associated with shortened survival in patients with NSCLCs, although sufficient confirmation in well designed multivariate analysis has not been obtained.[21,22] A similar tendency is seen in the analysis of *TP53* gene mutations, which is also thought to be important in pathogenesis of lung adenocarcinomas and many other types of human cancers.[23,24]

In this study, we examined for *EGFR*, *KRAS*, and *TP53* mutations among a large cohort of patients with lung adenocarcinomas who underwent pulmonary resection in a single institution and evaluated their prognostic implications.

PATIENTS AND METHODS

Patients

Primary tumor samples were obtained from 397 consecutive unselected patients with lung adenocarcinomas who underwent potentially curative pulmonary resection at the Department of Thoracic Surgery, Aichi Cancer Center Hospital from May 2000 through December 2005. Appropriate approval was given in advance by our institutional review board and all patients gave written informed consent. Two hundred and twenty-four patients from our previous report of *EGFR* mutational analysis were included in this cohort.[1] There were 201 males and 196 females with an age at diagnosis ranging from 26 to 89 years (median 64). Two hundred and forty-eight patients had stage I disease, 44 stage II, 96 stage III, and 9 stage IV. There were 189 never smokers and 208 ever smokers including current and former smokers. Fifty-six patients had received gefitinib treatment at a daily dose of 250 mg for their recurrent disease. The median follow-up period was 991 days (range, 4–2286).

EGFR Mutational Analysis of Lung Adenocarcinoma Specimens

Tumor samples were obtained at the time of surgery, rapidly frozen in liquid nitrogen, and stored at −80°C. Frozen tissues of the tumor specimens were grossly dissected to enrich tumor cells as much as possible by a surgical pathologist (Y.Y.). Total ribonucleic acid was isolated using RNeasy kits (Qiagen, Valencia, CA).

The first 4 exons (exons 18–21) of the 7 exons (exons 18–24) that code for the TK domain of the *EGFR* gene were amplified with primers F1 (5′-AGCTTGTGGAGCCTCTTACACC-3′) and R1 (5′-TAAAATTGATTCCAATGCCATCC-3′), in a one-step reverse transcription–polymerase chain reaction (RT-PCR) amplification using QIAGEN OneStep RT-PCR Kits (Qiagen, Valencia, CA), as previously described.[1] The cDNA sequence of *EGFR* gene was obtained from GenBank (accession number NM005228). RT-PCR conditions were: one cycle of 50°C for 30 minutes and 95°C for 15 minutes; 40 cycles of 94°C for 50 seconds, 62°C for 50 seconds, and 72°C for 1 minute; followed by one cycle of 72°C for 10 minutes. RT-PCR products were diluted and cycle-sequenced using the Big Dye Terminator v3.1/1.1 cycle sequencing kit (Applied Biosystems, Foster City, CA). The sequencing reaction products were electrophoresed using an ABI PRISM 3100 system (Applied Biosystems). Both forward and reverse sequences were analyzed with the basic local alignment search tool, and the chromatograms were analyzed by manual review.

KRAS and TP53 Gene Mutational Analysis

KRAS mutations and *TP53* mutations were analyzed as previously reported.[20,25] Briefly, *KRAS* gene (exons 1and 2) and *TP53* gene (exons 4 through 10) were amplified and sequenced directly using an ABI PRISM 310 Genetic Analyzer (Applied Biosystems). *KRAS* was analyzed for 254 tumors and *TP53* was analyzed for 376 tumors. Because this study represents a retrospective review of our cohort, we could not obtain the result of all 3 mutational statuses of 397 patients.

Statistical Analysis

For comparisons of proportions, the χ^2 test or Fisher exact test were used. The Kaplan-Meier method was employed to estimate the probability of survival as a function of time, and survival differences were analyzed by the log-rank test. The two-sided significance level was set at $p < 0.05$. The Cox proportional hazards modeling technique was applied for multivariate analysis of the overall survival. All analyses were performed using a StatView (version 5, SAS institute Inc., Cary, NC).

RESULTS

Gene Mutations in Unselected Lung Adenocarcinoma Specimens

EGFR gene mutations were detected in 196 of 397 patients (49.4%). There were 105 point mutations, 83 deletion mutations, and 12 duplication/insertion mutations. Details of these mutations are shown in Figure 1.

Ninety-two patients had L858R. Six patients had point mutations occurring at codon 719 in exon 18 resulting in the substitution of glycine with aranine, serine, or cysteine (G719X). Two patients had point mutations occurring at codon 768 in exon 20 and one had point mutation occurring at codon 861 in exon 21. Almost all of the 83 deletion mutations occurred around the 5 amino acid residues ELREA at codons 746–750 in exon 19. About half (39/83) of deletion mutations were simple deletions of ELREA. Thirty-four of the deletions were coupled with point mutations or insertions, yielding various changes in amino acid sequences. In 12 duplication/insertion mutations, 1 was in exon 19, and 11 were in exon 20.

In the 196 patients with mutations, 92 of the mutations were L858R (47%) and 83 were exon 19 deletions (42%), altogether accounting for about 90% of all the *EGFR* mutations found. The 4 major classes of mutations (i.e., L858R, deletions, mutations at codon 719, and duplications/insertions) never occurred simultaneously, confirming our previous observation.[1]

KRAS mutations were present in 32 of 254 patients (12.6%). Twenty-seven occurred in codon 12, 2 were in codon 13, and 3 were in codon 61. *TP53* mutations were present in 142 of 376 patients (37.8%). *KRAS* mutations were never found in tumors with *EGFR* mutations, showing a mutually exclusive relationship ($p < 0.0001$; Table 1). In

A Point mutations

exon	category	amino acid change	number
18	G719X	G719A	3
		G719S	2
		G719C	1
20	others	S768I	1
		S768I + V769L	1
21	L858R	L858R	89
		L858R + D761Y	1
		L858R + S768I	1
		L858R + T790M	1
	others	L861Q	1

B Deletions

Exon 19

```
740         750         760
KIPVAIKELREATSPKANKEILD
```

	number
KIPVAIK.....TSPKANKEILD	39
KIPVAIK...RPTSPKANKEILD	1
KIPVAIK.....ASPKANKEILD	1
KIPVAIK....IPSPKANKEILD	1
KIPVAIK....VASPKANKEILD	1
KIPVAIK......APKANKEILD	1
KIPVAIK......VPKANKEILD	1
KIPVAIKE...STSPKANKEILD	13
KIPVAIKE...PTSPKANKEILD	5
KIPVAIKE...QTSPKANKEILD	1
KIPVAIKE.....SPKANKEILD	3
KIPVAIKE....QSPKANKEILD	2
KIPVAIKE....PSPKANKEILD	1
KIPVAIKE......PKANKEILD	3
KIPVAIKE....QHPKANKEILD	2
KIPVAIKE....QRPKANKEILD	1
KIPVAIKELREA.....NK.ALD	1
KIPVAIKELREA.....NLD	1
KIPVAIKELREA.....SLD	1
KIPVAIKELREA.....LD	1

C Insertions/Duplications

Exon 19

```
740         750         760
KIPVAIKELREATSPKANKEILD
```

	number
KIPVAIKIPVAIKELREATSPKANKEILD	1

Exon 20

```
   760         770
KEILDEAYVMASVDNPHVCR
```

	number
KEILDEAFQEAYVMASVDNPHVCR	1
KEILDEAYVMATLASVDNPHVCR	1
KEILDEAYVMASVASVDNPHVCR	2
KEILDEAYVMASVGVDNPHVCR	1
KEILDEAYVMASVGFNPHVCR	1
KEILDEAYVMASVGYNPHVCR	1
KEILDEAYVMASVDSVPNPHVCR	1
KEILDEAYVMASVDNHPHVCR	1
KEILDEAYVMASVDNPDNPHVCR	1
KEILDEAYVMASVDNPHVHVCR	1

FIGURE 1. Details of amino acid changes of epidermal growth factor receptor (*EGFR*) gene mutations. *A*, Details of point mutations, *B*, Details of deletion mutations, *C*, Details of insertion/duplication mutations.

