly by the anti-delE746-A750 and anti-WT EGFR antibodies but not with the anti-L858R antibody; those carrying the L858R mutation were stained strongly by the anti-L858R and anti-WT EGFR antibodies but not with the anti-delE746-A750 antibody; those carrying only WT EGFR were only stained by the control anti-EGFR antibody (Fig. 3A).

Among the 45 cases of primary NSCLC, 12 samples had been shown to carry delE746-A750 deletion mutation, including a delE746-T751>A mutation (patient 5), and 75% (9 of 12) of these were stained by the deletion-specific antibody with a score of 2+ or 3+. Of the three samples that were identified by DNA sequencing as carrying rare exon 19 deletion mutations (patients 5, 13, and 14), the tumor sample carrying a delS752-I759 mutation (patient 13) and a delL747-T751>P mutation (patient 14) was not positively stained by the delE746-A750–specific antibody (Table 1).

All of the tumor samples from the 19 patients shown to carry the exon 21 L858R point mutation by direct DNA sequencing analysis (patients 15-33) were also stained by the anti-L858R antibody. Fifteen of 19 cases were positively stained with a score of 2+ or 3+ (Table 1). Twelve patients (patients 34-45), whose tumors carried WT EGFR according to DNA sequencing, were not stained by either of the mutation-specific antibodies. As shown in Fig. 3B, in one tumor (patient 23), the cancer cells and bronchial epithelial cells in the sample were strongly stained by the control EGFR antibody, but only the cancer cells were strongly stained by the anti-L858R antibody.

We further investigated whether these two mutation-specific antibodies can be useful for diagnosing rare exon 19 deletion mutations in 15 NSCLC patients who had been treated at the Aichi Cancer Research Hospital (patients 46-60). Paraffin-embedded tissue samples, which included four rare exon 19 deletions (patients 46-49), three delE746-A750 mutations (patients 50-52), four L858R mutations (patients 53-56), and four WT EGFR (patients 57-60), were examined immunohistochemically (Table 1). Of rare exon 19 deletion mutation, L747-P753>S (patient 47), L747-T751>Q (patient 48), and L747-A750>P (patient 49) were negatively stained by the anti-delE746-A750 antibody, and only T751-I759>NKA

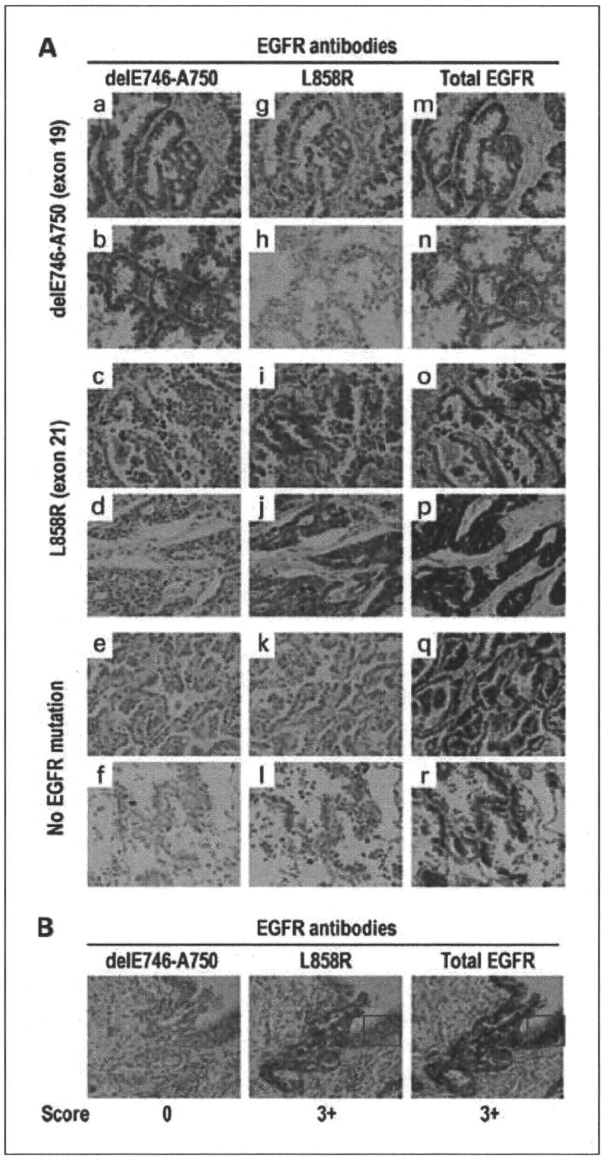

Fig. 3. A, immunohistochemical analysis of human NSCLC tumor samples. Control EGFR antibody stained all six tumor samples shown, the EGFR deletion-specific antibody stained cancer cells only in the two samples with delE746-A750 mutations (a and b), and the L858R-specific antibody stained only the cancer cells in the two samples with L858R mutations (i and j). B, differential diagnosis by immunohistochemical analysis of WT and mutant EGFRs in a NSCLC patient. In one tumor sample (Table 1, patient 23), labeling for total EGFR showed strong EGFR expression in both bronchial epithelial cells (red) and cancer cells. However, labeling with the anti-L858R antibody only stained the cancer cells and did not stain the bronchus.

(patient 46) was moderately stained (+2). None of these four samples carrying deletions were stained by the anti-L858R antibody, but tumor samples carrying L858R mutations (patients 53-56) by DNA sequencing were positively stained by the anti-L858R antibody.

The diagnostic data in Table 1 for EGFR mutations identified by immunohistochemistry are summarized in Table 2. We observed a high correlation between the results from DNA sequencing and immunohistochemistry. When staining +2 and +3 were determined as positive, EGFR mutation-specific antibodies detected delE746-A750 mutations in 79% (11 of 14) of cases identified by DNA sequencing, including patients from Kurume University Hospital and Aichi Cancer Research Hospital, and detected L858R mutations in 83% (19 of 23) of cases, indicating that this type of immunohistochemical analysis would be capable of diagnosing activating EGFR mutations. Thus far, rare exon 19 deletion mutations were examined using the anti-delE746-A750 antibody (Table 2); shorter (patients 14, 48, and 49) or longer (patients 13 and 47) deletion mutations than 15 bp were not positively stained. One (T751-I759>NKA, patient 46) harboring six-amino acid deletion, which was moderately (2+) stained and 1 (E746-T751>A, patient 5) was positively stained. Furthermore, of the samples without these EGFR mutations, immunohistochemistry with the two specific antibodies identified 100% (16 of 16) as negative for the deletion and point mutations in EGFR.

We next investigated whether these two mutation-specific antibodies can be useful in the small bronchial biopsies from stage IV NSCLC patients. Each stage IV patient harboring delE746-A750 or L858R showed strongly positive staining with the anti-delE746-A750 and anti-L858R, respectively (Fig. 4). In contrast, a stage IV patient without EGFR mutations showed strongly positive staining with anti-WT EGFR antibodies but not with both the anti-delE746-A750 and anti-L858R antibody.

Discussion

The ability to selectively administer EGFR-targeted drugs, such as gefitinib and erlotinib, to NSCLC patients carrying activating EGFR mutations is essential to the establishment of personalized anticancer therapy. A recent study has shown favorable clinical outcomes for patients with NSCLC adenocarcinoma carrying EGFR mutations after administering gefitinib compared with cisplatin-paclitaxel (17). The diagnosis of the activating EGFR mutations that are closely associated with the therapeutic efficacy of EGFR-targeted drugs is clearly essential to this strategy. The development of rapid and precise diagnostic techniques for activating EGFR mutations is particularly important for personalizing therapeutics in East Asian patients because these activating EGFR mutations (delE746-A750 and L858R) are significantly more frequent in this ethnic group. These EGFR mutations have been observed in 27.0% NSCLC patients in Japan, 36.8% in China, 19.2% in Korea, and 38.6% in Taiwan (8, 18–20).

The identification of EGFR mutations using mutation-specific antibodies would be a very useful diagnostic method for use in conjunction with DNA sequencing. Yu et al. (15) have generated antibodies specific for delE746-A750 and L858R mutations in EGFR and reported that the sensitivity of immunohistochemical assays using these antibodies was 92% in tests on 340 paraffin-embedded NSCLC tumor samples compared with a sensitivity of

Table 2. Summary of immunohistochemistry and DNA sequence analysis of NSCLC tumor samples

(A) Exon 19 deletions

Immunohistochemistry	DNA sequencing								
	delE746-A750	delE746-T751>A	delS752-I759	delL747-T751>P	delT751-I759>NKA	delL747-P753>S	delL747-T751>Q	delL747-A750>P	WT*
delE746-A750 (+)	11	1	0	0	1	0	0	0	0
delE746-A750 (–)	3	0	1	1	0	1	1	1	16

(B) L858R

Immunohistochemistry	DNA sequencing	
	L858R	WT*
L858R (+)	19	0
L858R (–)	4	16

*The same tumor samples were tested immuhistochemically in (A) and (B).

