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Dominant Papillary Subtype Is a Significant Predictor of the Response to Gefitinib in Adenocarcinoma of the Lung

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ABSTRACT

Purpose: **Gefitinib (IRESSA; AstraZeneca, Osaka, Japan) shows excellent antitumor activity against advanced non–small-cell lung cancer, especially for the treatment of adenocarcinoma. However, the predictive factors for the response to gefitinib are still controversial. The aim of this study was to identify the clinicopathological and immunohistochemical features that are favorable to the use of gefitinib in adenocarcinoma patients.**

Experimental Design: **Between June 2002 and October 2003, 36 adenocarcinoma patients who experienced a relapse after surgical resection were treated with gefitinib at our hospital. The histologic patterns of the tumors were divided into four distinctive subtypes according to the revised World Health Organization histologic classification, and the dominant histologic subtype for the maximum cut surface of each resected specimen was documented. Association between the response to gefitinib and the clinicopathological features or immunohistochemical expression of epidermal growth factor receptor (EGFR), phosphorylated EGFR, or c-erbB-2 were then investigated.**

Results: **A significant association between the response to gefitinib and dominant papillary subtype findings was observed ($P = 0.0021$); the survival time of papillary subtype patients was also significantly prolonged compared with that of non-papillary subtype patients ($P = 0.03$). No other clinicopathological features or the expression of EGFR, phosphorylated EGFR, or c-erbB-2 were associated with the response to gefitinib.**

Conclusions: **The results of the present study indicate that dominant papillary subtype findings of lung adenocarcinomas can be an important predictor of the response to gefitinib. Thus, this type of adenocarcinoma might be susceptible to postoperative adjuvant treatment with gefitinib.**

INTRODUCTION

Lung cancer is the leading cause of cancer deaths in many countries, and the global incidence is rising at a rate of 0.5% per annum (1, 2). Platinum-based combination chemotherapy has been shown to improve survival and quality-of-life in patients with advanced non–small-cell lung cancer (NSCLC; ref 3 and 4), which accounts for approximately 80% of all lung cancers. New chemotherapeutic agents, like gemcitabine, vinorelbine, docetaxel, paclitaxel, and irinotecan, were developed in the 1990s. However, chemotherapy for advanced NSCLC has been of limited benefit, with response rates of approximately 30% and a median survival period of about 8 months, and it seems to have reached a plateau (1, 5, 6). It is clear that additional treatment strategies are necessary.

Epidermal growth factor receptor (EGFR) has been shown to play an important role in the growth of many solid tumors and is overexpressed in approximately 40 to 80% of NSCLCs (7–9). Furthermore, the overexpression of EGFR has been associated with a poor prognosis in several studies on lung cancer (10, 11). EGFR activation occurs when ligands, such as epidermal growth factor, transforming growth factor-α, or amphiregulin, bind to its extracellular domain, resulting in cell proliferation, angiogenesis, metastasis, and antiapoptosis (8, 9). Gefitinib (IRESSA; AstraZeneca, Osaka, Japan) is an orally active, selective EGFR tyrosine kinase inhibitor that blocks downstream of the EGFR signal transduction pathway (12). In this context, gefitinib has a quite different profile from chemotherapeutic agents that have ever been used.

After phase I studies, two multicenter, randomized, double-blind phase II studies (IDEAL1, ref. 13; IDEAL2, ref. 14) were carried out to evaluate the tolerability and efficacy of gefitinib in patients with advanced NSCLC who had been treated previously with platinum-based combination chemotherapy. In total, 426 patients were enrolled in the two studies, and all of the patients had been treated previously with platinum-based combination chemotherapy. In both studies, the administration of two different gefitinib dosages (250 mg/day and 500 mg/day) were compared. No significant differences in efficacy were seen between the two dosages, but the 250 mg/day treatment was better tolerated than the 500 mg/day treatment in both studies. For the 250 mg/day gefitinib arms, the response rates were 18.4% and 12.0% in IDEAL1 and IDEAL2, respectively.

The results of IDEAL1 showed that gefitinib was significantly more effective for the treatment of adenocarcinomas than for other histologies (odds ratio, 3.45) and was also more effective in females than in males (odds ratio, 2.65). These

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findings were unexpected, given the mechanism of the drug, because squamous cell carcinomas are known to overexpress EGFR, the target of gefitinib, to a greater degree than adenocarcinomas. On the other hand, gefitinib-induced severe acute interstitial pneumonia resulting in a high mortality rate is a serious social problem in Japan (15). Although the precise mechanism of gefitinib's action is not yet completely understood, clinically it is more important to identify favorable characteristics in the treatment with gefitinib.

Gefitinib has been confirmed to be significantly effective for the treatment of adenocarcinomas, and some investigators have reported that gefitinib is especially effective in adenocarcinomas with bronchiolo-alveolar features (16, 17). To the best of our knowledge, however, these studies only examined biopsy specimens, and no studies examining surgical specimens have been done. Lung cancer is generally characterized by histologic heterogeneity; thus, studies examining histologic features should be done using surgically resected specimens.

In this study, we investigated the clinicopathological and immunohistochemical features of surgically resected adenocarcinoma specimens from patients who were subsequently treated with gefitinib after relapse to identify any characteristics that were associated with a favorable response to gefitinib.

PATIENTS AND METHODS

Patients. Between June 2002 and October 2003, 253 consecutive patients were treated with a 250 mg daily dosage of

Table 1 Patient characteristics ($N = 36$)

Patient no.	Age (year)	Gender (M/F)	PS	Smoking	Recurrent site		Previous treatment		Response	Current status
					Lung	Others		CT regimen (response)		
1	72	F	1	−	+	+	CT, RT	CDDP/VNR (PR)	PR	Continued
2	57	M	1	+	+	+	CT, bone RT	CDDP/VNR (SD)	PR	Continued
3	62	M	1	+	+	+	CT, bone RT	CDDP/VNR (PR)	PR	Ceased
4	60	M	1	+	+	−	CT	CDDP/VNR (SD)	PR	Ceased
5	61	F	1	−	+	−	CT	①CBDCA/PTX (CR) ②GEM/VNR (SD)	PR	Continued
6	64	M	2	−	+	+	brain RT		PR	Dead
7	66	M	1	+	+	+	CT	CDDP/VNR (SD)	PR	Continued
8	69	F	1	−	+	−	CT, OP	CDDP/VNR (PD)	PR	Continued
9	55	F	0	−	+	−	None		PR	Continued
10	66	F	1	−	+	−	CT	①GEM/VNR (SD) ②CBDCA (SD)	PR	Continued
11	61	F	0	−	+	−	CT	CDDP/VNR (SD)	PR	Ceased
12	69	M	0	+	+	−	None		PR	Continued
13	62	F	1	−	+	+	CT, OP, brain RT	CDDP/VNR (PR)	PR	Continued
14	67	M	0	+	+	−	None		PR	Continued
15	47	F	1	−	+	−	CT	CDDP/VNR (SD)	PR	Ceased
16	43	F	1	−	+	−	None		PR	Continued
17	73	F	1	−	−	+	None		PR	Continued
18	45	F	1	−	+	−	CT	CDDP/VNR (SD)	SD	Dead
19	45	F	0	−	+	−	None		SD	Ceased
20	59	M	0	+	+	−	CT	CDDP/VNR (SD)	SD	Continued
21	66	F	2	−	+	−	CT, RT, OP	CDDP/VDS+MMC (SD)	SD	Ceased
22	62	F	0	+	+	-	CT	GEM/VNR (SD)	SD	Ceased
23	60	M	1	+	+	+	CT, RT	①CDDP/GEM/VNR (SD) ②DTX (PD)	SD	Ceased
24	55	M	1	−	+	−	CT	CBDCA/PTX (SD)	SD	Ceased
25	71	F	2	−	−	+	bone RT		SD	Dead
26	82	F	1	−	+	−	OP		SD	Ceased
27	72	M	1	+	+	−	OP		SD	Unknown
28	67	F	1	+	+	−	None		SD	Dead
29	71	M	1	+	+	−	None		SD	Continued
30	57	M	1	+	+	−	None		SD	Ceased
31	74	M	1	+	+	−	None		SD	Dead
32	65	M	2	+	−	+	CT, brain RT	CDDP/VNR (SD)	SD	Continued
33	73	F	1	−	+	−	None		SD	Continued
34	72	F	1	−	+	+	RT		PD	Dead
35	67	M	1	+	+	+	CT	CDDP/VNR (SD)	PD	Dead
36	60	M	1	−	+	−	OP		PD	Unknown
$N = 36$	70 (43–82)	M/F: 17/19	0–1/2: 32/4	−/+: 20/16	33	12	Previous CT (+): 19	Number of regimen 1 regimen: 16 2 regimen: 3	OR: 47%	

