PDGF-BB, VEGF, HGF, and SDF-1 alpha) were assayed in a subgroup of patients and control individuals by using the Bio-Plex suspension array system (Bio-Rad, Hercules, CA), which allows the simultaneous identification of cytokines in a 96-well filter plate. In brief, the appropriate cytokine standards and diluted plasma samples were added to a 96-well filter plate and incubated at room temperature for 30 min with antibodies chemically attached to fluorescent-labeled micro beads. After 3 filter washes, premixed detection antibodies were added to each well and incubated for 30 min. After 3 more washes, premixed streptavidin-phycoerythrin was added to each well and incubated for 10 min, followed by 3 more washes. The beads were then resuspended in 125 μL of assay buffer and the reaction mixture was quantified using the Bio-Plex protein array reader. Data were automatically processed and analyzed with Bio-Plex Manager Software 4.1 by using the standard curve obtained using a recombinant cytokine standard.

Statistical analyses

The Mann–Whitney test was used to compare the distributions of clinical factors and marker concentrations between patients with progressive disease (PD) and those without PD, stages III and IV disease, or recurrence. The survival time (progression-free survival [PFS] and overall survival [OS]) and clinical factors (age, gender, and Eastern Cooperative Oncology Group [ECOG] performance status

Table 1 Patient characteristics and CEC detection

		Mean CEC level 166 cells/4 mL	Range (2–1195 cells/4 mL)	Total	P^a
		≥ 166 cells/4 mL	<166 cells/4 mL		
		CEC^{high}	CEC^{low}		
		12	25	37	
Age	Over 70	8	10	18 (49%)	0.17
	Below 70	4	15	19 (51%)	
Sex	Male	7	17	24 (65%)	0.72
	Female	5	8	13 (35%)	
Stage	III	3	11	14 (38%)	0.59
	IV	8	12	20 (54%)	
	Recurrence	1	2	3 (8%)	
ECOG PS	0	5	18	23 (62%)	0.09
	1	6	4	10 (27%)	
	2	1	3	4 (11%)	
Pancreatic tumor location	Head	5	12	17 (46%)	>0.9
	Body	5	9	14 (38%)	
	Tail	2	4	6 (16%)	
CA19-9 (U/mL)	≥10,000	3	5	8 (22%)	>0.9
	< 10,000	9	20	29 (78%)	
CRP (mg/dL)	≥1.0	7	3	10 (27%)	<0.01
	<1.0	5	22	27 (73%)	
Histology	Poorly differentiated	5	9	14 (38%)	0.62
	Moderately differentiated	4	10	14 (38%)	
	Adenosquamous	1	0	1 (2%)	
	N.E (cytology only)	2	6	8 (22%)	
Tumor response	Partial response	2	2	4 (11%)	<0.05
	Stable disease	4	18	22 (59%)	
	Progressive disease	6	5	11 (30%)	
Second line therapy	S-1	6	12	18 (49%)	1
	Oxaliplatin + S-1	0	2	2 (5%)	
	No	6	11	17 (46%)	

[a]*P* values were calculated for each variable using Fisher's exact test.
Abbreviations: CEC = circulating endothelial cell; ECOG = Eastern Cooperative Oncology Group; CA19-9 = carbohydrate antigen 19–9; CRP = C-reactive protein.

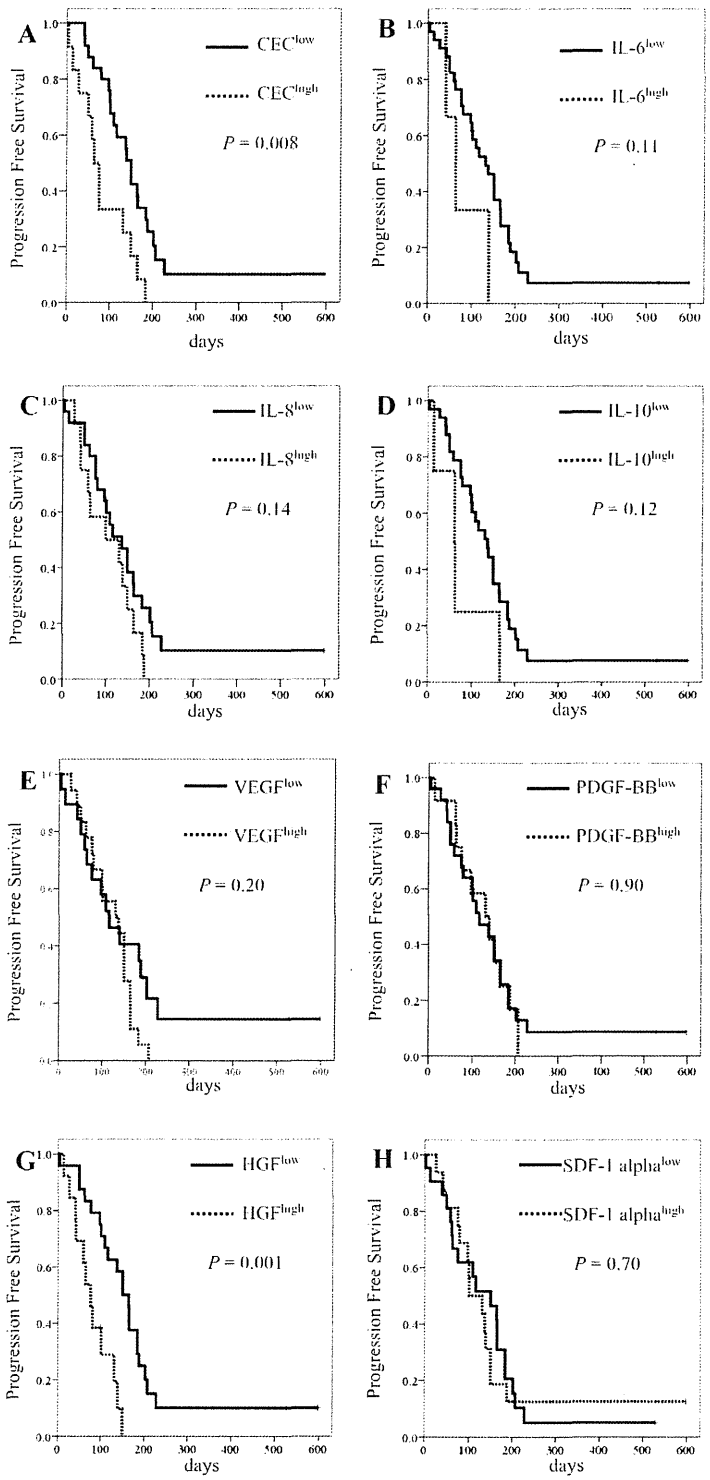

Figure 1 Kaplan-Meier curves for (A) progression-free survival with CEC counts, (B) progression-free survival with IL-6 levels, (C) progression-free survival with IL-8 levels, (D) progression-free survival with IL-10 levels, (E) progression-free survival with VEGF levels, (F) progression-free survival with PDGF-BB levels, (G) progression-free survival with HGF levels, and (H) progression-free survival with SDF-1 alpha levels. The cut-off points for the angiogenic factors were determined to be equal to or greater than these mean levels.

[PS], and clinical stage of the patients) were examined using the Cox proportional hazards model. The survival curves for PFS and OS were estimated using the Kaplan-Meier method. Kaplan-Meier curves were used only to determine the trends of the associations between the molecules and PFS/OS, as any determination of the optimal cutoff point for the molecules relative to PFS/OS was beyond the scope of the present study. All statistical analyses were performed using IBM SPSS Statistics 18 (IBM Corporation, Somers, NY, USA).

Results

Patient characteristics

A total of 37 patients with pancreatic carcinoma were prospectively enrolled in this study. Fourteen of these patients (38%) presented with locally advanced pancreatic carcinoma, 20 patients (54%) presented with metastases, and 3 patients (8%) were enrolled following recurrence after surgery. Twenty-three patients (62%) had ECOG PS0, 10 patients (27%) had ECOG PS1, and 4 patients (11%) had ECOG PS2. Histologically, 14 patients (38%) had poorly differentiated adenocarcinoma, 14 patients (38%) had moderately differentiated adenocarcinoma, 1 patient (2%) had an adenosquamous tumor, and 8 patients (22%) had cytological adenocarcinoma. No patient experienced a complete response to treatment. Four patients (11%) exhibited a partial response (PR) rate to treatment (11%), stable disease (SD) was observed in 22 patients (59%), and PD was observed in 11 patients (30%). Second-line therapy was administered to 20 patients (54%), whereby 18 patients (49%) received S-1 monotherapy and 2 patients (5%) received oxaliplatin and S-1 combination therapy (Table 1).

Baseline levels of CECs and angiogenic factors

The mean CEC level found in the pancreatic carcinoma patients was 166 cells/4 mL (range: 2–1195 cells/4 mL) while the median CEC level was 66 cells/4 mL. These CEC levels were higher than those of randomly-selected healthy volunteers ($P < 0.01$), as previously reported (n = 53, mean ± SD = 46.2 ± 86.3 cells/4 mL) [9]. In this study, the cut-off point of CEC^{high} was determined to be equal to or greater than 166 cells/4 mL while that of CEC^{low} was lower than 166 cells/4 mL. CEC^{high} was significantly associated with high levels of C-reactive protein (CRP) (over 1.0 mg/dL; $P < 0.01$). The median PFS was 64 days (95% confidence interval [CI], 45–83) in the CEC^{high} group, while that in the CEC^{low} group was 150 days (95% CI, 130–170; log-rank test; $P = 0.008$; Figure 1A). The median OS was 143 days (95% CI, 53–233) in the CEC^{high} group and 297 days (95% CI, 240–354) in the CEC^{low} group (log-rank test; $P < 0.001$; Figure 2A). Univariate analysis of CEC levels and clinical factors for OS was performed using the Cox proportional hazard model. The hazard ratio (HR) for CEC levels (CEC^{high} versus CEC^{low}) was 5.18 (95% CI, 2.23–12.03; $P < 0.001$).