99% for DNA sequencing. This suggested that this simple immunohistochemical approach can be useful for establishing a rapid, sensitive, and cost-effective method to identify NSCLC patients responsive to EGFR-targeted therapeutics (15). In one tumor sample (patient 23) from the series used in this study, tumor cells, but not normal bronchial epithelial cells, were specifically immunostained with anti-L858R antibody (Fig. 3B), indicating that differential diagnosis between the WT and mutant EGFR in a single pathologic section was possible using this immunohistochemical approach. This result also suggested that a somatic EGFR mutation was present in cancer cells but not in normal cells.

In this study we have confirmed the usefulness of EGFR mutation-specific antibodies for the identification of activating EGFR mutations. The sensitivity of the delE746-A750– and L858R-specific antibodies was found to be 79% to 83% when all samples from Kurume University Hospital and Aichi Cancer Research Hospital were scored.

Several other rare deletion mutations are also known to occur close to the E746-A750 deletion in exon 19 (21). In this study, to further investigate the presence of other EGFR mutations in detail, we also carried out direct DNA sequencing of exons 19 and 20 and confirmed that several deletion mutations occurred close to delE746-A750. We identified rare deletions in exon 19 of the *EGFR*

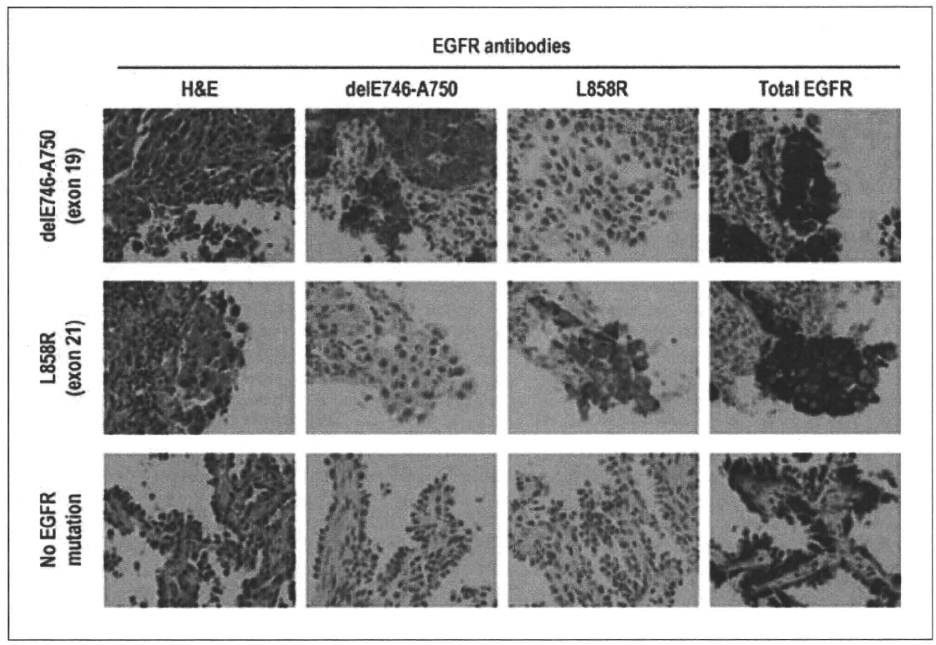

Fig. 4. Immunohistochemical analysis of bronchial biopsy samples of stage IV NSCLC patients. A sample with delE746-A750 mutation was stained with anti delE746-A750–specific antibody, and a sample with L858R mutation was stained with L858R-specific antibody. These samples were stained with WT antibody. No sample without EGFR mutations were stained with these two mutation-specific antibodies. Samples were stained with H&E.

gene in seven tumor samples: one 8–amino acid deletion (S752-I759, patient 13), two 6–amino acid deletion (T751-I759.NKA, patient 46; L747-P753>S, patient 47), one 5–amino acid deletion (E746-T751>A, patient 5), two 4–amino acid deletion (L747-T751>P, patient 14; L747-T751>Q, patient 48), and one 3–amino acid deletion (L747-A750>P, patient 49). Of these seven rare exon 19 deletion mutations, five samples (patients 13, 14, and 47-49) were not positively stained, one sample (patient 46) was moderately stained, and one sample (patient 5) that harbor 5–amino acid deletion with T751A was positively stained by the anti–delE746-A750 antibody (Tables 1 and 2), suggesting that the del E746-A750 antibody may not be useful for identification of these rare exon 19 deletion mutations. Yu et al. (15) also reported rare deletion mutations in exon 19, one of which (E746-T751) was stained by the anti–delE746-A750 antibody, whereas the other (L747-A750) was not. Further refinement of these mutation-specific antibodies will be required to encompass these rare exon 19 deletion mutations and to improve the sensitivity of molecular diagnosis using immunohistochemistry.

Our immunohistochemical data described here for EGFR mutation-specific antibodies suggest that this approach will be very useful for identifying the EGFR mutations that are known to be closely associated with the therapeutic efficacy of EGFR-targeted drugs. One particular merit of immunohistochemical diagnosis is that it provides a measure of the expression levels of mutant EGFRs in the cancer cells in tumors from individual patients. Combining DNA sequencing and immunohistochemistry will be very useful for further refining personalized diagnoses for patients with NSCLC.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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Effect of gefitinib on the survival of patients with recurrence of lung adenocarcinoma after surgery: A retrospective case-matching cohort study

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ABSTRACT

Background: Patients with lung adenocarcinoma who carry epidermal growth factor receptor (*EGFR*) gene mutations respond remarkably well to *EGFR* tyrosine kinase inhibitor (EGFR-TKI), gefitinib, or erlotinib. However, the effect of EGFR-TKI treatment on the prolongation of overall survival (OS) of these patients remains uncertain, although several recent studies have shown prolongation of progression free survival compared with cytotoxic chemotherapy.

Methods: A total of 304 patients with lung adenocarcinoma who had postoperative recurrent disease were studied. To eliminate potential biases as possible, the matching of four potential predictive factors of responsiveness to EGFR-TKI led to the identification of 81 pairs of patients (those who were treated with gefitinib and those who were not). A deletion mutation in exon 19 and a point mutation (L858R) in exon 21 of the *EGFR* gene were also analyzed. We compared the OS between the two groups.

Results: OS in the gefitinib group was significantly longer than in the control group (median, 63 vs. 41 months; $p = 0.015$). *EGFR* mutations were detected in 65 out of 129 patients (50%) in the whole sample. *EGFR* mutational status was not an independent prognostic factor of gefitinib benefit; rather, it was a predictive factor.

Conclusions: This study strongly suggested that gefitinib treatment improved OS of lung adenocarcinoma patients who had postoperative recurrence, especially those carrying *EGFR* mutations.

1. Introduction

Lung cancer is the leading cause of cancer-related mortality in Japan and in Western countries. However, standard platinum doublet chemotherapy for non-small cell lung cancer (NSCLC) appears to have reached a therapeutic plateau with a median survival of ~12 months [1,2]. Therefore, new treatment strategies that target specific molecular pathways are being actively sought.

Gefitinib and erlotinib are tyrosine kinase inhibitors (TKIs) specific for the epidermal growth factor receptor (*EGFR*). The response rate to TKIs was significantly high in NSCLC patients of East Asian origin and female sex, in patients with adenocarcinoma, and in patients who never smoked [3–6]. In 2004, somatic mutations in the tyrosine kinase domain of the *EGFR* gene were associated with high sensitivity to EGFR-TKIs [7,8]. According to published reports, the tumor response rates in patients with or without *EGFR* mutations are 81% and 12%, respectively [4]. Several prospective clinical studies confirmed that patients carrying *EGFR* mutations exhibit a high response to gefitinib [9–13]. Moreover, recent clinical trials indicated that this dramatic difference in response rate projected into prolonged progression free survival (PFS) [14,15]. However it is not certain whether overall survival (OS) is also prolonged, and it was possibly because of cross-over of treatment [16–20].

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In this study, we assessed whether gefitinib improved the survival of adenocarcinoma patients who experienced recurrence of the disease after potentially curative surgery. We also examined whether the benefit of gefitinib, when present, was limited to a certain patient subset, such as those carrying *EGFR* mutations.

2. Patients and methods

2.1. Patients

A total of 304 patients with lung adenocarcinoma who had recurrent disease after surgery were retrospectively reviewed. All patients underwent pulmonary resection at the Department of Thoracic Surgery, Aichi Cancer Center Hospital, from January 1995 through December 2006. Among them, 113 patients were treated with gefitinib after recurrence, 47 patients were given gefitinib as the first-line treatment, 41 were administered gefitinib as the second-line treatment, and 25 were given the drug as the third or later line of treatment. In contrast, 191 patients received other therapies than gefitinib, which included radiation, metastasectomy, anticancer agents, or best supportive care. For the molecular analysis of resected specimens, appropriate approval from the Institutional Review Board of the Aichi Cancer Center Hospital and patients' written informed consent were obtained.

2.2. Case-matching procedure

There was a significant bias in terms of potential prognostic factors between patients who were treated with or without gefitinib. Therefore, pairs of cases (patients with gefitinib treatment) and controls (patients without gefitinib treatment; control group) were created using a matching procedure on the computer that eliminated biases as much as possible. Matching factors included sex, age categories (40–49, 50–59, 60–69, ≥70), pathological stage (I or II–IV), and smoking status (never or ever). These factors were selected because they are closely associated with survival of patients with NSCLC and also correlate with the response rate to gefitinib treatment [21]. From the initial 304 patients, we were able to identify 81 case–control pairs (i.e., 162 patients in total) using this matching procedure.