TABLE 1. Relationship Between Three Gene Mutations and Clinicopathological Features

		EGFR			*KRAS*			*TP53*		
Variables	**Category**	**Mut**	**Wt**	***p***	**Mut**	**Wt**	***p***	**Mut**	**Wt**	***p***
n		196 (49%)	201		32 (13%)	222		142 (38%)	234	
Sex	Male	76 (38%)	125	<0.0001	24 (19%)	101	0.0018	91 (48%)	99	<0.0001
	Female	120 (61%)	76		8 (6%)	121		51 (27%)	135	
Age	<64	87 (46%)	103	0.1716	18 (15%)	100	0.2437	77 (42%)	105	0.0785
	≤64	109 (53%)	98		14 (10%)	122		65 (34%)	129	
Smoking status	Never	128 (68%)	61	<0.0001	8 (6%)	116	0.0114	49 (28%)	129	0.0001
	Current or former	68 (33%)	140		24 (18%)	106		93 (47%)	105	
Stage	I	127 (51%)	121	0.3443	17 (11%)	139	0.6986	77 (33%)	158	0.0098
	II–IV	69 (46%)	80		15 (15%)	83		65 (46%)	76	
Differentiation	Well to mod	148 (57%)	113	<0.0001	21 (12%)	148	0.8634	75 (30%)	174	<0.0001
	Poor	37 (32%)	80		11 (15%)	63		62 (57%)	46	
KRAS	Mut	0 (0%)	33	<0.0001	—	—	—	—	—	—
	Wt	127 (57%)	95		—	—	—	—	—	—
TP53	Mut	64 (45%)	78	0.1165	15 (15%)	86	0.4241	—	—	—
	Wt	125 (53%)	109		17 (11%)	132		—	—	—

Mod, moderately; Mut, mutation; *n*, number; Wt, wild-type; EGFR, epidermal growth factor receptor.

contrast, *TP53* mutations and *EGFR* or *KRAS* mutations appeared to occur independently ($p = 0.1165$ and 0.4241, respectively).

Relationships Between Mutations and Clinicopathological Features

EGFR mutations were significantly more frequent in females (61%) than in males (38%; $p < 0.0001$), in never-smokers (68%) than in smokers (33%; $p < 0.0001$), and in more prevalent among patients with well to moderately differentiated adenocarcinoma (57%) than in those with poorly differentiated adenocarcinoma (32%; $p < 0.0001$; Table 1). In contrast, *KRAS* mutations were significantly more frequent in males (19%) than in females (6%; $p = 0.0018$) and in smokers (18%) than in never-smokers (6%; $p = 0.0114$). The incidences of *TP53* mutations also contrasted with those for *EGFR* mutations. They were significantly more frequent in males (48%) than in females (27%; $p < 0.0001$), in smokers (18%) than in never-smokers (6%; $p = 0.0001$), in patients with poorly differentiated adenocarcinoma (57%) than in those with well to moderately differentiated adenocarcinoma (30%), and in those with advanced stage tumor (46%) than with early stage tumor (33%; $p = 0.0098$). There was no significant difference between the patients with the 2 major types of *EGFR* mutations (deletion and L858R) in clinicopathological features (Table 2).

When we divided smokers into three categories according to the amount of smoking exposure by pack-year, there was a trend showing that the higher the exposure, the lower the incidence of *EGFR* mutations (Figure 2). In contrast, the incidences of *KRAS* and *TP53* mutations increased along with increased smoking exposure.

Survival Analysis

Many studies have indicated that patients with *EGFR* mutations survived for a longer period than those without *EGFR* mutations after gefitinib treatment. Therefore, we performed survival analysis, excluding 56 patients who were treated with gefitinib when they had recurrent diseases. Univariate analysis showed that never-smokers, patients with early-stage and with well to moderately differentiated tumors survived significantly for a longer period, and females tended to survive longer (Figures 3*A–D*). Patients with *EGFR* mutations also survived significantly for a longer period than those without the mutations ($p = 0.0046$ by log-rank test;

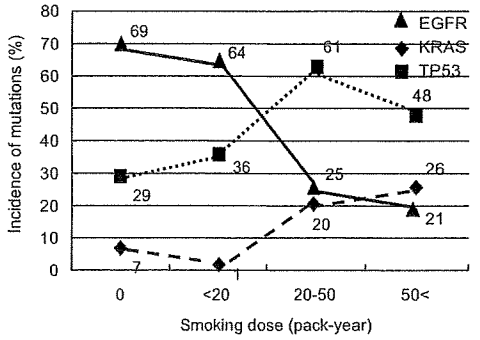

FIGURE 2. Incidence of epidermal growth factor receptor (*EGFR*), *KRAS*, and *TP53* gene mutations according to smoking dose in 250 patients for whom we performed mutational analyses of all three genes.

Figure 4*A*). The result of survival analysis using only 2 major mutations (deletions and L858R) compared with wild type was almost same as the result of all EGFR mutations ($p = 0.0075$; Figure 4*B*). There was no difference in overall survival between the patients with exon 19 deletions and those with L858R mutations ($p = 0.4144$; Figure 4*C*). In contrast, there was a tendency that patients with *KRAS* mutations survived for a shorter period than those without mutations, whereas there was no statistically significant difference ($p = 0.2183$; Figure 4*D*). Patients with *TP53* mutations survived significantly for a shorter period than those without the mutations ($p = 0.0230$; Figure 4*F*). There was no significant difference between patients with *EGFR* mutations and those with *KRAS* mutations in 208 patients who were able to be performed both mutational analyses ($p = 0.0713$; Figure 4*E*).

Multivariate analysis using Cox proportional hazards model revealed that being a never-smoker ($p = 0.0310$) and disease stage ($p < 0.0001$) were independent prognostic factors (Table 3). However, none of the gene mutations was an independent prognostic factor (*EGFR*, $p = 0.3225$; *KRAS*, $p = 0.8500$; *TP53*, $p = 0.3191$).

DISCUSSION

We found that none of the, *KRAS*, and *TP53EGFR*, *KRAS*, and *TP53* genes was an independent prognostic factor when tested by multivariate analysis, whereas they had sig-

TABLE 2. Relationship Between two Major Types of *EGFR* Mutations and Clinicopathological Features

Variables	Category	All Mutations	Exon 19 Deletion	L858R	*p*
n		196	83	92	
Sex	Male	76	32 (39%)	34 (37%)	0.8276
	Female	120	51 (61%)	58 (63%)	
Smoking status	Never	128	56 (67%)	63 (68%)	0.8865
	Current or former	68	27 (33%)	29 (32%)	
Differentiation	Well to mod	148	64 (82%)	72 (83%)	0.9051
	Poor	37	14 (18%)	15 (17%)	

Mod, moderately; *n*, number.