The mean levels of IL-6, IL-8, IL-10, PDGF-BB, VEGF, HGF, and SDF-1 alpha were found to be 19.3 pg/mL, 11.3 pg/mL, 7.82 pg/mL, 1127.5 pg/mL, 44.1 pg/mL, 471.3 pg/mL, and 110.6 pg/mL, respectively. The cut-off points for the angiogenic factors were determined to be equal to or greater than these mean levels, and the median PFS in HGF^{low} was longer than the HGF^{high} group ($P = 0.001$; Figure 1G). However, other factors were not found to have statistical significance with regard to PFS. The median OS was longer in the case of IL-10 (112 days [95% CI, 50–173] in $IL\text{-}10^{high}$ vs. 264 days [95% CI, 204–324] $IL\text{-}10^{low}$, log-rank test: $P = 0.003$; Figure 2d) and HGF (150 days [95% CI, 65–234] in HGF^{high} vs. 291 days [95% CI, 223–359] in HGF^{low}, log-rank test: $P = 0.01$; Figure 2G).

Among the clinical factors that were examined in this study, a poor PS (PS 1 and 2), advanced stage (stage IV and recurrence), and high levels of IL-10, HGF, and CRP were significantly correlated with poor OS in univariate cox analysis, with HRs of 2.72 (95% CI, 1.29–5.70; $P = 0.008$), 2.21 (95% CI, 1.03–4.71; $P = 0.04$), 5.05 (95% CI, 1.55–16.39; $P = 0.007$), 2.52 (95% CI, 1.22–5.21; $P = 0.01$), and 2.49 (95% CI, 1.14–5.42; $P = 0.02$), respectively. In a multivariate Cox analysis model that included clinical stage, PS, CRP levels, CEC levels, IL-10 levels, and HGF levels, the number of CECs detected remained statistically stable at 0.05. The resulting HRs were 2.04 (95% CI, 0.78–5.35; $P = 0.15$), 2.58 (95% CI, 0.98–6.76; $P > 0.05$), 2.04 (95% CI, 0.62–6.76; $P = 0.24$), 5.14 (95% CI, 1.83–14.45, $P = 0.002$), 5.26 (95% CI, 1.26–22.22; $P = 0.02$) and 1.34 (95% CI, 0.46–3.91; $P = 0.59$), respectively (Table 2).

Changes in CEC number during treatment

The number of CECs was analyzed in 22 of the 37 patients at 28 ± 7 days after the start of gemcitabine therapy. The mean number of CECs detected in these patients after 28 ± 7 days was 133 cells/4 mL (range: 15–664 cells/4 mL), while the median number of CECs was 68 cells/4 mL. The absolute counts of CECs did not change significantly between day 1 and day 28 ± 7 of treatment (Mann–Whitney test, $P = 0.11$). Furthermore, a change in CEC counts from baseline to after 28 ± 7 days of treatment was not statistically associated with tumor response (Mann–Whitney test, $P > 0.05$, Figure 3).

Association between CEC number and blood angiogenic factors

The numbers of CECs were compared between non-PD (PR and SD, n = 26) and PD patients (n = 11) for

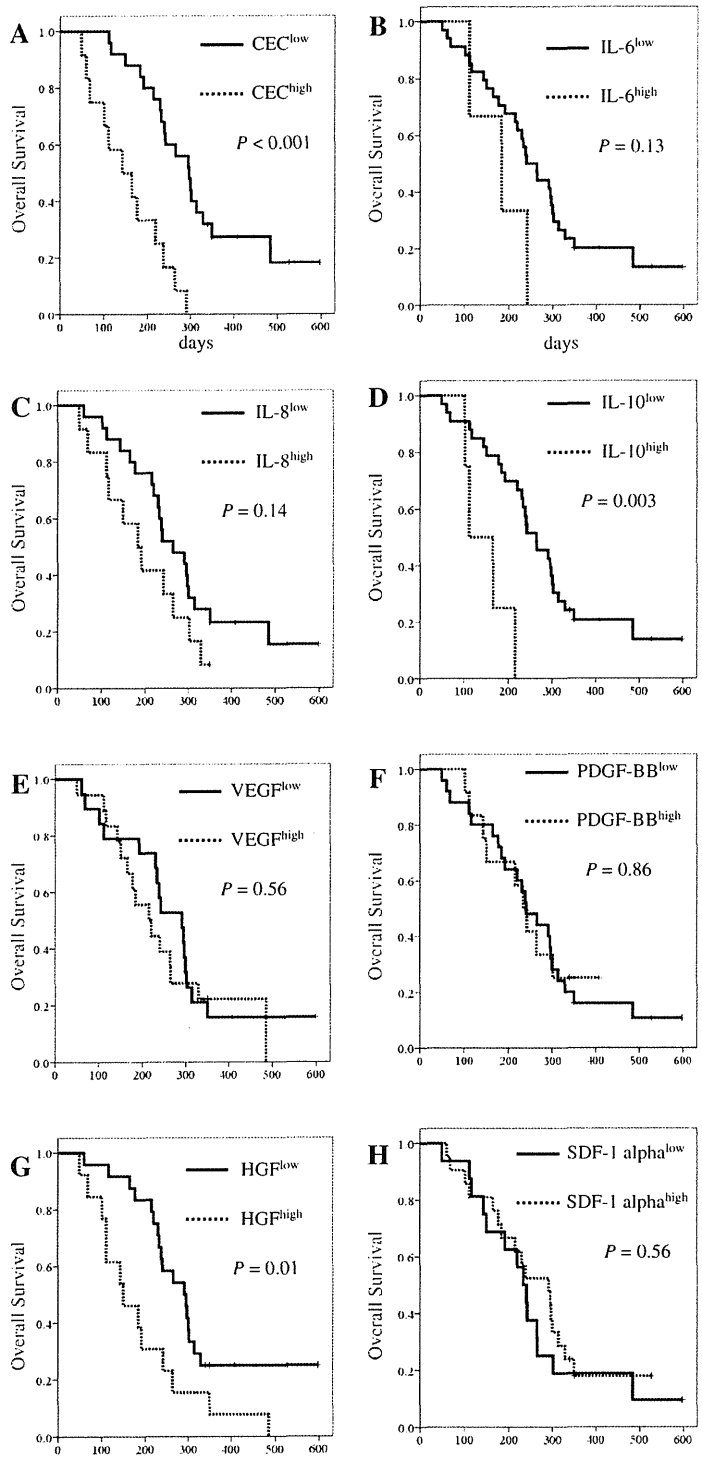

Figure 2 Kaplan-Meier curves for (A) overall survival with CEC counts, (B) overall survival with IL-6 levels, (C) overall survival with IL-8 levels, (D) overall survival with IL-10 levels, (E) overall survival with VEGF levels, (F) overall survival with PDGF-BB levels, (G) overall survival with HGF levels, and (H) overall survival with SDF-1 alpha levels. The cut-off points for the angiogenic factors were determined to be equal to or greater than these mean levels.

all markers. The baseline levels of CEC ($P = 0.03$), IL-6 ($P < 0.01$), and IL-10 ($P = 0.03$) were found to be significantly higher among patients with PD than among those with PR or SD. The blood concentrations of HGF ($P < 0.001$), IL-6 ($P < 0.01$), and IL-8 ($P < 0.001$) were also significantly higher among patients with clinical stage IV disease and recurrence than among those with stage III disease. When the association between CEC number and the expression of other angiogenic factors was examined, the number of CECs was found to correlate positively with the levels of VEGF (r = 0.34, $P = 0.04$), HGF (r = 0.37, $P = 0.02$), IL-8 (r = 0.38, $P = 0.02$), and IL-10 (r = 0.45, $P = 0.006$), suggesting that the number of CECs is related to the expression of these markers (Table 3).

Discussions

In most cases, CECs are apoptotic or necrotic cells that are released into circulation as a byproduct of vascular turnover. In some cancer patients, the level of CECs is significantly higher than that of healthy individuals, and this increased level has been identified as a surrogate marker of angiogenesis and anti-angiogenic drug activity [10,11]. The present study has shown that baseline CEC levels are markedly higher among pancreatic carcinoma patients than in healthy individuals. Our results also support the hypothesis that CEC levels are associated with clinical outcome in pancreatic carcinoma patients undergoing gemcitabine chemotherapy, and may be a prognostic factor for this disease. A previous study found that the baseline level of CECs, identified as $CD45^-CD31^+CD34^+$ by flow cytometry, was inversely associated with OS in patients who had gemcitabine-refractory metastatic pancreatic carcinoma and were treated with bevacizumab plus erlotinib [12]. CEC ($CD45^-CD31^+CD146^+$) detection by flow cytometry requires careful discrimination between blood cell populations with overlapping phenotypes showing hallmarks of T cells ($CD45^-CD31^-CD146^+$) and platelets ($CD45^-CD31^{high}CD146^-$). These cells populations show distinct regulation during cancer therapy, and their concomitant analysis may offer extended prognostic and predictive information [13].