Abbreviations: F, female; M, male; PS, performance status (ECOG, Eastern Cooperative Oncology Group); CT, chemotherapy; RT, radiation therapy; OP, operation; CDDP, cisplatin; CBDCA, carboplatin; VNR, vinorelbine; PTX, paclitaxel; GEM, gemcitabine; VDS, vindesine, MMC, mitomycine C; DTX, docetaxel; PR, partial response; SD, stable disease; PD, progressive disease; OR, overall response.

gefitinib at our hospital. To select fully treated patients, we defined assessable patients as follows. Response evaluation using chest computed tomography was done after receiving gefitinib at least for > 4 weeks. Among 253 patients, 222 satisfied these criteria. Of these 222 patients, 48 patients (36 patients with adenocarcinomas, 7 patients with squamous cell carcinomas, 3 patients with large cell carcinomas, 1 patient with adenosquamous carcinoma, and 1 patient with pleomorphic carcinoma) had previously undergone a lung resection for primary NSCLC at our hospital. In this study, we analyzed 36 surgically resected adenocarcinoma specimens. At the time of analysis, the mean gefitinib treatment period of these 36 patients was 172 days (range, 29–542 days).

Pathological Studies. All surgical specimens were fixed with 10% formalin or absolute methanol and embedded in paraffin. The tumors were cut at approximately 5-mm intervals, and serial 4-μm sections were stained with H&E, Alcian blue-periodic acid Schiff method to visualize cytoplasmic mucin production, or Verhoeff van-Gieson method (18) to visualize elastic fibers. Lymphatic permeation and pulmonary metastases were evaluated on sections stained with H&E. Vascular invasion and pleural invasion were evaluated with the Verhoeff van-Gieson method. Three observers (Y. K., G. I., and K. T.) who were unaware of the clinical data independently reviewed all pathologic slides. The histologic diagnoses were based on the revised World Health Organization histologic classification (19). In addition, the histologic subtypes and percentage of each subtype present in the tumor were evaluated with the maximum cut surface of the tumor. The histologic patterns were divided into four distinctive subtypes: bronchioloalveolar carcinoma (BAC), acinar subtype, papillary subtype, and solid adenocarcinoma with mucin. The dominant subtype of each tumor was then documented. Tumor size was measured as the maximal diameter on the cut section of the lung. The pathologic stage was determined according to the classification of the Union Internationale Contre le Cancer (20).

Immunohistochemistry. Tissue blocks were cut into 4-μm sections and mounted on silane-coated slides (Matsunami, Tokyo, Japan). The slides were then deparaffinized in xylene, dehydrated in a graded alcohol series, and blocked for endogenous peroxidase with 3% H_2O_2 in absolute methanol. After microwave pretreatment in citrate buffer (pH 6.0) at 95°C for 20 minutes, immunostaining was done at 4°C overnight with a mouse monoclonal antihuman EGFR (Novocastra, Newcastle, United Kingdom; ref. 21) at a dilution of 1:10, a mouse monoclonal antihuman phosphorylated EGFR (provided by Kyowa-hakko, Tokyo, Japan) that recognizes Try-1173 of the activated EGFR at a dilution of 1:10. As for the use of the antihuman phosphorylated EGFP (p-EGFR), a synthetic peptide (CG-STENAEpYLRVAPQSS), the amino acid sequence of which corresponds to COOH-terminal region of human EGFR, was used as an immunogen to generate a monoclonal antibody specific for the tyrosine-phosphorylated EGFR molecule. Obtained monoclonal antibody (KM2911) was further characterized by ELISA, Western blot assay, and immunohistochemical staining to verify the specificity and sensitivity. Furthermore, we compared the immunostaining of KM2911 with that of another monoclonal antibody against tyrosine-phosphorylated EGFR (MAB3052, Chemicon International, Inc., Temecula, CA) and confirmed the same specificity and sensitivity. The tissues were then exposed to DAKO EnVision+ (DAKO, Glostrup, Denmark) at room temperature for 30 minutes. Staining was visualized by exposure to 3,3′-diaminobenzidine for 3 to 5 minutes. For c-erbB-2, mouse monoclonal antihuman c-erbB-2 (Ventana, Frankfurt, Germany) and the NX/EX automatic stainer (Ventana) were used (22) As positive controls, lung adenocarcinoma specimen, which had been surgically resected at our hospital and had been determined previously to be strongly positive, was used for the EGFR and p-EGFR experiments. Breast cancer specimen, also surgically resected at our hospital and known to be strongly positive, was used for the c-erbB-2 experiment. Negative controls for each antibody were done with nonimmune serum instead of the primary antibodies. The expression of each receptor was scored as follows: − = no discernible staining, or <10% of cells stained; 1+ = >10% of

Table 2 Univariate analysis of clinicopathological factors (N = 36)

	Responder (N = 17)	Non-responder (N = 19)	P value
Age			
≥70	3	7	0.2742
<70	14	12	
Gender			
Male	7	10	0.5251
Female	10	9	
PS			
<2	16	16	0.6052
≥2	1	3	
Smoking history			
Smokers	6	10	0.3351
Never-smokers	11	9	
Previous chemotherapy			
Yes	11	8	0.2021
No	6	11	
Recurrent site			
Lung only	10	13	0.7301
Others	7	6	
Dominant histological subtype			
Papillary	13	4	0.0021 *
Non-papillary	4	15	
BAC	1	6	0.0918
Non-BAC	16	13	
Solid	2	5	0.4080
Non-solid	15	14	
Acinar	1	4	0.3420
Non-acinar	16	15	
Tumor size			
≤3.0 cm	6	7	>0.9999
>3.0 cm	11	12	
Lymph node metastasis			
+	10	13	0.7362
−	6	6	
Lymphatic permeation			
+	15	13	0.2357
−	2	6	
Vascular invasion			
+	14	15	>0.9999
−	3	4	
Pleural invasion			
+	13	8	0.0489 *
−	4	11	
Pulmonary metastases			
+	5	6	>0.9999
−	12	13	

cytoplasmic staining, or plasma membrane staining with weak intensity; 2+ = >10% of plasma membrane staining with moderate intensity; and 3+ = >10% of plasma membrane staining with strong intensity. Staining of 2+ and 3+ were evaluated as positive. As for EGFR and p-EGFR, no universal evaluation criteria exist at present; therefore, we applied the same criteria as c-erbB-2. Although this evaluation criteria basically followed HercepTest (23), we added some modification to evaluate cytoplasmic staining.

Statistical Analysis. All statistical analyses were done with the statistical program StatView, version 5.0 (Abacus Concepts, Berkley, CA). The significance of the relationships between individual clinicopathologic factors; the expression of EGFR, p-EGFR, and c-erbB-2; and a univariate analysis with the Fisher exact probability test was used to evaluate the response to gefitinib. A multivariate regression analysis was conducted according to the Cox proportional hazard model. Kaplan-Meier method was used to calculate survival rates, and a log-rank test was used to evaluate the statistical significance of any differences. A P value of less than 0.05 was considered significant.

