TABLE 1. Patient characteristics

	Lobectomy (n = 383)	Segmentectomy (n = 98)	*P* value
Age	66 (33-84)	67 (34-89)	.08
Gender			.75
Male	169 (44.1%)	45 (45.9%)	
Whole tumor size (cm)	2.2 (0.8-3.0)	1.7 (0.6-3.0)	<.001
Solid tumor size (cm)	1.5 (0-3.0)	0.5 (0-3.0)	<.001
SUVmax	2.1 (0-17)	1.2 (0-10)	<.001
Side			.005
Right	261 (68.4%)	52 (53.1%)	
Lobe			.001
Upper	200 (52.2%)	50 (51.0%)	
Middle	45 (11.7%)	0 (0%)	
Lower	138 (36.0%)	48 (49.0%)	
Lymphatic invasion	77 (20.1%)	6 (6.1%)	.001
Vascular invasion	89 (23.2%)	6 (6.1%)	<.001
Pleural invasion	51 (13.3%)	4 (4.1%)	.008
Lymph node metastasis	44 (11.5%)	1 (1.0%)	<.001

SUVmax, Maximum standardized uptake value.

a coefficient that was calculated using logistic regression analysis, and the sum of these values was taken as the propensity score for individual patients. C statistic of variables was 0.819 (95% confidence interval [CI], 0.776-0.863; $P < .0001$). After the calculation of their propensity scores, the subjects were divided into 3 groups according to tertile to compare characteristics between lobectomy and segmentectomy in each tertile. For matching, lobectomy and segmentectomy pairs with an equivalent propensity score were selected by a 1-to-1 match. Statistical Package for the Social Sciences (SPSS) software (version 10.5; SPSS Inc, Chicago, Ill) was used to statistically analyze the data.

RESULTS

Table 1 summarizes the characteristics of the 481 patients analyzed in this study. Of these, 383 patients underwent lobectomy and 98 patients underwent segmentectomy. There was no 30-day postoperative mortality in this population. The median follow-up period after surgery was 43.2 months, during which the tumor recurred in 50 patients. There were 20 local-only recurrences, including mediastinal lymph node metastasis, and 30 distant ± local recurrences. Age and gender were not significantly different between patients who underwent lobectomy and those who underwent segmentectomy. Lobectomy was performed significantly more often for patients with large whole and solid tumor size, high SUVmax, pathologically invasive tumors (presence of lymphatic, vascular, or pleural invasion), and lymph node involvement. Tumor location was significantly different between patients who underwent lobectomy and those who underwent segmentectomy. Detailed procedures in segmentectomy were shown in Table 2.

Local recurrence occurred in 17 patients who underwent lobectomy (2 involving the bronchial stump, 1 involving the hilar lymph nodes, 11 involving the mediastinal lymph nodes, and 3 involving the pleura) and 3 patients who underwent segmentectomy (1 involving the residual lobe, 1 involving the surgical stump, and 1 involving the pleura).

TABLE 2. Details of segmentectomy (n = 98)

Site	No.	Site	No.
Right		Left	
S1	4	S1 + 2	7
S2	12	S3	3
S3	3	S1 + 2 + 3	10
S6	23	S1 + 2 + 3c	1
S8	5	S4	2
S7 + 8	1	S5	1
S8 + 9	3	S4 + 5	7
S7 + 8 + 9 + 10	1	S6	10
		S8	1
		S9	3
		S6 + 8 + 9 + 10	1

Table 3 shows the multivariate analyses of distant and local RFS. Gender, solid tumor size, and SUVmax were significant independent prognostic factor for distant RFS, whereas whole tumor size was not. Regarding local RFS, solid tumor size and SUVmax were independent prognostic factors, but whole tumor size was not. RFS was not significantly different between patients who underwent lobectomy (3-year RFS, 87.3%) compared with segmentectomy (3-year RFS, 91.4%; hazard ratio [HR], 0.57; 95% CI, 0.27-1.20; $P = .14$, Figure 1, *A*). OS was not significantly different between patients who underwent lobectomy (3-year OS, 94.1%) compared with segmentectomy (3-year OS, 96.9%; HR, 0.49; 95% CI, 0.17-1.38; $P = .18$; Figure 1, *B*).

After the calculation of the propensity score, the subjects were divided into 3 groups according to tertile (Table 4). The numbers of patients in tertiles 1, 2, and 3 according to the operative procedures (lobectomy; segmentectomy) were 79 and 66, 118 and 27, and 141 and 5, respectively. Solid tumor size was smaller and SUVmax was lower in the lowest tertile group, indicating that segmentectomy trended to be performed in patients with a tumor of smaller solid tumor size and lower SUVmax. There were some differences in background characteristics, especially in the lowest tertile group. Therefore, we performed propensity score matching to compare the survival between lobectomy and segmentectomy groups.

When propensity score matching was used and variables such as age, gender, solid tumor size, SUVmax, side, and lobe were included, lobectomy and segmentectomy pairs were well matched (81 patients each) without significant differences in clinical and pathologic factors (Table 5).

Among propensity score–matched patients, no difference in RFS was identified between patients who underwent lobectomy (3-year RFS, 92.9%) compared with segmentectomy (3-year RFS, 90.9%; Figure 1, *C*). In addition, similar OSs were observed between patients who underwent

TABLE 3. Multivariate analyses for distant or local RFS

Variables	HR (95% CI)	*P* value
Multivariate analysis for distant RFS		
Model 1		
Age	1.00 (0.96-1.04)	.86
Gender		
Male vs female	2.62 (1.15-5.95)	.022
Whole tumor size (cm)	1.17 (0.60-2.27)	.65
SUVmax	1.26 (1.14-1.39)	<.001
Procedure		
Lobectomy vs segmentectomy	1.44 (0.41-5.00)	.57
Model 2		
Age	1.00 (0.96-1.03)	.80
Gender		
Male vs female	2.57 (1.14-5.78)	.023
Solid tumor size (cm)	1.86 (1.09-3.16)	.023
SUVmax	1.19 (1.06-1.34)	.003
Procedure		
Lobectomy vs segmentectomy	0.90 (0.24-3.36)	.88
Multivariate analysis for local RFS		
Model 1		
Age	1.04 (0.99-1.10)	.15
Gender		
Male vs female	0.59 (0.24-1.46)	.26
Whole tumor size (cm)	1.44 (0.66-3.12)	.94
SUVmax	1.17 (1.03-1.33)	.015
Procedure		
Lobectomy vs segmentectomy	1.06 (0.29-3.86)	.36
Model 2		
Age	1.04 (0.98-1.09)	.19
Gender		
Male vs female	0.58 (0.24-1.43)	.24
Solid tumor size (cm)	2.89 (1.52-5.50)	.001
SUVmax	1.09 (0.94-1.27)	.26
Procedure		
Lobectomy vs segmentectomy	0.54 (0.14-2.13)	.38

RFS, Recurrence-free survival; *HR*, hazard ratio; *CI*, confidence interval; *SUVmax*, maximum standardized uptake value.

lobectomy (3-year OS, 93.2%) compared with segmentectomy (3-year OS, 95.7%; Figure 1, *D*).

DISCUSSION

The current study compared oncologic outcomes between patients who underwent lobectomy and segmentectomy for clinical stage IA lung adenocarcinoma. In all cohorts, when preoperative clinical factors were not adjusted, RFS and OS of the segmentectomy group were not significantly different from those of the lobectomy group. The survival curves of the segmentectomy group appeared to be better than those of the lobectomy group. However, each patient group was different in terms of solid tumor size and SUVmax, which could affect the patient's survival.[8,9,13-16] In addition, the number of patients who had lymph node metastasis was inevitably larger in the lobectomy group than in the segmentectomy group, which also could affect the survival. To minimize patient selection bias, we used propensity score matching analyses. In the model that matched for potentially confounding variables such as age, gender, solid tumor size, SUVmax, tumor location, in lobectomy and segmentectomy pairs, there were no significant differences in clinical features or pathologic factors such as lymphatic, vascular, pleural invasion, or lymph node metastasis. Even in our matched model, RFS and OS in the segmentectomy group was similar to the lobectomy group, indicating that segmentectomy could be an optimal surgical procedure for clinical stage IA lung adenocarcinoma selected on the basis of HRCT and FDG-PET/CT.

The strength of this study was that variables such as findings from HRCT (solid tumor size) and FDG-PET (SUVmax) were included in the propensity score–matched analysis. We reported that solid tumor size on HRCT and SUVmax on FDG-PET/CT had higher predictive values with respect to pathologic invasiveness such as lymphatic, vascular, pleural invasion, and prognosis compared with whole tumor size.[8,9] In addition, once matching for solid tumor size and SUVmax, pure solid tumor and solid tumor with GGO showed equivalent survivals.[17] Indeed, whole tumor size was not an independent factor for distant or local RFS in this study, whereas solid tumor size and SUVmax were. Solid tumor size does represent tumor malignancy compared with whole tumor size. Therefore, we did not include whole tumor size in matching variables. Inasmuch as SUVmax on FDG-PET/CT was a prognostic indicator for lung adenocarcinoma, not for squamous cell carcinoma in our previous study,[16] the database included only adenocarcinoma, which is a major histologic type for NSCLC. Although several studies have indicated equivalent survivals for segmentectomy and lobectomy in patients with clinical stage IA lung cancer, to our knowledge, this is the first study adjusting for preoperative HRCT and FDG-PET/CT findings, both of which should be considered when selecting patients for limited resections such as segmentectomy. Furthermore, we used an anthropomorphic body phantom to minimize the interinstitutional variability in SUV, which may be influenced by factors such as preparation procedures, scan acquisition, image reconstruction, and data analysis.