Table 2 Univariate and multivariate Cox analyses of prognosis

Univariate analysis	**HR**	**95% CI**	***P***
Age: Over 70 vs. Below 70	0.52	0.25–1.13	0.1
Sex: Male vs. Female	1.00	0.48–2.08	0.99
Stage: IV + Recurrence vs. III	2.21	1.03–4.71	0.04
ECOG PS: 2 + 1 vs. 0	2.72	1.29–5.70	0.008
Pancreatic tumor location: Head vs. Others	0.94	0.46–1.90	0.86
CA19-9 (cut-off: 10,000 U/mL): $CA19\text{-}9^{high}$ vs. $CA19\text{-}9^{low}$	1.77	0.75–4.15	0.19
CRP level (cut-off: 1.0 mg/dL): CRP^{high} vs. CRP^{low}	2.49	1.14–5.42	0.02
Histology: Poorly differentiated vs. Others	1.09	0.52–2.27	0.82
Second line therapy: Yes vs. No	0.61	0.30–1.24	0.17
CEC level (cut-off: 166 cells/4 mL): CEC^{high} vs. CEC^{low}	5.18	2.23–12.03	<0.001
IL-6 (cut-off: 19.3 pg/mL): $IL\text{-}6^{high}$ vs. $IL\text{-}6^{low}$	2.52	0.73–8.64	0.14
IL-8 (cut-off: 11.3 pg/mL): $IL\text{-}8^{high}$ vs. $IL\text{-}8^{low}$	1.74	0.82–3.67	0.15
IL-10 (cut-off: 7.82 pg/mL): $IL\text{-}10^{high}$ vs. $IL\text{-}10^{low}$	5.05	1.55–16.39	0.007
VEGF (cut-off: 44.1 pg/mL): $VEGF^{high}$ vs. $VEGF^{low}$	1.22	0.60–2.47	0.59
PDGF-BB (cut-off: 1127.5 pg/mL): $PDGF\text{-}BB^{high}$ vs. $PDGF\text{-}BB^{low}$	0.93	0.43–2.04	0.86
HGF (cut-off: 471.3 pg/mL): HGF^{high} vs. HGF^{low}	2.52	1.22–5.21	0.01
SDF-1 alpha (cut-off: 110.6 pg/mL): SDF-1 $alpha^{high}$ vs. SDF-1 $alpha^{low}$	1.23	0.60–2.53	0.56
Multivariate analysis	**HR**	**95% CI**	***P***
Stage: IV + Recurrence vs. III	2.04	0.78–5.35	0.15
ECOG PS: 2 + 1 vs. 0	2.58	0.98–6.76	>0.05
CRP level (cut-off: 1.0 mg/dL): CRP^{high} vs. CRP^{low}	2.04	0.62–6.76	0.24
CEC level (cut-off: 166 cells/4 mL): CEC^{high} vs. CEC^{low}	5.14	1.83–14.45	0.002
IL-10 (cut-off: 7.82 pg/mL): $IL\text{-}10^{high}$ vs. $IL\text{-}10^{low}$	5.26	1.26–22.22	0.02
HGF (cut-off: 471.3 pg/mL): HGF^{high} vs. HGF^{low}	1.34	0.46–3.91	0.59

Abbreviations: HR = hazard ratio; CI = confidence interval; ECOG PS = Eastern Cooperative Oncology Group performance status; CEC = circulating endothelial cells; IL = interleukin; PDGF-BB = platelet-derived growth factor-B chain; VEGF = vascular endothelial growth factor; HGF = hepatocyte growth factor; CA19-9 = carbohydrate antigen 19–9; CRP = C-reactive protein; CEA = carcinoembryonic antigen.

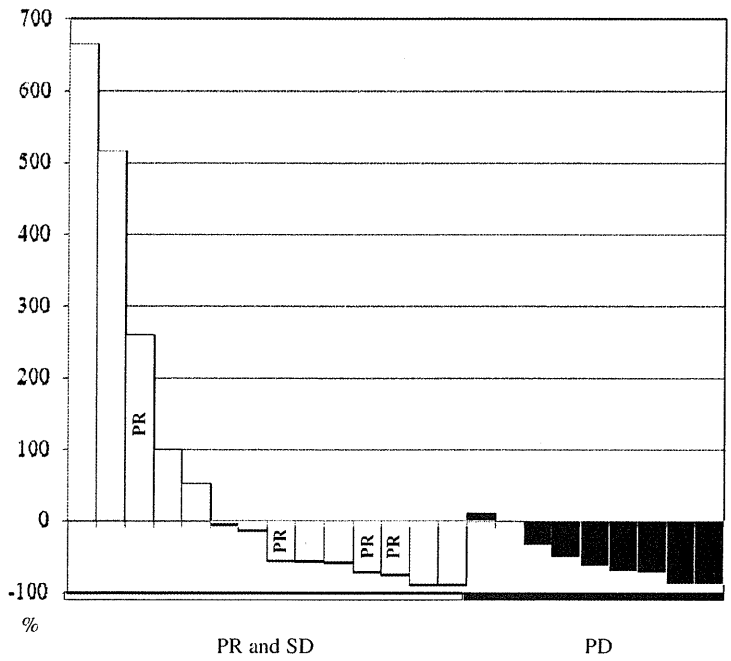

Figure 3 Waterfall plot showing the changes in CEC counts and tumor response in patients without progressive disease (PD) (those with partial response [PR] or stable disease [SD]) and patients with PD, after 28 ± 7 days of gemcitabine treatment.

Our study also found the baseline level of CECs, as well as the levels of HGF, IL-6, and IL-10, which are associated with gemcitabine resistance or stemness, to be significantly higher among PD patients. Univariate Cox model analysis further demonstrated that PS, clinical stage, CRP levels, and CEC levels are all associated with the survival of pancreatic carcinoma patients, while multivariate Cox analysis showed that CEC and IL-10 levels are strongly associated with survival.

The number of CECs detectable in individuals has previously been found to be associated with the plasma levels of VCAM-1 and VEGF in cancer patients [14] [15]. Our findings further show that, in addition to VEGF, CEC levels are strongly associated with the expression levels of IL-8, IL-10, and HGF in pancreatic carcinoma patients. These molecules, among others, play important roles in tumor biology and have been implicated in several cellular phenotypes. Chemokines,

Table 3 Association between CECs and other factors

	Mean ± SD	Spearman's rank correlation coefficient	*P*
CEC (cells/4 mL)	166.2 ± 228.9	1	-
IL-6 (pg/mL)	19.3 ± 52.4	0.17	0.30
IL-8 (pg/mL)	11.3 ± 10.1	0.38	0.02
IL-10 (pg/mL)	7.82 ± 26.9	0.45	0.006
VEGF (pg/mL)	44.1 ± 38.8	0.34	0.04
PDGF-BB (pg/mL)	1,127.5 ± 941.5	0.24	0.16
HGF (pg/mL)	471.3 ± 249.0	0.37	0.02
SDF-1alpha (pg/mL)	110.6 ± 43.7	0.15	0.37
CRP (mg/dL)	1.9 ± 3.9	0.31	0.06
CA19-9 (U/mL)	18,229.1 ± 55,377.8	0.11	0.50
CEA (ng/mL)	18.3 ± 51.0	0.03	0.88

Abbreviations: CEC = Circulating endothelial cell; IL = interleukin; PDGF-BB = platelet-derived growth factor-B chain; VEGF = vascular endothelial growth factor; HGF = hepatocyte growth factor; CA19-9 = carbohydrate antigen 19–9; CRP = C-reactive protein; CEA = carcinoembryonic antigen.

including IL-8 and IL-10, are small peptides involved in controlling cell migration, particularly in leukocytes, during inflammation and the immune response. Chemokines are also important in tumor biology as they influence tumor growth, invasion, metastasis, and angiogenesis. For instance, VEGF, HGF and IL-8 significantly stimulate the proliferation, migration, and invasion of cancer cells. CEC are shed from vessels and this process may be amplified by an aberrant vascular turnover/remodeling associated with high local levels of VEGF required for CEC survival [16]. The chemokine SDF-1 has likewise been found to enhance the production of IL-8 by pancreatic cells in a paracrine manner [17]. Although our results did not indicate that SDF-1 levels were associated with CEC or IL-8 levels in the pancreatic cancer patients examined, it is likely that several of the proangiogenic factors examined in this study interact with each other to promote vascular turnover and remodeling, thereby leading to a higher number of CECs in the peripheral blood of cancer patients.

Drugs targeting angiogenesis, such as those that inhibit the VEGF pathway, have had a major impact in the treatment of many types of cancer. The VEGF pathway is also an independent prognostic factor for patient survival in pancreatic carcinoma. Although preclinical models have suggested that VEGF-VEGF receptor inhibitors would be effective in the treatment of pancreatic carcinoma, patients who received bevacizumab and axitinib therapy in addition to gemcitabine have not shown a survival advantage when compared to those treated with gemcitabine alone [6,18]. These results add to the increasing evidence that suggests that targeting VEGF signaling is an ineffective strategy in the treatment of pancreatic carcinoma. However, many antiangiogenic therapies modulate the expression levels of proangiogenic factors [19], and many factors are associated with tumor angiogenesis. Therefore, there are a variety of potential therapeutic targets that may be exploited in order to target angiogenesis, potentially including those examined in this study.

In advanced non-small cell lung cancer (NSCLC), patients with higher baseline CEC counts have PR/SD and longer PFS. It has also previously been reported that the elevated CEC numbers exhibited in NSCLC patients decrease following treatment with carboplatin in combination with paclitaxel [9]. Paclitaxel and docetaxel are categorized as mitotic spindle agents with potent antiangiogenic properties [20-22]. Therefore, it seems that the baseline CEC count is a promising predictor of clinical response to the carboplatin plus paclitaxel regimen, as well as of survival. However, although several other clinical studies that have examined CECs have also found chemotherapy to be associated with either an increase or decrease in CEC number [23,24], no association was detected between gemcitabine treatment and CEC number in the pancreatic carcinoma patients in our study. Although gemcitabine has anti-angiogenic properties, higher baseline CEC levels were associated with PD in pancreatic carcinoma patients receiving gemcitabine therapy, and patients with high CEC counts exhibited poor clinical condition. It is therefore likely that the tumor type, anti-cancer drugs being administered, and the amount of time between the start of treatment and the time when CEC counts are obtained influence the number of CECs detected in cancer patients after treatment. In this study, we measured CEC levels before starting chemotherapy and at 28 ± 7 days after starting chemotherapy, the time of sampling might influence the changes of CEC level. Moreover, the diversity in literature regarding CEC up-or down-regulation during cancer therapy and the associated prognostic and predictive evidence might in part be explained by a differential focus on or by the lack of discrimination between these cell populations [13].