2.3. Mutational analysis

Among these 162 patients, 129 specimens were available for RNA or DNA analysis. We extracted RNA or DNA from tumor samples and detected *EGFR* mutations as described previously [22,23]. Briefly, we performed direct sequencing of the products of the reverse transcriptase–polymerase chain reaction (RT–PCR) of exons 18–21 of the *EGFR* gene [23]. The cycleave real-time PCR method was used for DNA analysis to limit detection to the deletions around codons 746–750 (DEL) in exon 19 and to the point mutation at codon 858 (L858R) in exon 21[22]. We defined these two mutations as "*EGFR* mutation-positive", because they account for over 90% of all *EGFR* mutations and are known to have a clinically strong correlation with antitumor response when compared with other mutations [3]. Some of the patients reported in our previous report were included [21].

2.4. Statistical analysis

The OS was defined as the time span between the date of surgery and the date of death. We also defined the time span between the date of surgery and the date of recurrence as the recurrence-free survival (RFS) and the time span between the date of recurrence and the date of death as the postrecurrence survival (PRS). Hence, RFS and PRS sum up to OS. The survival rate was evaluated using the Kaplan–Meier method and intergroup differences were assessed using the Log rank test. Multivariate and interaction analyses were performed using the Cox regression model. The chi-squared test was used to compare the proportions of the two groups. Differences were considered significant when the p value was < 0.05. All analyses were carried out using the JMP software (version 5; SAS Institute, Cary, NC).

3. Results

3.1. Clinicopathological background

Patient characteristics are listed in Table 1. All patients were Japanese. Platinum therapy was performed more often in the gefitinib group than in the control group. The median recurrence date in the gefitinib group was delayed by approximately 10 months compared with the control group (December 3, 2003 vs. February 6, 2003). This reflects the date of gefitinib approval in Japan (July, 2002).

3.2. Survival analysis

The OS in the gefitinib group was significantly longer than in the control group (Fig. 1A). The median survival time (MST) in the gefitinib and control groups was 62.6 and 40.9 months, respectively ($p = 0.015$). On the other hand, no significant difference of RFS was observed between the gefitinib and control groups (Fig. 1B), with a median of 14.7 months vs. 12.0 months, respectively ($p = 0.34$).

Although the number of those who were received treatment with platinum-based chemotherapies in the gefitinib group was more than those in the control group, we found no significant difference in OS between the two groups distinguished by the presence of treatment with platinum-based chemotherapy in the gefitinib group and control group ($p = 0.69$ and $p = 0.08$, respectively).

3.3. Mutational analysis

One hundred and twenty-nine samples (71 from the gefitinib group and 58 from the control group) were available for *EGFR* mutational analysis. *EGFR* mutations were detected in 65 patients (55.3%). The DEL and L858R mutations were detected in 33 and 32 patients, respectively. *EGFR* mutations were more frequently

Table 1
Patient characteristics.

		Gefitinib ($n = 81$)	Control ($n = 81$)	p value
Age (median ± SD, years)[a]		62 ± 9.3	62 ± 8.6	0.84
Sex[a]	Female	40	40	1
	Male	41	41	–
Smoking[a]	Never	44	44	1
	Ever	37	37	–
Adenocarcinoma (%)		100	100	1
pStage[a]	I	31	31	–
	II	9	9	–
	III	39	39	–
	IV	2	2	1
Platinum-based chemotherapy		50	34	0.03
Metastasectomy		13	13	1
Radiation therapy		45	44	0.91
No other treatment		5	13	0.019

SD, standard deviation.
[a] Matching factor.

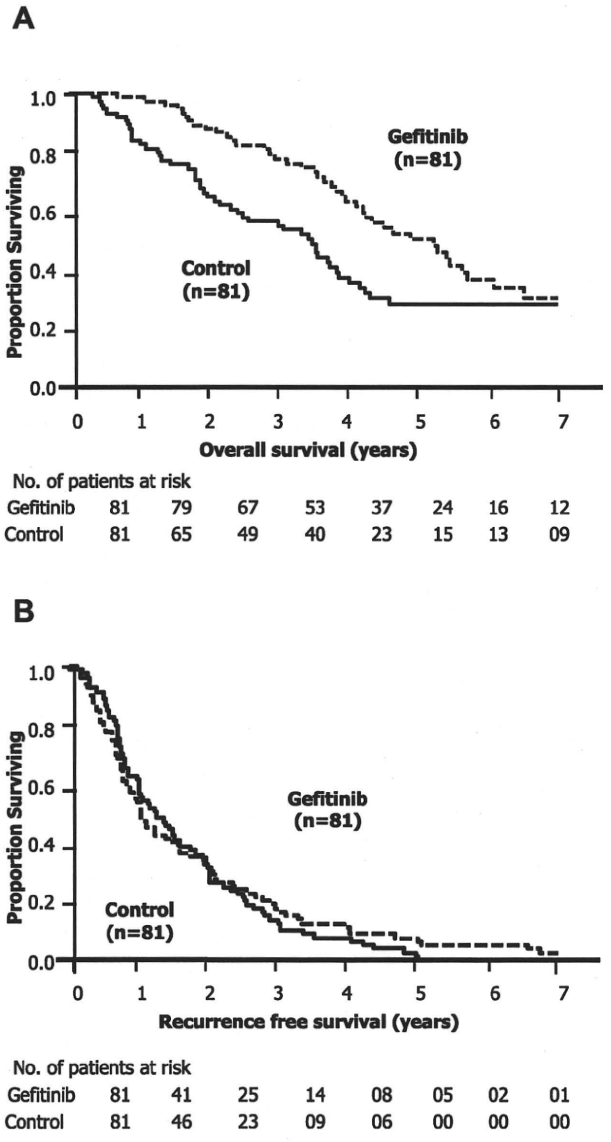

Fig. 1. *A*, Comparison of OS between the two groups. The five-year survival rate in the gefitinib treatment and control groups was 54% and 30%, respectively. The HR was 0.78 (95% CI, 0.63–0.95; $p = 0.0018$; Log rank test). *B*, Comparison of RFS between the groups. The HR was 0.90 (95% CI, 0.74–1.11; $p = 0.34$; Log rank test).

detected in the gefitinib group (44/71) than in the control group (21/58; $p = 0.03$). This imbalance was due to the fact that gefitinib treatment was chosen on the basis of *EGFR* mutational status in some patients after discovery of *EGFR* mutation in 2004.

3.4. Subset analysis of overall survival and multivariate analysis

Subset analyses of OS led to the detection of several significant differences. Female individuals in the gefitinib group survived for a longer period than those in the control group (MST, 78.3 vs. 42.4 months; $p = 0.03$; Fig. 2A and B). Similarly, in never smokers, or in those with *EGFR* mutation, the gefitinib group survived for a longer period than those in the control group (MST, 78.3 vs. 42.4 months; $p = 0.01$; Fig. 2C and D), and (MST, 63.4 vs. 42.4 months; $p = 0.02$; Fig. 2E), respectively. In contrast, we found no significant difference in OS for male patients, ever smokers, or patients without *EGFR* mutations (Fig. 2A, C, and E).

Multivariate analysis revealed that the pathological stage and gefitinib treatment were independent prognostic factors, with a hazard ratio (HR) of 0.423 and 0.537, respectively. The *EGFR* mutational status showed a marginally significant association with OS ($p = 0.11$; Table 2).

3.5. Effect of EGFR mutation and gefitinib treatment on survival

We evaluated the survival rate in four groups stratified according to *EGFR* mutational status and gefitinib administration (Fig. 3). The comparison between the gefitinib and control groups revealed a significant improvement in survival in patients with *EGFR* mutations (HR = 0.38; $p = 0.008$), whereas no difference was observed in patients without *EGFR* mutations ($p = 0.14$). Regarding the *EGFR* mutational status, a significant difference was detected in the gefitinib group (HR = 0.47; 95% CI, 0.24–0.92; $p = 0.02$), but not in the control group ($p = 0.57$) (Fig. 3).

A possible interaction was observed between the mutational status of *EGFR* and survival improvement in the gefitinib group ($p = 0.43$) (Table 3). If these two factors were independent, the HR in patients with *EGFR* mutations in the gefitinib group would be expected to be 0.48 (i.e., 0.64 × 0.75). However, the HR observed in this group was 0.38, which was lower than expected.