Most previous studies that showed favorable outcomes with segmentectomy indicated this procedure for T1 N0 M0 NSCLC of 2 cm or less.[4-6] We included patients with a whole tumor size of 2 to 3 cm (ie, clinical T1b tumor) in this study. We[9] have reported that patients with T1b lung adenocarcinomas selected on the basis of HRCT and FDG-PET/CT findings could be candidates for sublobar resection with a sufficient surgical margin. Inasmuch as clinical T1b N0 M0 lung adenocarcinomas occasionally show large GGO components and/or low SUVmax (signs of

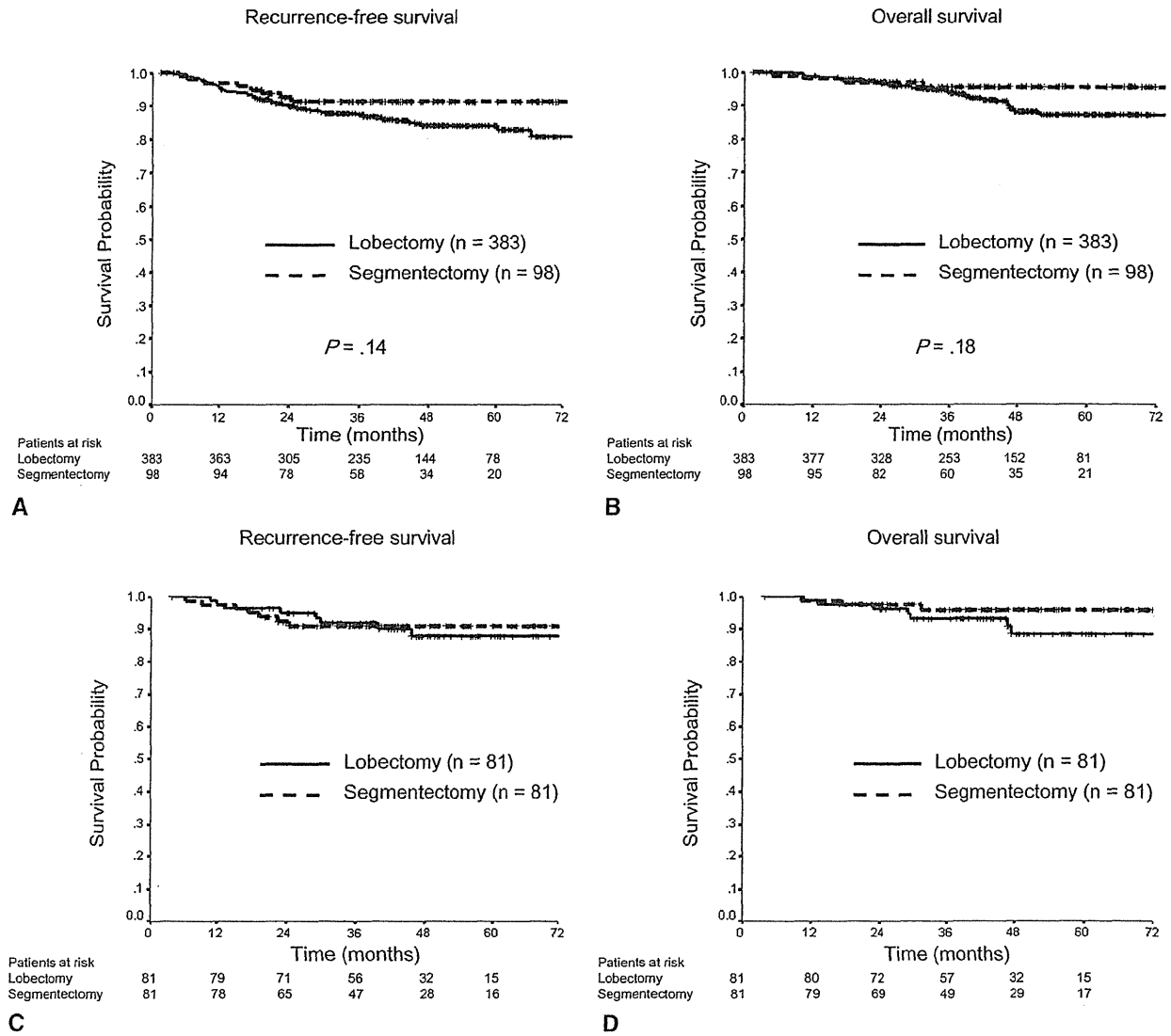

FIGURE 1. Recurrence-free survival (RFS) curves and overall survival (OS) curves for patients who underwent lobectomy and segmentectomy. A, In all cohorts, 3-year RFSs of 87.3% (mean RFS, 66.8 months; 95% confidence interval [CI], 64.6-69.4 months) and 91.4% (mean RFS, 70.3 months; 95% CI, 66.9-73.8 months) were identified for patients who underwent lobectomy and segmentectomy, respectively (hazard ratio [HR], 0.57; 95% CI, 0.27-1.20; $P = .14$). B, In all cohorts, 3-year OSs of 94.1% (mean OS, 70.4 months; 95% CI, 68.7-72.1 months) and 96.9% (mean OS, 72.9 months; 95% CI, 70.3-75.4 months) were identified for patients who underwent lobectomy and segmentectomy, respectively (HR, 0.49; 95% CI, 0.17-1.38; $P = .18$). C, In propensity score–matched patients, 3-year RFSs of 92.9% (mean RFS, 68.6 months; 95% CI, 64.9-72.2 months) and 90.9% (mean RFS, 70.2 months; 95% CI, 66.4-73.9 months) were identified for patients who underwent lobectomy and segmentectomy, respectively. D, In propensity score–matched patients, 3-year OSs of 93.2% (mean OS, 69.3 months; 95% CI, 65.8-72.7 months) and 95.7% (mean OS, 73.2 months; 95% CI, 70.6-75.8 months) were identified for patients who underwent lobectomy and segmentectomy, respectively.

low malignant behavior), such tumors could be treated with lesser resection.[9]

This study has several limitations. Because this study was retrospective, patients who underwent segmentectomy were possibly highly selective. In addition, we could not match intended procedures in the study because the database included only performed surgical procedures, not intended procedures, and patients with R1 or R2 resection were never included in the database. Most patients who underwent segmentectomy in this study tended to have relatively low-malignancy tumors, with small solid tumor size and/or low SUVmax, and thus low pathologic invasiveness. The present study revealed that large solid tumor size on HRCT and high SUVmax on FDG-PET/CT were significantly associated with both local and distant recurrences. The outcome of segmentectomy for relatively high-malignancy clinical stage IA lung adenocarcinomas with large solid tumor size and high SUVmax is unclear.

TABLE 4. Patient characteristics divided into 3 groups according to tertile based on the propensity score

	Tertile 1			Tertile 2			Tertile 3		
	L (n = 79)	S (n = 66)	*P* value	L (n = 118)	S (n = 27)	*P* value	L (n = 141)	S (n = 5)	*P* value
Age	68 (48-82)	68.5 (42-89)	.53	65 (40-83)	65 (34-86)	.92	65 (33-84)	64 (53-83)	.5
Gender			.87			.28			1.0
Male	36 (45.6%)	29 (43.9%)		46 (39.0%)	14 (51.9%)		65 (46.1%)	2 (40.0%)	
Whole tumor size (cm)	2.0 (0.8-3.0)	1.7 (0.9-3.0)	.01	1.8 (1.0-3.0)	1.6 (0.6-2.9)	.048	2.5 (1.2-3.0)	2.4 (1.5-3.0)	.51
Solid tumor size (cm)	0.5 (0-2.0)	0.3 (0-1.0)	.056	1.4 (0-2.0)	1.2 (0-2.0)	.03	2.3 (1.0-3.0)	2.2 (1.0-3.0)	.71
SUVmax	1.2 (0-4.9)	1.0 (0-4.1)	.002	1.9 (0.6-8.3)	1.9 (0.4-9.8)	.77	3.9 (0.7-16.9)	2.1 (1.5-4.3)	.13
Side			.41			1.0			1.0
Right	44 (55.7%)	32 (48.5%)		69 (58.5%)	16 (59.3%)		103 (73.0%)	4 (80.0%)	
Lobe			.51			.53			1.0
Upper	41 (51.9%)	30 (45.5%)		66 (55.9%)	17 (63.0%)		93 (66.0%)	3 (60.0%)	
Lower	38 (48.1%)	36 (54.5%)		52 (44.1%)	10 (37.0%)		48 (34.0%)	2 (40.0%)	

L, Lobectomy; *S*, segmentectomy; *SUVmax*, maximum standardized uptake value.

Although surgical procedure did not correlate with local or distant recurrence in this study, segmentectomy for such tumors (ie, with large solid tumor size or high SUVmax) should be carefully considered. A clinical trial is being conducted by the Japanese Clinical Oncology Group/West Japan Oncology Group (JCOG0802/WJOG4607L), which aims to compare the surgical results between lobectomy and segmentectomy for T1 N0 M0 NSCLC measuring 2 cm or less.[18] This prospective study includes patients with radiologically invasive tumors, such as solid dominant tumors, that have large solid tumor size on HRCT. The results of this trial may provide an important insight into this issue.

Segmentectomy is beneficial because it preserves lung function. Although the database used in this study did not incorporate lung function data, several reports have demonstrated that segmentectomy has functional advantages over lobectomy.[5,19,20] If similar oncologic outcomes are expected, segmentectomy should be considered for patients with clinical stage IA lung adenocarcinoma.

In conclusion, the oncologic outcomes of segmentectomy are similar to those of standard lobectomy for patients with clinical stage IA lung adecnocarcinoma, as determined by the matched model adjusting for preoperative clinical factors such as HRCT and FDG-PET/CT findings. Segmentectomy could be favorable for selective patients with stage IA lung adenocarcinoma.

TABLE 5. Propensity score–matched comparison of clinical and pathologic factors between patients who underwent lobectomy and segmentectomy

	Lobectomy (n = 81)	Segmentectomy (n = 81)	*P* value
Clinical factors			
Age	66 (48-82)	65 (34-86)	.68
Gender			.74
Male	37 (45.6%)	34 (42.0%)	
Whole tumor size (cm)	2.0 (1.0-3.0)	1.7 (0.6-3.0)	.11
Solid tumor size (cm)	0.7 (0-2.0)	0.8 (0-3.0)	.17
SUVmax	1.4 (0-7.0)	1.2 (0-9.8)	.23
Side			.63
Right	33 (40.7%)	37 (45.6%)	
Lobe			.23
Upper	51 (63.0%)	43 (53.1%)	
Lower	30 (37.0%)	38 (46.9%)	
Pathologic factors			
Lymphatic invasion	10 (12.3%)	6 (7.4%)	.42
Vascular invasion	6 (7.4%)	6 (7.4%)	1.0
Pleural invasion	7 (8.6%)	4 (4.9%)	.45
Lymph node metastasis	3 (3.7%)	1 (1.2%)	.63

SUVmax, Maximum standardized uptake value.