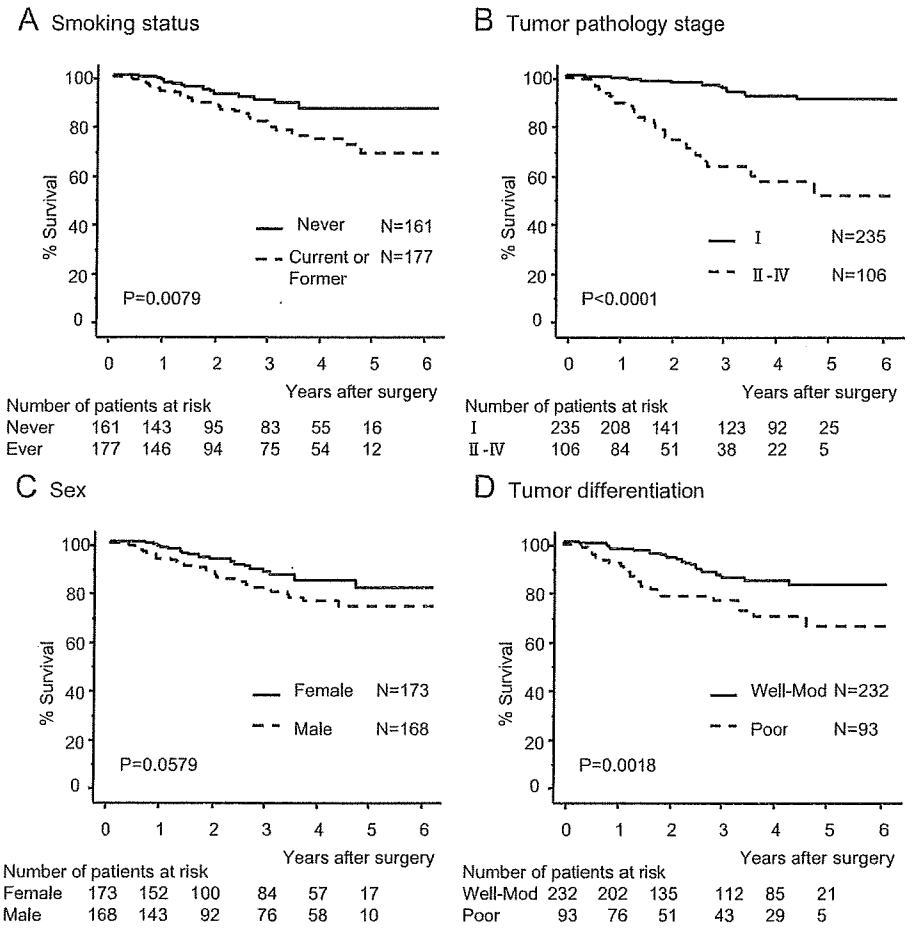

FIGURE 3. Effect of clinicopathological features on the survival of the patients with pulmonary adenocarcinoma without gefitinib treatment. *A*, Overall survival in relation to smoking status, *B*, Overall survival in relation to tumor pathology stage, *C*, Overall survival in relation to sex, *D*, Overall survival in relation to tumor differentiation.

nificant prognostic value (except for *KRAS*) by univariate analysis. We consider that statistical significance in the univariate analysis might have been caused by confounding with other prognostic factors such as sex, smoking status, and tumor differentiation. *EGFR* mutations were more prevalent in females, in never smokers, or in well to moderately differentiated tumors, which are thought to be predictors of better survival among patients with NSCLCs. In contrast, *KRAS* and *TP53* mutations were more prevalent in males or in smokers, which are thought to be predictors of worse survival. When we adjusted for these prognostic factors, the three genes lost their prognostic impact by univariate analysis.

In this study, we focused on the prognostic implications of EGFR mutation, not the predictive implications. Therefore we excluded the patients who received gefitinib from the current survival analysis. However, there is possibility that removal of these patients introduced an adverse bias as they were patients who recurred therefore may be more likely to have had worse survival. The mutation frequencies of *EGFR*, *KRAS*, and *TP53* in patients with gefitinib treatment were 64%, 10%, and 49%, respectively (those of patients without gefitinib treatment were 49%, 13%, and 38%, respectively). Patients with gefitinib had a higher prevalence of *EGFR* mutations (64% versus 49%). This was due to the tendency to select patients with favorable characteristics; i.e., adenocarcinoma, female, and never-smokers.

Recently, Marks et al.[26] have reported a prognostic analysis of *EGFR* and *KRAS* mutations in 296 patients who underwent resection in their institution for stage I–III lung adenocarcinomas, without any treatment with TGFR-TKIs. They found by univariate analysis that patients with *EGFR* mutations survived for a longer period than those without mutations ($p = 0.031$), and the patients with *KRAS* mutations survived for a shorter period (the statistical value was not shown). These results are consistent with our results. They described that the survival difference approached significance on multivariate analysis, whereas there was no detailed description about the p value, or which factors they used for multivariate analysis. Why such a difference on multivariate analysis occurred was not clear. The difference between races or the difference of the mutation frequency might be related about it.

We confirmed that the incidence of *EGFR* mutations decreased along with smoking exposure, as indicated in our previous report.[1] We found that the incidences of *KRAS* and *TP53* mutations increased along with the increasing of smoking exposure, in contrast to the tendency for *EGFR* mutations.

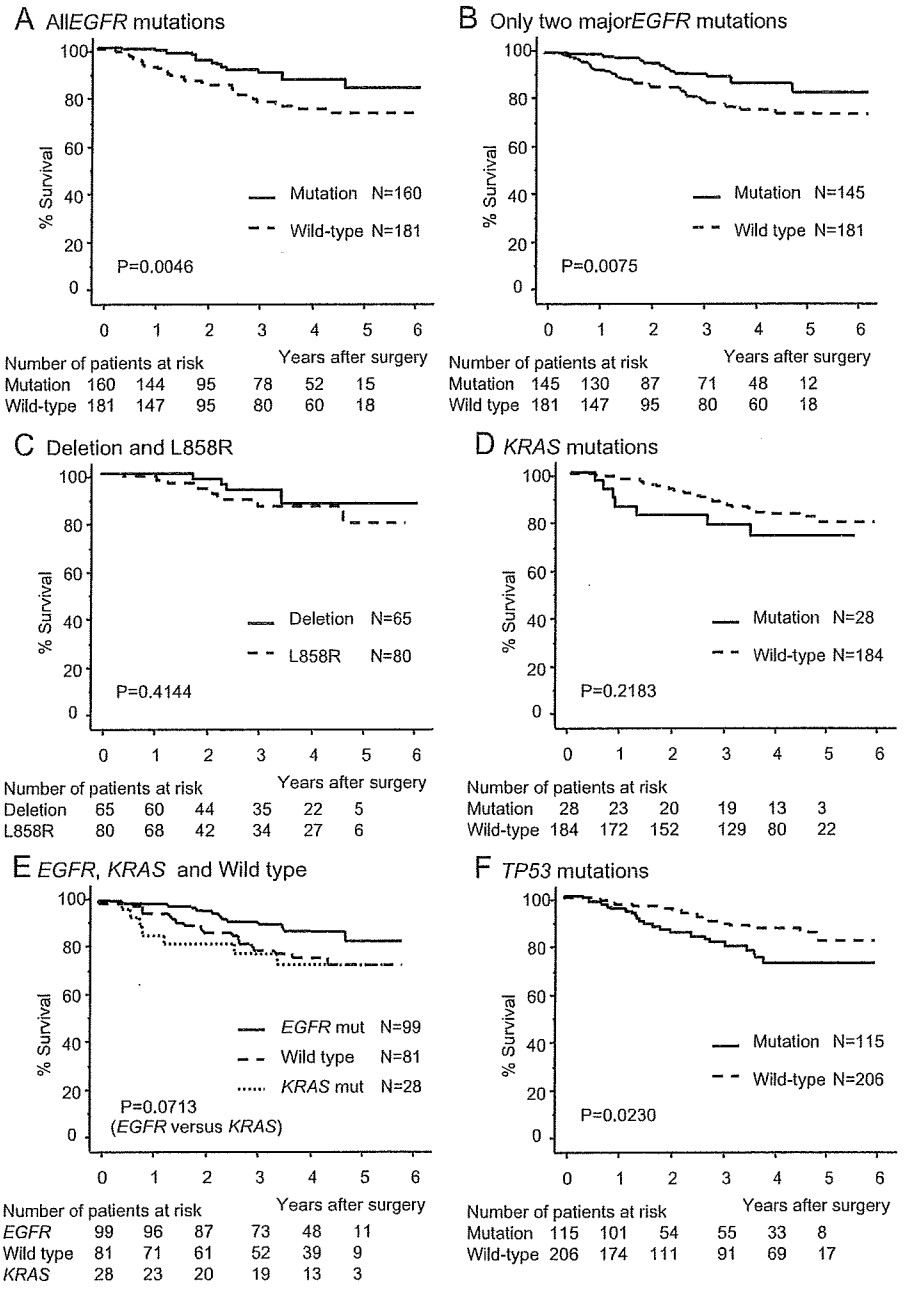

FIGURE 4. Effect of gene mutations for the survival of the patients with pulmonary adenocarcinoma without gefitinib treatment. *A*, Overall survival in relation to epidermal growth factor receptor (*EGFR*) mutations, *B*, Overall survival among patients with major two types of *EGFR* mutations (exon 19 deletions and L858R mutations) and with no mutations, *C*, Overall survival among patients with an exon 19 deletion compared with those harboring an L858R mutation, *D*, Overall survival in relation to *KRAS* mutations, *E*, Overall survival among patients with *EGFR* mutation, *KRAS* mutation, and wild type for both mutations, *F*, Overall survival in relation to *TP53* mutations.