4. Discussion

We have previously reported that gefitinib treatment in patients with *EGFR* mutations improves their survival better than those patients without *EGFR* mutations [24]. Because this report did not include patients with *EGFR* mutations who were not treated with gefitinib, one possible criticism is that *EGFR* mutations may just predict better prognosis irrespective of treatment modalities and that *EGFR* may not predict better treatment outcome by EGFR-TKI [24]. Indeed, our recent analysis showed that patients with *EGFR* mutations who received potentially curative surgery but not gefitinib treatment survived for a longer time than those without *EGFR* mutations, using univariate analysis. However, a multivariate analysis did not indicate that *EGFR* mutations was an independent prognostic factor [25,26]. The present study aimed to gain insights on whether *EGFR* mutations are predictive of gefitinib treatment outcome, and not just prognostic, irrespective of treatment modalities, by applying a case-matching procedure that simulates randomized clinical trials by adjusting for confounding factors. The presence of treatment with platinum-based chemotherapy did not significantly affect OS of the respective groups. We found no significant difference in RFS between the two groups. This implies that the tumor aggressiveness was similar in both groups, which supports the comparability of the two groups. Yet, OS was significantly better in the gefitinib group, which suggests the overall efficacy of gefitinib in this patient subset.

Exploratory subset analyses showed that the OS in the gefitinib group was longer than that observed in the control group in female patients, nonsmokers, and *EGFR* mutation carriers. This is in good agreement with the patient subsets that had a high response rate to EGFR-TKI treatment. Multivariate analysis showed that pathological stage and gefitinib treatment were independent prognostic factors, whereas *EGFR* mutation was not. In the same way, *EGFR* mutation was not a prognostic factor compared with the OS in the four groups stratified by *EGFR* mutational status and gefitinib administration. The interaction analysis between the two revealed the presence of a positive trend. Furthermore, a significant improvement in survival was observed in patients with *EGFR* mutations when comparing the gefitinib and control groups. *EGFR* mutation may be a predictive factor of gefitinib treatment outcome.

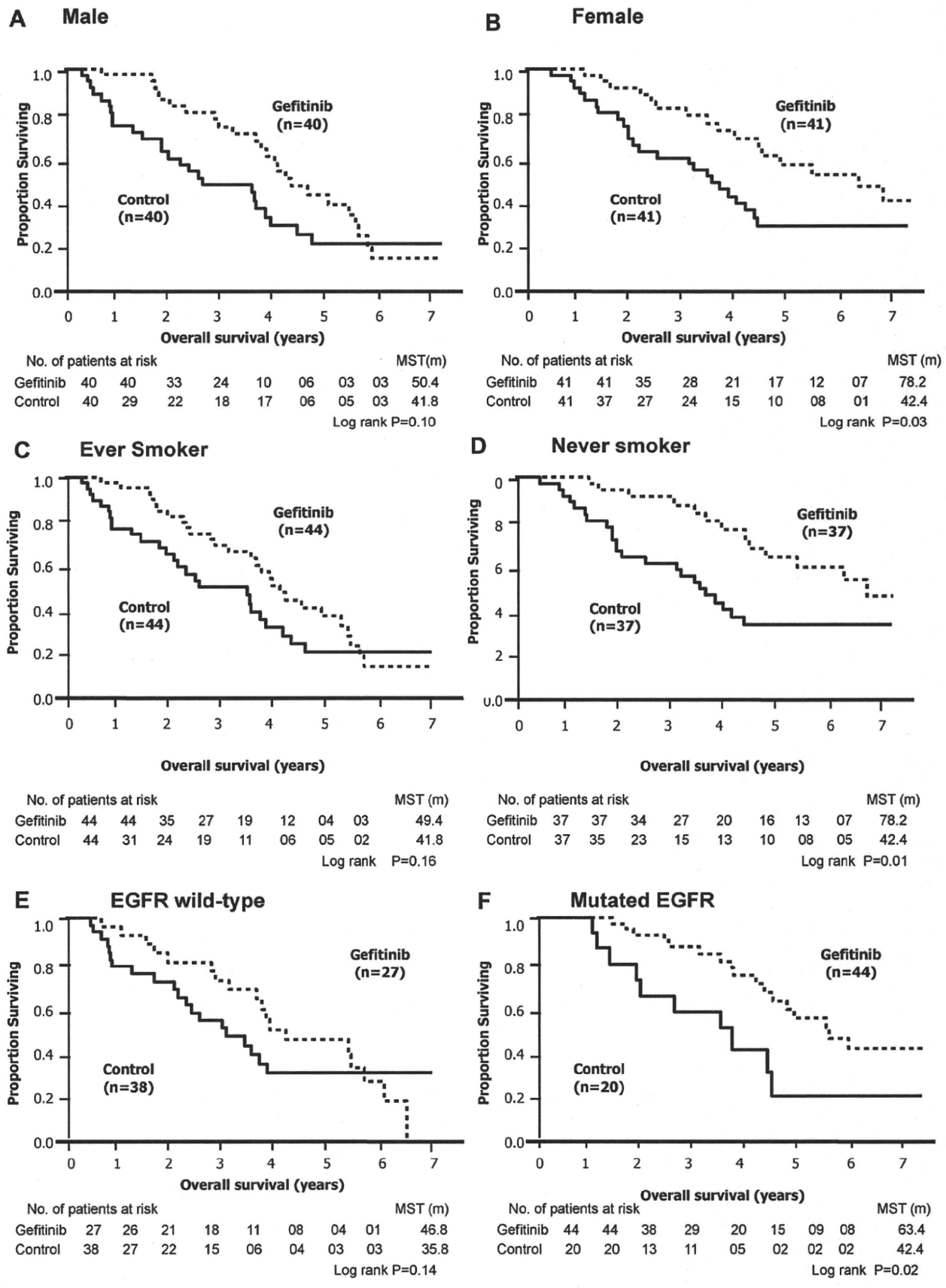

Fig. 2. Kaplan–Meier plots showing the OS of the subset of two groups. *A*, Male (MST 50.4 vs. 41.8 months; $p = 0.10$). *B*, Female (MST, 78.2 vs. 42.4 months; $p = 0.03$). *C*, Ever smoker (MST, 49.8 vs. 41.8 months; $p = 0.16$). *D*, Never smoker (MST, 78.2 vs. 42.4 months; $p = 0.01$). *E*, Wild-type *EGFR* (MST, 46.8 vs. 35.8 months; $p = 0.14$). *F*, Mutated *EGFR* (MST, 63.4 vs. 42.4 months; $p = 0.02$).

Table 2
Multivariate cox regression analysis.

		HR	95% CI	*p* value
Sex[a]	Male/Female	1.054	0.685–1.692	0.831
Smoking[a]	Never/Ever	1.236	0.760–1.897	0.493
Age[a]	≥66/<65	1.390	0.905–2.123	0.299
pStage[a]	II–IV/I	2.364	1.381–4.132	0.001
Gefitinib	No/Yes	1.862	1.175–2.659	0.01
EGFR	Wild-type/Mutated	1.515	0.900–2.577	0.11

HR, hazard ratio.
[a] Matching factor.

Table 3
Interaction hazard ratio of Gefitinib treatment and *EGFR* mutational status.

	EGFR Wild-type	*EGFR* Mutated
Other Treatment	1	0.75 (0.50–1.66) $p = 0.57$[a]
Gefitinib Treatment	0.64 (0.56–1.61) $p = 0.20$	0.38[b] (0.21–0.69) $p < 0.01$[a]

[a] Log rank test.
[b] Under the assumption that two factors were independent and their impact was multiplicative, the HR would be expected to be 0.48 (i.e., 0.75 × 0.64).

This result also showed that the selection of patients according to *EGFR* mutational status was an appropriate approach.

Takano et al. were the first to show a significant interaction between *EGFR* mutation and a survival benefit from EGFR-TKI therapy [27]. In the present study, an interaction analysis did not reveal a significant interaction; however, a trend toward interaction was observed. This issue should be investigated in the prospective study using a large patient cohort.

The survival analysis of 148 mutation-positive patients combined from seven prospective trials conducted in Japan[14] revealed that gefitinib prolongs survival in this selected NSCLC population. In addition, the phase III IRESSA Pan-Asia study (IPASS) comparing gefitinib monotherapy with carboplatin/paclitaxel in never or light smokers revealed an improvement in progression free survival (PFS) in the gefitinib arm[15]. Furthermore, a prespecified subset analysis showed that gefitinib significantly increases PFS in patients harboring *EGFR* mutations. However, the effect on the prolongation of OS of these patients remains uncertain to date.

The present study was a retrospective analysis. It was imperative that the evidence of the effect of EGFR-TKI treatment on survival benefit be validated in ongoing clinical trials by the West-Japan Oncology Group, the North-East Japan (NEJ) Gefitinib Study Group, and the Spanish Lung Cancer Group, which were performed in patients selected according to *EGFR* mutational status. While preparing this manuscript, NEJ Gefitinib Study Group reported a significant prolongation in PFS in the gefitinib arm rather than carboplatin/paclitaxel arm but again effect on OS is not certain at this moment [28]. In addition, clinical behavior of survival in patients with postoperative recurrence is superiorly different from that those with stage IV disease, although these two groups showed comparable responses to chemotherapy [29].