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GTS

Methotrexate and gemcitabine combination chemotherapy for the treatment of malignant pleural mesothelioma

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Abstract. Malignant pleural mesothelioma (MPM) is an aggressive tumor of serosal surfaces with a poor prognosis. Methotrexate and gemcitabine have exhibited single-agent activity in MPM. We evaluated the feasibility of sequential administration of these agents in the treatment of MPM. A total of 21 patients with MPM received a 30-min infusion of 100 mg/m^2 methotrexate and, 30 min later, a 30-min infusion of 800 mg/m^2 gemcitabine. Twenty-four hours following the administration of methotrexate, leucovorin rescue therapy was initiated (10 mg/m^2 leucovorin administered 4 times at 6-h intervals). These treatments were administered weekly, with 4 weekly administrations constituting a cycle of therapy. A total of 88 cycles were administered to the 21 patients, with each patient receiving 1-10 cycles (median, 4.2 cycles). Eight patients (38.1%) exhibited a partial response, 10 patients (47.6%) had stable disease and 3 patients (14.3%) had progressive disease. The median overall survival was 19.4 months (range, 02-41 months). One-year and 2-year survival rates were 61.9 and 38.1%, respectively. Hematological toxicity was considered acceptable, with grade 3-4 toxicities occurring in 3 (14.3%) patients. Non-hematologic toxicity was generally mild. There was no treatment-related mortality. Our results suggest that methotrexate and gemcitabine combination therapy is feasible and effective in the treatment of MPM. This regimen may offer an alternative to platinum-based chemotherapy and a prospective trial including a larger cohort of patients is recommended to confirm these results.

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Introduction

Malignant pleural mesothelioma (MPM) arises from the mesothelial surface of the pleural cavity and is a locally invasive tumor with poor prognosis (1,2). In >70% of patients, the tumor is associated with exposure to asbestos fibers following a long latent period of 20-50 years (3). The incidence of mesothelioma is rare in the general population; however, it is expected to increase in the next 20 years in industrialized countries as a result of past asbestos use (4,5).

MPM is refractory to the currently available treatment options. The efficacy of surgical therapy has not been precisely defined (6) and radiotherapy may be palliative but does not prolong survival (7). For the majority of patients with MPM, systemic chemotherapy remains the standard of care (8). Prior to 2003, the majority of studies on chemotherapy for MPM were conducted using either single agents or combination regimens in the setting of small phase II trials. The results demonstrated <20% of tumor regression with no significant effect on patient survival, which was 6-9 months (8,9). Since 2003, the combination of cisplatin and pemetrexed (PTX) has been used as standard chemotherapy for MPM (10). This was based on a randomized phase III study in which PTX plus cisplatin achieved a response rate of 41.3% and a median survival of 12.1 months, compared to 16.7% response rate and 9.3-month median survival achieved by cisplatin alone (10).

In this study, a non-platinum-based combination therapy with two anti-metabolites (methotrexate and gemcitabine) was devised. Methotrexate is an analogue of folic acid known to be effective against breast cancer, lymphoblastic leukemia and osteosarcoma (11,12). Gemcitabine is a pyrimidine analogue, effective against a wide range of solid tumors, including pancreatic carcinoma and non-small cell lung carcinoma (13). Methotrexate and gemcitabine have been reported to exhibit single-agent activity in MPM (8,9); however, the combined administration of these agents has not yet been investigated.

In the present study, we evaluated the feasibility and efficacy of methotrexate and gemcitabine combination therapy in the treatment of MPM, through the analysis of toxicity, response and survival data.

Patients and methods

Patients. Patients with histologically confirmed MPM who had previously received 0-1 chemotherapy cycles, not including gemcitabine and methotrexate, were considered eligible for this single-center study. Tumor extension was classified according to the tumor-node-metastasis (TNM) staging system developed by the International Mesothelioma Interest Group (IMIG) (14). Patients were 18-75 years of age, with an Eastern Cooperative Oncology Group (ECOG) performance status of 0-2, had adequate bone marrow function (hemoglobin concentration ≥10 g/dl, total leukocyte count ≥3.0x10^9/l, granulocyte count ≥1.5x10^9/l and platelet count ≥100x10^9/l), adequate renal function (serum creatinine level <1.5 mg/dl) and adequate hepatic function (total bilirubin level ≤1.5 times the upper limit of normal and serum alanine transferase and alkaline phosphatase levels ≤3 times the upper limit of normal). Patients with a concurrent malignancy of another type or symptoms and/or signs of metastases in the central nervous system were excluded. Patients with prior surgery were considered eligible. This study was approved by the Institutional Review Board of Hyogo College of Medicine and informed consent was obtained from each patient.

Treatment. Patients received a 30-min intravenous (i.v.) infusion of 100 mg/m^2 methotrexate and, 30 min later, a 30-min i.v. infusion of 800 mg/m^2 gemcitabine. For leucovorin rescue, calcium leucovorin (10 mg/m^2, p.o. or i.v.) was administered 4 times at 6-h intervals, initiated 24 h after the administration of methotrexate. These treatments were administered weekly, with 4 treatments constituting a cycle of therapy. A maximum of 6 cycles were administered, unless therapy was terminated due to tumor progression, patient death or wish of treatment discontinuation, or in the presence of convincing evidence that further treatment was not beneficial. Antiemetic and symptomatic treatments were permitted. Analyses of blood cell count and chemistry were performed weekly. Treatment was delayed in the case of i) absolute neutrophil count <1.5x10^9/l and/or platelet count <100x10^9/l; ii) any grade 3 or 4 non-hematological toxicity (except for nausea/vomiting) that did not resolve to grade 1 or less. If these toxicities were not resolved within the cycle, the dose was reduced to 75% of the previous dose level for the next cycle.

Response and toxicity criteria. Chest imaging by computed tomography (CT) was performed at baseline, following completion of every other treatment cycle and every 8weeks following completion of therapy. Objective response was evaluated and calculated using the modified Response Evaluation Criteria in Solid Tumors (RESIST) criteria for MPM (15). Treatment-related toxicities were evaluated according to the National Cancer Institute Common Toxicity Criteria version 3.0 (16).

Statistical analysis. Survival was calculated as the time period from treatment initiation to death, using the Kaplan-Meier method (17).

Results

Patient characteristics. The characteristics of the 21 eligible patients are listed in Table I. There were 16 males and 5 females, with a median age of 63 years (range, 51-75 years). The histological pattern of MPM was epithelial in 17 cases, sarcomatous in 3 cases and biphasic in 1 case. Nineteen patients (90.4%) had stage III and IV disease according to the IMIG staging system at the time of enrollment. Thirteen patients (61.9%) had an ECOG performance status of 0 or 1.

Table I. Patient characteristics.

Characteristics	No. (%)
Gender	
Male	16 (76.2)
Female	5 (23.8)
Age, years	
Median	63
Range	51-75
Performance status	
0	1 (4.8)
1	12 (57.1)
2	8 (38.1)
IMIG stage	
Ib	1 (4.8)
II	1 (4.8)
III	4 (19.0)
IV	15 (71.4)
Histological subtype	
Epithelial	17 (81.0)
Sarcomatous	3 (14.3)
Biphasic	1 (4.7)
Previous treatment	
None	10 (47.6)
Surgery	2 (9.5)
Chemotherapy	9 (42.9)
Asbestos exposure	
Yes	13 (61.9)
No	8 (38.1)

IMIG, International Mesothelioma Interest Group.

Responses to treatment. A total of 88 cycles were administered to the 21 patients. Each patient received a median 4.2 cycles (range, 2-10 cycles). Response to chemotherapy is shown in Table II. No patients exhibited a complete response. Eight patients (38.1%) exhibited a partial response. According to the histological pattern, a PR was observed in 6 out of the 17 patients with epithelial type and in 2 out of the 3 patients with sarcomatous type MPM. Out of the total 21 patients, 10 (47.6%) had stable disease and 3 (14.3%) had progressive disease with no period of stabilization.

Toxicity. The toxicity observed in each patient is shown in Table III. There was no treatment-related mortality. The most frequently observed hematological side effects were neutropenia and thrombocytopenia. Grade 3-4 hematologic

Table II. Response to chemotherapy and histologic subtype.

Response	Overall no. (%)	Histologic subtype		
		Epithelial no. (n)	Sarcomatous no. (n)	Biphasic no. (n)
Complete response	0	0	0	0
Partial response	8 (38.1)	6	2	0
Stable disease	10 (47.6)	8	1	1
Progressive disease	3 (14.3)	3	0	0

Table III. Chemotherapy-related toxicity in eligible patients.

Toxicity	Grade 1 no. (%)	Grade 2 no. (%)	Grade 3 no. (%)	Grade 4 no. (%)
Hematologic	5	2 (9.5)	2 (9.5)	1 (4.8)
Gastrointestinal	5 (23.8)	1 (4.8)	0 (0)	0 (0)
Hepatobiliary	4 (19.0)	3 (14.3)	0 (0)	0 (0)
Pulmonary	0 (0)	2 (9.5)	0 (0)	0 (0)

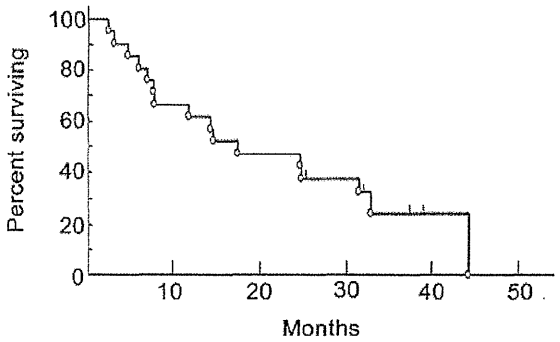

Figure 1. Overall survival.

toxicities were observed in 3 patients (14.3%), which, however, were manageable and did not result in life-threatening complications. Six patients (28.6%) experienced grade 1-2 gastrointestinal toxicities (nausea, vomiting and anorexia) and 7 patients (33.3%) developed grade 1-2 liver dysfunction. Two patients developed interstitial pneumonitis (grade 2) and were administered glucocorticosteroid therapy.