Conclusions

Although the number of patients examined in this study was small, and patients were recruited prospectively, this study, along with others, has shown the clinical importance of CEC number as a prognostic factor in advanced pancreatic carcinoma treated with gemcitabine chemotherapy, whereby high CEC counts are associated with poor prognosis. This study also found that elevated CEC counts are associated with the high expression levels of several chemokines and proangiogenic factors involved in the regulation of tumor immunological and angiogenic factors. Although this correlation between blood parameters is not proof of a causal relationship, these factors may provide viable therapeutic targets for the treatment of pancreatic carcinoma in the future. Further studies in a larger population will be required to confirm our findings.

Abbreviations

CEC: circulating endothelial cell; ECOG: Eastern Cooperative Oncology Group; CA19-9: Carbohydrate antigen 19–9; CRP: C-reactive protein; IL: Interleukin; PDGF-BB: Platelet-derived growth factor-B chain; VEGF: Vascular endothelial growth factor; HGF: Hepatocyte growth factor; PD: Progressive disease; PR: Partial response; HR: Hazard ratio; CI: confidence interval; SD: Stable disease.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

SK and KT designed and participated in all stages of the study. SK and JH performed most of the experiments. FK and CM participated in CEC analysis, as well as the statistical analyses and discussion of the results. HU and TO recruited the patients, collected the tumor biopsy samples, and helped to draft the manuscript. All authors read and approved the final manuscript.

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Case Reports

Gemcitabine-induced Pleuropericardial Effusion in a Patient with Pancreatic Cancer

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Pleuropericardial effusion is an extremely rare complication of gemcitabine chemotherapy. The patient was a 56-year-old woman administered systemic chemotherapy with gemcitabine for local recurrence of pancreatic cancer and lymph node metastasis developing 4 years after pancreaticoduodenectomy. Four months after the start of the chemotherapy, she presented with exertional dyspnea and edema in both her legs and face. Echocardiography and computed tomography revealed pericardial and bilateral pleural effusion. A pericardiocentesis was immediately performed to prevent the development of cardiac tamponade as well as to examine the cause of the pericardial effusion. As a result, the patient's exertional dyspnea and edema resolved. No metastases to the thorax or mediastinum were noted. A cytological study of the pericardial and pleural effusions revealed no malignant cells. Cultures for bacteria, mycobacteria and fungi were negative. Tests for autoantibodies indicating autoimmune disease were also negative, and hormonal assays for the detection of endocrine disease were normal. She was followed up after discontinuation of the gemcitabine treatment, and no further episodes of pericardial or pleural effusion occurred. Thus, it is speculated that the pericardial effusion and bilateral pleural effusion may have been caused by gemcitabine.

Key words: pericardial effusion – pleural effusion – chemotherapy – gemcitabine

INTRODUCTION

Pleuropericardial effusion can develop in patients with acute pericarditis or acute pleuritis, or in association with a variety of systemic disorders including drug adverse effects. Procainamide (1,2), hydralazine (3,4), isoniazid (5,6) and minoxidil (7) are well-known causative agents of pleuropericardial effusion. In addition, several reports have also described pleuropericardial effusion induced by anticancer drugs, such as dasatinib (8), imatinib (9) and docetaxel (11). However, drug-induced pleuropericardial effusion has seldom been reported with gemcitabine. Here, we report a patient who developed pleuropericardial effusion possibly caused by gemcitabine treatment. This is the first report of pleuropericardial effusion induced by treatment with gemcitabine alone.

CASE REPORT

The patient was a 56-year-old woman. Her past medical history included gastritis and insomnia, and she had been under treatment with ranitidine hydrochloride and alprazolam. She had no history of allergy. At the age of 51 years, she underwent a pancreaticoduodenectomy for the treatment of pancreatic cancer at another hospital. Gross examination of the resected specimen revealed a tumor (2 cm × 1.5 cm × 1.5 cm) arising from the head of the pancreas. Microscopic

examination revealed a moderately differentiated tubular adenocarcinoma with lymphatic and venous invasion. The edge of the resected specimen was negative. A regional lymph node metastasis was found in 1 out of 38 dissected lymph nodes. She underwent a laparotomy based on a diagnosis of ileus 2 years after the pancreaticoduodenectomy. Since recovering from the ileus, she had been followed up without any further anticancer treatment. Four years after the pancreaticoduodenectomy, a laboratory examination revealed an elevation of her serum carbohydrate antigen 19-9 (CA19-9) level to 101.8 ng/ml and she was referred to our hospital for the first time. A computed tomographic (CT) examination of the abdomen performed at our hospital revealed a local recurrence (15 mm in diameter) and also two abdominal lymph node metastases. The patient was asymptomatic, and her ECOG performance status was 0 at the time of detection of the recurrence. A blood examination showed no abnormalities, except for mild elevation of the serum amylase level (199 IU/l) and the serum CA19-9 level (49 ng/ml). Systemic chemotherapy using gemcitabine was started for the treatment of the recurrence. During the first 4 months of treatment, the only adverse effects of gemcitabine were mild nausea and mild fatigue. Oral intake was sufficient. However, at 4 months after the start of the chemotherapy, she presented with complaints of exertional dyspnea and edema in both her legs and face. The edema steadily worsened over the course of the following 2 months. CT examinations revealed pericardial and bilateral pleural effusion, and she was admitted to our hospital with the diagnosis of pleuropericardial effusion. Upon admission, her blood pressure was 142/90 mmHg, pulse rate was 110 min^{-1}, regular, and body temperature was 37.6°C. Her peripheral blood arterial oxygen saturation level was 94% under room air. Her ECOG performance status had worsened to 2 because of the exertional dyspnea. The first heart sound and second heart sound were distant; however, there was no audible murmur or pericardial friction rub. The breath sounds were normal vesicular, except for a decrease over the right lung areas, presumably on account of the pleural effusion. An electrocardiogram performed at admission revealed a sinus rhythm, low-voltage complexes and no ST elevations in any of the leads. Laboratory examination revealed slight anemia, proteinuria (2+) and hematuria (3+), which were not observed before the initiation of gemcitabine (Tables 1 and 2). The daily urinary protein excretion level was 1.92 g/day. The serum creatinine level was of normal value throughout the entire course of this episode (Table 1). The serum C-reactive protein level was 2.2 mg/dl. The thyroid hormone profile was normal. Complement-fixation tests were performed in paired serum specimens for antibodies against Coxsackie virus, adenovirus and echovirus, which are well-known causes of pleuropericardial effusion. At the time of admission, the antibody titers for all of these viruses were 1:32 or less. A paired sample taken 4 weeks later showed a less than 4-fold increase in the titers when compared with the titers recorded at the time of admission (Table 3). A rapid influenza diagnostic test yielded negative results for influenza A and B. Although the rheumatoid factor test was positive, the tests for other autoantibodies were normal (Table 2). The possibility of collagen vascular disease was ruled out by a rheumatologist based on the absence of the characteristic arthralgia, skin sclerosis or antinuclear antibody in the serum. An X-ray of the chest revealed cardiac enlargement (CTR, 60%) and increased pulmonary markings. A chest CT revealed pericardial effusion and bilateral pleural effusion; no evidence of metastatic tumors was

Table 1. The time course for changes in laboratory data

	Normal value	Initiation of GEM (May 2009)	Pericardial effusion occurred (Oct 2009)	Two months after the discontinuation of GEM (Dec 2009)
Leukocyte (μl^{-1})	3900–6300	5400	3700	5900
Hemoglobin (g/dl)	11.3–14.9	13.6	8.7	10.0
Hematocrit (%)	33.6–44.6	41.3	27.1	31.2
Platelet ($\times 10^4$ μl^{-1})	12.5–37.5	22.5	21.6	15.8
Albumin (g/dl)	3.7–5.2	4.7	3.5	3.9
Creatinine (mg/dl)	0.4–0.7	0.6	0.9	0.8
C-reactive protein (mg/l)	<0.1	0.32	0.09	0.42
CEA (ng/ml)	<5.0	0.8	0.8	4.3
CA19-92 (U/ml)	<37	49	80	2130
Protein (urine)	—	—	2+	2+
Occult blood (urine)	—	—	3+	3+

GEM, gemcitabine; CEA, carcinoembryonic antigen; CA19-9, carbohydrate antigen 19-9.

Table 2. Laboratory data obtained upon admission for the treatment of pericardial effusion

Coagulation		**Tumor marker**	
Prothrombin time (INR)	1.08	Carcinoembryonic antigen	0.8 ng/ml
APTT	79 s	Carbohydrate antigen 19-9	80 ng/ml
Endocrine function tests		**Autoimmunity**	
Brain natriuretic peptide	558.5 pg/ml	CH50	47 U/ml
Thyroid-stimulating hormone	0.56 μU/ml	C3c	117 mg/dl
Free triiodothyronin	1.76 ng/ml	C4	22.3 mg/dl
Free thyroxin	1.15 ng/ml	Antinuclear antibody	<40 mg/dl
		Anti-DNA antibody	—
		Anti-Sm antibody	—
		Rheumatoid factor	160-fold
		Proteinase-3-ANCA[d]	<1.3 U/ml
		Myeloperoxidase ANCA[d]	<1.3 U/ml

INR, international normalized ratio; APTT, activated partial thromboplastin time; CH50, 50% hemolytic unit of complement; ANCA, anti-neutrophil cytoplasmic antibody.