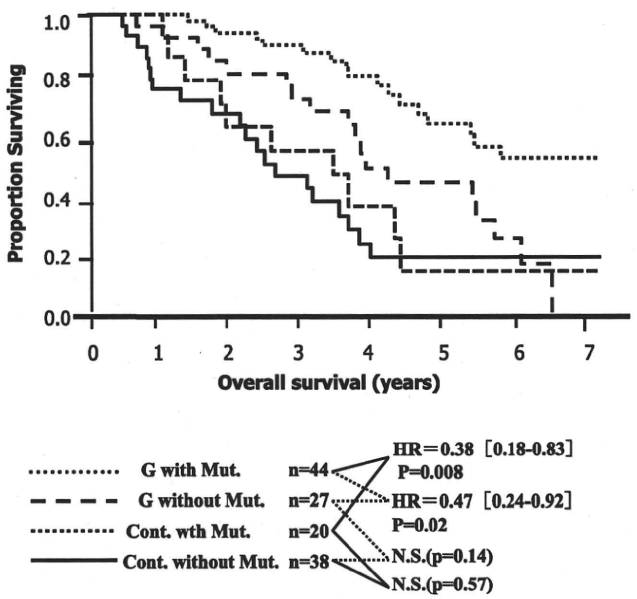

Fig. 3. Kaplan–Meier plots showing the OS in four groups, i.e., gefitinib and control groups segregated according to *EGFR* mutational status.

In conclusion, our study strongly suggested that gefitinib improve OS of Japanese lung adenocarcinoma patients with post-operative recurrence, especially in those with *EGFR* mutations.

Conflict of interest statement

Dr. Mitsudomi was paid an honorarium as a speaker in the professional meeting from AstraZeneca, Chugai pharm, Daiichi-Sankyo, Bristol-Meyers, Astells, and Taiho. He also provided testimony at theJapanese court in relation to the efficacy and toxicity of gefitinib. The other authors declare no conflict of interest. This work was supported in part by a Grant-in-Aid for Scientific Research (B) from the Japan Society for the Promotion of Science (20903076) and a grant from the Kobayashi Institute for Innovative Cancer Chemotherapy.

Authorship statement

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Data analysis: Tatsuya Katayama
Statistical analysis: Tatsuya Katayama
Manuscript preparation: Tatsuya Katayama
Manuscript editing: Tatsuya Katayama
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Effect of selective lymph node dissection based on patterns of lobe-specific lymph node metastases on patient outcome in patients with resectable non–small cell lung cancer: A large-scale retrospective cohort study applying a propensity score

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Objective: Lobectomy with systematic complete mediastinal lymph node dissection is standard surgical treatment for localized non–small cell lung cancer. However, selective mediastinal lymph node dissection based on lobe-specific metastases (selective dissection) has often been performed. This study was designed to evaluate the validity of the selective lymph node dissection.

Methods: From 1995 through 2003, 625 patients in our hospital had surgery for complete mediastinal lymph node dissection and 147 for selective dissection. We evaluated whether selective dissection adversely affected overall survival. To minimize possible biases due to confounding by treatment indication, we performed a retrospective cohort analysis by applying a propensity score. The propensity score was calculated by logistic regression based on 15 factors available that were potentially associated with treatment indication. Patients were divided into 4 groups according to quartile, and comparison between selective dissection and complete mediastinal lymph node dissection was made using propensity score quartile-stratified Cox proportional hazard models.

Results: Comparison of baseline characteristics between patients having selective dissection and patients having complete mediastinal lymph node dissection according to propensity score quartile supported comparability of the 2 groups. The 5-year overall survival rates were 76.0% for selective dissection versus 71.9% for complete mediastinal lymph node dissection. The 5-year survival probabilities stratified by propensity score quartile consistently showed no marked difference. In multivariate models, there was no significant difference between the 2 groups (hazard ratio = 1.17, $P = .500$) as also seen in the analysis without propensity score (hazard ratio = 1.06; 95% confidence interval, 0.68–1.64; $P = .810$). Therefore, selective dissection showed no significant impact on poor survival compared with complete mediastinal lymph node dissection.

Conclusions: Selective lymph node dissection did not worsen the survival of patients with non–small cell lung cancer.

GTS

The standard surgical treatment for patients with localized non–small cell lung cancer (NSCLC) is lobectomy or pneumonectomy with complete systematic mediastinal as well as hilar lymphadenectomy, known as radical complete lymph node dissection (CD).[1,2] However, the significance of lymphadenectomy is controversial. Some authors advocate the benefit of lymphadenectomy on histologic staging of lymph node spread but found no influence on overall survival (OS) or disease-free survival.[3,4] Dissection of lymph nodes without cancer cells is considered to be futile and can potentially increase perioperative complications or may require longer operative times.[3-7] In contrast, others claim that lymphadenectomy is important for therapeutic purposes as well as for staging.[8-11] Despite this controversy, there have been only 2 randomized controlled trials (RCTs) comparing CD with mediastinal lymph node sampling.[4,9] Izbicki and colleagues[4] concluded that there was no difference between the 2 groups in terms of both disease-free survival and OS. On the other hand, Wu and associates[9] reported that CD has a prognostic impact on survival. However, these results are not conclusive because of limited sample size and lack of intention-to-treat analysis. In this regard, we have to wait for the results of an ongoing randomized trial (ACOSOG Z0030) in North America.[12]

It is clear that the location of primary tumor in the lobes influences mode and extent of nodal spread.[13-15] For example, Okada and colleagues[13] reported that among patients with skip N2 metastases (no N1 nodes involved) with an upper-lobe lesion, none had positive subcarinal nodes. Only 1 of 13 patients with lower-lobe lesions (7.7%) showed nodal

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Abbreviations and Acronyms

CD = complete lymph node dissection
NSCLC = non–small cell lung cancer
OS = overall survival
PS = propensity score
RCT = randomized controlled trial
SD = selective dissection

spread to the upper mediastinum. Okada and colleagues[13] suggested that lower mediastinal lymphadenectomy was dispensable if hilar and upper mediastinal nodes were tumor-free in upper-lobe tumors. For lower-lobe tumors, upper mediastinal lymphadenectomy was dispensable when the hilar and subcarinal nodes were tumor-free. These studies suggest validity of selective lymphadenectomy based on patterns of lobe-specific lymph node metastases.

From the above-mentioned data, selective dissection (SD) has often been performed for patients with no apparent lymph node metastasis or with poor pulmonary reserve, or for elderly patients, although there were no predefined criteria for type of lymphadenectomy. It should be noted, however, that SD is different from lymph node sampling mentioned above, in that lymph nodes that should be removed according to patterns of lymph node metastases are radically dissected.

There is currently no evidence from RCTs regarding the validity of SD compared with CD. Large RCTs would take a long time and have great cost and therefore cannot be easily performed. The second best evidence should exist in a retrospective study comparing the 2 approaches. However, a serious concern with a retrospective analysis is that results might be biased by confounding for patient selection,[5,6,10] because patients with earlier diseases, those with poor pulmonary reserve, or elderly patients are likely to receive SD.

To eliminate these biases as much as possible, we conducted a retrospective cohort analysis using a propensity score (PS) to evaluate validity of SD compared with CD. A PS is defined as the conditional probability of exposure to a treatment given preoperatively observed covariates. Hypothetically, patients with the same PS have the same probability of receiving SD or CD. Therefore, patients receiving SD and patients receiving CD with the same PS provide similar comparability. Hence, results obtained by a retrospective study using a PS are assumed almost similar to those obtained by prospective RCT.[16]

PATIENTS AND METHODS

Patients

Approval for this study was obtained from and the need for individual patient consent was waived by the institutional review board. From 1995 through 2003, 893 patients with NSCLC had pulmonary resection at the Department of Thoracic Surgery, Aichi Cancer Center Hospital. Of them, 772 patients had potentially curative lobectomy, bilobectomy, or pneumonectomy, excluding 121 patients who had lesser resection (partial resection, segmentectomy, lobectomy without mediastinal node dissection, as shown in Figure 1). Patients who had neoadjuvant or adjuvant treatment were also excluded from this study.

Surgical Technique

Surgical techniques for resection of affected lobes were the same in both groups, consisting either of lobectomy, bilobectomy, or pneumonectomy. Tumors that exhibited adherence to neighboring structures were treated by extended resections with en bloc removal of the lobe or lung with adjacent organs. Locations of lymph nodes were described according to the lymph node map for lung cancer described by Naruke and associates.[17]

In the CD group, resection was combined with a radical systematic en bloc mediastinal lymphadenectomy as described by Naruke and colleauges[1] and Martini and coworkers.[2]

In the SD group, lymph node dissection was performed based on patterns of lobe-specific lymph node metastases. When the tumor was located in the right upper lobe, the upper mediastinal lymph nodes (superior mediastinal nodes, paratracheal nodes, pretracheal nodes, and tracheobronchial nodes) were systematically removed. When the tumor was located in the left upper lobe, aortopulmonary window nodes and aortic nodes in addition to tracheobronchial nodes were resected. In these cases, dissection of lower mediastinum was not performed when the nodes in both the hilum and the upper mediastinum or aortic nodes were free from metastases as shown by intraoperative diagnosis. Intraoperative frozen section analyses were performed when lymph node metastases were suspected macroscopically, and when positive, all patients had CD. Alternatively, when the tumor was located in the lower lobe, subcarinal and lower mediastinal nodes were dissected, and dissection of the superior mediastinum was omitted when the intraoperative diagnosis was negative. By such definition, 625 patients belonged to the CD group and 147 to the SD group. All operations were performed via thoracotomy.