Smoking status was a significant prognostic factor shown by multivariate analysis in this study. The confounding of mutational status and smoking status is very important for survival analysis. However, the apparent negative correlation with *EGFR* mutations and smoking dose arose from the statistical dilution of *EGFR*-mutated tumors with the increased of tumors with wild-type *EGFR* that occurs along with increasing rate of smoking dose. We found this effect in our recent case-control study.[27] The odds ratio for the patients with wild-type *EGFR* increased significantly with the increased smoking exposure. In contrast, no significant change in risk was observed for the patients with *EGFR* mutations. Cumulative exposure to smoking showed a linearly increased risk for *EGFR*-wild-type NSCLCs only. These results indicate that *EGFR* mutations are caused by carcinogens other than those found in tobacco smoke.

Many reports have revealed that the response rates of patients with *EGFR* mutations for EGFR-TKIs treatment are high, and that patients with *EGFR* mutations survived for longer periods than those without mutations.[8] Considering these results along with our limited current analyses, the presence of *EGFR* mutations would be a predictive factor for

TABLE 3. Cox Proportional Hazards Model for Survival Analysis

		Univariate Analysis			Multivariate Analysis		
Variables	**Category**	**HR**	**95% CI**	***p***	**HR**	**95% CI**	***p***
Sex	Female/male	0.577	0.325–1.026	0.0611	1480	0.583–3.759	0.4095
Smoking status	Never/current or former	0.447	0.242–0.822	0.0097	0.288	0.093–0.893	0.0310
Differentiation	Well to mod/poor	0.417	0.236–0.734	0.0024	1.215	0.553–2.666	0.6276
Stage	I/II–IV	0.145	0.078–0.270	<0.0001	0.162	0.075–0.349	<0.0001
EGFR	Mut/Wt	0.419	0.225–0.779	0.0060	0.665	0.297–1.492	0.3225
KRAS	Mut/Wt	1.667	0.732–3.798	0.2234	1.091	0.443–2.685	0.8500
TP53	Mut/Wt	1.978	1.086–3.602	0.0257	1.471	0.689–3.141	0.3191

CI, confidence intervals; HR, hazard ratio; CI, confidence interval; Mod, moderately; Mut. mutation; Wt, wild-type; EGFR, epidermal growth factor receptor.

the gefitinib treatment, but is not an independent prognostic factor for pulmonary adenocarcinomas without gefitinib treatment. To determine whether *EGFR* mutations indeed have a predictive impact for treatment with EGFR-TKIs and that they do not have a prognostic impact without treatment, randomized prospective clinical trials are necessary. The West Japan Thoracic Oncology Group launched a phase III clinical trial (WJTOG3405) comparing gefitinib monotherapy with cisplatin plus docetaxel in lung-cancer patients with *EGFR* mutation. Primary end point is progression-free survival, to avoid confounding by possible crossover between 2 arms and the sample size is 200 patients with EGFR mutations.

Shigematsu et al.[15] reported that patients with NSCLCs harboring L858R mutations survive for significantly longer than those with exon 19 deletions who did not receive EGFR-TKI. However, we found no such significant difference, in agreement with Sugio et al. and Marks et al.[16,26] One possible explanation for this discrepancy could be the association between the types of mutations and clinicopathological features. There was almost no difference in the incidence of predictors of better survival—sex, smoking status, or tumor differentiation—between exon19 deletions and L858R mutations in our cohort. For patients treated with RGFR-TKI, several authors claims that patients with exon 19 deletions have better prognosis than those with L858R.[28,29] It is suggested that the treatment with EGFR-TKI contributes to the prognosis of patients with EGFR mutations, and the degree of contribution might be different according to the types of mutation.

In conclusion, we found that none of the mutations commonly found in adenocarcinoma of the lung; i.e., *EGFR*, *KRAS* and *TP53* mutations, was independently associated with prognosis of patients, when adjusted by clinical factors such as smoking history, stage, differentiation grade, and sex.

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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

Futoshi Ishiguro, MD,[a] Keitaro Matsuo, MD,[b] Takayuki Fukui, MD,[a] Shoichi Mori, MD,[a,c] Shunzo Hatooka, MD,[a] and Tetsuya Mitsudomi, MD[a]

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. (J Thorac Cardiovasc Surg 2010;139:1001-6)

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

From the Department of Thoracic Surgery,[a] Aichi Cancer Center Hospital; Division of Epidemiology and Prevention,[b] Aichi Cancer Center Research Institute; and Department of Thoracic Surgery,[c] Japanese Red Cross Nagoya First Hospital, Nagoya, Japan.

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Address for reprints: Futoshi Ishiguro, MD, Department of Thoracic Surgery, Aichi Cancer Center Hospital, 1-1 Kanokoden, Chikusa-Ku Nagoya 461-8681, Japan (E-mail: hishi@aichi-cc.jp).

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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,

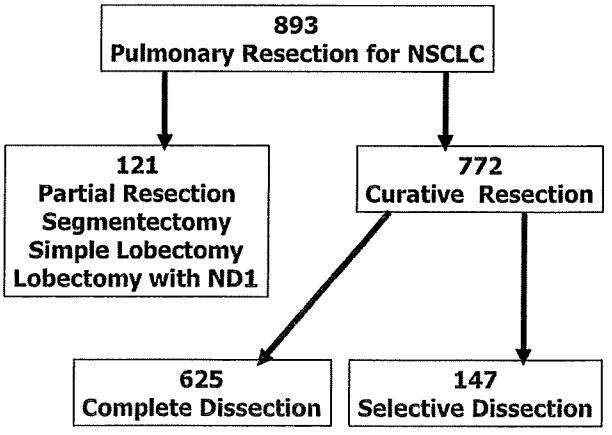

FIGURE 1. Presentation of the cohort and inclusion and exclusion criteria and the number of patients. *NSCLC*, Non–small cell lung cancer.

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

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.

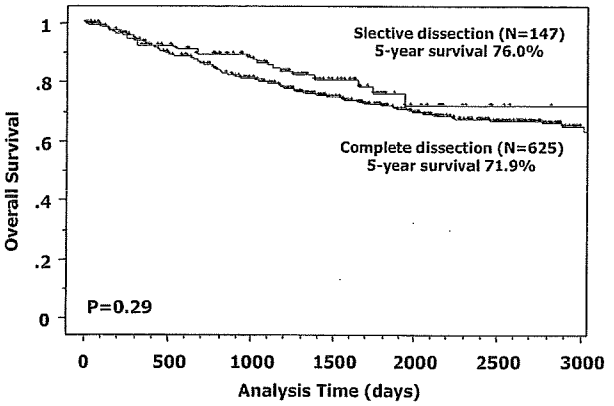

FIGURE 2. Unadjusted overall survival curves of patients stratified by the type of mediastinal dissection (crude).

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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Clinicopathological features of small-sized non-small cell lung cancer with mediastinal lymph node metastasis

Takayuki Fukui*, Tatsuya Katayama, Simon Ito, Tetsuya Abe, Shunzo Hatooka, Tetsuya Mitsudomi

Department of Thoracic Surgery, Aichi Cancer Center Hospital, 1-1 Kanokoden, Chikusa-Ku, Nagoya 464-0021, Japan

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ABSTRACT

Introduction: In clinical practice, peripheral small-sized lung cancers with positive mediastinal lymph nodes are sometimes detected. To understand the characteristics of these aggressive tumors, we reviewed the clinicopathological features of small-sized non-small cell lung cancer patients with mediastinal lymph node metastasis resected in our institution.

Methods: We studied 360 patients with small-sized lung lesions with a maximum diameter of 2 cm or less. The clinicopathological characteristics of each patient were reviewed and compared among the subgroups, which were stratified according to pathological nodal status.

Results: 21 patients (5.8%) had a positive mediastinal lymph node. Among them, 17 patients had lung lesions larger than 1.5 cm. No mediastinal nodal involvement was found in patients with squamous cell carcinomas. In contrast, mediastinal nodal involvement was significantly common in patients with poorly differentiated carcinoma ($P = 0.004$) and high serum carcinoembryonic antigen levels detected during preoperative evaluation ($P = 0.006$). None of the 14 patients with upper lobe tumor had a positive subcarinal lymph node. Lower lobe tumors frequently developed extensive multiple-level involvement, which included the upper mediastinum. Radiographic evaluation of pN2 patients using computed tomography revealed a total absence of ground-glass opacity, or the presence of a small area of ground-glass opacity.

Conclusions: Most small-sized non-small cell lung cancer cases with mediastinal lymph node metastasis were invasive adenocarcinoma with poor differentiation, which usually showed a solid shadow without ground-glass opacity on computed tomography.