RESULTS

Clinical Characteristics. The patient characteristics are shown in Table 1. All clinical data were retrieved from medical records. The mean age of the patients was 70 years (range, 43–82 years). Seventeen patients were male and 19 were female. The Eastern Cooperative Oncology Group performance status was 0 for 7 patients, 1 for 25 patients, and 2 for 4 patients. Sixteen patients were current or ex-smokers. Nineteen patients had been treated previously with chemotherapeutic agents for postoperative recurrences. The Response Evaluation Criteria in Solid Tumors (24) was used to evaluate the response of the patients. Seventeen patients experienced a partial response (PR) to gefitinib, 16 patients had a stable disease (SD), and 3 patients had a progressive disease (PD); the overall response (OR) rate was 47%. No clinical differences were observed between the responders and the non-responders (Table 2). Mean duration of response was 258 days; however, 11 of 17 responded patients were continuing gefitinib at the time of analysis.

Pathologic Findings of Surgical Specimens. The details of the pathologic findings for the surgical specimens are sum-

Table 3 Pathological findings of adenocarcinoma cases (N = 36)

Patient no.	Pathological stage		Histological subtype(%)				Dominant histological subtype	Tumor size	Lymph node metastases	Ly	v	P	pm
			BAC	Acinar	Papillary	Solid							
1	$T_2N_2M_0$	IIIA	20	20	60	0	Papillary	4.5 cm	+	+	+	+	−
2	$T_4N_2M_0$	IIIB	20	30	50	0	Papillary	2.9 cm	+	+	+	+	−
3	$T_2N_2M_0$	IIIA	60	40	0	0	BAC	3.3 cm	+	+	+	+	−
4	$T_2N_1M_0$	IIB	10	0	0	90	Solid	3.5 cm	+	+	+	+	−
5	$T_4N_xM_0$	ND	0	40	0	60	Solid	1.5 cm	ND	−	+	−	−
6	$T_4N_2M_0$	IIIB	30	20	50	0	Papillary	4.3 cm	+	+	+	+	+
7	$T_3N_2M_0$	IIIA	20	30	50	0	Papillary	6.0 cm	+	−	+	+	−
8	$T_4N_0M_0$	IIIB	0	0	100	0	Papillary	4.2 cm	−	+	−	+	+
9	$T_3N_0M_0$	IIIA	30	0	70	0	Papillary	2.8 cm	−	+	+	−	−
10	$T_4N_2M_0$	IIIB	0	0	100	0	Papillary	3.2 cm	+	+	+	+	+
11	$T_4N_1M_0$	IIIB	30	10	60	0	Papillary	2.8 cm	+	+	+	+	−
12	$T_2N_0M_0$	IB	30	10	60	0	Papillary	3.2 cm	−	+	+	+	+
13	$T_1N_0M_0$	IA	0	80	10	10	Acinar	1.5 cm	−	+	+	-	−
14	$T_2N_2M_0$	IIIA	20	10	70	0	Papillary	2.0 cm	+	+	−	+	−
15	$T_4N_0M_0$	IIIB	0	0	100	0	Papillary	4.8 cm	−	+	−	−	+
16	$T_2N_0M_0$	IB	20	10	70	0	Papillary	3.2 cm	−	+	+	+	−
17	$T_2N_1M_0$	IIB	0	10	80	10	Papillary	3.5 cm	+	+	+	+	−
18	$T_4N_2M_0$	IIIB	0	30	0	70	Solid	3.2 cm	+	+	+	−	+
19	$T_2N_2M_0$	IIIA	60	0	40	0	BAC	2.5 cm	+	+	+	+	−
20	$T_4N_2M_0$	IIIB	20	0	80	0	Papillary	2.8 cm	+	+	+	−	+
21	$T_3N_1M_0$	IIIA	0	60	0	40	Acinar	2.8 cm	+	+	+	+	−
22	$T_2N_0M_0$	IB	60	0	40	0	BAC	3.5 cm	−	−	−	−	−
23	$T_2N_2M_0$	IIIA	0	10	0	90	Solid	4.5 cm	+	+	+	+	−
24	$T_1N_0M_0$	IA	80	0	20	0	BAC	2.2 cm	−	−	−	−	−
25	$T_2N_0M_0$	IB	80	10	10	0	BAC	3.2 cm	−	−	−	−	−
26	$T_1N_0M_0$	IA	80	20	0	0	BAC	1.6 cm	−	−	−	+	−
27	$T_2N_1M_0$	IIB	10	0	90	0	Papillary	3.3 cm	+	+	+	−	−
28	$T_2N_0M_0$	IB	0	100	0	0	Acinar	1.8 cm	−	+	+	+	−
29	$T_1N_2M_1$	IV	0	70	0	30	Acinar	2.2 cm	+	+	+	+	+
30	$T_2N_2M_0$	IIIA	0	20	50	30	Papillary	8.4 cm	+	+	+	−	−
31	$T_2N_2M_0$	IIIA	30	0	0	70	Solid	3.9 cm	+	−	+	−	−
32	$T_4N_2M_0$	IIIB	0	10	0	90	Solid	4.0 cm	+	+	+	+	+
33	$T_4N_2M_0$	IIIB	0	20	80	0	Papillary	8.0 cm	+	+	+	−	+
34	$T_2N_2M_0$	IIIA	0	50	20	30	Acinar	6.5 cm	+	+	+	+	−
35	$T_4N_2M_0$	IIIB	0	20	0	80	Solid	7.5 cm	+	+	+	−	+
36	$T_2N_0M_0$	IB	80	10	10	0	BAC	5.5 cm	−	−	+	−	−

Abbreviations: BAC, bronchioloalveolar carcinoma; Acinar, acinar subtype; Papillary, papillary subtype; Solid, solid adenocarcinoma with mucin; ly, lymphatic permeation; v, vascular invasion; p, pleural invasion; pm, pulmonary metastases; ND, not determined.

marized in Table 3. The pathologic stage was IA in 3 cases, IB in 6 cases, IIA in 0 cases, IIB in 3 cases, IIIA in 11 cases, IIIB in 11 cases, and IV in 1 case. The stage IV disease was caused by pulmonary metastasis to another lobe. The pathologic stage could not be determined in one case, because only a partial resection had been done. All but 3 specimens were adenocarcinomas of mixed subtype. The dominant histologic subtype was BAC in 7 cases, acinar subtype in 5 cases, papillary subtype in 17 cases, and solid adenocarcinoma with mucin in 7 cases. Both a dominant papillary subtype ($P = 0.0021$) and the presence of pleural invasion ($P = 0.0489$) were significantly associated with the response to gefitinib when examined with a univariate analysis (Table 2), but a multivariate analysis revealed that a dominant papillary subtype was the only significant factor (Table 4). In addition, the survival period of the dominant papillary subtype patients was longer than that of the non-papillary subtype patients (Fig. 1). The representative histologic features of the papillary subtype are shown in Fig. 2.

Immunohistochemistry. The immunohistochemical evaluation was done according to the scoring system described in Patients and Methods. Immunohistochemistry was not done in one patient, because a tissue block was not available. Nine patients (28%) were positive for EGFR, and 14 (40%) were positive for p-EGFR. None of the patients were positive for c-erbB-2. No substantial association was observed between the immunohistochemical expression of each receptor and the response to gefitinib. The results of immunohistochemistry are summarized in Table 5.

DISCUSSION

Adenocarcinomas were known to be significantly sensitive to the treatment with gefitinib, despite their lower expression rates of EGFR compared with squamous cell carcinomas (13). Some investigators have reported that gefitinib is particularly

Table 4 Multivariate analysis

Parameter	Odds ratio	95% CI	*P* value
Dominant histological subtype (papillary subtype)	14.902	2.497–88.916	0.0030 *
Pleural invasion (present)	0.167	0.027–1.044	0.0556

Abbreviation: CI, confidence interval.

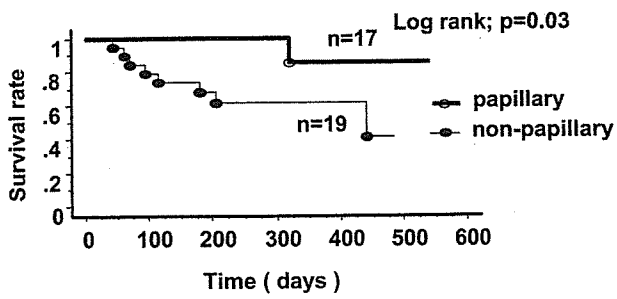

Fig. 1 Survival curves of the adenocarcinoma patients treated with gefitinib.