Survival. The median overall survival was 19.4 months (range, 2-41 months), with a 1- and 2-year survival rates of 61.9 and 38.1%, respectively (Fig. 1). As regards the histological pattern, the median survival was 19.6 months for the epithelial, 22.6 months for the sarcomatous and 7.1 months for the biphasic type of MPM.

Discussion

MPM is notoriously refractory to the majority of treatments and the standard first-line treatment is currently cisplatin and PTX chemotherapy (10). In the present study, we evaluated the feasibility of a non-platimum regimen for MPM, involving the sequential administration of the anti-metabolites, methotrexate and gemcitabine.

Methotrexate, an antifolate, has long been used as an anticancer agent and exerts its action through the inhibition of dihydrofolate reductase (DHFR) (12). High-dose methotrexate (1500 mg/m^2) has been reported to be effective in the treatment of MPM, with a response rate of 37% (18). However, high-dose methotrexate was associated with severe toxicity and this method of treatment has been abandoned. The efficacy of low- or medium-dose methotrexate has not been assessed in MPM. In the treatment of gastric cancer (19) and head and neck cancer (20), weekly administration of medium-dose methotrexate (100-200 mg/m^2) combined with sequential administration of 5-fluorouracil (5-FU) (600 mg/m^2) has been reported to be effective and of low toxicity. In this study, a moderate dose of methotrexate (100 mg/m^2) was administered weekly in combination with gemcitabine.

PTX is a newly developed antifolate that targets multiple enzymes involved in DNA synthesis and folate metabolism. Single use of PTX has been reported to be moderately effective against MPM (21). Following combination therapy with 1,250 mg/m^2 gemcitabine administered on days 1 and 8 and 500 mg/m^2 PTX administered on day 8 or 1, chemotherapy-naïve MPM patients exhibited a response rate of 17-26%, with a median survival of 8-10 months (22). Hematologic toxicities included grade 3-4 neutropenia (60%) and febrile neutropenia (10%). These results indicated that the combination of PTX and gemcitabine was moderately effective in MPM patients but was associated with a notably high incidence of neutropenia (22).

In this study on the methotrexate and gemcitabine doublet regimen, 3 patients (14.3%) exhibited grade 3-4 hematologic toxicity, with no sepsis or hemorrhage. There was no observed grade 3-4 non-hematological toxicity. Two patients developed interstitial pneumonitits (grade 2) which responded well to

steroid therapy. Thus, the tolerability and toxicity profiles were considered acceptable.

The response rate with the methotrexate and gemcitabine combination chemotherapy was 38.1%, which is within the range of 20-50% observed with other 'active' agents for MPM (8,10). Median survival was 19.4 months. Antifolates may be one of the key agents for MPM, since the majority of mesothelioma cells of all histological MPM subtypes express high-affinity α folate receptor (23). In our combination regimen, we observed that methotrexate, an old-type antifolate, exhibited desirable efficacy. Methotrexate has also been reported to be more efficient compared to PTX, a newly developed antifolate, against osteosarcoma cells (24), indicating that methotrexate possesses a therapeutic potential.

In the present study, 10 out of the 21 patients were chemotherapy-naïve and their response rate to this regimen was similar to the overall response rate described above. This suggests that methotrexate plus gemcitabine may be beneficial as the first-line treatment for MPM. Eleven patients who had been previously treated also exhibited a response rate similar to the overall response rate. Although the optimal regimen constituting the second-line chemotherapy remains to be determined, results of the present study suggest that methotrexate plus gemcitabine may also be beneficial as a second-line treatment.

In conclusion, the present study demonstrated that the methotrexate and gemcitabine combination therapy is feasible, with a more favorable toxicity profile and efficient in the treatment of MPM. Further clinical evaluation is required, with prospective trials including a larger cohort of patients.

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Combined serum mesothelin and carcinoembryonic antigen measurement in the diagnosis of malignant mesothelioma

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Abstract. Malignant mesothelioma (MM) is a highly aggressive tumor associated with asbestos exposure. The identification of a marker specific for MM may be of considerable value for the early detection of this tumor and may be used in particular to screen groups with a history of asbestos exposure. The aim of this study was to evaluate serum soluble mesothelin-related peptide (SMRP) levels as a diagnostic marker for MM and investigate whether its diagnostic value is enhanced by combination with other biomarkers. Serum SMRP levels were measured using a quantitative enzyme-linked immunosorbent assay in 96 patients with MM, 55 patients with lung cancer and 39 individuals with a history of asbestos exposure. Receiver operating characteristic curves were constructed for performance evaluation. Stepwise logistic regression analysis was used to select marker combinations (MCs). Serum SMRP levels in patients with MM were significantly higher compared to those in the other groups (P<0.001). The sensitivity of SMRP levels in diagnosing MM was 56% and its specificity for MM vs. lung cancer and individuals with asbestos exposure was 87 and 92%, respectively. The area under the curve (AUC) was 0.76 [95% confidence interval (CI): 0.68-0.83] for the differentiation between MM and lung cancer and 0.78 (95% CI: 0.71-0.86) for the differentiation between MM and individuals with asbestos exposure. For the MC of presence of effusion, SMRP and carcinoembryonic antigen (CEA) levels, the AUC for the differentiation between MM and lung cancer (0.92; 95% CI: 0.88-0.97) and the differentiation between MM and individuals with asbestos exposure (0.93; 95% CI: 0.87-1.0) was significantly higher compared to that for SMRP alone (P=0.0001 and 0.0058, respectively). While the specificity of this MC was comparable to SMRP alone, its sensitivity was ~20% higher compared to that of SMRP alone. Therefore, combining SMRP and CEA improves the diagnostic performance of SMRP alone. A combination of serum biomarkers, including SMRP, may facilitate the non-invasive diagnosis of MM.

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Key words: malignant mesothelioma, mesothelin, soluble mesothelin-related peptide, Mesomark™, marker combinations

Introduction

Malignant mesothelioma (MM) is a tumor that develops from the serous membranes that line the body cavities and it may arise in the pleura, peritoneum and pericardium; in addition, although extremely rare, it may also develop in the tunica vaginalis testis. The most common form of this disease is the malignant pleural mesothelioma (MPM). MM was previously considered as being extremely rare; however, its incidence and associated mortality rate exhibited a sharp increase worldwide over the last 50 years, due to the close association of MM with asbestos exposure. The prognosis of MPM is poor, with a median survival of ~9-17 months (1). However, in selected patients with epithelioid tumor histology, early-stage disease, who undergo trimodality treatment (combination of chemotherapy, postoperative radiotherapy and extrapleural pneumonectomy), median overall survival of 51 months and 5-year survival rates of 46% have been reported (2). Recent phase II trials reported a median survival of ~30 months for the patients who completed the trimodality treatment (3,4). Therefore, early diagnosis may play a vital role in the improvement of therapeutic outcomes. Together with the advances in imaging studies and endoscopic examinations, the development of biomarkers useful for serum or effusion diagnosis is crucial for the early diagnosis of MM. Currently known biomarkers for diagnosing MM include cytokeratin 19 fragment (CYFRA) (5-7), tissue polypeptide antigen (TPA) (5,6,8), hyaluronic acid (8), carbohydrate antigen (CA125) (8,9) and osteopontin (10-15). However, these markers have low specificity for MM.

Mesothelin is a 40-kDa cell surface glycoprotein that is overexpressed in cells of pancreatic and ovarian cancer, mesothelioma and other malignancies. The mesothelin gene encodes a 69-kDa glycoprotein, the mesothelin precursor protein, which is cleaved by a furin-like protease and its N-terminal region is released in the blood as a 31-kDa protein, the megakaryocyte

potentiating factor (MPF). The 40-kDa C-terminal region of this glycoprotein binds to the cell membrane as mesothelin. Three distinct variants of mesothelin have been identified, one of which has a modified C-terminus and becomes detached from the cell membrane since it lacks a glycosylphosphatidylinositol (GPI) anchor. This soluble isoform corresponds to the soluble mesothelin-related peptide (SMRP) (16). SMRP and MPF may be highly specific biomarkers for MM and have an equivalent diagnostic performance (17-19). SMRP is currently the most extensively investigated and is considered to be the best available blood protein biomarker of MM (20).

However, the diagnostic performance of SMRP alone is not considered to be sufficiently high, as it appears to exhibit insufficient sensitivity for MM (20,21). In diagnosing malignant tumors, such as ovarian or prostate cancer, the diagnostic performance of individual serum biomarkers was improved by combining data obtained using multiple biomarkers (22,23).

In the present study, we evaluated the performance of serum SMRP levels in the diagnosis of MM and investigated whether its diagnostic value could be improved through its combination with other biomarkers.

Materials and methods

Study design. The subjects of this study were patients who satisfied the following inclusion criteria: i) age ≥20 years; ii) pathologically proven MM or lung cancer; and iii) except for ii), individuals with asbestos exposure proven on the basis of their history or from the medical viewpoint. Only patients who personally provided written informed consent for the measurement of their serum biomarkers were enrolled in this study. Subjects who satisfied the above inclusion criteria during the study period were retrospectively enrolled. The pathological diagnosis was based on standard histological and immunohistochemical criteria (24,25). The subjects were classified into three groups: individuals with a history of asbestos exposure, patients with lung cancer and patients with MM. This study was approved by the Institutional Review Board of the Hyogo College of Medicine.