Pre- and Postoperative Measurements

Survival was determined by institutional database, which is updated with an annual institutional census or each patient visit. Serum carcinoembryonic antigen levels were measured using a chemiluminescent immunoassay kit (Abbott, Tokyo, Japan). Blood gas analyses were performed during rest in room air. Clinical and postsurgical staging was determined according to the TNM classification of the International Union Against Cancer.[18] Spirometry testing was performed by medical technicians of the specialty using a spirometer. Trained medical staff asked about smoking history in detail,

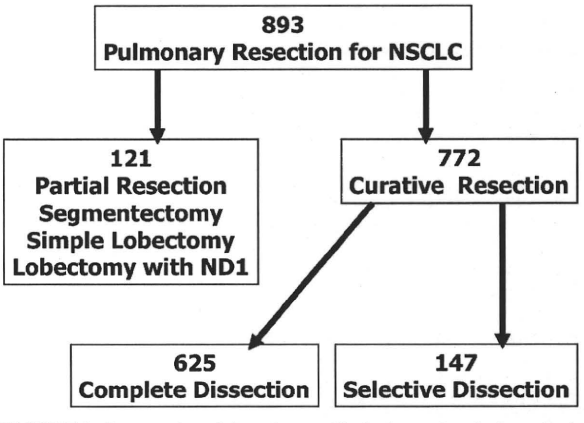

FIGURE 1. Presentation of the cohort and inclusion and exclusion criteria and the number of patients. *NSCLC,* Non–small cell lung cancer.

TABLE 1. Covariates that are considered to concern selection of the types of lymph node dissection

Covariates	Category
Age at diagnosis (y)	<40, 40–59, 60–69, and ≥70
Sex	Male vs female
CEA at diagnosis	Continuous value
Arterial blood gas	
Pao_2, $Paco_2$	Continuous value
Pulmonary function	
% VC, % FEV1.0, % DLCO, and FEV1.0	Continuous value
Clinical stage	
T factor, N factor	Ordinal variable
Smoking index	Continuous value
Histologic type	Adeno, squamous, and others
Operator	Surgeon 1, 2, 3, and 4
Operative procedures	Lobectomy, extended lobectomy, bilobectomy, and pneumonectomy

CEA, Carcinoembryonic antigen; *DLCO*, diffusing capacity for carbon monoxide; *FEV 1.0*, forced expiratory volume in 1 second; *VC*, vital capacity.

and the Brinkman index, defined by the cigarettes smoked per day × total years of smoking, was recorded. Resected specimens were examined histopathologically, and histologic classification was performed according to the World Health Organization classification as shown in Table 1.[19]

Statistical Methods

Propensity score calculation. We calculated the PS using logistic regression based upon factors available that were thought to be potentially associated with patient selection,[20] using the pscore command in STATA version 10 (STATA, College Station, Tex).[21] Fifteen such factors included for calculation of the PS are summarized in Table 1. The number of blocks in the PS calculation was set as 5. After the calculation of their PS, subjects were divided into 4 groups according to quartile.

Survival analysis. Our primary end point was OS, which was defined as the interval between the date of operation and final date of observation or date of death. Comparison of the CD and SD groups was conducted using a log-rank test and a Cox proportional hazard model coupled with forward stepwise covariate selection (threshold P values for removal and inclusion were .20 and .10, respectively) with stratification by PS quartile. The latter aimed to remove residual confounding after PS stratification. Factors examined in the stepwise Cox proportional hazard model were the 15 factors used to calculate PS (Table 1). Comparison of baseline characteristics between SD and CD were examined by the Wilcoxon rank sum test for continuous variables and the Fisher exact test or chi-square test for categorical variables as appropriate. All survival analyses were conducted with STATA version 10.[21]

A total of 772 subjects provided statistical power of more than 88% (1-sided $\alpha = .05$) and 80% (2-sided $\alpha = .05$) to detect a 0.3 difference in the hazard ratio of SD relative to CD, when final failure probability was assumed to be 40%.

RESULTS

Characteristics of subjects in the CD and SD groups are shown in Table 2. Younger patients, patients in earlier stages, patients with adenocarcinoma, and those who had lobectomy were more frequently observed in the SD group, as expected. Therefore, one may assume that direct comparison between SD and CD may be confounded by patients' treatment indication based upon background characteristics. Table 3 shows a comparison of these characteristics between CD and SD according to PS quartile. The number of subjects in quartiles 1, 2, 3, and 4 according to the mode of lymph node dissection (CD; SD) were (188; 5), (172; 21), (157; 36), and (108; 85), respectively. This demonstrates equivalent distribution of background characteristics in each PS quartile between the 2 groups, except that age at operation was significantly higher in the SD group in the highest quartile group.

TABLE 2. Patient characteristics

Variables	CD (%)	SD (%)	*P* value
All patients	625	147	
Sex			
Male	390 (62)	84 (57)	.22*
Female	235 (38)	63 (43)	
Age (y)	19–80 (median 62)	34–82 (median 69)	.0001†
Clinical stage			
IA	276 (44)	94 (64)	.0001*
IB	182 (29)	46 (31)	
IIA	7 (1)	0 (0)	
IIB	73 (12)	6 (4)	
IIIA	76 (12)	1 (1)	
IIIB	11 (2)	0 (0)	
Histology			
AD	436 (70)	117 (80)	.02*
SQC	128 (20)	16 (11)	
Others	61 (10)	14 (9)	
Operation			
Lobectomy	522 (84)	140 (95)	.002*
Bilobectomy/ pneumonectomy/ extended lobectomy	66 (11)	6 (4)	
Lobectomy with adjacent organ resection	37 (5)	1 (1)	
Operator			
1	189 (30)	51 (35)	.001*
2	149 (24)	15 (10)	
3	264 (42)	79 (54)	
4	23 (4)	2 (1)	

CD, Complete dissection; *SD*, selective dissection; *AD*, adenocarcinoma; *SQC*, squamous cell carcinoma. *Fisher exact test or chi-square test. †Wilcoxon rank-sum test.

Figure 2 shows OS after surgery for the CD and SD groups. The 5-year survival probabilities were 71.9% (95% confidence interval [CI]: 68.0–75.5) for the CD group and 76.0% (95% CI: 65.3–83.9) for the SD group. There was no significant difference in OS between the 2 groups ($P = .29$) without stratification by PS. After consideration of PS, difference in survival between the 2 groups was decreased ($P = .8098$). The 5-year survival probabilities stratified by PS quartile are shown in Table 4. This also indicates that the 5-year OSs are consistently comparable across each PS quartile.

In the Cox proportional hazard model not considering PS, a crude hazard ratio (HR) for SD relative to CD was 1.06

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TABLE 3. Patient characteristics stratified by PS quartile

Variables	Quartile 1			Quartile 2			Quartile 3			Quartile 4		
	CD	SD	*P* value	CD	SD	*P* value	CD	SD	*P* value	CD	SD	*P* value
No. of patients	188	5		172	21		157	36		108	85	
Sex												
Male	139	5	.186*	101	12	.890*	87	18	.556*	64	49	.821*
Female	49	0		71	9		70	18		44	36	
Age (y), median	58	59	.773†	59	57	.446†	63	61	.803†	70	73	<.001†
Clinical stage												
IA	23	1	.876*	83	10	.885*	95	26	.417*	75	57	.993*
IB	33	1		60	9		57	9		32	27	
IIA	4	0		3	0		0	0		0	0	
IIB	42	2		25	2		5	1		1	1	
IIIA	76	1		0	0		0	0		0	0	
IIIB	10	0		1	0		0	0		0	0	
Histology												
AD	92	4	.356*	125	17	.517*	128	17	.765*	91	66	.428*
SQC	66	1		34	2		20	2		8	8	
LA	30	0		13	2		9	2		9	11	
Operation												
Lobectomy	115	3	1.0*	149	20	.500*	152	35	.895*	106	82	.767*
Bilobectomy/ pneumonectomy/ extended lobectomy	42	1		17	1		5	1		2	3	
Lobectomy with adjacent organ resection	31	1		6	0		0	0		0	0	
Operator												
1	43	0	.528*	51	6	.987*	53	15	.832*	42	30	.447*
2	64	2		63	8		22	4		0	1	
3	69	3		51	6		78	16		66	54	
4	12	0		7	1		4	1		0	0	

PS, Propensity score; *CD*, complete dissection; *SD*, selective dissection; *AD*, adenocarcinoma; *SQC*, squamous cell carcinoma; *LA*, large cell carcinoma. *Fisher exact test or chi-square test. †Wilcoxon rank-sum test.

(95% confidence interval, 0.68–1.64; $P = .810$). Results of stepwise multivariate analyses adjusted by PS are shown in Table 5. Similar to the crude model, no significant risk change was observed in final multivariate model (HR = 1.17; 0.74–1.85, $P = .500$). Other factors significantly associated with poor prognosis in the model were pathologic N score (2.12 for 1 unit increase, $P < 0.001$) and T score (HR = 1.32 for 1 unit increase, $P = .006$), histology other than adenocarcinoma and squamous cell carcinoma (HR = 2.63 relative to adenocarcinoma, $P < 001$), age (1.72 for 1 age category increase, $P < .001$), percent diffusing capacity for carbon monoxide (0.99 for 1 unit increase, $P = .037$), and lobectomy with adjacent organ resection (HR = 2.26 relative to lobectomy, $P = .004$). Therefore, considering propensity to SD and impact of other prognostic factors, SD showed no significant impact on poor survival compared with CD.