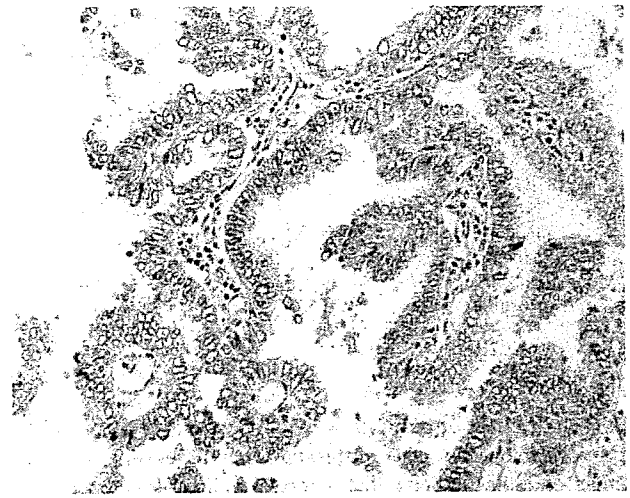

Fig. 2 Representative case of papillary adenocarcinoma (HE× 100). Tumor cells are growing with their own fibrovascular stroma and displaying complicated secondary and tertiary papillary branches. Abbreviation: HE, hematoxylin and eosin.

effective especially in BAC (16, 17), and clinical trials targeting BAC are now under way (25). In fact, as Hirsch *et al.* (8) reported, a high expression level of both EGFR and c-erbB-2 was seen in the BAC in a preclinical study. However, the association between the overexpression of EGFR or c-erbB-2 and the sensitivity to gefitinib is still controversial (26–28), and no data supporting an association has been obtained in clinical studies (29). Moreover, none of the previous studies examined surgical specimens, although BAC cannot be diagnosed with small biopsy specimens (19). In the present study, we investigated the clinicopathological and immunohistochemical features of surgically resected specimens from adenocarcinoma patients who were treated with gefitinib for postoperative recurrences. The surgical specimens were used to determine the dominance of the histologic subtypes according to the revised World Health Organization classification and to precisely evaluate the expressions of EGFR, p-EGFR, and c-erbB-2.

Clinical factors, including age, gender, performance status, smoking history, previous chemotherapy, and recurrence site, were not associated with the response to gefitinib in this study. The immunohistochemical expression profiles of EGFR, p-EGFR, and c-erbB-2 were also not associated with the response. However, both the dominant histologic subtype and the presence of pleural invasion differed significantly between responders and non-responders according to a univariate analysis, whereas a multivariate analysis revealed that only the dominant histologic subtype was a significant factor. In other words, a dominant papillary subtype was the feature that most favored a response to gefitinib, and the survival period of patients with this feature was significantly longer than that of patients with non-papillary subtypes.

The finding that gefitinib is more effective in papillary subtype lesion is of great interest. Drug delivery might be more effective in this histologic subtype, because cancer cells with a papillary structure usually line the fibrovascular stroma. In an *in vitro* and *in vivo* study, Hirata *et al.* (30) showed that the

Table 5 Results of immunostaining ($N = 35$)

	Score	Responders; $N = 17$ (%)	Non-responders; $N = 18$ (%)	Total (%)	P value
EGFR					
Negative	−/1+	11 (65)	15 (85)	26 (72)	0.2642
Positive	2+/3+	6 (35)	3 (15)	9 (28)	
p-EGFR					
Negative	−/1+	10 (59)	11 (61)	21 (60)	>0.9999
Positive	2+/3+	7 (41)	7 (39)	14 (40)	
c-erbB2					
Negative	−/1+	17 (100)	18 (100)	35 (100)	
Positive	2+/3+	0 (0)	0 (0)	0 (0)	

antitumor effect of gefitinib was partly mediated by the inhibition of tumor angiogenesis through direct effects on microvascular endothelial cells that express EGFR. In the papillary subtype, this direct effect on microvascular endothelial cells might be more efficient than in other subtypes.

The results of the present immunohistochemical study suggest that EGFR expression is not a useful predictor of the response to gefitinib. Recently, Paez *et al.* (31) and Lynch *et al.* (32) originally showed that EGFR mutations may predict sensitivity to gefitinib. These epoch-making studies arouse an interest about association of EGFR mutations with histologic subtypes.

In the present study, 9 patients (28%) were positive for EGFR and 14 (40%) were positive for p-EGFR. It seems somewhat strange that the positive rate of p-EGFR surpassed that of EGFR; however, we consider that it is simply because the p-EGFR antibody was more sensitive than the EGFR antibody.

The response rate of our study was high even for adenocarcinoma patients; however, patients were not selected at a point of administration of gefitinib for the most likely respond and patient's selection in the present study strictly followed the definition described in the Patients and Methods section. The relatively high proportion of female (53%) and never-smoker (56%) might lead to this result.

A micropapillary pattern (MPP) of lung adenocarcinoma, which was not included in the revised World Health Organization histologic classification, was first described by Silver and Askin (33). Lung adenocarcinomas characterized by MPP are thought to be more likely to metastasize and have a poor prognosis (34, 35). Most MPP-positive adenocarcinoma cases were included in the papillary subtype in the present study. Miler *et al.* (16) reported that a never-smoker status was a significant predictor of the response to gefitinib, whereas Wu *et al.* (36) reported that all of their patients who achieved a complete response with gefitinib had bilateral diffuse small pulmonary metastases. Both a never-smoker status and diffuse pulmonary metastases are frequently observed in MPP-positive adenocarcinoma (35). These reports, combined with the results of the present study, suggest that gefitinib might be effective against MPP-positive adenocarcinoma. In fact, MPP-positive adenocarcinomas (12 cases) were more sensitive to gefitinib than MPP-negative lesions (24 cases) in the present study ($P = 0.0328$).

In conclusion, the results of the present study indicate that a dominant papillary adenocarcinoma subtype can be an important predictor of the response to gefitinib. Even in patients with pathologic stage IA NSCLC who undergo a complete resection, the 5-year survival rate is about 70% at best (2). Therefore, adenocarcinoma with a dominant papillary subtype might be susceptible to postoperative adjuvant treatments with gefitinib. However, the precise mechanism of how this agent works is still obscure. Additional studies are needed to reveal the relation between the sensitivity to gefitinib and the histology of papillary subtype.

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ORIGINAL ARTICLE

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Pilot phase II study of weekly chemotherapy with paclitaxel and carboplatin for refractory or relapsed small-cell lung cancer

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Abstract *Purpose*: The safety and efficacy of weekly chemotherapy with paclitaxel and carboplatin for the treatment of patients with refractory or relapsed small-cell lung cancer (SCLC) were evaluated. *Patients and methods*: Paclitaxel (100 mg/m^2) and carboplatin (with a target area under the concentration versus time curve of 2 mg min/ml using the Calvert formula) were administered to patients with previously- treated SCLC on days 1 and 8 at every 3–4 weeks. *Results*: A total of 29 patients (pts) [male/female, 26/3 pts; median age 62.7 years (43–74); performance status 0/1/2, 9/10/10 pts] were enrolled between March 2000 and June 2002. The mean number of cycles administered per pt was 3 (1–7). The overall response rate was 69% (95% confidence interval 52–86%), and 83% (15/18) in sensitive pts and 45% (5/11) in refractory pts ($P<0.01$). The overall median survival time was 29.6 weeks with a 1-year survival rate of 37% [34.1 weeks in sensitive pts and 23.1 weeks in refractory pts ($P=0.085$), 46.9 weeks in PS 0–1 and 16.3 weeks in PS 2 ($P<0.001$)]. The median time to progressive disease was 16.4 weeks [21.7 weeks in sensitive pts and 15.3 weeks in refractory pts ($P=0.32$)]. Hematologic toxicities observed included grade ≥3 neutropenia in 55%, grade ≥3 anemia in 36%, and grade ≥3 thrombocytopenia in 3%. Non-hematologic toxicities were mild except for grade 3 diarrhea in three pts and grade 3 pneumonitis in one pt. *Conclusion*: Weekly chemotherapy with paclitaxel and carboplatin was well-tolerated and gave a high-response rate in pts with refractory or relapsed small-cell lung cancer.