Table 3. Virological examination of blood samples (neutralizing antibody titer)

	Acute phase at the time of admission (Oct 2009)	Convalescence phase 4 weeks after the acute phase (Dec 2009)
Adenovirus type7	Negative	Negative
Echovirus type6	Negative	Negative
Echovirus type9	Negative	Negative
Coxsackie B1	4-fold	Negative
Coxsackie B2	16-fold	16-fold
Coxsackie B3	16-fold	32-fold
Coxsackie B4	32-fold	32-fold
Coxsackie B5	4-fold	8-fold
Rapid influenza diagnostic test		
Influenza A	—	
Influenza B	—	

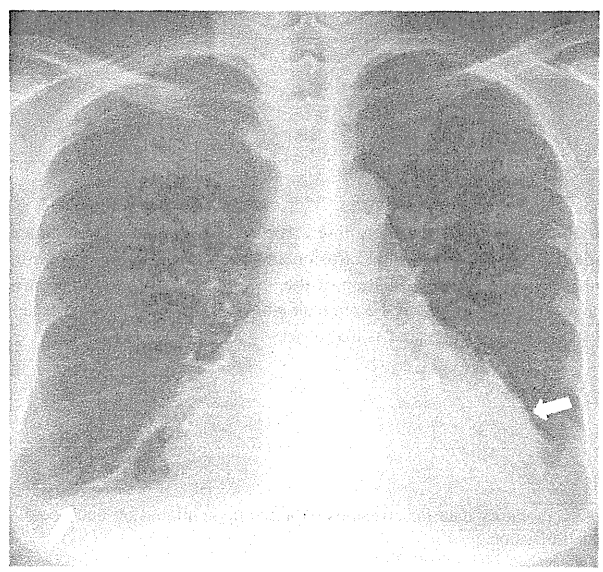

Figure 1. Chest X-ray obtained upon admission shows cardiac enlargement (60%), increased pulmonary markings and bilateral pleural effusion.

observed (Figs 1 and 2). An emergency echocardiography demonstrated a large amount of pericardial effusion (left ventricle: 15 mm) and a slightly pendular left ventricular wall motion. The ejection fraction was 59%. An abdominal CT revealed local recurrence in the remnant stump of the pancreas; the tumor size was slightly decreased when compared with that before the start of gemcitabine therapy. The serum level of CA19-9 had decreased to 31 ng/ml. Pericardiocentesis was immediately performed to prevent the development complication of cardiac tamponade and to examine the cause of the pericardial effusion. An indwelling drain yielded ~700 ml of fluid on the first day, which resulted in the improvement of the patient's hemodynamic condition and marked alleviation of both the exertional dyspnea and the edema; however, no evidence of decrease in the size of the bilateral pleural effusion was noted. Cytology of the pericardial and pleural fluid samples was negative for malignant cells, and both the pericardial and pleural fluid

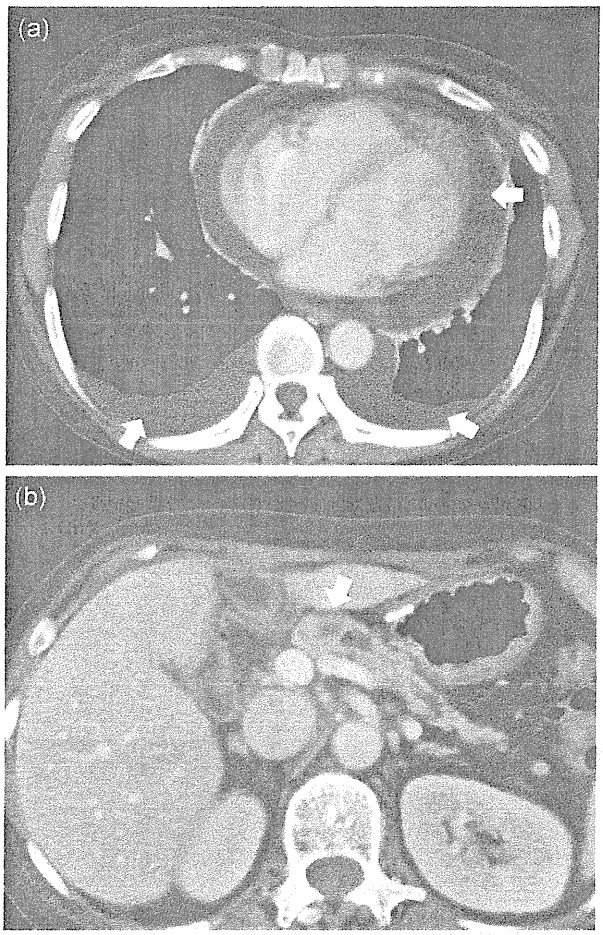

Figure 2. (a) Computed tomographic examination of the chest obtained upon admission shows pericardial effusion and bilateral pleural effusion. (b) Computed tomographic examination of the abdomen obtained upon admission shows local recurrence in the remnant stump of the pancreas; the recurrence was almost the same size as that observed 2 months previously.

Table 4. Laboratory data for effusions obtained upon admission because of pericardial effusion

	Pleural effusion (right)	Pleural effusion (left)	Pericardial effusion
Total protein (g/dl)	2.4	2.4	3.8
LDH (IU/l)	224	237	1796
Glucose (mg/dl)	145	140	55
CEA (ng/ml)	0.3	0.3	3.4
CA19-9 (ng/ml)	11	9	32
Culture	Negative	Negative	Negative
Cytology	No malignant cell	No malignant cell	No malignant cell

LDH, lactate dehydrogenase.

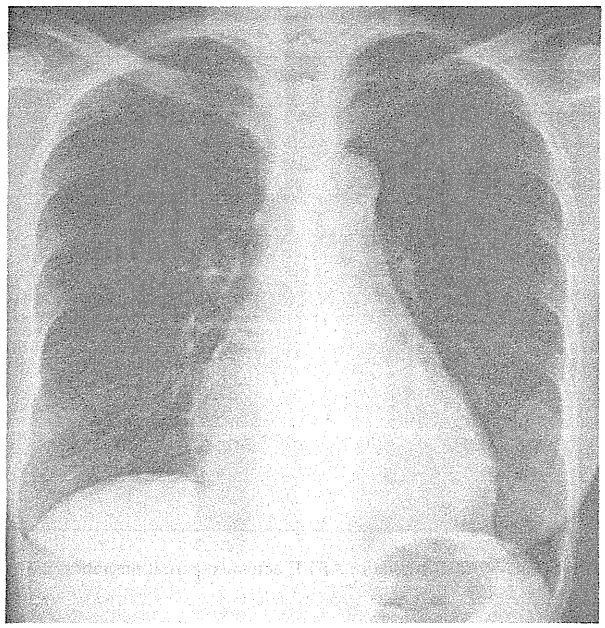

Figure 3. Chest X-ray obtained 2 months after discharge showing a normal cardiac shadow.

samples were clear, pale yellow in color and classified as exudates. The lactate dehydrogenase level of the pericardial aspirate was elevated, and the glucose level was low. Cultures of both the pericardial and pleural fluid specimens for bacteria, mycobacteria and fungi were negative (Table 4). To treat the residual bilateral pleural effusion, the patient was given furosemide 20 mg for 4 days and also a preparation of human serum albumin (8.8 g) for 3 days to counter the possible contribution of hypoalbuminemia, which may cause the pleuropericardial effusion to worsen. Thereafter, the bilateral pleural effusion completely resolved. The daily drainage volume of the pericardial effusion fluid decreased to <100 ml on the 12th day after the pericardiocentesis procedure, and the drainage tube was removed. Echocardiography demonstrated the dramatic decrease in pericardial effusion (left ventricle: <5 mm) and improvement of the ejection fraction to 76%. The patient was discharged from the hospital 20 days after the emergency admission. Two months later, an X-ray of the chest showed a normal cardiac shadow (Fig. 3), and no evidence of pleural/pericardial effusion. In view of the risk of relapse of the pleuropericardial effusions, re-administration of gemcitabine was avoided. Although we proposed other anticancer agents as second-line chemotherapy, she refused any additional anticancer treatment. Therefore, she received only supportive care thereafter and died 4 months later from hepatic metastasis and failure.

DISCUSSION

The main causes of pleuropericardial effusion are infection (viral, pyogenic, tuberculosis, fungal etc.), acute idiopathic,

uremia, neoplasia, myxedema, acute myocardial infarction, post-radiation reactions, drug-induced reactions, collagen vascular disease, inflammatory bowel disease, aortic dissection and trauma.

In our case, a differential diagnosis between malignant effusion and a benign cause of the effusion was essential in view of the diagnosis of cancer recurrence. The fluid samples were found to be exudates, which by itself is not sufficient to rule out the possibility of malignant effusion. However, cytological examinations of the fluid samples revealed no malignant cells. Furthermore, although liver metastasis was diagnosed in our patient after she was discharged from our hospital, she did not have a relapse of the effusion for a long time after the removal of the drainage tube despite the absence of anticancer treatment. Therefore, a malignant effusion was thought to be unlikely. At the time of the diagnosis of pleuropericardial effusion, the patient's oral intake was sufficient and her serum albumin level was 3.5 g/dl. Therefore, hypoalbuminemia did not cause the pleuropericardial effusion. Although proteinuria (2+) and hematuria (3+) were observed at the time of the diagnosis of pleuropericardial effusion, the serum creatinine level was normal. The renal dysfunction may have been caused by hypertension and the chemotherapy. The severity of the renal dysfunction was too low to be a possible cause of the pleuropericardial effusion. Bacteriologic and mycobacteriologic cultures of the blood, pericardial and pleural fluid (both sides) samples were all negative. Complement fixation tests of paired serum samples to detect an elevation in the antibodies to major causative viruses of pleuropericardial effusion were negative. Chest pain, high fever and ST elevation on the electrocardiogram, which are typical findings of acute pericarditis induced by viral infection, were absent. The patient did not have any history of injury, radiation or thoracic surgery. Other causative diseases, such as collagen vascular diseases, cardiovascular diseases, renal failure and hypothyroidism, were excluded based on the results of the physical examination, laboratory examination and imaging studies. Although the use of common medicines was continued, with the discontinuation of gemcitabine, after the diagnosis of the pleuropericardial effusion, the effusion did not recur. In view of the above-mentioned clinical information, we concluded that the most probable cause of the pleuropericardial effusion in our patient was the gemcitabine treatment. Although the re-administration of gemcitabine with follow-up might have improved the reliability of our conclusion, such treatment was not ethically acceptable, especially as the patient refused any further chemotherapy.