Table 6 shows comparisons of operative time, blood loss, and length of hospital stay in all patients and in those who had muscle-sparing thoracotomy. For patients with SD, operative time was shorter (202 minutes for CD vs 169 minutes for SD), blood loss was smaller (220 g for CD vs 65 g for SD) and length of hospital stay was shorter (15 days vs 13 days). When we limited the analysis to patients who had muscle-sparing thoracotomy, eliminating those who had bilobectomy and pneumonectomy, there were also significant differences for each measurement.

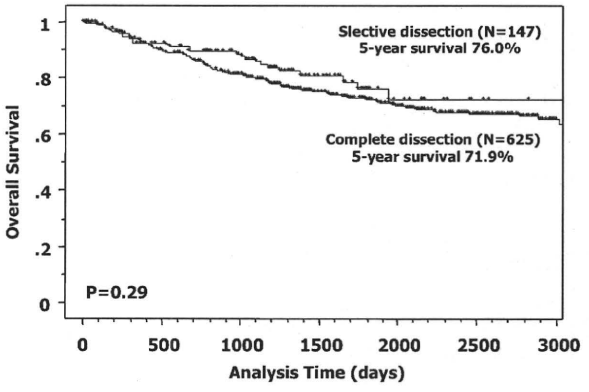

FIGURE 2. Unadjusted overall survival curves of patients stratified by the type of mediastinal dissection (crude).

TABLE 4. The 5-year survival probabilities stratified by PS quartile

	CD	SD	
	5-year survival (95% CI)	5-year survival (95% CI)	*P* value
Total	71.9% (68.0–75.5)	76.0% (65.3–83.9)	.29
Quartile 1	52.3% (44.0–59.9)	60.0% (12.6-88.2)	.83
Quartile 2	74.8% (66.9–81.1)	73.8% (24.4–93.7)	.36
Quartile 3	83.9% (76.6–89.0)	81.1% (62.5–91.9)	.55
Quartile 4	78.3% (68.8–85.2)	74.9% (60.1–84.9)	.56

PS, Propensity score; *CD*, complete dissection; *SD*, selective dissection.

DISCUSSION

To date, a number of retrospective or prospective studies for assessment of mediastinal lymph node dissection (CD or sampling) have been performed.[3-15] Two prospective RCTs compared CD with sampling,[4,9] but the results were not consistent and the question whether mediastinal lymphadenectomy improved survival was still unresolved.

Several investigators reported that there were distinct patterns of metastatic lymphatic spread based on location of the primary tumors. Watanabe and colleagues[14] reported that the metastatic prevalence of patients with pN2 nodes where no N1 nodes were involved was 7% to 11% from upper-lobe tumors to the lower part of the mediastinum. Asamura and colleagues[15] found that the most common site of metastasis for tumors with pN2 located in right upper lobe or tumors in the left superior division was the superior mediastinal station, whereas metastases to the subcarinal station were seen in only 12% to 13% of cases. Indeed, they proposed that subcarinal lymphadenectomy is not always necessary for tumors located there.[15] There is a report that suggests that 3 stations (10, 11, or 12) of N1 lymph nodes or 1 station of N2 nodes (4 for upper-lobe tumors, 5 for left upper-lobe tumors, and 7 for lower-lobe tumors) are sentinel lymph nodes of lung cancer like in breast cancer.[5] Based on these reports, we take lobe-specific lymph node metastases into consideration for omitting lymph node dissection. Besides, patients with unusual lymph node metastases (ie, patients with subcarinal metastases from upper-lobe tumor, or patients with superior mediastinal metastases from lower-lobe tumor) generally had very poor outcome even when these lymph nodes were systematically dissected.

TABLE 5. A final stepwise multivariate analysis model for overall survival

Factor	HR	*P* value	95% LCI	95% UCI
Lymph node dissection (selective vs complete)	1.17	.500	0.71	1.79
pN (continuous)	2.12	<.001	1.80	2.50
pT (continuous)	1.32	.006	1.08	1.60
Pathology				
Adenocarcinoma	1.00			
Squamous cell carcinoma	1.14	.523	0.76	1.71
Others	2.63	<.001	1.74	3.98
Age categories (70–, 60–69, 40–59, and 40–)	1.72	<.001	1.39	2.12
% DLCO (continuous)	0.99	.037	0.99	1.00
Operation				
Lobectomy	1.00			
Middle lobe lobectomy	0.81	.562	0.39	1.68
Bilobectomy/ pneumonectomy/ extended lobectomy	1.19	.426	0.77	1.83
Lobectomy with adjacent organ resection	2.26	.004	1.23	3.74
$Paco_2$ (continuous)	1.00	.063	1.00	1.01

HR, Hazard ratio; *LCI*, lower confidence interval; *UCI*, upper confidence interval; *DLCO*, diffusing capacity for carbon monoxide.

For example, Asamura and associates[15] reported that right lower-lobe tumors with superior mediastinal metastasis carried a particularly poor 5-year survival of only 4.1%.

From the above-mentioned data, SD has been often performed by Japanese surgeons especially when the patients were of poor risk and had earlier diseases. In addition, prognostic difference between CD and SD is expected to be even smaller than that between CD and sampling. Okada and colleagues[5] reported that SD did not worsen prognosis of patients with clinicosurgical stage I NSCLC in their retrospective analysis. The 5-year OS rate was 79.7% for CD

TABLE 6. Intraoperative parameters

	CD	SD	*P* value
All patients (n)	625	147	
Operative time, min (range)	201.9 ± 54.7 (97–482)	169.3 ± 52.2 (90–441)	<.001*
Blood loss, g (median range)	220 (15–1445)	65 (10–1630)	<.001†
Length of stay, d (median range)	15 (6–346)	13 (8–117)	<.001†
Anteroaxillary thoracotomy, vertical muscle-sparing thoracotomy (n)	410	121	
Operative time, min (range)	192.1 ± 48.9 (97–405)	163.3 ± 44.4 (90–371)	<.001*
Blood loss, g (median range)	110 (15–1170)	65 (10–770)	<.001†
Length of stay, days (median range)	15 (6–151)	13 (8–117)	<.003†

Patients who received lobectomy only (except bilobectomy, pneumonectomy or more). *Unpaired *t* test. †Mann–Whitney *U* test.

and 81.9% for SD ($P = .149$). The type of lymph node dissection did not affect OS in the multivariate analysis. However, histologically controlled studies have inherent potential biases in nature.

In this study, we used PS to eliminate such biases as much as possible. We found that there was no significant difference in terms of OS between the 2 groups. However, we admit that the number of covariates to calculate PS was limited. It is clear that firm conclusions must await an adequately designed RCT whose results would be the most important evidence for supporting SD. However, this RCT is almost impossible, and therefore the carefully designed analysis presented here is of great importance.

We also showed that patients who had SD also had significantly shorter operative time, less blood loss, and shorter hospital stay than those who had CD, indicating that SD is less invasive than CD. Okada and associates[5] reported the morbidity rates (dysrhythmia, pneumonia, prolonged air leak, chylothorax, etc) were significantly less for patients with SD (17.3% for CD vs 10.1% for SD, $P = .005$).

In conclusion, SD did not have significantly impact poor survival compared with CD by our analysis applying PS. In addition, it was suggested that SD was associated with less invasiveness. From the practical point of view, it is reasonable to perform SD especially for patients with no apparent lymph node metastases, those with poor pulmonary reserve, or elderly patients.

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Original Article

Serum Carcinoembryonic Antigen Level as a Surrogate Marker for the Evaluation of Tumor Response to Chemotherapy in Nonsmall Cell Lung Cancer

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Purpose: Carcinoembryonic antigen (CEA) is a tumor marker widely used for nonsmall cell lung cancer (NSCLC). The aim of this study was to evaluate changes in serum CEA levels as a surrogate marker for tumor response to chemotherapy in NSCLC.

Methods: From 1995 through 2005, we retrospectively analyzed 24 NSCLC patients who had high serum CEA levels (>5 ng/ml) and who received chemotherapy followed by surgery. We compared serum CEA levels with tumor response, as defined by Response Evaluation Criteria in Solid Tumors (RECIST) or World Health Organization (WHO) criteria, as well as with histological response.

Results: Serum CEA levels after chemotherapy significantly decreased in patients who achieved partial response, defined by RECIST or WHO criteria ($p = 0.004$ and $p = 0.008$, respectively), when compared with the CEA levels before chemotherapy. In contrast, there was no significant difference in CEA levels in patients with either stable disease or no response to chemotherapy. They decreased significantly, however, in patients in whom less than one-third of tumor cells was viable by pathological examination, but not in patients in whom more than a third was viable ($p = 0.008$). Using the receiver-operating characteristic (ROC) curve analysis, we found that a 60% reduction of CEA levels was an appropriate cutoff value in predicting a good response to chemotherapy. When the value was set at that level, the sensitivity of CEA for RECIST was 82%, and the specificity was 69%.