Measurement of serum biomarker levels. At the time of confirmation of the diagnosis, blood samples were collected from the subjects and, following prompt separation of the serum, the samples were stored at -80˚C. The serum SMRP levels were measured using an ELISA kit (Mesomark™; Fujirebio Diagnostics Inc., Malvern, PA, USA) according to the manufacturer's instructions. The serum levels of CYFRA and carcinoembryonic antigen (CEA) were measured using commercially available immunoassay systems according to the manufacturer's instructions: the serum CEA levels were determined using a chemiluminescent immunoassay (Abbott Japan Co., Ltd., Tokyo, Japan) and the serum levels of CYFRA were determined using a solid-phase sandwich immunoradiometric assay (CIS Bio International, Gif-sur-Yvette, France). The manufacturer suggests 3.5 ng/ml for CYFRA and 5.0 ng/ml for CEA as the cut-off values to differentiate between non-malignant disease and malignant tumors.

Statistical analysis. Summary statistics were used (median and 25th and 75th percentiles) to evaluate the distribution of serum SMRP levels. The Steel's test, a non-parametric form of the Dunnett's test, was used for comparing MM to the other groups. The sensitivity and specificity of SMRP for diagnosing MM were calculated, along with the corresponding 95% exact confidence intervals (CIs). The above analyses were also performed for CYFRA and its performance was compared to that of SMRP by using the McNemar's test. To compare the serum SMRP levels between each histological subtype of MM, the Steel-Dwass test, a non-parametric form of the Tukey's test, was performed. Subsequently, a stepwise logistic regression analysis was used to select marker combinations (MCs) that were more effective for diagnosing MM. The criterion for assessing whether a difference was significant in the variable selection was 5%. The diagnostic performance of SMRP and the MC was assessed by constructing a receiver operating characteristic (ROC) curve and calculating the area under the curve (AUC). The AUC for SMRP and that for the MC were compared using the theory on generalized U-statistics to generate an estimated covariance matrix and the χ^2 test (26). For each test, two-sided $P<0.05$ was considered to indicate a statistically significant difference. Data were analyzed using the statistical software SAS, version 9.1.3 (SAS Institute Inc., Cary, NC, USA) and Stata, version 11.0 (StataCorp College Station, TX, USA). The GraphPad Prism software, version 4.00 for Windows (GraphPad Software, San Diego, CA, USA) was used to prepare the figures.

Results

Patient characteristics. A total of 190 subjects were enrolled in this study. A summary of the clinical characteristics of these subjects, together with a breakdown of each group by age, gender, history of asbestos exposure and presence of effusion (pleural or peritoneal) is presented in Table I. Among the 39 individuals with asbestos exposure, pleural plaque was present in 16, benign asbestos pleurisy in 7, asbestosis in 3 patients, asbestosis plus benign asbestos pleurisy in 5, round atelectasis in 2 and no imaging abnormalities in 6 patients. The histological subtype in the 55 patients with lung cancer was adenocarcinoma in 24, squamous cell carcinoma in 14 and small-cell carcinoma in 17 patients. Among the 96 patients with MM, the primary tumor site was the pleura in 91 and the peritoneum in 5 patients (Table II). The histological subtype was epithelioid in 57 patients, sarcomatoid in 12, biphasic in 6, desmoplastic in 4 and unspecified in the remaining 7 patients (Table II). Of the 91 patients with MPM, 74 were diagnosed with clinical stage IV disease according to the staging classification proposed by the International Mesothelioma Interest Group (IMIG). Only 5 patients had either stage I or II disease (Table II).

Performance of serum SMRP in diagnosing MM. Fujirebio Diagnostics, Inc., the developer of the Mesomark assay, recommends a cut-off value of 1.5 nM, which was the 99th percentile of the normal serum SMRP concentration in a population of 409 healthy Americans (27). An investigation in a population of healthy Germans revealed a cut-off value of 1.5-1.6 nM, which was the 95th percentile of the serum SMRP concentration (28). In our study, we performed a preliminary investigation of the distribution of serum SMRP levels among

Table I. Characteristics of the study subjects.

Characteristics	AE (n=39)	LC (n=55)	MM (n=96)
Age (years)			
Mean ± SD	68.1±8.1	64.7±10.6	61.2±9.5
Range	44-90	39-84	33-83
Gender			
Male	36	45	75
Female	3	10	21
Asbestos exposure			
Occupational	26	1	55
Environmental	13	1	27
None	0	53	14
Presence of effusion	12	16	78

AE, asbestos exposure; LC, lung cancer; MM, malignant mesothelioma; SD, standard deviation.

Table II. Demographic data of MM patients.

Characteristics	Patient no. (%)
Primary site	
Pleura	91 (94.8)
Peritoneum	5 (5.2)
Histological subtype	
Epithelioid	57 (59.4)
Sarcomatoid	12 (17.4)
Biphasic	16 (16.7)
Desmoplastic	4 (5.8)
NOS	7 (7.3)
Staging classification[a]	
I	3 (3.3)
II	2 (2.2)
III	12 (13.2)
IV	74 (81.3)

[a]Proposed by the International Mesothelioma Interest Group (IMIG), peritoneal mesothelioma (n=5) was excluded. MM, malignant mesothelioma; NOS, not otherwise specified.

72 healthy individuals without a history of asbestos exposure. Since this investigation revealed that 69 individuals (96%) had serum SMRP levels of <1.5 nM, we selected 1.5 nM, the 96th percentile, as the cut-off value.

The distributions of serum SMRP levels in each group are shown in Fig. 1. The serum SMRP levels in MM patients were significantly higher compared to those in the other groups (P<0.001) (Table III). The sensitivity of SMRP for diagnosing MM was 56% (95% CI: 46-66%) and its specificity for MM vs. lung cancer and individuals with asbestos exposure was 87% (95% CI: 76-95%) and 92% (95% CI: 79-98%), respectively (Table IV). By contrast, the sensitivity of CYFRA for diagnosing MM was 63% (95% CI: 52-72%) and its specificity for MM vs. lung cancer was 49% (95% CI: 35-63%) (Table IV). The sensitivity of SMRP and CYFRA did not differ significantly (P=0.157), although the specificity of SMRP for MM vs. lung cancer was significantly higher compared to that of CYFRA (P<0.001). The serum SMRP levels in epithelioid disease [median, 2.47 nM; interquartile range (IQR): 0.97-4.86] were significantly higher compared to those in sarcomatoid disease (median, 0.8 nM; IQR: 0.38-1.15) (P=0.04). However, there were no significant differences when compared to the other histological subtypes. There was no significant association between the serum SMRP levels and MPM stages (data not shown).

The diagnostic performance of SMRP was evaluated using ROC curves (Fig. 2). For the differentiation between MM and lung cancer, the AUC was 0.76 (95% CI: 0.68-0.83) (Fig. 2A) and for the differentiation between MM and individuals with asbestos exposure, the AUC was 0.78 (95% CI: 0.71-0.86) (Fig. 2B). For CYFRA, the AUC for the differentiation between MM and lung cancer was 0.55 (data not shown). Therefore, the diagnostic performance of SMRP for differentiating between MM and lung cancer was superior to that of CYFRA.

Investigation of MCs and their performance in diagnosing MM. To improve the performance of serum biomarkers in diagnosing MM, we investigated the optimal MCs. The measured variables common to patients with MM and lung cancer were age, gender, presence of effusion, clinical stage and the levels of SMRP, CYFRA and CEA. The measured variables common to patients with MM and individuals with a history of asbestos exposure were age, presence of effusion and the levels of SMRP, CYFRA and CEA. Since the distributions of all the biomarkers were significantly skewed to the right, the variables were logarithmically transformed using common logarithms. A stepwise logistic regression analysis was used to select the variables. To differentiate between MM and lung cancer, SMRP levels, presence of effusion and CEA levels were selected (Table V). From the signs of the estimates, we determined that the probability of a diagnosis of MM was higher for elevated SMRP levels, presence of pleural

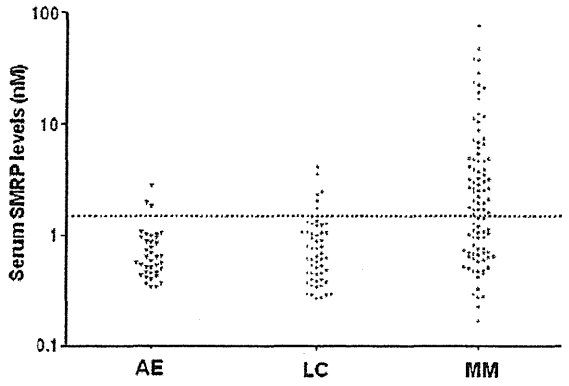

Figure 1. Distribution of serum soluble mesothelin-related peptide (SMRP) levels in each group. The serum SMRP levels in patients with malignant mesothelioma (MM) are compared to those in patients with lung cancer (LC) and individuals with a history of asbestos exposure (AE). The cut-off value is denoted by the horizontal dotted line.

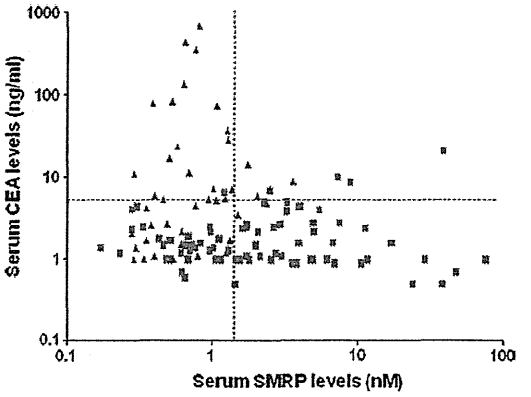

Figure 3. Scatter diagrams of serum biomarker levels in patients with malignant mesothelioma (■) and lung cancer (▲). Carcinoembryonic antigen (CEA) levels plotted against soluble mesothelin-related peptide (SMRP) levels. Each cut-off value is denoted by horizontal or vertical dotted lines.