Although pulmonary toxicity is a well-known side effect of gemcitabine, there have been only a few reports of pleural effusion developing as a complication secondary to the pulmonary toxicity of this drug (17–20). With regard to pericardial effusion, only one previous report describing four cases of pericardial effusion caused by gemcitabine-induced radiation recall reactions was identified (10). Therefore, our case is the first report of pleuropericardial effusion induced by gemcitabine treatment alone.

The mechanism underlying the development of gemcitabine-induced pleuropericardial effusion is unknown. With regard to reports of pleuropericardial effusion caused by other anticancer agents, this has often been reported in patients treated with docetaxel, dasatinib or imatinib. Docetaxel is a cytotoxic agent that is toxic to the microtubule assembly in the cells. Docetaxel-induced pleuropericardial effusion is reported to be associated with systemic fluid retention caused by the capillary protein leak syndrome (11,12). Although no cases of pleuropericardial effusion have been reported, some cases of gemcitabine-induced systemic capillary leak syndrome have been reported previously (13–15). Favorable effects of corticosteroids, which significantly delay the onset of docetaxel-induced fluid retention, have been reported (16), and this treatment could also be considered for the treatment of gemcitabine-induced pleuropericardial effusion. The colloid osmotic pressure of edema, the interstitial fluid pressure and the interstitial hydrostatic pressure were measured before and after treatment to explain the theory of treatment-induced capillary protein leakage as the mechanism responsible for the fluid retention in patients treated with docetaxel (12). On the other hand, imatinib and dasatinib, molecular-targeted agents categorized as multitargeted tyrosine kinase inhibitors, have been reported to cause pleuropericardial effusion. The underlying mechanism is still unknown, but may involve an immune-mediated pathway or off-target inhibition of the platelet-derived growth factor receptor, β-polypeptide (8). Gemcitabine, a novel deoxycytidine analog antimetabolite, does not exert off-target kinase inhibition.

If the above-mentioned discussions are taken into consideration, the pleuropericardial effusion in our case could have been associated with the capillary leak syndrome induced by gemcitabine.

Complications of pleuropericardial effusion, especially cardiac tamponade, complicating pericardial effusion, and acute respiratory failure complicating pleural effusion are life-threatening and might have a rapid clinical course. Therefore, it should be kept in mind during chemotherapy with gemcitabine, especially when patients complain of dyspnea, tachycardia or edema.

CONCLUSION

We encountered a case of gemcitabine-induced pleuropericardial effusion in a patient with recurrent pancreatic cancer. Physicians should be aware of the possibility of gemcitabine-induced pleuropericardial effusion.

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Conflict of interest statement

Takuji Okusaka has received research findings and honoraria from Eli Lilly Japan. Hideki Ueno has received honoraria from Eli Lilly Japan.

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Research Article

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Comparison of resection and ablation for hepatocellular carcinoma: A cohort study based on a Japanese nationwide survey

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Background & Aims: The treatment of choice for early or moderately advanced hepatocellular carcinoma (HCC) with good liver function remains controversial. We evaluated the therapeutic impacts of surgical resection (SR), percutaneous ethanol injection (PEI), and radiofrequency ablation (RFA) on long-term outcomes in patients with HCC.

Methods: A database constructed on the basis of a Japanese nationwide survey of 28,510 patients with HCC treated by SR, PEI, or RFA between 2000 and 2005 was used to identify 12,968 patients who had no more than 3 tumors (≤3 cm) and liver damage of class A or B. The patients were divided into SR (n = 5361), RFA (n = 5548), and PEI groups (n = 2059). Overall survival and time to recurrence were compared among them.

Results: Median follow-up was 2.16 years. Overall survival at 3 and 5 years was respectively 85.3%/71.1% in the SR group, 81.0%/61.1% in the RFA, and 78.9%/56.3% in the PEI. Time to recurrence at 3 and 5 years was 43.3%/63.8%, 57.2%/71.7%, and 64.3%/76.9%, respectively. On multivariate analysis, the hazard ratio for death was significantly lower in the SR group than in the RFA (SR *vs.* RFA:0.84, 95% confidence interval, 0.74–0.95; $p = 0.006$) and PEI groups (SR *vs.* PEI:0.75, 0.64–0.86; $p = 0.0001$). The hazard ratios for recurrence were also lower in the SR group than in the RFA (SR *vs.* RFA:0.74, 0.68–0.79; $p = 0.0001$) and PEI groups (SR *vs.* PEI:0.59, 0.54–0.65; $p = 0.0001$).

Conclusions: Our findings suggest that surgical resection results in longer overall survival and shorter time to recurrence than either RFA or PEI in patients with HCC.

Keywords: Hepatectomy; Surgical resection; Radiofrequency ablation; Percutaneous ethanol injection.

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Abbreviations: HCC, hepatocellular carcinoma; SR, surgical resection; RFA, radiofrequency ablation; PEI, percutaneous ethanol injection; TACE, transcatheter hepatic arterial chemoembolization.

Introduction

Hepatocellular carcinoma (HCC) is the fifth most common cancer in men and the seventh in women, worldwide [1]. Outcomes remain disappointing, despite recent progress in the techniques of diagnosis and therapy. Japanese [2], European [3] and American [4] clinical practice guidelines strongly recommend surgical resection (SR) and percutaneous ablation, including radiofrequency ablation (RFA) and percutaneous ethanol injection (PEI), for the management of early or moderately advanced HCC (i.e., up to 3 tumors 3 cm or less in diameter) in patients with adequately maintained liver function. Although comparative studies of these treatments have been conducted previously [5–7], the most suitable treatment strategy still remains controversial.

By nationwide surveys initiated in 1965, the Liver Cancer Study Group of Japan has prospectively collected data on patients with HCC in Japan. The Group conducted two retrospective analyses to define the treatment with the best outcomes [8,9]. However, each of the analyses was flawed, and had several problems: data on RFA were not included in the first report [8], and the follow-up period was short in the second one [9]. Although the second analysis demonstrated that surgical resection was superior to RFA and PEI for preventing recurrence [9], no apparent difference in the overall survival could be discerned between surgery and percutaneous ablation therapies (RFA and PEI). Thus, the treatment of choice for less advanced HCC still remains under debate.

Before starting this study, the results of 2 randomized controlled trials (RCT) were available [10,11]. As we pointed out in a previous report [12], however, the study designs of these 2

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Research Article

trials were critically flawed by factors such as insufficient sample size, excessively optimistic hypotheses, and high conversion ratios. Because of these problems, the results of the two RCTs do not allow firm conclusions to be drawn concerning the important clinical question: is surgery or percutaneous ablation the treatment of choice for early or moderately advanced HCC? To answer this question, we conducted this cohort study based on the latest data available from a Japanese nationwide survey.

Patients and methods

Patients and settings

The Liver Cancer Study Group of Japan has performed nationwide surveys of patients with primary liver cancer since 1965. Patients are registered and followed up, as reported previously [9]. Although this study protocol was not submitted to the Institutional Review Board of each institution participating in the nationwide survey, the collection and registration of data of patients with HCC were performed with the approval of each institution. Because RFA has been available for clinical use since 1999 in Japan, we set the study period from 2000 to 2005, to exclude preliminary experiences with RFA. During this period, a total of 28,510 patients with HCC were registered and received surgical resection, RFA or PEI as the primary treatment with curative intent for HCC. We identified 12,968 patients who met the following criteria: (1) liver function classified as liver damage A or B defined by the Liver cancer Study Group of Japan [13]; (2) number of tumors 3 or less; (3) maximum tumor diameter $\leqslant$3 cm. The 12,968 patients were divided into 3 groups according to the treatment received: SR group (n = 5361, 41.3%), RFA group (n = 5548, 42.8%), and PEI group (n = 2059, 15.9%). The diagnostic criteria and details of follow-up were described previously [8]. Because it has been unusual for biopsies to be performed in cases treated by percutaneous ablation in Japan, histological findings such as microscopic vascular invasion, tumor grading, and microscopic intrahepatic metastasis were not evaluated in this study. Relevant clinical data were collected and analyzed.

Statistical analyses

The baseline characteristics of the three groups (Table 1) were compared by analysis of variance for continuous variables and by Chi-square or Mantel-trend tests for categorical variables. Consistent with our preliminary report [9], the SR group had a higher proportion of younger patients and male patients than the RFA and PEI groups. Hepatitis C virus infection was less prevalent in the SR group than in the RFA and PEI groups. Based on the liver damage class, serum albumin and total bilirubin levels, platelet counts, and the indocyanine green retention rate at 15 min, liver function was better in the SR group than in the RFA and PEI groups, consistent with our previous report [9]. As for tumor-related factors, the number of tumors was smaller, and the maximum tumor diameter was larger in the SR group than in the RFA or PEI group. The SR group had the lowest proportion of patients with abnormally elevated alpha-fetoprotein levels ($\geqslant$15 ng/ml) and the highest proportion of patients with abnormally elevated des-γ-carboxy prothrombin levels ($\geqslant$40 AU/ml).

Overall survival and time to recurrence curves were plotted using the Kaplan–Meier method and compared with the use of the log-rank test. Recurrence was diagnosed on the basis of imaging studies, clinical data, and/or histopathological studies at each institution [9].