Conclusion: Serum CEA concentration was a useful surrogate marker for the evaluation of tumor response to chemotherapy and seemed to be comparable with RECIST in NSCLC patients who had elevated CEA levels prior to treatment. (Ann Thorac Cardiovasc Surg 2010; 16: 242–247)

Key words: tumor marker, WHO, RECIST

Introduction

The first criterion for the evaluation of tumor response was established in the 1960s. In 1979, the World Health Organization (WHO) devised a series of criteria that codify the evaluation method of tumor response[1]; however, several modifications brought to the WHO criteria raised objectivity and universality concerns. As an effort to clarify and simplify the rules of the assessment of tumor shrinkage, the final version of the Response Evaluation Criteria in

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Solid Tumors (RECIST) was proposed in 2000.[2] RECIST is based on the unidimensional measurement of tumors, which is simpler than the bidimensional measurement stipulated by WHO criteria. RECIST is now regarded as a standard method for evaluating tumor response, which is required for clinical trials, and its validity and reproducibility have also been demonstrated for nonsmall cell lung cancer (NSCLC).[3-5]

The carcinoembryonic antigen (CEA) is a significant tumor marker for malignant tumors, including NSCLC.[6-8] Several studies have revealed the role of serum CEA concentration as a prognostic factor or as a marker for early detection of postoperative recurrence in NSCLC.[9-11] Although evaluation criteria based on imaging modalities, including computed tomography (CT), have become standard in the evaluation of tumor response, serum levels of tumor markers are sometimes used in clinical settings to estimate tumor response, especially in patients with pleural dissemination or effusion and multiple pulmonary metastases, in which measuring tumor size is difficult. To date, the concordance between changes in the levels of tumor markers and tumor reduction evaluated by imaging approaches has not been analyzed extensively. Salgia et al. reported that the response and change in CEA with surgical therapy or chemotherapy in NSCLC.[12] They found a significant decrease in the CEA levels after treatment and suggested that it would be useful to compare changes of tumor marker concentrations with the levels of response to chemotherapy, especially in late-stage NSCLC.

The aim of this study was to determine the role of serum CEA levels in the evaluation of tumor response to preoperative chemotherapy in NSCLC by comparing serum CEA levels with WHO criteria and RECIST. We also analyzed the relationship between these criteria and histological response, which should most precisely reflect the effects of chemotherapy.

Materials and Methods

1. Patients

Approval for this study was obtained from the institutional review board, which waived the need for individual patient consent. During the 11-year period from 1995 through 2005, a total of 65 patients with primary NSCLC received preoperative chemotherapy at the Aichi Cancer Center Hospital in Japan. Among them, 24 with high-serum CEA levels (cutoff, 5 ng/ml) at presentation were analyzed, including 20 males and 4 females ranging in age from 40 to 70 years (median, 55). There were 16 (66.7%) adenocarcinomas, 3 (12.5%) squamous cell carcinomas, and 5 (20.8%) large-cell carcinomas. The cohort included 1 patient with clinical stage IB disease, 5 with IIB disease, 15 with IIIA disease, and 3 with IIIB disease. Platinum doublet chemotherapy was performed in 23 patients, and docetaxel was administered to 1 (Table 1).

Table 1. Patient characteristics

		No. of patients
Gender	Male	20
	Female	4
Age (years)	Median (range)	55 (40–70)
Clinical stage	IB	1
	IIB	5
	IIIA	15
	IIIB	3
Histology	Adeno ca.	16
	Squamous ca.	3
	Large-cell ca.	5
Chemotherapy	CBDCA + PTX	5
	CBDCA + DOC	8
	CBDCA + VNR	1
	CBDCA + VP-16	1
	CDDP + VP-16	2
	MVP	6
	DOC	1

CBDCA, carboplatin; PTX, paclitaxel; DOC, docetaxel; VNR, vinorelbine; VP-16, etoposide; CDDP, cisplatin; MVP, mitomycin C + vindesine + cisplatin
Nine patients received preoperative radiation therapy.

The serum CEA concentration was measured by using a chemiluminescent immunoassay kit (Abbott, Japan). Time intervals between first measurement and the initiation of chemotherapy ranged from 49 to 65 days (median, 58.5 days), and intervals between postchemotherapeutic CEA measurement and the completion of chemotherapy ranged from 17 to 43 days (median, 29). The evaluation of tumor response on CT was performed by a thoracic surgeon (Futoshi Ishiguro). A confirmation of response for 4 weeks guided by RECIST was not mandated because the patients were scheduled for pulmonary resection just after chemotherapy.

2. Histological examination

The histological response to chemotherapy was evaluated by the following criteria from "General Rule for Clinical and Pathological Record of Lung Cancer" (6th edition, The Japan Lung Cancer Society): EF0, no histological response; EF1, more than one-third of the tumor cells

viable; EF2, less than one-third viable; EF3, no viable cells.

3. Statistical analysis

A comparison of the two groups was analyzed by a two-sided Wilcoxon signed rank test. A p value <0.05 was considered significant. Correlation between reduction rate and RECIST, WHO criteria, changes of serum CEA levels, and histological response were investigated by Spearman rank correlation.

Results

Using RECIST, we obtained a partial response (PR) in 11 patients, and stable disease (SD) was observed in 13. The response rate was 46%. Using WHO criteria, we obtained PR in 9 patients, no changes (NC) were observed in 14, and progressive disease (PD) was established in 1. The concordance between RECIST and WHO criteria was 83% (Table 2).

Table 2. Evaluation of tumor response according to RECIST and WHO criteria

Criteria	No. of patients				Response rate (%)
RECIST	CR	PR	SD	PD	45.8
	0	11	13	0	
WHO	CR	PR	NC	PD	37.5
	0	9	14	1	

CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease; NC, no change.

Comparison of serum CEA levels before and after chemotherapy and correlation with tumor response

A comparison of CEA levels before chemotherapy with those obtained after chemotherapy revealed a significant decrease of serum CEA concentration in patients with PR defined by RECIST (median, 17.3 ng/ml to 4.4 ng/ml, $p = 0.004$) or WHO criteria (median, 26.3 ng/ml to 4.3 ng/ml, $p = 0.008$). In contrast, no significant differences were observed in patients with SD (median, 16.8 ng/ml to 19.6 ng/ml, $p = 0.24$) or NC (median, 16.8 ng/ml to 9.9 ng/ml, $p = 0.24$) (Figs. 1A and 1B). Only one patient with PR defined by RECIST showed increased CEA levels after chemotherapy (9.3 ng/ml to 22.6 ng/ml). In that patient, brain metastases appeared 3 months after surgery, and the patient died 17 months later (20 months after surgery).

In regard to pathological findings, CEA levels decreased significantly in patients in whom less than one-third of the tumor cells were viable after surgery (median, 17.0 ng/ml to 4.4 ng/ml, $p = 0.008$), but not in patients in whom more than a third of tumor cells were viable (16.8 ng/ml to 7.9 ng/ml, $p = 0.06$) (Fig. 1C).

Definition of the CEA cutoff value by receiver operating characteristic (ROC) curve

To assess the applicability of CEA concentration as a marker for the evaluation of tumor response to chemotherapy, we next tried to determine the cutoff value of relevant CEA reduction ratio. It was defined as follows: CEA concentration before treatment minus the concentration after treatment divided by the concentration before treatment.

We set the cutoff values of the CEA reduction ratio to 15, 20, 30, 50, 60, 70, and 80%. A patient was considered a good responder when the ratio was superior to each cutoff value, and a poor responder when this parameter was inferior to each value. An ROC curve analysis was carried out based on the sensitivity and specificity of the reduction of the CEA ratio for RECIST or WHO criteria, respectively (Figs. 2A and 2B). The analysis revealed that a 60% reduction in CEA levels was an appropriate cutoff value for the prediction of a good response to chemotherapy, using both RECIST and WHO criteria. At a 60% reduction, the sensitivity of CEA for RECIST was 82.8%, the specificity was 69.2%, the positive predictive value was 69.2%, and the negative predictive value was 75.0%.

A correlation between histological response and CEA reduction ratio was evaluated by RECIST, WHO criteria, or changes in serum CEA levels.

We examined the correlation between histological response and CEA reduction ratio evaluated by RECIST, WHO criteria, and changes in serum CEA levels. The contribution ratio evaluated by RECIST was $r^2 = 0.396$ ($p = 0.003$), by WHO criteria it was $r^2 = 0.426$ ($p = 0.001$), and by CEA reduction ratio it was $r^2 = 0.212$ ($p = 0.04$). The correlation between histological response and CEA reduction ratio evaluated by RECIST, WHO criteria, and changes in CEA values was not significant (Figs. 3A, B and C).

Discussion

The purpose of cancer chemotherapy is to improve the overall survival of patients. In clinical settings, tumor response evaluated by RECIST is an important surrogate marker for survival; however, it cannot be applied in patients with no measurable lesions, including those with