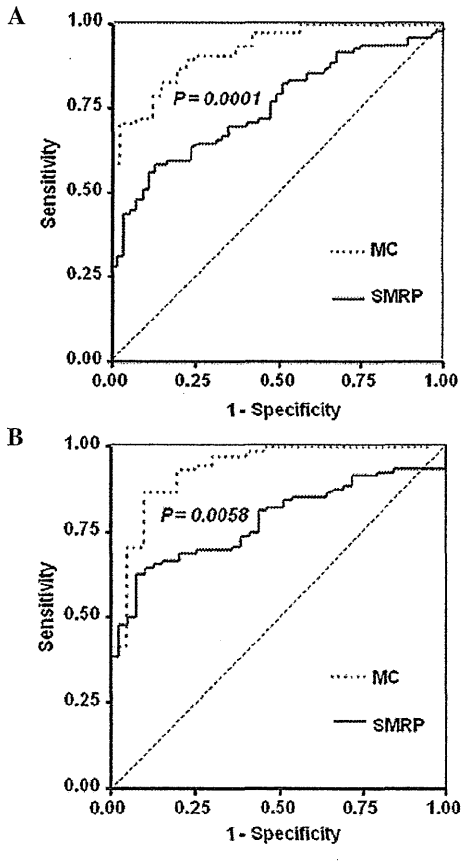

Figure 2. (A) Receiver operating characteristic (ROC) curves for soluble mesothelin-related peptide (SMRP) and the marker combination (MC) for differentiating between patients with malignant mesothelioma and lung cancer. The area under the curve (AUC) for the MC is significantly higher compared to that for SMRP alone (P=0.0001). (B) ROC curves for SMRP and the MC for differentiating between patients with malignant mesothelioma and individuals with a history of asbestos exposure. The AUC for the MC is significantly higher compared to that for SMRP alone (P=0.0058).

effusion and lower CEA levels. It was concluded that the selected markers were reasonable from the clinical standpoint. Subsequently, the markers selected to differentiate between MM and individuals with a history of asbestos exposure were age and CYFRA (data not shown). However, this model was composed of a single marker rather than multiple markers. Therefore, it was excluded from further investigation.

To further evaluate the models in Table V, the association between SMRP and CEA was analyzed using scatter diagrams (Fig. 3). The scatter diagrams demonstrated that the majority of patients with high CEA levels were those with lung cancer. In addition, the majority of patients with high SMRP levels were those with MM. Therefore, the combination of SMRP and CEA resulted in only a minor overlap of the diagnostic findings of MM and lung cancer, suggesting that the diagnostic performance for MM was improved. By contrast, since the combination of SMRP and CYFRA resulted in a significant overlap of the diagnostic findings of MM and lung cancer, it was inferred that the diagnostic performance was scarcely improved (data not shown).

The MC was composed using the results of Table V. Since the ratio of the estimates for SMRP, presence of effusion and CEA was ~3:1:5, the following MC was selected: MC=1xI(presence of effusion) + 3 x $\log_{10}$(SMRP) - 5 x $\log_{10}$(CEA), where I (presence of effusion) was defined as an indicator function with a value of 1 when effusion was present and 0 when effusion was absent. Wherein -1 was selected as the cut-off value to maximize the sum of the sensitivity and specificity, the sensitivity of MC for diagnosing MM was 76% (95% CI: 64-85%) and its specificity for MM vs. lung cancer and individuals with asbestos exposure was 88% (95% CI: 74-96%) and 90% (95% CI: 68-99%), respectively. While the specificity of MC was comparable to SMRP alone, its sensitivity was ~20% higher compared to that of SMRP alone. In addition, three of the five MPM patients with stage I-II disease were above the cut-off value, although none exhibited elevated serum levels of SMRP alone. The ROC curves for MC are shown in Fig. 2. The AUC for the differentiation between MM and lung cancer was 0.92 (95% CI: 0.88-0.97), which was significantly higher compared to that for SMRP alone (P=0.0001) (Fig. 2A). The AUC for the differentiation between MM and individuals with a history of asbestos exposure was 0.93 (95%CI: 0.87-1.0), which was also significantly higher compared to that for SMRP alone (P=0.0058) (Fig. 2B). These results indicate that combining CEA with SMRP improves the performance

Table III. Diagnostic findings based on the serum SMRP levels.

Serum SMRP levels (nM)	AE (n=39)	LC (n=55)	MM (n=96)
Mean ± SD	0.78±0.50	0.93±0.77	5.77±11.1
Median	0.64	0.65	1.88[a]
QR25-QR75	0.49-0.96	0.40-1.08	0.71-4.79
Min-max	0.30-2.80	0.30-4.10	0.30-75.4

[a]P<0.001, MM vs. AE or LC (by Steel's test). SMRP, soluble mesothelin-related peptide; AE, asbestos exposure; LC, lung cancer; MM, malignant mesothelioma; SD, standard deviation; QR25, 25th percentile; QR75, 75th percentile; min, minimum; max, maximum.

Table IV. Sensitivity and specificity of biomarkers for diagnosing MM.

Biomarkers	AE (n=39)	LC (n=55)	MM (n=96)
SMRP (%)			
Sensitivity	8	13	56
95% CI	2-21	5-24	46-66
Specificity	92	87	
95% CI	79-98	76-95	
CYFRA (%)			
Sensitivity	8	51	63
95% CI	2-21	37-65	52-72
Specificity	92	49	
95% CI	79-98	35-63	
CEA (%)			
Sensitivity	64	57	9
95% CI	41-83	41-72	4-17
Specificity	36	43	
95% CI	17-59	28-59	

MM, malignant mesothelioma; AE, asbestos exposure; LC, lung cancer; SMRP, soluble mesothelin-related peptide; CYFRA, cytokeratin 19 fragment; CEA, carcinoembryonic antigen; CI, confidence interval.

Table V. Results of stepwise logistic regression analysis (MM vs. LC).

Parameter	DF	Estimate	SE	Wald χ^2	P-value
Intercept	1	3.08	0.79	15.45	<0.001
SMRP[a]	1	2.83	0.92	9.48	0.002
Presence of effusion	1	1.28	0.42	9.15	0.003
CEA[a]	1	-5.52	1.46	14.20	<0.001

[a]The levels of SMRP and CEA were logarithmically transformed. MM, malignant mesothelioma; LC, lung cancer; DF, degree of freedom; SE, standard error of estimate; SMRP, soluble mesothelin-related peptide; CEA, carcinoembryonic antigen.

of SMRP alone in diagnosing MM and may facilitate early detection of MPM.

Discussion

The recent development of Mesomark, a quantitative ELISA kit using two monoclonal antibodies (OV569 and 4H3) that recognize SMRP, has enabled the measurement of serum SMRP levels. The findings of key studies on the performance of SMRP in diagnosing MM by using the Mesomark kit demonstrated that serum SMRP levels were significantly higher in MM patients compared to those in controls, such as healthy individuals, subjects with a history of asbestos exposure, or patients with asbestos-related benign pleural disease or lung cancer (9,11-21,27-35). In the present study, also undertaken using the Mesomark kit, the serum SMRP

levels were found to be significantly higher in MM patients compared to those in lung cancer patients and individuals with asbestos exposure. These findings are consistent with those first reported by Robinson *et al* (36), suggesting that the use of serum SMRP levels for diagnosing MM has excellent universality and reproducibility. Based on previous studies, including our own, SMRP is considered to be a highly specific biomarker for MM; however, its sensitivity, ranging from 48-80%, is moderate (9,11-21,27-35). To improve the performance of SMRP in diagnosing MM, there is a need to increase the sensitivity while maintaining a high degree of specificity.

One way of improving the sensitivity may be by lowering the cut-off value; however, this is not recommended, since it may result in a simultaneous reduction of specificity (26,28). Another approach may be to improve the diagnostic performance by combining data obtained using multiple biomarkers. The accuracy of the histopathological diagnosis of MM has markedly improved. One reason for this improvement has been the introduction of immunohistochemical analysis involving the combination of a positive marker that is highly expressed in MM and a negative marker that has a low frequency of expression in MM (37,38). A systemic review of markers for diagnosis of MM demonstrated that positive staining for CEA and epithelial antigen (clone Ber-EP4) and negative staining for epithelial membrane antigens and calretinin may confirm that a patient does not have MM (21). In addition, based on biomarker measurements in the pleural effusion, algorithms for the diagnosis of malignant pleural diseases were established. The CEA level achieved a greater accuracy in the differential diagnosis of MPM through its combination with other markers. For example, an elevated CYFRA level with a low CEA level in pleural effusion was shown to be highly suggestive of MPM (7).

To date, whether the combination of blood biomarkers, including SMRP, is able to improve the performance of SMRP alone in diagnosing MM remains controversial. A previous study by van den Heuvel *et al* (34) reported that the combination of two serum markers (CEA and SMRP) was the most accurate in differentiating MPM from non-small-cell lung cancer. The AUC of this marker combination demonstrated a significant improvement compared to the inverse levels of CEA alone. However, in that study, a direct comparison of diagnostic performance between this combination and SMRP alone was not performed.

Amati *et al* (31) evaluated the combination of two hematological biomarkers: 8-hydroxy-2'-deoxyguanosine (8-OHdG), an indicator of oxidative DNA damage and vascular endothelial growth factor β (VEGFβ), an angiogenic molecule. The results of that study indicated that the diagnostic performance of this combination in differentiating between healthy individuals and those with a history of asbestos exposure was superior to that of each biomarker alone. Although it was also mentioned that a combination of SMRP, 8-OhdG and VEGFβ was optimal for distinguishing between individual groups, including the MM group, that study provided no specific measures of diagnostic performance or any further details.