1. Introduction

The use of recently developed methods of radiographic investigation, particularly low-dose helical computed tomography (CT) for lung cancer, has increased the incidence of detection of small peripheral lung lesions. Among these small lesions, bronchioloalveolar carcinoma (BAC), which is classified as a noninvasive carcinoma with no evidence of stromal, vascular, or pleural invasion in the revised histological classification of the World Health Organization (WHO), shows better outcomes when compared with invasive adenocarcinomas [1–3]; thus, patients with BAC detected as a peripheral small-sized lesion may be good candidates for limited resection.

In contrast, mediastinal lymph node involvement is sometimes detected in patients with small-sized peripheral lung cancer, even when the tumor size is smaller than 2 cm in diameter, and the risks of limited resection for these patients have been documented [4,5]. An inadequate limited resection can lead to an incomplete resection or misdiagnosis of nodal staging, thus depriving these patients of the opportunity to be cured or to receive adjuvant chemotherapy; therefore, the management and determination of the appropriate operative mode for each patient with small-sized lung cancer requires an understanding of the characteristics of these aggressive tumors, which cannot be inferred from the size of the tumor alone.

In this study, we reviewed the clinicopathological data of small-sized (2 cm or less) non-small cell lung cancer (NSCLC) patients with mediastinal lymph node metastasis treated in our institution.

2. Materials and methods

2.1. Patient cohort

Approval for this study was obtained and the need for individual patient consent was waived by the institutional review board. During an 11-year period from 1997 to 2007, 1513 patients with primary lung cancers underwent pulmonary resection at the Aichi Cancer Center. In this study, we reviewed 360 of these patients presenting with a small-sized lesion with a maximum diameter of 2.0 cm or less. Data extracted from each patient's medical record included age, sex, smoking history, tumor histology and location,

* Corresponding author. Tel.: +81 52 762 6111; fax: +81 52 764 2967.
E-mail address: tfukui@aichi-cc.jp (T. Fukui).

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tumor size, surgical procedure, preoperative serum carcinoembryonic antigen (CEA) level, date of last follow-up, and death from any cause. The maximum diameter of the primary tumor was measured on formalin-fixed surgical specimens by pathologists. The patient's cohort included 162 men (45%) and 198 women (55%) ranging in age from 26 to 83 years (median, 62 years). There were 326 (91%) adenocarcinomas, 21 (6%) squamous-cell carcinomas, nine (2%) large-cell carcinomas, and four (1%) other types of cancers. No patient has received preoperative therapy such as chemotherapy or radiotherapy. When limited for the period of 2004 through 2007, 81 of 166 patients (49%) were preoperatively diagnosed among this cohort.

2.2. Evaluation of lymph node metastasis

The criterion for lymph node enlargement assessed during preoperative evaluation using chest CT is a size larger than 1.0 cm in the short axis of each mediastinal node. Here, lymph nodes were dissected from the adipose connective tissue of the corresponding anatomic regions, as subdivided by the surgeon immediately during the operation. The lymph nodes classified in this way were sent for histopathological examination after hematoxylin and eosin staining. The absence or presence as well as the anatomic extent of nodal metastasis (N categories) were recorded, as defined by the Tumor-Node-Metastasis classification according to Naruke's lymph node mapping.

2.3. Statistical analyses

The Student's t test was used to compare the numbers of lymph nodes with metastasis between each group. The Kaplan–Meier method was used to plot the survival curves and the log–rank test was used to evaluate differences between subgroups. The threshold of significance was set at $P<0.05$. Statistical calculations were performed using a statistical package (StatView version 5.0; SAS Institute Inc., Cary, NC, USA).

3. Results

3.1. Characteristics of the patients with mediastinal lymph node metastases

The breakdown by pathological N category was 322 (89%) pN0, 17 (5%) pN1, and 21 (6%) pN2 patients. The comparison of the clinicopathological characteristics between pN0 and pN2 patients is shown in Table 1. Mediastinal lymph node metastasis was found in only 1 (5%) and 3 (14%) patients with tumors sized less than 1.0 cm and from 1.1 to 1.5 cm in diameter, respectively, whereas positive mediastinal lymph nodes were found in 17 (81%) patients with a tumor larger than 1.5 cm ($P=0.01$). The detailed distribution of pN2 patients according to tumor size is shown in Fig. 1.

Interestingly, no mediastinal nodal involvement was found in patients with squamous cell carcinomas, although this finding was of borderline significance ($P=0.06$). The analysis of tumor differentiation mainly among adenocarcinoma patients revealed that mediastinal nodal involvement was significantly common in patients with poorly differentiated carcinoma ($P=0.004$). In addition, the mean preoperative serum CEA level in patients with mediastinal lymph node metastasis was significantly higher than in those without metastasis (5.0 ± 10.9 and 2.7 ± 2.6, respectively; $P=0.006$).

Preoperative radiographic evaluation of pN2 patients using CT showed a total absence ground-glass opacity (GGO), or the presence of a small (25% or less) GGO area (three representative cases are shown in Fig. 2).

Table 1
Comparison of patient characteristics according to pN status.

	pN status		P
	pN2 ($n=21$)	pN0 ($n=322$)	
Age			
Median (range)	61 (46–79)	63 (26–83)	0.56
Sex			
Male	8	142	0.66
Female	13	180	
Smoking history			
<20 pack-years	14	211	0.96
≥20 pack-years	7	108	
Size			
~10 mm	1	54	0.01
11–15 mm	3	117	
16–20 mm	17	151	
Histology			
AD	19	291	0.06
(BAC)	(0)	(16)	
SQ	0	19	
LA	2	5	
Others	0	7	
Surgical procedure			
Lobectomy	20	269	0.32
Segmentectomy	1	30	
Wedge resection	0	23	
Differentiation			
Well	0	107	0.004
Moderate	6	28	
Poor	12	127	
Preoperative CEA			
Mean ± SD	5.0 ± 10.9	2.7 ± 2.6	0.006

AD, adenocarcinoma; BAC, bronchioloalveolar carcinoma; CEA, carcinoembryonic antigen; LA, large-cell carcinoma; SD, standard deviation; and SQ, squamous-cell carcinoma.

3.2. Survival analysis

The overall five-year survival rate for patients with NSCLC tumors with a diameter of 2.0 cm or less was 83%, whereas that for patients with tumors of 1.0 cm or less, 1.1–1.5 cm, and 1.6–2.0 cm was 91%, 88%, and 80%, respectively (Fig. 3A). The five-year survival rate for patients with mediastinal lymph node metastasis (pN2) was 23%, which was significantly worse when compared with patients without nodal involvement (pN0), who had a survival rate of 89% ($P=0.0001$, Fig. 3B).

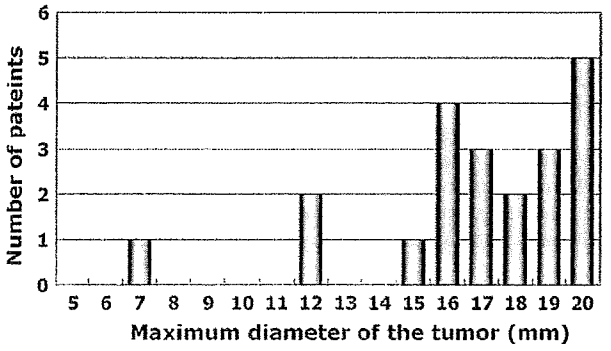

Fig. 1. Detailed distribution of patients with mediastinal lymph node metastasis according to tumor size. Among 21 patients, 17 patients (81%) with a tumor larger than 1.5 cm had positive mediastinal lymph nodes.