The therapeutic impacts of surgical resection, RFA and PEI were estimated using a Cox proportional hazards model including the following 10 covariates: age, gender, liver damage class, hepatitis C virus antibody, hepatitis B surface antigen, platelet count, number of tumors, tumor size, and serum alpha-fetoprotein and des-γ-carboxy prothrombin levels. The results of multivariate analysis were expressed as hazard ratios with 95% confidence intervals. p values of <0.05 were considered to indicate statistical significance.

For the subgroup analyses, the study populations were classified into 8 subgroups according to the tumor size ($<$ or $\geqslant$2 cm), tumor number (single or multiple), and liver damage class (A or B). Macroscopic vascular invasion was excluded from the subgroup analyses because its presence is a contraindication to percutaneous ablation therapies. The therapeutic impacts of the three treatments were evaluated in each of these subgroups, and hazard ratios with 95% confidence intervals and p values were calculated according to the above three factors (tumor size, number of tumors, and liver damage class).

Results

The median follow-up after treatment was 2.16 years, and the 5th and 95th percentiles were 0.14 and 5.19 years, respectively. The overall survival rates at 3/5 years were 85.3%/71.1% in the SR group, 81.0%/61.1% in the RFA group, and 78.9%/56.3% in the PEI group (Fig. 1). The median survival times were 8.4, 5.9, and 5.6 years in the three groups, respectively. The time to recurrence rates at 3/5 years in the 3 groups were 43.3%/63.8%, 57.2%/71.7%, and 64.3%/76.9%, respectively (Fig. 2).

According to the results of the multivariate analysis, the hazard ratio for death in the SR group was 0.84 (0.74–0.95, $p = 0.006$) relative to that in the RFA group, and 0.75 (0.64–0.86, $p = 0.0001$) relative to that in the PEI group (Table 2A). The hazard ratios for recurrence in the SR group were 0.74 (0.68–0.79, $p = 0.0001$) and 0.59 (0.54–0.65, $p = 0.0001$) relative to those in the RFA and PEI groups, respectively (Table 2B). These results indicated that the overall survival and time to recurrence rates were both significantly better in the SR group than in the RFA and PEI groups.

The overall survival rates following surgical resection, RFA and PEI in the 4 subgroups with a single tumor are shown in Fig. 3A–D. The results of the subgroup analyses (summarized in Fig. 4A) showed that the overall survival was significantly longer in the SR group than in the RFA group in 2 subgroups of patients, namely, those who had a single tumor smaller than 2 cm in diameter with liver damage class A, and those who had a single tumor 2 cm or larger in diameter with liver damage class B.

As shown in Fig. 4B, the time to recurrence was shorter in the SR group than that in the RFA group in the 4 following subgroups: patients with a single tumor with liver damage class A (regardless of the tumor size), those with multiple tumors 2 cm or larger in diameter with liver damage class A, and those with a single tumor 2 cm or larger in diameter with liver damage class B.

Discussion

Our study showed that surgical resection was associated with significantly lower risk of both death and recurrence as compared to RFA and PEI in patients with early or moderately advanced HCC. Our previous preliminary report [9] suggested that surgery reduces the risk of recurrence, but failed to demonstrate any difference in the overall survival between surgery and percutaneous ablation therapies in patients with early or moderately advanced HCC. The present study reconfirms that surgery is associated with a reduced recurrence rate and newly shows that surgery yields a longer overall survival than percutaneous ablation therapies.

Differences in the results between the present study and previous investigations are most likely related to the sample size and length of follow-up. The total number of subjects increased markedly from 7185 in our previous study to 12,968 in this study, and the median follow-up period increased from 10.4 months to 2.16 years (25.9 months). These factors are considered not only to have enhanced the reliability of our findings, but also to have strengthened our conclusions. We believe that our results, which are, of course, subject to the inherent drawbacks of the study design, are meaningful, given the current lack of credible data derived from well-designed RCTs.

The large sample size and prolonged follow-up period also allowed us to perform several subgroup analyses, which were not feasible in our previous study [9]. We classified the patients

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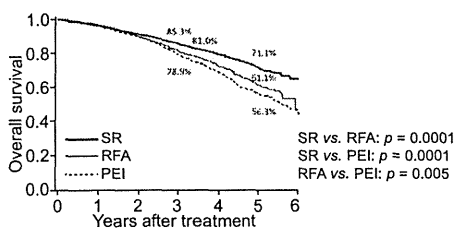
Table 1. Baseline characteristics.

Variables	SR n = 5361	RFA n = 5548	PEI n = 2059	*p* value
Age, median (5, 95 percentile), yr	66 (48, 77)	69 (52, 80)	69 (52, 80)	<0.0001[a]
Sex				<0.0001[b]
Male, No. (%)	3967 (74.0)	3569 (64.3)	1303 (63.3)	
Female, No. (%)	1394 (26.0)	1979 (35.7)	756 (36.7)	
Hepatitis virus infection				<0.0001[b]
HBs Ag(+)/HCV-Ab(-), No. (%)	908 (16.9)	462 (8.3)	141 (6.8)	
HBs Ag(-)/HCV-Ab(+), No. (%)	3393 (63.3)	4263 (76.8)	1632 (79.3)	
HBs Ag(+)/HCV-Ab(+), No. (%)	106 (2.0)	87 (1.6)	32 (1.6)	
HBs Ag(-)/HCV-Ab(-), No. (%)	760 (14.2)	512 (9.2)	160 (7.8)	
Unknown	194 (3.6)	224 (4.0)	94 (4.6)	
Liver damage				<0.0001[b]
A, No. (%)	4000 (74.6)	3349 (60.4)	1204 (58.5)	
B, No. (%)	1361 (25.4)	2199 (39.6)	855 (41.5)	
Serum albumin, median (5, 95 percentile), g/dl	3.9 (3.1, 4.6)	3.7 (2.9, 4.4)	3.7 (2.8, 4.4)	<0.0001[a]
Serum total bilirubin, median (5, 95 percentile), mg/dl	0.8 (0.4, 1.5)	0.9 (0.4, 1.9)	0.9 (0.4, 2.2)	<0.0001[a]
Platelet count, median (5, 95 percentile), x 10^4/μl	12.6 (5.8, 24.0)	9.9 (4.5, 20.4)	9.5 (4.4, 19.6)	<0.0001[a]
ICG R15, median (5, 95 percentile), %	15 (5, 35)	22 (7, 51)	24 (8, 51)	<0.0001[a]
Tumor number				<0.0001[c]
Single, No. (%)	4458 (83.2)	4068 (73.3)	1449 (70.4)	
Two, No. (%)	706 (13.2)	1096 (19.8)	443 (21.5)	
Three, No. (%)	197 (3.7)	384 (6.9)	167 (8.1)	
Tumor size, median (5, 95 percentile), mm	23 (12, 30)	20 (10, 30)	17 (10, 30)	<0.0001[a]
Alpha-fetoprotein				<0.0001[b]
≥15 ng/ml, No. (%)	2726 (50.9)	3028 (54.6)	1125 (54.6)	
<15 ng/ml, No. (%)	2457 (45.8)	2301 (41.5)	828 (40.2)	
Unknown, No. (%)	178 (3.3)	219 (3.9)	106 (5.2)	
Des-γ-carboxy prothrombin				<0.0001[b]
≥40 AU/ml, No. (%)	2182 (40.7)	1593 (28.7)	541 (26.3)	
<40 AU/ml, No. (%)	2651 (49.5)	3322 (59.9)	1169 (56.8)	
Unknown, No. (%)	528 (9.9)	633 (11.4)	349 (17.0)	

HBsAg, hepatitis B virus antigen; HCV-Ab, hepatitis C virus antibody; ICG R15, indocyanine green retention rate at 15 min.
[a]ANOVA.
[b]Chi-square.
[c]Mante-trend test.



Patients at risk

SR	5361	3833	2570	1680	894	400	29
RFA	5548	3780	2328	1264	569	160	5
PEI	2059	1595	1112	718	444	247	58

Fig. 1. Overall survival curves after surgical resection (SR), radiofrequency ablation (RFA), and percutaneous ethanol injection (PEI).

into 8 subgroups according to 3 factors (liver damage class, tumor size, and number of tumors), which have repeatedly been shown to be clinically relevant prognostic factors. The results of the subgroup analyses indicated that surgical resection would effectively prevent recurrence in patients with relatively advanced HCC (2–3 cm in diameter) among the study populations, irrespective of liver damage class or number of tumors. This finding suggests that surgery might be superior to percutaneous ablation therapies in patients with a more advanced tumor stage. As for the subgroups with a single tumor, surgical resection yielded better overall survival and time to recurrence rates than RFA or PEI. Especially in the subgroup with a single tumor smaller than 2 cm in diameter, both the overall and time to recurrence rates were statistically significantly better after surgery than after RFA, whereas no such statistically significant differences in these two parameters between the two treatment groups were detected in a few subgroups with a single tumor, maybe due to the insufficient sample size of the subgroups. Thus, surgical resection would be considered as the treatment modality of first choice for a single HCC, as recommended by the Japanese clinical practice guideline [2]. Overall, there was a trend toward superior

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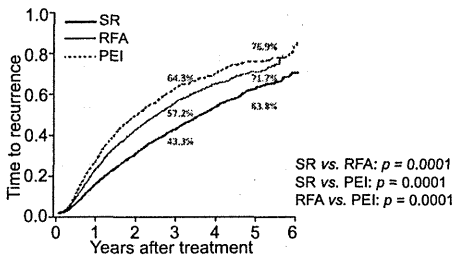

Patients at risk							
SR	5361	3265	1844	1039	451	189	15
RFA	5548	2954	1396	591	225	62	4
PEI	2059	1154	583	304	172	90	15

Fig. 2. Time to recurrence curves after surgical resection (SR), radiofrequency ablation (RFA), and percutaneous ethanol injection (PEI).

overall and time to recurrence rates after surgery than after RFA and PEI.