Several previous studies evaluated the diagnostic performance of combined SMRP and osteopontin measurements in MM. Creaney *et al* (12) demonstrated that the combination of SMRP, serum osteopontin and MPF did not exhibit increased sensitivity for detecting MM compared to that of SMRP alone. A recent study investigated serum SMRP and plasma osteopontin levels in 66 patients with MPM, 47 patients with non-malignant asbestos-related lung or pleural diseases, 42 patients with other benign pleural and lung diseases and 21 patients with lung cancer, as plasma osteopontin was proven to be more stable compared to serum osteopontin (14). A logistic regression analysis revealed that the combined marker model had an AUC of 0.912 and a sensitivity of 76%, with a 95% specificity (14). The AUC for this marker combination did not differ from that for serum SMRP alone. In previous studies, the majority of osteopontin-positive MM patients were also found to be positive for SMRP. This high degree of concordance may result in the finding that a combination of these two markers does not improve the performance of SMRP alone in diagnosing MM (12,14). Cristaudo *et al* (15) also measured serum SMRP and plasma osteopontin levels in 93 healthy subjects, 111 individuals with benign respiratory disease and 31 patients with MPM. That study was the first to demonstrate that a combination of these two markers was more efficient in MPM diagnosis compared to each marker used alone by means of the combined risk index, a new statistical approach of a logistic regression analysis. In that study, however, a small number of patients with MPM were enrolled and its histological subtype was limited to the epithelioid type. To confirm those findings, larger-scale studies are required. The combination of SMRP with CA125 (9), or MPF (12,18) has also been investigated. However, none of those studies demonstrated that the diagnostic performance of SMRP in combination with other markers outperformed that of SMRP alone.

The present study demonstrated that combining SMRP and CEA improved the diagnostic performance of SMRP alone, since these two markers act in a complementary manner. However, since we used the same data for selecting and assessing the performance of MC, it is possible that our evaluation of the MC may have been optimistic. Furthermore, in our study, data were collected from a single center; validation of the diagnostic performance of this particular MC by a multicenter study is recommended in the future.

It is difficult to determine whether pleural effusion developing in individuals with a history of asbestos exposure represents benign asbestos pleurisy or is an initial symptom of MPM and misdiagnosis at this stage may hinder the early detection of MPM. Future prospective research is required to confirm whether a combination of serum biomarkers, including SMRP, may be useful in diagnosing early-stage MPM.

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Carboplatin plus Either Docetaxel or Paclitaxel for Japanese Patients with Advanced Non-small Cell Lung Cancer

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Abstract. *Aim: Assessment of the efficacy of docetaxel plus carboplatin vs. paclitaxel plus carboplatin in Japanese patients with advanced non-small cell lung cancer (NSCLC). Patients and Methods: Chemotherapy-naïve patients were randomly assigned at a ratio of 2 to 1 to receive six cycles of either docetaxel (60 mg/m^2) plus carboplatin [area under the curve (AUC)=6 mg/ml min] or paclitaxel (200 mg/m^2) plus carboplatin (same dose), on day 1 every 21 days. The primary end-point was progression-free survival (PFS). Results: A total of 90 patients were enrolled. Overall response rate, median PFS and median survival time in the docetaxel-plus-carboplatin group and the paclitaxel-plus-carboplatin group were 23% vs. 33%, 4.8 months vs. 5.1 months, and 17.6 months vs. 15.6 months, respectively. The docetaxel-plus-carboplatin group had a higher incidence of grade 3 or 4 neutropenia (88% vs. 60%). Conclusion: Both regimens were similarly effective in Japanese patients with advanced NSCLC.*

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Key Words: Non-small cell lung cancer, chemotherapy, randomized phase 2, ethnicity, docetaxel, paclitaxel carboplatin.

Lung cancer is one of the most common malignancies and is the leading cause of cancer-related death worldwide (1). Non-small cell lung cancer (NSCLC) accounts for 85% of all cases of lung cancer. Platinum-based chemotherapy has been considered a standard treatment for advanced NSCLC. In addition, molecular-targeted therapy, including vascular endothelial growth factor (VEGF) inhibitors such as bevacizumab, epidermal growth factor receptor (EGFR) inhibitors such as gefitinib or erlotinib, and anaplastic lymphoma kinase (ALK) inhibitors, has recently become a treatment option for specific subsets of patients, especially those with non-squamous cell lung cancer (2-5). These molecular targeted therapies have led to a paradigm shift of

treatment. Unfortunately, all patients with *EGFR*-mutant or ALK-positive lung cancer who receive EGFR or ALK inhibitors eventually experience disease relapse and require chemotherapy at some point during the course of treatment (4). Chemotherapy thus continues to play an important role in the management of NSCLC.

Docetaxel has been demonstrated to be effective against previously-untreated advanced NSCLC. Results of a large phase III trial found that docetaxel plus cisplatin was significantly superior to vindesine plus cisplatin in terms of overall response rate and overall survival (6). Carboplatin has shown broad equivalence to cisplatin in combination with chemotherapy for advanced NSCLC. To our knowledge, however, no clinical trial has directly compared docetaxel + carboplatin (DCarb) with paclitaxel plus carboplatin (PCarb) in patients with advanced NSCLC.

Fossella *et al.* reported a phase III study comparing docetaxel plus a platinum agent with vinorelbine plus cisplatin, performed by the TAX 326 Study Group (7). Docetaxel with cisplatin led to a better overall response and higher survival rate than docetaxel plus carboplatin, with a median survival time (MST) of 11.3 months, as compared with 9.4 months, respectively. However, that study was not designed to directly compare docetaxel plus cisplatin with docetaxel plus carboplatin. The therapeutic value of docetaxel with carboplatin as a front-line regimen for advanced NSCLC, thus remains unclear.

Millward *et al.* conducted a phase II study of docetaxel plus carboplatin in white and Asian patients with advanced NSCLC (8). The MST was 12.9 months, and multivariate analysis showed that ethnicity was a significant independent predictor of response and survival. Two clinical trials have evaluated docetaxel with carboplatin in Japanese patients with advanced NSCLC (9, 10). These trials reported a good MST of 12 months and 12.9 months, respectively. However, randomized phase II studies comparing docetaxel plus carboplatin with a standard regimen have yet to be performed on Asian patients with NSCLC. We therefore designed a randomized phase II study to compare the newer combination of DCarb with PCarb as standard treatment in patients with advanced NSCLC.

Patients and Methods

All patients enrolled in this study had cytologically- or histologically-confirmed diagnoses of NSCLC (adenocarcinoma, squamous cell carcinoma, large cell carcinoma, or NSCLC not otherwise specified) with advanced stage IIIB or stage IV disease or relapse after surgical resection of NSCLC (regarded as stage IV). Other eligibility criteria were as follows: chemotherapy-naïve status; an Eastern Cooperative Oncology Group performance status (PS) of 0 or 1; a neutrophil count of at least 2.0×10^9 cells/l; a platelet count higher than 100.0×10^9 cells/l; a hemoglobin concentration of at least 90 g/l; serum aspartate aminotransferase (AST) or alanine aminotransferase (ALT) concentrations of less than two-times the upper limit of normal (ULN); serum total bilirubin and creatinine concentrations of less than the ULN; a creatinine clearance of 50 ml/min or higher (as calculated by the Cockcroft-Gault equation) (11); and an alveolar partial pressure of oxygen (PaO_2) of 70 Torr or higher or an oxygen saturation on pulse oximetry (SpO_2) of 94% or higher (while breathing room air). Patients were excluded if they had any of the following conditions: severe infection, pregnancy or breastfeeding; a previous malignancy within the previous five years (except for patients with cured carcinoma *in situ*); another active cancer; an allergy to polysorbate 80 or polyoxyethylene castor oil; evidence of interstitial lung disease on a plain chest x-ray film; uncontrolled co-morbidities such as malignant hypertension, congestive heart failure, myocardial infarction within the previous six months, arrhythmia requiring treatment, bleeding tendency, or diabetes mellitus; pleural or pericardial effusion requiring drainage; symptomatic brain metastasis; or peripheral neuropathy of more than grade 1.

All patients provided written informed consent. The study protocol was approved by the Institutional Review Boards of all participating institutions and by the Japan Multinational Trial Organization (JMTO) ethical committee. This study was conducted in accordance with the Declaration of Helsinki and was registered with UMIN 000001225 on June 30, 2008.

Study design and treatment. This was a randomized, phase II, open-label study. The primary end-point was the determination of progression-free survival (PFS). The secondary end-points were tumor response, survival (1-year survival rate, overall survival), and toxic effects. Patients were randomly assigned at a ratio of 2 to 1 to receive either DCarbo or PCarbo. Central randomization to each arm was performed with the use of Pocock and Simon's method (12). Stratification factors were PS (0 or 1), more than 5% weight loss within the previous six months (yes or no), and serum lactic dehydrogenase (LDH) concentration (abnormally high or not).

Patients in the DCarbo group received intravenous docetaxel (60 mg/m^2) over the course of 60 to 90 min and carboplatin [area under the curve (AUC) 6 mg/ml min] over the course of three hours on day 1 every 21 days for six cycles. Pre-medication, such as anti-emetic agents or corticosteroids, was given as required. In the PCarbo group, patients received intravenous paclitaxel (200 mg/m^2) and carboplatin (AUC 6 mg/ml min, same as in the DCarbo group) on day 1 every 21 days for six cycles. Creatinine clearance was calculated using the Cockcroft-Gault equation. The serum creatinine level (mg/dl) used in this equation was modified by adding 0.2 mg/dl, because an enzyme assay is used in Japan, whereas Jaffe's non-enzyme assay was used to develop this equation. Patients in the PCarbo group were given pre-medication with dexamethasone, diphenhydramine, and ranitidine or cimetidine. The use of additional antiemetics was left at the physician's discretion. Use of granulocyte-colony stimulating factor (G-CSF) was permitted any time during the study (except for prophylactic use) in both groups. In the absence of progressive disease or intolerable toxicity, patients in both groups received six cycles of chemotherapy.

Treatment could be delayed for up to 14 days if the neutrophil count was less than 1.5×10^9 cells/l and the platelet count was less than 75×10^9 cells/l on day 1 of each course. In the event of prolonged or complicated grade 4 neutropenia or thrombocytopenia, the dose of docetaxel was reduced by 10 mg/m^2, that of paclitaxel by 25 mg/m^2, or that of carboplatin by AUC 1 mg/ml min for the subsequent cycle of chemotherapy. Dose reduction was allowed

twice. Treatment could be delayed for up to 14 days if AST or ALT (or both) was more than 2.5-times higher than the ULN, the serum creatinine concentration was more than 1.5-times higher than the institutional ULN, or nonhematological toxicity of grade 2 or higher developed (except for nausea, vomiting, fatigue, loss of appetite, mild electrolyte abnormalities, and alopecia) developed.