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Keywords Small-cell lung cancer · Second line chemotherapy · Weekly chemotherapy · Carboplatin · Paclitaxel

Introduction

Small-cell lung cancer (SCLC) accounts for 15–20% of the total number of lung cancer patients. It grows more rapidly and shows a higher incidence of remote metastasis than non-small-cell lung cancer (NSCLC). It is apparently more sensitive to chemotherapy and radiotherapy than NSCLC, but is cured only in a small number of patients and recurs in a great majority of them. Recurrent SCLC is less responsive to chemotherapy, and the median survival time from recurrence to death is 2–3 months [3]. Chemotherapy has been reported to contribute to the improvement of symptoms and prolongation of the survival time in patients with recurrent SCLC [2, 6]. In general, first-line chemotherapy is conducted for sensitive disease (relapse ≥90 days after completion of first-line chemotherapy). For refractory disease (relapse during first-line chemotherapy or less than 90 days after completion of initial chemotherapy), however, salvage chemotherapy is undertaken due to the lack of a standard chemotherapy regimen. However, no standard chemotherapy has been established for recurrent SCLC [17].

In recent years, a number of institutions have undertaken weekly chemotherapy for lung cancer and reported the outcome [11, 14]. Weekly chemotherapy is being reported to be useful for recurrent SCLC as well [1, 4, 7, 10]. It is considered to be more suitable than the standard chemotherapy conducted every 3–4 weeks for recurrent cases with impaired bone marrow due to initial chemotherapy because it uses smaller doses of anticancer drugs in each administration cycle and it is possible to titrate their doses after starting the treatment depending on hemotoxicity and the patients' physical condition.

When used alone, paclitaxel was reported to produce good therapeutic results in patients with refractory SCLC with a response rate of 29% and a median survival time of 100 days [15]. When coadministered with carboplatin, paclitaxel showed even better results with a response rate of 73.5% and a median survival time of 31 weeks [5]. This report prompted us to conduct the present study to evaluate the efficacy and safety of weekly chemotherapy using carboplatin and paclitaxel in recurrent SCLC patients.

Patients and methods

Patient selection

All patients with histologically or cytologically confirmed SCLC with documented progression after chemotherapy were eligible for this phase II trial. Patients with either limited- or extensive-stage disease were allowed. The trial was initiated after a rest period of at least 4 weeks following previous chemotherapy (2 weeks in the case of radiotherapy). Patients were required to have recovered completely from prior therapy, with no ongoing toxicity greater than grade 1.

Other eligibility criteria included expected survival of 12 weeks, age $\leq$ 75 years, Eastern cooperative oncology group performance score of 0–2, measurable lesions, and adequate hematological function. Primary refractory disease was defined as relapse during first-line chemotherapy or less than 90 days after completing initial chemotherapy, and sensitive disease was defined as relapse $\geq$90 days after completion of first-line chemotherapy.

The ethical committee of the Tochigi cancer center approved the protocols. Written informed consent stating that the patient was aware of the investigational nature of this treatment regimen was obtained in every case.

Treatment

Paclitaxel was administered at a dose of 100 mg/m^2 intravenously during a 1-h infusion on days 1and 8 of the treatment cycle. Carboplatin was given at a dose designed to give an area under the curve (AUC) of 2 on days 1 and 8 with the use of the Calvert formula: 2 $\times$ (creatinine clearance + 25). Prior to each treatment, patients were given 50 mg diphenhydramine orally, and an H2 blocker intravenously along with 16 mg dexamethasone . Intrvenously administered antiemetics, 3 mg graniston, were used. The length of each chemotherapy cycle was 21 days. Patients who experienced grade 4 leukopenia or neutropenia that lasted for three days or more , or who experienced grade 4 thrombocytopenia, reversible grade 2 neurotoxicity, or liver dysfunction, received reduced doses of both paclitaxel and carboplatin (paclitaxel 80 mg/m^2, carboplatin AUC1.5) for the next cycle. If non-hematologic toxicities of grade 3 or more occurred, treatment was stopped. Subsequent courses of chemotherapy were started after 3–4 weeks when the leukocyte count was 3,000/mm^3 or more, the neutrophil count 1,500/mm^3 or more, the platelet count 75,000/mm^3 or more, serum creatinine less than 1.5 mg/dl, GOT and GPT less than twice the upper limit of the normal range, and neurotoxicity was grade 1 or less. If these variables did not return to adequate levels by the first day of the next course of chemotherapy, treatment was withheld until full recovery. If more than 6 weeks passed from the time of the last treatment before these criteria were satisfied, or if more than dose reduction were indicated, the patient was taken off the study at that time, but still included in the analysis.

Evaluation of response and toxicity

Pretreatment evaluation included medical history, physical examination, complete blood count, bone marrow examination, serum biochemical analyses, chest roentgenogram, electrocardiogram, and urinalysis. All patients underwent radionuclide bone scan, bone marrow aspiration or biopsy, magnetic resonance or computerized tomography (CT) of the brain, and CT of thorax and abdomen. Complete blood count, biochemical tests, serum electrolytes, urinalysis, and chest roentgenograms were obtained weekly during this phase II trial.

Response and toxicity were evaluated on the basis of tumor images obtained by CT and other techniques, laboratory data and subjective/objective symptoms before, during, and after administration of the study drugs and during the period from completion of treatment to final analysis. Measurable disease parameters were determined every 4 weeks by various means such as CT. Evaluation was made in compliance with response evaluation criteria in solid tumors (RECIST) guidelines [16] for anti-tumor activity, and with NCI common toxicity criteria Version 2 for safety. Patients were withdrawn from the study if evidence of tumor progression was observed. The Institutional Ethical Review Committee approved the study.

Statistical analyses

Time to progression was measured as a period from the start of this treatment to the identifiable time for progression. Survival time was measured from the start of the present treatment until death or last follow-up. The Kaplan–Meier method was used to calculate survival curves. Survival differences between subgroups were compared using the log-rank test. The chi-square test was used to compare the percentage of patients in each group.

Primary endpoints were response rate and toxicity; secondary endpoints were survival and time to pro-

gression. We chose a 50% response rate as a desirable target level and a 25% response rate as an undesirable target. Our design had a power in excess of 95% and less than 20% type I error, requiring 26 patients. Considering the percentage of probable dropout cases, 29 patients were required.

Results

Patient characteristics

Twenty-nine patients were enrolled in this study from March 2000 to June 2002. All patients were assessed for toxicity, response and survival. Characteristics of the 29 patients are listed in Table 1. There were 11 refractory cases and 18 sensitive cases against the first-line chemotherapy.

Efficacy of treatment

The mean number of cycles administered per patient was three, and ranged from one to seven. There were no cycles of dose reduction. One patient achieved a complete response (CR) and 19 patients showed partial response (PR). Overall response rate was 69% (20/29) [95% confidence interval (CI) 52–86%]. The response rate was 83% (15/18, 95% CI: 66–100%) in sensitive cases and 45% (5/11, 95% CI: 16–75%) in refractory cases, with significant differences between the two groups ($P < 0.01$). The median time to progressive disease was 16.4 weeks [21.7 weeks in sensitive pts and 15.3 weeks in refractory pts ($P = 0.32$)]. The overall median survival time was 29.6 weeks (Fig. 1) with no significant differences between sensitive cases (34.1 weeks) and refractory cases (23.1 weeks) ($P = 0.085$). The median survival time differed significantly between PS 0 or 1 patients (46.9 weeks) and PS 2 patients (16.3 weeks) ($P < 0.001$). The 1-year survival rate was 38% (11/29).