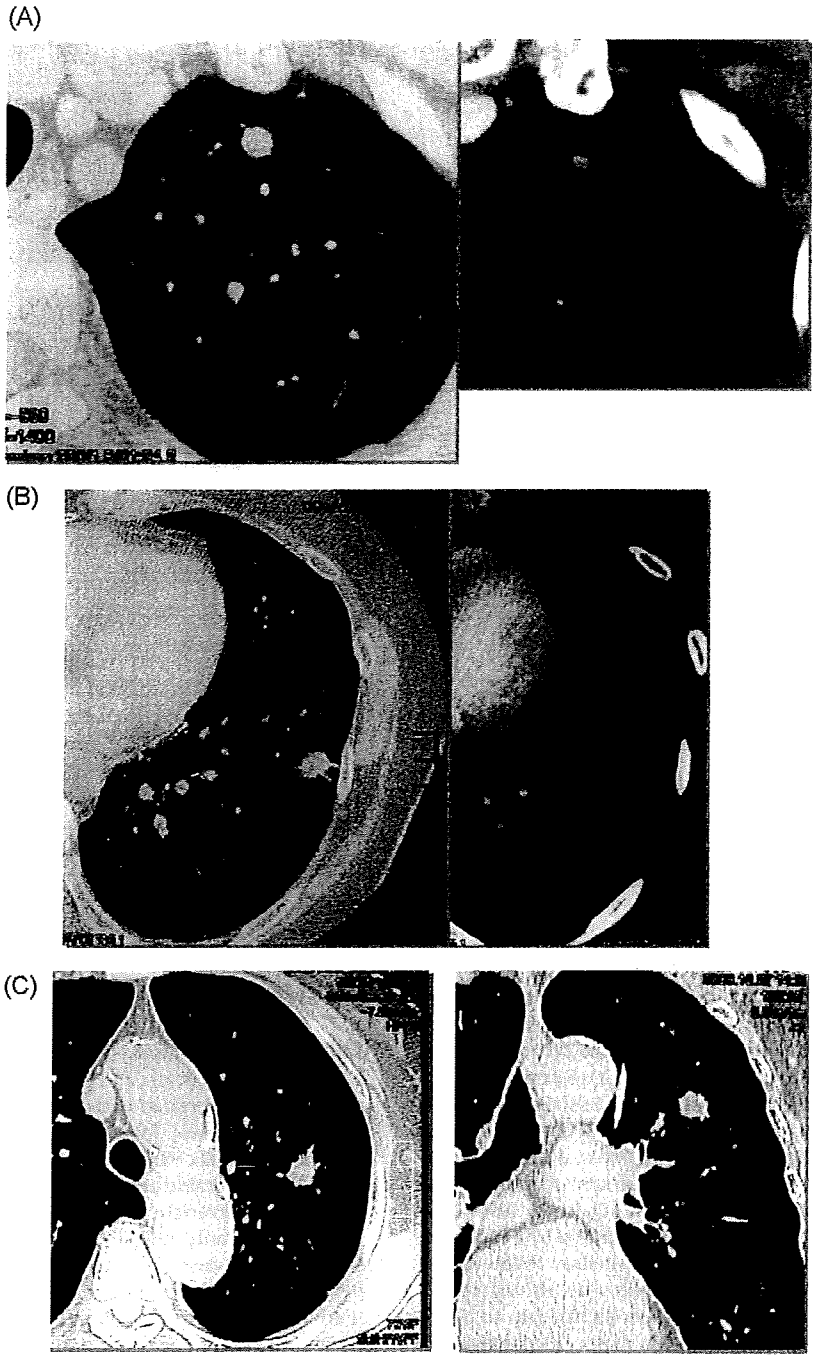

Fig. 2. Computed tomography (CT) from three representative patients with mediastinal lymph node metastasis. (A) CT images from a 55-year-old man. (B) CT images from a 53-year-old woman. (C) CT images from a 76-year-old woman. All three tumors showed a solid pattern without GGO on CT.

3.3. *Distribution of metastatic stations*

The distribution of mediastinal lymph node metastasis grouped by lobe of primary tumor is shown in Fig. 4. Metastasis was found at a single station in 12 patients, whereas nine patients had multistation metastasis. The most frequent site of metastasis in the mediastinum differed according to the location of the primary tumor. Right upper lobe tumors mainly developed extensive involvement within the upper mediastinum, especially in the pretracheal (#3) nodes (7/7). Three of four right-lower lobe primary cases presented upper mediastinal nodal involvement, and one these cases showed no involvement of the subcarinal node (#7). On the left side, the Botallo's (#5) and para-aortic (#6) nodes were mainly involved in tumors of the upper lobe (7/7 and 4/7, respectively), whereas the subcarinal (#7) nodes were involved in tumors of the lower lobe (3/3).

4. Discussion

The time frame of progression from small-sized lung lesions to invasive tumors remains unclear. Although it has been suggested that the adenoma–carcinoma sequence also applies to the tumori-

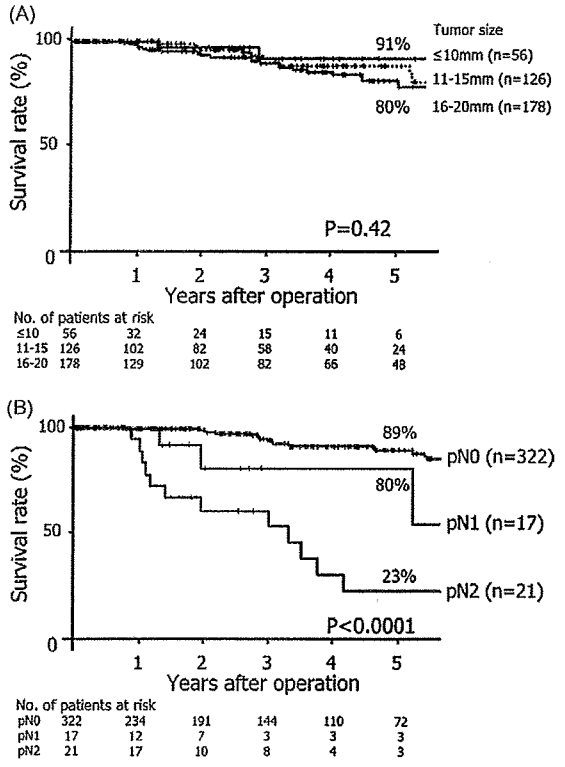

Fig. 3. Survival curves for patient subgroups stratified according to tumor size and pathological nodal status. The five-year survival rate and the number of patients in each subgroup are indicated. (A) No survival rate difference was observed between each tumor size subgroup ($P=0.42$). (B) The survival curves showed a significant stepwise deterioration as the number increased ($P<0.0001$).

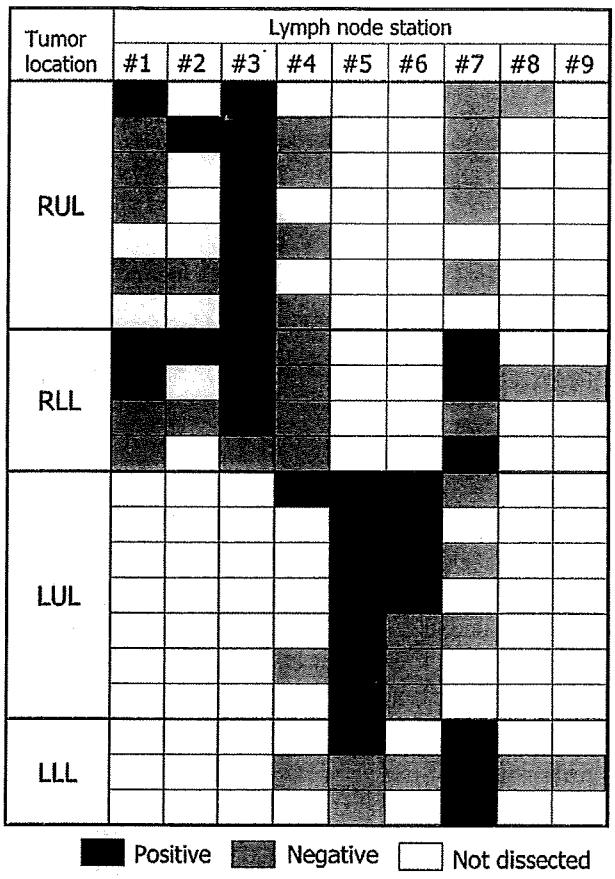

Fig. 4. Distribution of mediastinal lymph node metastasis grouped by lobe of primary tumor. The number of lymph node stations was based on Naruke's lymph node mapping. None of the 14 patients with upper lobe tumors showed positive subcarinal lymph nodes. Lower lobe tumors frequently developed extensive multiple-level involvement, which included the upper mediastinum.

genesis of adenocarcinomas of the lung, no persuasive data have been published to date [6]. In clinical practice, peripheral lung lesions that never change in size over a long-term period are sometimes detected, as are lesions with positive mediastinal lymph nodes, even when the tumor size is smaller than 2 cm in diameter.