The reason why the long-term outcomes of the SR group were better than those of the PEI and RFA groups cannot be definitely clarified from the results of this study, however, in theory, surgical resection has the advantage of offering better local control of HCC over PEI and RFA, both of which have some potential risks of local recurrence associated with insufficient ablation. In addition, anatomic resection to remove minute tumor satellites [14] might have decreased the recurrence rate in the SR group, although this remains a speculation.

Recently, the latest trial from China [15], which had an adequate sample size (total 230 patients), reported that surgical resection yielded significantly better long-term outcomes than RFA. Although the study design was better than that of the two previously reported RCTs [10,11], it appeared to have limitations with respect to the results, such as drop in the overall survival in the RFA group as compared with that in the surgery group during the early period after treatment. The early deaths in the RFA group could have been treatment-related rather than cancer-related. Thus, no conclusion can be drawn from the three currently available RCTs.

One of the limitations of our study is the diversity of demographic factors in the study population, which would have been

Table 2. Hazard ratios for death and recurrence adjusted by multivariate analysis.

A For death

Variables		Hazard ratio	95% CI	*p* value
Treatments	SR *vs.* RFA	0.84	0.74, 0.95	0.006
	SR *vs.* PEI	0.75	0.64, 0.86	0.0001
	RFA *vs.* PEI	0.88	0.77, 1.01	0.08
Age	<65 *vs.* ≥65	0.71	0.63, 0.79	0.0001
Sex	Female *vs.* male	0.87	0.78, 0.98	0.03
HBsAg	Positive *vs.* negative	0.91	0.74, 1.11	0.34
HCV Ab	Positive *vs.* negative	0.93	0.79, 1.10	0.40
Liver damage	A *vs.* B	0.62	0.56, 0.69	0.0001
Platelet count	$\geq 10^4$ *vs.* $<10^4/\mu l$	0.76	0.68, 0.85	0.0001
Tumor size	<2 *vs.* ≥2 cm	0.82	0.73, 0.92	0.0007
Tumor number	Single *vs.* multiple	0.72	0.64, 0.80	0.0001
AFP	<15 *vs.* ≥15 ng/ml	0.66	0.59, 0.74	0.0001
DCP	<40 *vs.* ≥40 AU/ml	0.59	0.53, 0.66	0.0001

B For recurrence

Variables		Hazard ratio	95% CI	*p* value
Treatments	SR *vs.* RFA	0.74	0.68, 0.79	0.0001
	SR *vs.* PEI	0.59	0.54, 0.65	0.0001
	RFA *vs.* PEI	0.81	0.74, 0.88	0.0001
Age	<65 *vs.* ≥65	0.83	0.78, 0.89	0.0001
Sex	Female *vs.* male	0.88	0.82, 0.95	0.0001
HBsAg	Positive *vs.* negative	1.04	0.92, 1.17	0.53
HCV Ab	Positive *vs.* negative	1.15	1.04, 1.27	0.007
Liver damage	A *vs.* B	0.87	0.81, 0.93	0.0001
Platelet count	$\geq 10^4$ *vs.* $<10^4/\mu l$	0.92	0.86, 0.98	0.02
Tumor size	<2 *vs.* ≥2 cm	0.84	0.79, 0.90	0.0001
Tumor number	Single *vs.* multiple	0.69	0.64, 0.74	0.0001
AFP	<15 *vs.* ≥15 ng/ml	0.71	0.67, 0.76	0.0001
DCP	<40 *vs.* ≥40 AU/ml	0.72	0.67, 0.77	0.0001

HBsAg, hepatitis B virus surface antigen; HCV, hepatitis C virus; Ab, antibody; AFP, alpha-fetoprotein; DCP, des-γ-carboxy prothrombin; SR, surgical resection; RFA, radiofrequency ablation; PEI, percutaneous ethanol injection; CI, confidence interval.

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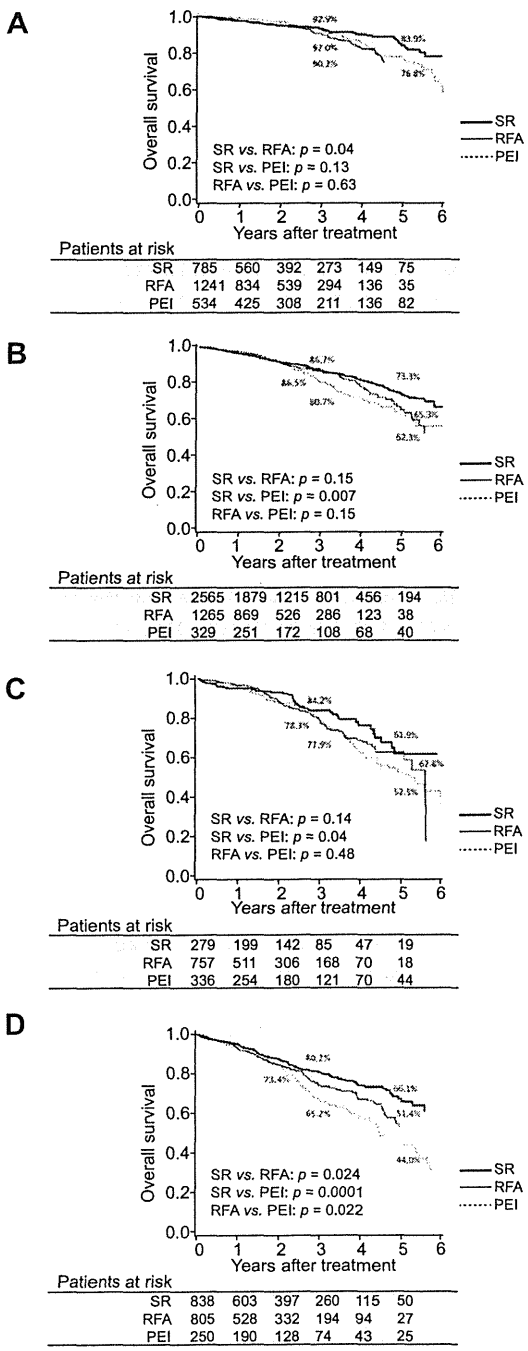

Fig. 3. Overall survival rates after surgical resection (SR), radiofrequency ablation (RFA), and percutaneous ethanol injection (PEI) in the subgroup of cases with single tumor and liver damage class A and B. (A) Liver damage class A, a single tumor (<2 cm); (B) liver damage class A, a single tumor (2–3 cm); (C) liver damage class B, a single tumor (<2 cm); (D) liver damage class B, a single tumor (2–3 cm).

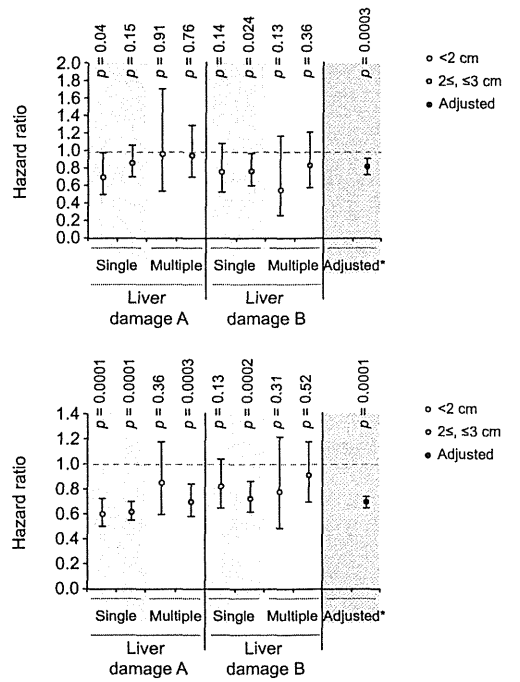

Fig. 4. Hazard ratios for death and recurrence with 95% confidence intervals and *p* values after surgical resection relative to those after radiofrequency ablation in the 8 subgroups. *The adjusted values for death and recurrence were calculated according to the three factors (tumor size, number of tumors, and liver damage class), as done in each subgroup. (A) Hazard ratios for death; (B) hazard ratios for recurrence.

caused by the selection process of treatment modalities. As similar to the previous retrospective studies [5–9], the patients amenable to surgery had had younger age, less prevalence of hepatitis C virus infection, better liver function, less association with portal hypertension, fewer number of tumors and lower alpha-fetoprotein level, whereas their tumor size was larger and their des-γ-carboxy prothrombin level was higher. To minimize potential effects of confounding factors, we studied patients who had similar tumor-related and liver function-related factors and performed multivariate analysis using 10 clinically important factors, similar to our previous study [9]. Although it is impossible to completely eliminate potential negative impacts of demographic diversity, we believe that our results are clinically meaningful, because of the large sample size of our study. In Japan, a nationwide RCT in patients with HCC is now ongoing, and the results are expected to lead to more definitive conclusions [16].

Another potential limitation of our study is the lack of data on liver function during the follow-up, which precluded assessment of the relationship between the liver function status and the choice of treatment at recurrence. In HCC, the influence of the first treatment is considered to be smaller than that in other primary malignant diseases, because the liver function remarkably affects the recurrence rate. Further investigations, particularly prospective clinical trials, are needed to address these issues.

In conclusion, this large cohort study based on data obtained by a nationwide survey in Japan, suggests that surgical resection may offer some advantage over RFA and PEI in terms of both overall survival and time to recurrence in patients with less advanced HCC. Although our results are considered as being more reliable than those of previous studies comparing the treatment

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outcomes in HCC, our conclusions need to be confirmed by future RCTs.

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Conflicts of interest

The authors who have taken part in this study declared that they do not have anything to disclose regarding funding or conflict of interest with respect to this manuscript.

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