Toxicities

Table 2 lists the toxicities observed during this study. Hematological and blood biochemical reactions included a high incidence of leukopenia and neutropenia, leukopenia, and neutropenia of grade 3 or higher occurred in 55 and 55%, respectively. All neutropenia patients recovered upon treatment with G-CSF. Anemia and thrombocytopenia of grade 3 or higher occurred in 27 and 3%, respectively. Subjective and objective symptoms observed included grade 3 diarrhea in three patients who all showed improvement after administration of anti-cholinergic drugs, and grade 3 pneumonitis in one, who showed rapid recovery following administration of steroids. Other subjective and objective symptoms observed were of grade 2 or less and included nausea in 34%, vomiting in 10%, alopecia in 59%, neuropathy in 28%, and flushing in 17%. All of these toxicities disappeared or improved by symptomatic treatment. There were no toxic deaths.

Discussion

No standard chemotherapy for recurrent SCLC has been established since only two Phase III clinical studies have been reported to date on chemotherapy for this disease [13, 17]. In contrast, many studies have been undertaken on salvage chemotherapy for recurrent SCLC, with monotherapy with new third-generation anti-cancer agents and platinum-based multi-drug chemotherapy being the mainstay in recent years [1, 4, 5, 8–10, 14, 15]. Some institutions administer anti-cancer drugs on a weekly basis (weekly chemotherapy) [1, 4, 7, 10]. This treatment regimen makes it possible to titrate the dose of anti-cancer drugs depending on adverse reactions and the patients' physical condition after starting the treatment by dividing the dose into some installments.

The results reported with weekly chemotherapy are summarized in Table 3 [1, 4, 7, 10]. While the study by Goto et al. [4] included only sensitive cases, all other studies included 35–64% of refractory cases. The overall response rate ranged between 31% and 88%: 37–91% in sensitive cases and 23–83% in refractory cases. No study, apart from ours, reported any significant difference between sensitive and refractory cases. The overall median survival time was 6.1–11.8 months with no significant differences between sensitive and refractory cases [10]. In our study, the median survival time was 46.9 weeks in PS 0 or 1 patients and 16.3 weeks in PS 2 patients ($P < 0.001$). Naka et al. [10] reported significant differences between PS 0 or 1 patients (6.9 months) and PS 2 patients (3.8 months) [10]. Hemotoxicity was the main adverse reaction in all studies. Thrombocytopenia was milder in our study than in other studies. Diarrhea also showed a high incidence in regimens including CPT-11.

Groen et al. [5] reported therapeutic results similar to ours with carboplatin and paclitaxel therapy: overall response rate of 73.5% and overall median survival time of 31 weeks. They administered carboplatin and paclitaxel at AUC 7 and 175 mg/m^2, respectively at an interval of 3 weeks. These doses were 1.7 and 0.88 times that obtained by us. . The main adverse reaction was hemotoxicity in both studies, but thrombocytopenia was milder in our study. In the study by Groen et al., 22 and 4 of 34 patients received RBC transfusions and platelet transfusions, respectively [5].

In a phase III trial, which compared topotecan versus cyclophosphamide, doxorubicin and vincristine (CAV) in patients with recurrent SCLC [17], the response rate was 24.3 and 18.3%, respectively; time to progression 13.3 and 12.3 weeks; median survival time 25.0 and 24.7 weeks; 1-year survival rate 14.2 and 14.4%. In our study, the response rate was 69%, time to progression 16.4 weeks,

Table 1 Patient characteristics

Eligible patients	29
Gender	
Male	26
Female	3
Age (years)	
Median	63
Range	43–74
Performance status	
0	9
1	10
2	10
Disease extent at relapse	
Limited disease	7
Extensive disease	22
Relapse type	
Refractory case	11
Sensitive relapse case	18
Prior therapy	
Chemotherapy alone	21
Chemotherapy and irradiation	8
Prior chemotherapy regime	
CBDCA + ETOP	3
CDDP + ETOP(PE)	11
CODE + PE	1
CDDP + CPT-11(PI)	9
CDDP + ETOP + CPT-11	3
PE + PI	2
Response to prior chemotherapy	
Complete response	4
Partial response	21
Stable disease	3
Progressive disease	1

CBDCA carboplatin, *ETOP* etoposide, *CDDP* cisplatin, *CODE* cisplatin/vincristine/doxorubicin/etoposide, *CPT-11* irinotecan

median survival time 29.6 weeks, and 1-year survival rate 37%, and our study showed better therapeutic performance in terms of all four parameters although ours was a pilot study and direct comparisons cannot be made.

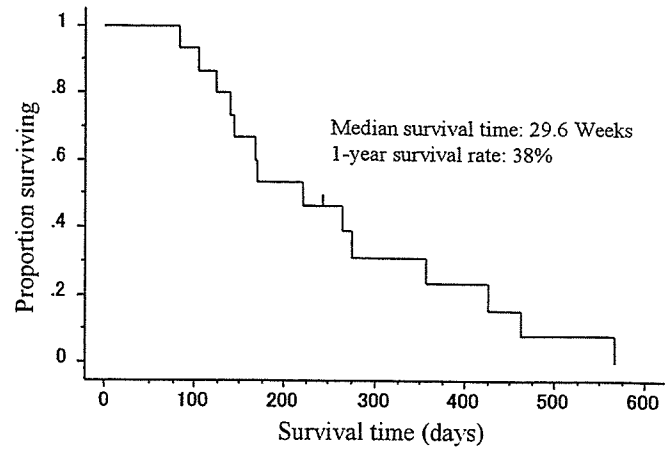

Fig. 1 Kaplan–Meier estimated overall survival curves. Median survival time, 29.6 weeks; 1-year survival rate, 38%

In Japan, cisplatin and irinotecan chemotherapy is the standard therapy for untreated patients in extensive SCLC. Only 8 of 40 patients in the study by Goto et al. [4] and 14 of 29 in our study received irinotecan-based regimens in initial therapy, and no other weekly chemotherapy studies included in Table 3 used such regimens. Carboplatin and paclitaxel combination chemotherapy appears rational in patients with recurrence following initial therapy with cisplatin and irinotecan because the two regimens are not cross resistant.

Conclusion

Weekly chemotherapy with paclitaxel and carboplatin is tolerable and an active regimen for patients with refractory or relapsed SCLC. It is to be recommended as a candidate regimen in planning a phase III clinical study in refractory or relapsed SCLC, and this regimen will ultimately be evaluated in a phase III clinical study.

Table 2 Toxicities ($n = 29$)

	Grade (common toxicity criteria)				Grade ≤ 3 (%)
	1	2	3	4	
Leukopenia	1	7	14	2	16 (55%)
Neutropenia	1	5	9	7	16 (55%)
Anemia	5	8	6	2	8 (27%)
Thrombocytopenia	8	3	1	0	1 (3%)
Diarrhea	7	0	3	0	3 (10%)
Pneumonitis	0	0	1	0	1 (3%)
Nausea	9	1	0	–	
Vomiting	3	0	0	–	
Fatigue	3	3	0	0	
Alopecia	17	0	–	–	
Neuropathy	8	0	0	0	
Flushing	5	–	–	–	
Edema	4	0	0	0	
Arthralgia	3	0	0	0	
Rash	3	0	0	0	
Arrythmia	2	0	0	0	

Table 3 Weekly chemotherapy studies for relapsed small-cell lung cancer

References	Regimen	No. of pts	% of ref pts (%)	RR	RR in sen pts (%)	RR in ref pts (%)	MST (months)
7	CODE	17	35	88	91	83	8.2
10	CPT-11/CBDCA	28	46	31	37	23	6.1
1	CPT-11/CDDP	25	64	80	78	81	7.9
4	CPT-11/CDDP/ETOP	40	0	78	78	–	11.8
Present study	CBDCA/PTX	29	38	69	83	45	7.4

pts patients, *ref* refractory, *sen* sensitive, *RR* response rate, *MST* median survival time, *CODE* cisplatin/vincristine/doxorubicin/etoposide, *CPT-11* irinotecan, *ETOP* etoposide, *CDDP* cisplatin, *PTX* paclitaxel, *CBDCA* carboplatin

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CT-guided needle biopsy of lung lesions: A survey of severe complication based on 9783 biopsies in Japan

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Abstract

Purpose: The aim of our study was to update the rate of severe complications following CT-guided needle biopsy in Japan via a mailed survey.