The review of the natural history of pure GGOs through the long-term observation of 19 patients revealed that the size of pure GGOs did not change in 8 patients, increased slightly (up to 5 mm) in 6 patients, and increased by >5 mm in 5 patients, during a follow-up of two or more years. The authors suggested that some pure GGOs will never progress to clinical disease [7]. In our institution, we reviewed 13 patients with small GGOs detected by CT who had no intervention for more than two years (median follow-up, 48 months; range 24–96 months). During follow-up, the size of the lesions either did not change or increased slightly (up to 5 mm) in all but four of the 13 patients. In two of the 13 patients, the lesion became larger without the appearance of solid component. In another 2 patients, the lesion became slightly larger and multiple new GGOs appeared (detailed data not shown) [8].

In contrast, the comprehensive analysis of hilar and mediastinal lymph nodes dissected systematically showed nodal micrometastasis in 20% of the patients with adenocarcinoma of 1.1–2.0 cm in diameter and in 4 of 11 patients with adenocarcinoma of 1.0 cm or less [9]. The frequency of lymph node metastasis in small-sized lung cancer with a diameter of 1 cm or less reported by other authors ranged from 2% to 7% of the patients [5,10–12]. In our cohort, 1 of 56 patients (1.8%) with a tumor of 1 cm or less, and 20 of 304 patients (6.6%) with a tumor of 1.1–2 cm, showed positive mediastinal lymph nodes.

Previous reports have often suggested that the management of GGO lesions should differ from the management of noncalcified solitary nodules showing soft-tissue density. In general, increased lymph node metastasis and pleural and vascular invasion are suggested to be associated with greater scarring and fibrotic focus in the tumor [3,13–16]. Takashima et al. reported that a lesion size of 1.5 cm, GGO areas >57%, and the presence of BAC histology correlate with a favorable prognosis, as assessed by univariate analysis, although multivariate analysis showed that the percentage of GGO areas was the only independent prognostic factor [15]. In addition, Okada et al. showed a correlation between tumor shadow disappearance rate, which is defined as the ratio of the tumor area of the mediastinal window to that of the lung window on high-resolution CT, and the percentage of favorable BAC histology in resected specimens in tumors smaller than 3 cm [1]. In our cohort, lesions with mediastinal lymph node metastasis showed either no or few GGO areas on CT. In this way, a classification that takes into account not only the tumor size but also the percentage of GGO areas seems to be more suitable for the selection of patients for limited surgery (described below).

During surgical treatment for small-sized lung cancer, the requirement for lobectomy remains controversial. According to the only prospective randomized trial available, which was performed by the Lung Cancer Study Group in 1995, the appropriate surgical procedure for stage IA NSCLC is lobectomy with systematic mediastinal lymph node dissection [17]. This study demonstrated that a lesser resection, which includes wedge or segmentectomy, leads to three times more local recurrence than a lobectomy; however, it may not be appropriate to simply apply these results to

the treatment of recent small-sized lung cancers detected by high-resolution CT. Limited resection, which includes wedge resection without lymph node dissection, is one of the minimum invasive surgeries, which surgeons try to apply in the treatment of small peripheral lung cancer. Comparisons of limited resection with conventional lobectomy for this small-sized cancer have been performed and reported by several investigators [10,12,18–21]. Some of these results show satisfactory five-year survival rates. A prospective phase II study of limited surgical resection for peripheral lung cancers with a diameter of 2.0 cm or less is currently planned by the Japan Clinical Oncology Group. This study is based on the results of exploratory analysis of the study, which indicates that a radiographic noninvasive cancer is defined as a tumor having consolidation of less than half of the maximum tumor dimension [22]. The demonstration that some types of lung cancer can be resected using such procedures without an increase in local recurrence would promote the use of this method as a future standard treatment for small lung cancers.

Essentially, radical resection by lobectomy and hilar–mediastinal lymph node dissection should be indicated for tumors with a present or possible risk of nodal invasion. A pathologic noninvasive cancer that is a candidate for limited surgery is defined as a tumor with no lymph node metastasis nor lymphatic or vascular invasion [22]. As reported previously by Asamura et al. [23], patients with squamous cell carcinoma did not have mediastinal lymph node metastasis, which was confirmed in our cohort. In addition, we suggest that a poor differentiation of the tumor and relatively higher levels of serum CEA may be risk factors for mediastinal nodal involvement; thus, to avoid an incomplete resection or misdiagnosis of nodal staging, careful consideration is warranted before performing limited resection in patients who exhibit these characteristics.

Recently, a phase III study to compare segmentectomy with standard lobectomy for peripheral small lung carcinoma <2 cm are planned both in the US and Japan. In our opinion, segmentectomy might be adequate for this type of small lesion. However, in what concerns lymphadenectomy, complete mediastinal dissection should be recommended for lower lobe tumors, even when the lesions are small in size because lower lobe tumors frequently developed extensive multiple-level involvement within the upper and middle mediastinum in our series. In contrast, Watanabe et al. suggested that a thorough mediastinal dissection should be performed for upper lobe tumors [24]; however, the necessity for subcarinal lymph node dissection in upper lobe tumors seemed to be low, as none of the 8 patients with upper lobe tumor who underwent subcarinal node dissection showed positive nodes in our cohort.

In conclusion, most small-sized NSCLCs with mediastinal lymph node metastasis were invasive adenocarcinomas with poor differentiation that usually showed a solid shadow without GGO on CT. As small-sized NSCLCs are a heterogeneous subgroup, a treatment suitable for the specific feature of each tumor should be administered.

Conflict of interest statement

None declared.

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Phase II study of oxaliplatin plus S-1 as first-line treatment for advanced gastric cancer (G-SOX study)

W. Koizumi[1]*, H. Takiuchi[2], Y. Yamada[3], N. Boku[4], N. Fuse[5], K. Muro[6], Y. Komatsu[7] & A. Tsuburaya[8]

[1]*Department of Gastroenterology/Gastrointestinal Oncology, Kitasato University School of Medicine, Sagamihara;* [2]*Department of Gastroenterology, Osaka Medical College, Takatsuki;* [3]*Gastrointestinal Medical Oncology Division, National Cancer Center Hospital, Tokyo;* [4]*Division of Gastrointestinal Oncology, Shizuoka Cancer Center, Sunto-gun;* [5]*Division of Gastrointestinal Oncology and Digestive Endoscopy, National Cancer Center Hospital East, Kashiwa;* [6]*Department of Clinical Oncology, Aichi Cancer Center Hospital, Nagoya;* [7]*Department of Cancer Chemotherapy, Hokkaido University Hospital Cancer Center, Sapporo and* [8]*Department of Gastrointestinal Surgery, Kanagawa Cancer Center, Yokohama, Japan*

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Background: The efficacy and safety of oxaliplatin combined with S-1 (SOX regimen) for unresectable advanced or recurrent gastric cancer were investigated.

Patients and methods: Oxaliplatin was administered i.v. (100 mg/m^2) on day 1, while S-1 was administered orally (80 mg/m^2/day, b.i.d.) for 14 days followed by a 7-day rest. This schedule was repeated every 3 weeks.

Results: Among 55 patients enrolled, one patient received oxaliplatin for the other study, and three patients were considered unsuitable against the inclusion criteria. Accordingly, 51 patients were assessable for efficacy. The response rate was 59%, and the disease control rate was 84%. The median progression-free survival time was 6.5 months, the 1-year survival rate was 71%, and the median survival time was 16.5 months. In 54 patients assessed for safety, the major grade 3/4 toxic effects were neutropenia (22%), thrombocytopenia (13%), anemia (9%), anorexia (6%), fatigue (6%), and sensory neuropathy (4%).

Conclusion: These findings indicate that SOX regimen with oxaliplatin at a dose of 100 mg/m^2 is feasible and shows promising efficacy against advanced gastric cancer.

Key words: advanced gastric cancer, oxaliplatin, phase II, S-1, SOX

introduction

Chemotherapy for advanced gastric cancer was proven to be superior to best supportive care in terms of survival and quality of life [1–3]. Phase III studies have been carried out to compare epirubicin/cisplatin/5-fluorouracil (5-FU) with 5-FU/doxorubicin/methotrexate, cisplatin/5-FU with docetaxel/cisplatin/5-FU, and 5-FU/cisplatin with capecitabine/cisplatin [4–6]. On the basis of the results of these studies, advanced gastric cancer is mainly treated with combination chemotherapy that includes fluoropyrimidine derivatives and platinum compounds.