Patients were assessed every two cycles, and the objective response was evaluated according to the Response Evaluation Criteria in Solid Tumors (RECIST), version 1.0 (13). The best response in individual patients was derived from investigator-reported data. Objective response rates were confirmed by at least one sequential tumor assessment. Toxic effects were graded in accordance with the National Cancer Institute Common Toxicity Criteria, version 2.0 (14). The numbers and frequencies of each adverse event were respectively summarized for any grade and for grade 3 or higher in each treatment group. The MST with 95% confidence intervals (CI) and the probability of 1-year survival with 95% CI were calculated by the Kaplan-Meier method for each group.

Statistical plan and analysis. The primary end-point was PFS. The main objective of the study was to estimate the PFS rate at six months in the DCarbo group. The median PFS in the DCarbo group was predicted to be about 150 days on the basis of the results of previous studies. The PFS rate at six months was thus assumed to be 45%. Given that the range of the 90% CI at six months is 0.1 or less, we estimated that at least 60 patients would be required in the DCarbo group. Because patients were randomly assigned to either the DCarbo group or PCarbo group at a ratio of 2:1, the target number of patients in the latter group (calibration group) was 30. Hazard ratios (HR) and 95% CIs were calculated with a Cox proportional-hazards model.

Results

Patients' characteristics. A total of 90 patients were enrolled between June 2007 and September 2008 at 15 institutions in Japan. All patients were eligible for analysis. Sixty patients were assigned to the DCarbo group and 30 were assigned to the PCarbo group (Figure 1). The patients' characteristics for both groups were shown in Table I. The baseline characteristics of patients in the DCarbo group were similar to those in the PCarbo group.

Tumor response and survival. The total number of administered cycles of chemotherapy was 230 in the DCarbo group and 139 in the PCarbo group. The median follow-up time was 15.8 months.

Sixty patients began chemotherapy in the DCarbo group, and 19 completed six cycles according to protocol. The mean number of administered cycles of chemotherapy was 4.0 (range, 1 to 6). Dose modification was carried out once in 17 patients (28%) and more than once in 23 patients (38%). Treatment was delayed in 11 patients (18%). The reasons for treatment discontinuation before the completion of six cycles of DCarbo were disease progression (n=18), dose modification necessitated by adverse events more than twice (n=12), and withdrawal of treatment by the patient (n=6) or investigator (n=5). In the PCarbo group, 30 patients began chemotherapy, and 14 completed six cycles. The mean number of administered cycles was 4.6 (range, 1 to 6). Dose modification was carried out once in seven patients (23%) and more than once in seven patients (23%). Treatment was delayed in 10 patients (33%). The reasons for discontinuation of PCarbo before the completion of six cycles were disease progression (n=6), withdrawal of treatment by the patient (n=5), dose modification necessitated by adverse events more than twice (n=4), and withdrawal of treatment by the investigator (n=1).

The overall response rate (based on the best confirmed response during study treatment) was 23% [14 out of 60 patients with partial response (PR); 95% CI=13%-36%] in the DCarbo group and 33% (10 out of 30 patients with PR; 95% CI=17%-53%) in the PCarbo group (Table II). No patient had a complete response. Stable disease was obtained in 31 patients (52%; 95% CI=38%-65%) in the DCarbo group and 15 patients (50%; 95% CI=31%-69%) in the PCarbo group. The Median PFS was 4.8 months (95% CI=3.9-7.2 months) in the DCarbo group and 5.1 months (95% CI=4.4-6.4 months) in the PCarbo group. The PFS rate at six months was 42% (90% CI=31%-52%) in the DCarbo group and 40% (90% CI=25%-54%) in the PCarbo group (Figure 2). The hazard ratio of DCarbo referenced to PCarbo was 0.86 (95% CI=0.55-1.36). The MST was 17.6 months (95% CI=10.2-22.9 months) in the DCarbo group and 15.6 months (95% CI=9.3-20.8 monts) in the PCarbo group (Figure 3). The 1-year survival rate was 60% in both groups (90% CI=49%-70% in the DCarbo group and 44%-73% in the PCarbo group). The hazard ratio of DCarbo compared to PCarbo was 0.77 (95%CI=0.47-1.26).

Toxicity. All patients were assessable for toxicity (Table III). Patients in the DCarbo group had a higher incidence of grade 3 or 4 neutropenia than those in the PCarbo group (88% *vs*. 60%, 95% CI=77%-95% *vs*. 41%-77%). The PCarbo group had a higher incidence of grade 2 or more sensory neuropathy (37% *vs*. 3%, 95% CI=20%-56% *vs*. 0%-12%), myalgia (13% *vs*. 0%, 95% CI=4%-31% *vs*. 0%-6%), and arthralgia (20% *vs*. 2%, 95% CI=8%-39% *vs*. 0%-9%) than the DCarbo group. There were no major differences between the two groups regarding any other toxic effects (Table III).

One treatment-related death was reported in the DCarbo group. Acute respiratory distress syndrome (ARDS) developed in a 76-year-old woman two months after the end of the fifth, final cycle of treatment. Five days after the onset of respiratory failure, the patient had an acute myocardial infarction and died two days later. The patient's attending physician judged that the relation to treatment was "not definite." An independent data monitoring committee judged

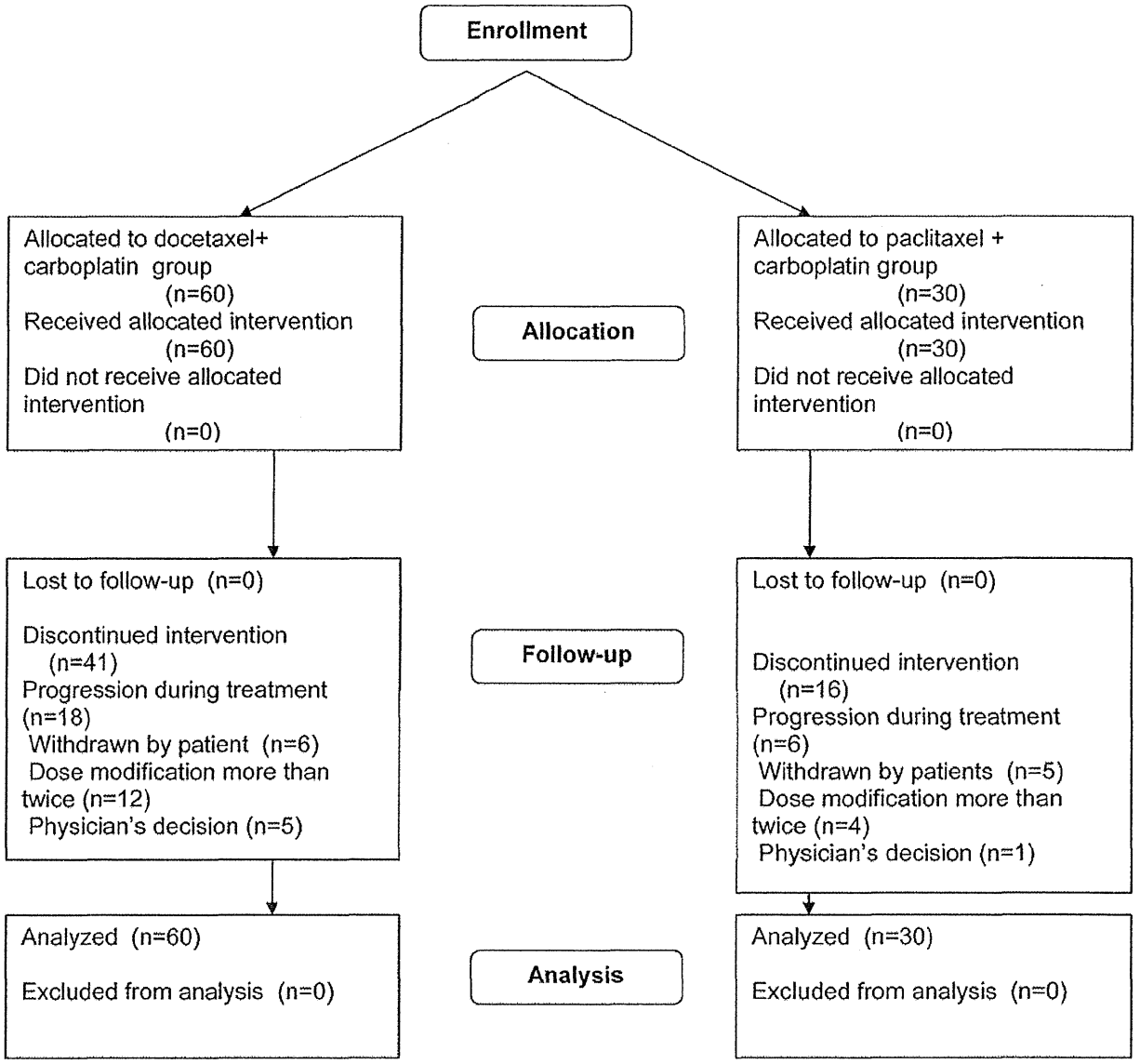

Figure 1. *Study design and patient flow. n: Number of patients.*

that the relation of death to the study treatment was not definite, but possible.

Discussion

This randomized phase II trial comparing DCarbo with PCarbo is the first of this kind to be performed in Asia. Our results suggest that both regimens are similar in terms of PFS and overall survival. The PFS of 4.8 (95% CI=3.9-7.2) months and MST of 17.6 (95% CI=10.2-22.9) months in the DCarbo group were favorable.

Asian ethnicity may contribute to some degree to better results in patients who receive DCarbo, as reported by Millward *et al.* (8). Three large phase III trials performed on Japanese patients with advanced NSCLC have included paclitaxel + carboplatin as one treatment arm (15-17). In these studies, the number of patients who received PCarbo was 281 (Okamoto *et al.*) (15), 197 (JMTO LC 00-03 study) (16), and 145 (Four-Arm Cooperative Study) (17), respectively. The dose of carboplatin was AUC 6 mg/ml min, with paclitaxel given at a dose of 200 mg/m^2 in two studies (15, 17) and 225 mg/m^2 in the other (16). The median PFS