Materials and methods: Postal questionnaires regarding CT-guided needle biopsy were sent out to multiple hospitals in Japan. The questions regarded: the total number and duration of CT-guided lung biopsies performed at each hospital, and the complication rates and numbers of pneumothorax, hemothorax, air embolism, tumor seeding, tension pneumothorax and other rare complications. Each severe complication was followed with additional questions.

Results: Data from 9783 biopsies was collected from 124 centers. Pneumothorax was the most common complication, and occurred in 2412 (35%) of 6881 cases. A total of 39 (35%) hospitals reported 74 (0.75%) cases with severe complications. There were six cases (0.061%) with air embolism, six cases (0.061%) with tumor seeding at the site of the biopsy route, 10 cases (0.10%) with tension pneumothorax, six cases (0.061%) with severe pulmonary hemorrhage or hemoptysis, nine cases (0.092%) with hemothorax, and 27 cases (0.26%) with others, including heart arrest, shock, and respiratory arrest. From a total of 62 patients with severe complications, 54 patients (0.55%) recovered without sequela, however one patient (0.01%) recovered with hemiplegia due to cerebral infarction, and the remaining seven patients (0.07%) died.

Conclusions: This is the first national study documenting severe complications with respect to CT-guided needle biopsy in Japan. The complication rate in Japan is comparable to internationally published figures. We believe this data will improve both clinicians as well as patients understanding of the risk versus benefit of CT-guided needle biopsy, resulting better decisions.

Keywords: CT-guided needle biopsy; Complication; Lung nodule

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1. Introduction

Transthoracic needle biopsy is a common procedure used mainly to elucidate the nature of pulmonary nodules [1,2]. CT has rapidly become the guidance modality of choice for performing transthoracic needle biopsy due to technical advances in CT and its better detection of pulmonary lesions, which sometimes cannot be identified on chest radiograph [3].

CT-guided needle biopsy is generally regarded as a safe procedure, although pneumothorax and other rare complications can sometimes occur [4]. There have been occasional reports of deaths due to severe complications, such as, air embolism following lung biopsy [5]. Fortunately, these complications are generally very rare; previously published data shows wide variations in complication rates, making them difficult to generalize [5–8].

The aim of our study was to update the rate of severe complications following CT-guided needle biopsy in Japan via a mailed survey.

2. Materials and methods

Postal questionnaires regarding CT-guided needle biopsy were sent out to named radiologists at 101 university hospitals and cancer centers in Japan in August 2001. The radiologists at these hospitals were asked to pass duplications of the questions to other associate hospitals. The questions required information regarding: the total number and duration of CT-guided lung biopsies performed at each hospital, and the complication rates, numbers of pneumothorax, hemothorax, air embolism, tumor seeding, tension pneumothorax, severe pulmonary hemorrhage or hemoptysis which was treated with drugs for hemostasis and other rare complications, and mortalities and morbidities after that.

We defined a case as having a severe complication when one of the following criteria was met: (1) the duration of hospital stay was prolonged due to the biopsy, (2) a special technique or treatment was required to treat the complication, (3) a special procedure was required for resuscitation, and (4) shock or pre-shock developed. Each severe complication was followed with additional questions, including diagnosis of the complication, the position of the pulmonary lesion, the distance of the pulmonary lesion from the peripheral pleura, whether the lesion was located near the hilum or large pulmonary vessel, whether there was any reasonable factor causing the complication such as cough during biopsy, biopsy technique (CT-fluoroscopy or Co-axial method), the number of biopsies for each case, type and size of the needle, and presence of significant sequela from the complication.

Furthermore, the questionnaire included the following enquiries: whether emergency medication was prepared for resuscitation in the operating room, whether the patient was treated by the intravenous route and monitors, such as automatic sphygmomanometer, pulse oximetry, and electrocardiography. Finally, availability of access to other departments in case of emergency was questioned. Postal replies of questionnaire had been received for a year, and these answers were analyzed.

3. Results

A total of 9783 biopsy data were collected from 124 centers. The average number of biopsies performed per center was 79 cases, and that per center per year was 21 cases. The number of institutions in which hyperbaric oxygen recompression can be performed was 41 of 114 (37%) hospitals. Patients were kept on peripheral intravenous drip infusion in 86 of 92 (93%) hospitals, automatic sphygmomanometer in 38 of 92 (41%) hospitals, pulse oximetry in 32 of 92 (35%) hospitals, and electrocardiography in 8 of 92 (9%) hospitals.

Pneumothorax was the most common complication, and occurred in 2412 (35%) of 6881 cases. The number of centers that reported severe complications was 39 (35%) of 114 centers. The total number of overall severe complications was 74 (0.75%) cases. Of these, details of the complications in 64 cases are described in Table 1. There were six cases (0.061%) with air embolism, six cases (0.061%) with tumor seeding at the site of the biopsy route, 10 cases (0.10%) with tension pneumothorax, six cases (0.061%) with severe pulmonary hemorrhage or hemoptysis, 10 cases (0.10%) with hemothorax, and 26 cases (0.26%) with others. The others included 14 cases of pneumothorax requiring temporal drainage of the pneumothorax or chest tube insertion, three cases of heart arrest, and so on. There was no report of coughing during needle placement into the thorax in any of the cases with air embolism. Two of six pulmonary lesions were complicated with air emboli located near the large pulmonary vessel, and one lesion contained a cavity (Table 2). Tumor seeding occurred in two cases following CT-guided biopsy performed

Table 1
Summary of 64 cases of severe complications

Severe complications	No.
Pneumothorax requiring drainage of air	14
Tension pneumothorax	10
Hemothorax	10
Air embolism	6
Tumor seeding	6
Pulmonary hemorrhage of hemoptysis	6
Heart arrest	3
Respiratory arrest	1
Shock	1
Cyanosis	1
Cardiac tamponade	1
Pneumomediastinum	1
Mediastinal hematoma	1
Loss of consciousness	1
Severe pain of biopsied site	1
disseminated intravascular coagulation (DIC)	1
Total	64

Table 2
Summary of cases of air embolism

No.	Age	Sex	Size (mm)	Location (lobe)	Distance from pleura (mm)	Large vessel near the nodule	Cavity	CT-fluoloscopy	Co-axial method	No. of biopsy	Technique of biopsy	Size of the needle	Sequela
1	72	F	20	Left lower	40	Yes	No	Yes	No	2	Core biopsy	18G	Death
2	59	M	10	Left lower	20	No	No	NA[a]	Yes	1	Core biopsy	18G	Totally improved
3	57	F	7	Right middle	25	No	No	Yes	No	1	Core biopsy	18G	Totally improved
4	74	M	20	Right upper	25	Yes	No	Yes	No	2	Core biopsy	20G	Partially improved
5	57	M	12	Right lower	3	No	No	No	Yes	1	Core biopsy	20G	Totally improved
6	75	M	25	Right lower	18	No	Yes	No	No	1	Core biopsy	18G	Totally improved

[a] NA, information was not available.

by the Co-axial method (Table 3). In one of these two cases, the tip of the outer cannula was placed within the chest wall, so that seeding obviously occurred by direct contact of the inner needle with the biopsy route.

From a total of 62 cases with severe complications, 54 cases (0.55%) were recovered without sequela, and one case (0.01%) recovered but with hemiplegia due to cerebral infarction. Unfortunately, four (0.04%) of the remaining seven cases died just after the CT-guided biopsy procedure; these consisted of one case of air embolism, one case of DIC, and two cases of heart arrest. Three cases (0.03%) of the remaining seven cases died several years later due to tumor seeding. Four cases complicated with air embolism, three of which were treated with hyperbaric oxygen recompression, were recovered without sequela out of a total of six cases. In 23 (50%) of 46 centers, an emergency team was able to attend when a severe complication occurred.