Oxaliplatin is a third-generation platinum compound that was developed to improve tolerability and ease of administration compared with cisplatin [7]. The non-inferiority of oxaliplatin-based regimens to cisplatin-based regimens was demonstrated in the Revised European-American Lymphoma (REAL)-2 phase III study [8]. In addition, the result of phase III study comparing 5-FU/leucovorin/cisplatin with 5-FU/leucovorin/oxaliplatin showed that oxaliplatin was at least as effective as cisplatin [9].

S-1 is an orally active prodrug of 5-FU that contains tegafur (which is continuously metabolized to 5-FU) blended with two modulators, gimeracil and potassium oxonate [10]. In Japan, advanced gastric cancer is mainly treated with S-1 alone or S-1 combined with other drugs. The SPIRITS phase III study demonstrated the superiority of S-1 plus cisplatin to S-1 alone [11]. The S-1 plus cisplatin regimen was also investigated by the FLAGS phase III study carried out in Western countries, which demonstrated that S-1 plus cisplatin was at least as effective as 5-FU plus cisplatin and less toxic [12].

We conducted a multicenter phase II study to evaluate the efficacy and safety of the combination regimen of S-1 and oxaliplatin (SOX regimen) in advanced gastric cancer as first-line therapy.

patients and methods

patients' eligibility

The following criteria were used to enroll patients for the present study. All patients had unresectable advanced or recurrent gastric cancer excluding the esophagus and gastroesophageal junction, confirmed by histological or

Correspondence to: Dr W. Koizumi, Department of Gastroenterology/Gastrointestinal Oncology, Kitasato University School of Medicine, 2-1-1 Asamizodai, Sagamihara, Kanagawa 228-8520, Japan. Tel: +81-42-748-9111; Fax: +81-42-748-5120; E-mail: koizumi@med.kitasato-u.ac.jp

cytological examination. They had survived at least 4 weeks if extended or standard surgery had been carried out (or at least 2 weeks after minor surgery) and were able to take oral drugs. They were aged ≥20 years, had an Eastern Cooperative Oncology Group performance status (PS) of zero to two, and were expected to survive for at least 2 months. In general, they had not received prior chemotherapy, but those who had completed postoperative adjuvant therapy at least 180 days before enrollment were eligible. They had at least one measurable lesion according to RECIST guidelines [13]. They also had adequate bone marrow function (hemoglobin level ≥ 80 g/l, white blood cell count of 3–12 × 10^9/l, neutrophil count ≥ 1.5 × 10^9/l, and platelet count ≥ 100 × 10^9/l), liver function (total bilirubin ≤ 1.5 × the institutional upper limit of normal, aspartate aminotransferase/alanine aminotransferase ≤ 2.5 × the institutional upper limit of normal, and alkaline phosphatase ≤ 2.5 × the institutional upper limit of normal), and renal function (serum creatinine level ≤ 1.5 mg/dl and creatinine clearance ≥ 50 ml/min). All patients provided written informed consent.

This study was carried out in accordance with the Helsinki declaration and Good Clinical Practice guidelines and was approved by the institutional review boards of all participating medical institutions.

treatment plan

Oxaliplatin was administered i.v. at a dose of 100 mg/m^2 on day 1. S-1 was administered orally at a dose of 80 mg/m^2/day b.i.d. for 14 days (from the evening on day 1 until the morning on day 15), followed by a 7-day rest period in the 3-weekly schedule. Treatment was repeated until there was disease progression, unacceptable toxicity, or withdrawal of consent.

In the event of grade 4 neutropenia or febrile neutropenia or grade 3 diarrhea or stomatitis, the doses of oxaliplatin and S-1 were reduced by one dose level from the next cycle. If grade 2 sensory neuropathy not recovering by the end of the cycle or grade 3 sensory neuropathy occurred, the dose of oxaliplatin was reduced by one dose level from the next cycle after recovering to grade 2 or less. If grade 2 thrombocytopenia continued ≥8 days after the scheduled day for starting the next cycle or if platelet transfusion was required, oxaliplatin was reduced by one dose level from the next cycle. Oxaliplatin and S-1 could be reduced by two dose levels, but treatment was discontinued if subsequent reduction was indicated. The doses of oxaliplatin and S-1 could be reduced by 25 mg/m^2 and 10–30 mg/day, respectively, for each level. Treatment was discontinued if grade 4 diarrhea, stomatitis, or sensory neuropathy occurred, if grade 3 sensory neuropathy failed to recover by the time when the next cycle was scheduled, if grade 2 thrombocytopenia continued ≥15 days after the scheduled day for starting the next cycle, or if the rest period of S-1 was over 21 days.

evaluation

The data on the patients' characteristics, a 12-lead electrocardiogram, computed tomography (CT) scans, and tumor marker levels (CA19-9 and carcinoembryonic antigen) were obtained within 14 days of enrollment, while hematology tests, biochemistry tests, and assessment of symptoms and signs were carried out within 7 days before enrollment. During the study, hematology tests, biochemistry tests, and assessment of symptoms and signs were carried out every week until the end of the fourth cycle and subsequently every 3 weeks. CT scans were carried out and tumor markers were measured every 6 weeks (every 2 months after the best overall response was achieved).

Responses were evaluated according to the RECIST guidelines. To confirm partial response (PR) (30% or greater decrease in the sum of the longest diameter of target lesions, referenced against the baseline sum of the longest diameter of target lesions together with stabilization or decrease in size of nontarget lesions) or complete response (CR) (disappearance of all target and nontarget lesions together with normalization of tumor marker levels), tumor measurements were repeated no <4 weeks after objective response was firstly obtained. Responses were assessed by the independent review committee. Overall survival (OS) was defined as the time from treatment initiation to death from any cause. Progression-free survival (PFS) was the time from treatment initiation to first documentation of disease progression detected by the review committee or death from any cause (censored at second-line chemotherapy). Time-to-treatment failure (TTF) was the time from treatment initiation to discontinuation of treatment, first documentation of disease progression by the review committee, or death from any cause. Toxic effects were evaluated according to the Common Terminology Criteria for Adverse Events V3.0.

statistical analysis

The primary end point was the response rate (RR), while the secondary end points were OS, PFS, TTF, and safety. The required sample size was calculated to be at least 49 patients on the null hypothesis of the RR of ≤40% versus the alternative hypothesis of the RR of >60%, power 80%, and α 2.5% (one sided). The 95% confidence interval (CI) was calculated for the RR, PFS, and TTF. OS, PFS, and TTF were calculated by the Kaplan–Meier method. Safety was analyzed in all patients who received at least one dose of study medication.

The cut-off date for RR, PFS, TTF, and safety was 27 May 2008, while that for OS was 13 July 2009.

results

patients' characteristics

Fifty-five patients were enrolled from April to December in 2007. Among them, one patient who received oxaliplatin for the other study by mistake was excluded from all analyses. Three other patients were excluded from efficacy analysis because of prior chemotherapy (methotrexate), severe interstitial pneumonia, or absence of measurable lesions (one patient each). Accordingly, 51 patients formed the efficacy analysis set (Table 1), while 54 patients were analyzed for safety. The median age of the 51 patients was 63 years (range 30–77 years) and the PS was zero or one in 50 patients. Prior adjuvant chemotherapy with S-1 had been carried out in one patient, while 50 patients had received no prior chemotherapy.

treatment

At the data cut-off date, treatment was ongoing in eight patients. The major reasons for discontinuation of treatment in 46 patients were disease progression (63%), adverse events (28%), and withdrawal of consent (2%).

The median number of treatment cycles was 6.0 (range 1–16+). The median dose intensity was 88 mg/m^2/3 weeks for oxaliplatin and 867 mg/m^2/3 weeks for S-1, and the median relative dose intensity was 87.5% and 85.7%, respectively. The median total dose was 600 mg/m^2 for oxaliplatin and 5966 mg/m^2 for S-1.

efficacy

The response was assessed as PR, stable disease (SD) (less than a 30% reduction and less than a 20% increase in the sum of the longest diameter of target lesions, referenced against the baseline sum of the longest diameter of target lesions together with stabilization or decrease in size of nontarget lesions), and progressive disease (PD) in 30, 13, and 5, respectively, of the 51