4. Discussion

Recently, many small pulmonary lesions, which cannot be detected on chest radiograph, have been easily visualized by CT examination in daily clinical work. These lesions are usually followed with CT, or in some cases these are biopsies using CT-guided technique. CT-guided needle biopsy is a widely accepted technique and is one of the principal methods for evaluating a pulmonary lesion [9]. Although it is not rare to have minor complications due to CT-guided needle biopsy, such as, a small amount of pneumothorax and pulmonary hemorrhage, these complications improve without any treatment [5]. On the other hand, it is well known that potentially life-threatening complications such as air embolism and tumor seeding can occur. Fortunately, the frequency of these complications is considered very rare [5]. However, the number of published reports has shown that the incidence of air embolism has been increasing over the last several years. Only seven cases with air embolism were documented in the 20 years before 1995 [10–16], whereas six cases have already been published in the last 10 years [17–22].

This is the first national research study demonstrating the incidence rate of severe complications with respect to CT-guided needle biopsy based on a large number of biopsy cases using a multi-center survey.

The most common complication of transthoracic percutaneous needle biopsy is pneumothorax, with a frequency rate of 0–61%, whereas the incidence of pneumothorax requiring chest tube drainage ranges from 1.6% to 17% [23]. In the present study, the rate of pneumothorax was 35.1%, which is considered comparable to the previous studies.

Sinner's review of the literature determined that there were two cases suspected of air embolism in 2726 patients [5]. He estimated that the relative risk of air embolism per patient was about 0.07%. In the present study of 9783 biopsies, air embolism occurred in six patients, resulting in an incidence

Table 3
Summary of cases of tumor seeding

No.	Age	Sex	Size (mm)	Location	Distance from pleura (mm)	Co-axial method	No. of biopsy	Technique of biopsy	Size of the needle
1	72	M	30	Right upper	0	No	1	Core biopsy	18G
2	73	M	30	Left lower	30	Yes	3	Core biopsy	18G
3	71	M	10	Right upper	20	No	2	Aspiration biopsy	22G
4	30	F	28	Left upper	76	No	2	Core biopsy	18G
5	69	M	15	Right lower	0	No	2	Core biopsy	21G
6	77	M	12	Right upper	30	Yes	2	Core biopsy	20G

rate of 0.06%, which also shows no major difference from the previously reported complication rate. However, in the present study, there were several cases of severe complications including cardiac and respiratory arrest, and shock, which can be secondary to air embolism, although it is very difficult to confirm air embolism in the coronary artery in cases of myocardial infarction when the patient has not been scanned at the level of the heart. It is speculated that concurrent cough during the procedure has a high possibility of an air embolism misplacing the biopsy needle into the large vessel adjacent to the pulmonary lesion. Among the total of six cases with air emboli in the present study, two cases demonstrated biopsied pulmonary lesions located close to the large vessels, however the remaining four cases have no close relation to the large vessels. There were no reports of coughing during the procedure in any of the cases complicated by air embolism. Air embolism even occurred in a case in which the nodule was very near the pleura (case no. 5). In our study, all cases with air emboli had undergone CT-guided biopsy using a core biopsy needle of 18–20 gauge, which is greater in diameter than the usually used fine aspiration needles. Having said that, in the previous reviews, most cases with air emboli were biopsied by fine aspiration needles, and there are two prior reports of air embolism following CT-guided lung needle marking using thin needles without recent biopsy [24–26].

Tumor seeding into the needle tract seems to be a rare possibility in several case reports [27–34]. There were six cases (0.06%) of tumor seeding in our study, which is a relatively high frequency compared to previous studies [5,35]. The true incidence of tumor seeding along the needle may be underestimated as not all cases can be diagnosed, and many patients die before these metastases become clinically apparent. Tumor seeding appears to depend on the size of the needle, therefore large-bore needles carry a relatively greater risk of tumor seeding, however tumor seeding following a fine needle aspiration was reported in one case of our study. It is thought that CT-guided biopsy performed using the Co-axial method has less frequency of tumor seeding as the outer cannula minimizes direct contact of the tumor cells with the biopsy route. Surprisingly, tumor seeding occurred in two cases using the Co-axial method. We speculate that the outer cannula was not appropriately placed.

Unfortunately, there were seven patients (0.07%) who died in our study due to complications in the CT-guided needle biopsy. Greene [6] estimated the mortality rate associated with fine needle aspiration to be 0.02%, however Richardson et al. [8] reported eight deaths (0.15%) in their study due to complications in CT-guided needle biopsy. Most of the deaths in the present study were attributed to fatal air embolism. Three cases of air embolism that were treated with hyperbaric oxygen recompression were recovered without sequela, which may suggest hyperbaric oxygen recompression therapy is effective for treatment of air embolism, and for reducing the mortality rate.

Our study has several limitations, including selection bias, the long period of the study, multi-center analysis with a large variety of techniques and CT scanners, and the possibility of missing or misdiagnosing significant complications such as the number of air emboli and tumor seeding. Moreover, our study is a retrospective questionnaire-based analysis rather than a prospective survey.

In conclusion, this is the first nation-wide study documenting severe complications with respect to CT-guided needle biopsy in Japan. The complication rate in Japan is comparable to internationally published figures. We believe this data will improve both clinicians as well as patients understanding of the risk versus benefit of CT-guided needle biopsy, resulting better decisions.

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Schedule-Dependent Interactions Between Pemetrexed and Cisplatin in Human Carcinoma Cell Lines In Vitro

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The combination of pemetrexed and cisplatin shows good clinical activity against mesothelioma and lung cancer. In order to study the potential cellular basis for this, and provide leads as to how to optimize the combination, we studied the schedule-dependent cytotoxic effects of pemetrexed and cisplatin against four human cancer cell lines in vitro. Tumor cells were incubated with pemetrexed and cisplatin for 24 h at various schedules. The combination effects after 5 days were analyzed by the isobologram method. Both simultaneous exposure to pemetrexed and cisplatin for 24 h and sequential exposure to cisplatin for 24 h followed by pemetrexed for 24 h produced antagonistic effects in human lung cancer A549, breast cancer MCF7, and ovarian cancer PA1 cells and additive effects in colon cancer WiDr cells. Pemetrexed for 24 h followed by cisplatin for 24 h produced synergistic effects in MCF7 cells, additive/synergistic effects in A549 and PA1 cells, and additive effects in WiDr cells. Cell cycle analysis of MCF7 and PA1 cells supported these findings. Our results suggest that the simultaneous clinical administration of pemetrexed and cisplatin may be suboptimal. The optimal schedule of pemetrexed in combination with cisplatin at the cellular level is the sequential administration of pemetrexed followed by cisplatin and this schedule is worthy of clinical investigations.

Key words: Pemetrexed; Cisplatin; Isobologram; Synergism; Antagonism

INTRODUCTION

Pemetrexed (multitargeted antifolate) is a novel antifolate that inhibits multiple points in folate metabolism including thymidylate synthase, dihydrofolate reductase, and glycinamide ribonucleotide formyl transferase (1–3). Preclinical studies of pemetrexed have demonstrated antitumor activity against a variety of human cancer cells in preclinical models (4). The optimal dose and schedule of pemetrexed was considered to be 500 mg/m^2 in a 10-min infusion once every 3 weeks (5,6). Clinical trials of pemetrexed showed a broad activity against a variety of solid tumors including malignant mesothelioma, and colorectal, pancreas, lung, head and neck, gastric, bladder, and breast cancers (6–14). Dose-limiting toxicities included neutropenia, mucositis, diarrhea, and severe nausea and vomiting (5,6). Patients with a folate-deficient state were associated with severe toxicity, and folate and cobalamin administration before pemetrexed has been introduced in clinical trials (9,13).

Combination chemotherapy has become a standard in the treatment of cancer, based upon theoretical advantages and on proven clinical efficacy. The clinical studies of pemetrexed and platinums (e.g., cisplatin, carboplatin, and oxaliplatin) in combinations have been used against malignant mesothelioma and non-small cell lung cancer, and the promising activity of this combination has been observed (15–19). The wide range of antitumor activity of pemetrexed and platinums (20), their different cytotoxic mechanisms and different toxic profiles, and the absence of cross-resistance provide a rationale for using combinations of these agents.

The cytotoxic action of cisplatin is considered to be the result of the formation of cisplatin–DNA adducts

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