Table 6 Results of univariate analysis of survival after salvage chemoradiotherapy

Factors	No. of patients	Median survival time (months)	6-month survival (%)	1-year survival (%)	2-year survival (%)	*p*-value
All patients	30	8.8	77	33	26	
Age						
< 65	14	8.1	79	29	14	
≥ 65	16	9.2	75	38	38	0.2
Gender						
Male	16	8.1	75	31	25	
Female	14	9.2	79	36	29	0.6
Karnofsky performance status						
≥ 80	28	9.1	79	36	28	
< 80	2	4.8	50	0	0	0.03
Primary tumor location						
Head	15	9.4	93	40	33	
Body / tail	15	8.5	60	27	18	0.5
Number of regimens of primary chemotherapy						
1	25	9.4	80	40	32	
2	5	6.1	60	0	0	0.006
Best response to primary chemotherapy						
PR	9	9.2	89	33	33	
SD or PD	21	8.5	71	33	24	0.6
Pre-chemoradiotherapy tumor diameter (cm)						
≤ 4	12	10.8	83	50	50	
> 4	18	8.5	72	22	0	0.04
Pre-chemoradiotherapy serum CA19-9 level (U/ml)						
≤ 1,000	29	10.8	90	47	42	
> 1,000	11	6.4	54	9	0	0.002
Local progression before starting chemoradiotherapy						
Absent	4	NA	80	60	60	
Present	26	8.8	76	28	19	0.15
Time from the start of primary chemotherapy to chemoradiotherapy						
≤ 6 months	12	8.5	75	33	25	
> 6 months	18	8.8	78	33	28	0.9
Combined chemoradiotherapy agents						
5-FU	14	7.2	64	21	14	
S-1	16	9.9	88	44	37	0.09

PR partial response, *SD* stable disease, *PD* progressive disease, *NA* not available.

salvage CRT ($p = 0.15$) and concomitant use of S-1 during salvage CRT ($p = 0.09$) were not significant prognostic factors. The time from the start of primary chemotherapy to salvage CRT was not associated with survival ($p = 0.73$). Using multivariate analysis, a lower pre-CRT serum CA-19-9 level (≤ 1000 U/ml; $p = 0.009$) and a single regimen of primary chemotherapy ($p = 0.004$) were found to be independent prognostic factors for survival after salvage CRT (Table 7).

Discussion

In the present study, the MST of the entire patient population from the start of salvage CRT was 8.8 months. The median time to local progression from the commencement of salvage CRT was 8.9 months. Before starting CRT, all of the patients experienced failure of the primary chemotherapy. However, the MST of 8.8 months for this cohort is comparable to the historical MST achieved after primary CRT combined with 5-FU

Table 7 Results of multivariate analysis of survival after salvage chemoradiotherapy

Variables	Factors	Hazard rate (95% CI)	*p*-value
Pre-chemoradiotherapy serum CA19-9 level (U/ml)	≤ 1000 versus > 1000	1 4.38 (1.45-13.22)	0.009
Number of regimens of primary chemotherapy	1 versus 2	1 6.28 (1.78-22.18)	0.004
Local progression before chemoradiotherapy	absent versus present	1 1.58 (0.34-7.18)	0.6
Pre-chemoradiotherapy tumor diameter (cm)	≤ 4.0 versus > 4.0	1 1.11 (0.35-3.46)	0.9

[2,14,19]; the median time to local progression was also similar [13]. In addition, the frequency of grade 3–4 non-hematological toxicity observed in the current study was also similar to that reported in previous studies. These findings show that CRT combined with S-1 or 5-FU had moderate anti-tumor activity and an acceptable toxicity profile in patients with LAPC, even after failure of GEM-based primary chemotherapy.

In the literature, the representative MST of patients with LAPC who were included in prospective clinical trials was reported to be 8.4-11.4 months for 5-FU-based CRT [2,3,14,19], 9.2-15.0 months for GEM monotherapy [15,20] and 10.3-11.1 months for GEM-based CRT [20,21]. Generally, only a few patients with LAPC survive for 3 years or more. The MST from salvage CRT in our cohort seems to be inferior to those reported in recent studies involving primary therapy for LAPC. However, if we consider primary chemotherapy and salvage CRT as a combined treatment strategy, the MST of 17.8 months from the start of primary chemotherapy is a promising result. Additionally, long-term survivors from the start of primary chemotherapy in our cohort seem to be distinct, with 22% achieving a 3-year overall survival. In our cohort, only patients who underwent primary chemotherapy and progressed locally without distant metastases were selected to receive salvage CRT. Because of the strong selection bias, we should not compare this outcome to that of prospective clinical trials in the literature. However, the existence of long-term survivors in our cohort suggests that salvage CRT should have some benefit in selected patients with LAPC, even after failure of the primary chemotherapy. The strategy of using chemotherapy alone as a primary treatment for LAPC, followed-by CRT for salvage intent, should be further investigated in prospective clinical trials.

Combined with radiotherapy, S-1 has been demonstrated to exert a synergistic effect against 5-FU-resistant cancer xenografts [22]. We previously conducted a phase I trial to determine the maximum tolerated dose of S-1 with concurrent radiotherapy for LAPC [4]. This dose was 80 mg/m^2/day, which is the same as the full dose of S-1 when administered alone. The toxicity of CRT combined with S-1 for LAPC was generally mild and manageable with conservative treatment. Several phase II clinical trials of CRT combined with S-1 for LAPC achieved MSTs in the range 14.3-16.2 months [7,8]. These MSTs compare favorably with the historical MSTs reported for CRT combined with 5-FU of 8.4-11.4 months [2,14]. In the current study, either S-1 or 5-FU was combined with radiotherapy. Univariate analysis of survival after subsequent CRT showed a non-significant trend towards better results when CRT was combined with S-1 (Table 6). The occurrence of grade 3–4 non-hematological toxicity during and after CRT was less frequent among the patients who had received CRT combined with S-1, as compared with 5-FU (6% versus 43%). Because of the retrospective nature of this study, a difference in baseline characteristics may inhibit a fair comparison between the two agents. Although a direct comparison between S-1 and 5-FU has not yet been undertaken in a prospective clinical trial, CRT combined with S-1 is an attractive alternative to 5-FU-based CRT.

The value of S-1 in pancreatic cancer is not limited to its sensitizing effect during CRT. Single agent S-1 has excellent activity regarding chemo-naïve metastatic pancreatic cancer, with a response rate of 37.5% and a MST of 9.2 months [23]. S-1 is the first agent that has not proved inferior to GEM as a single agent for the treatment of advanced pancreatic cancer in a phase III randomized-controlled trial [16]. S-1 also retains its activity in relation to advanced pancreatic cancer even after the failure of GEM, with a response rate of 21% [24]. Accordingly, in the current study, the activity of salvage CRT with S-1 should be related to the excellent systematic effect of the agent on subclinical distant metastasis, as well as its local sensitizing effect.

Recently, induction chemotherapy has become a major component in the treatment strategy for LAPC. Two well-designed retrospective studies have shown that induction chemotherapy followed by CRT yielded a survival benefit over primary CRT or continued chemotherapy alone for LAPC [12,25]. More recently, several phase II prospective clinical trials have been conducted to evaluate the value of induction chemotherapy followed by CRT, which resulted in MSTs in the range 12.6-19.2 months [26-28]. The optimum duration of induction chemotherapy for LAPC continues to be a matter of debate. Recent prospective clinical trials that included induction chemotherapy for LAPC had chosen to evaluate the effects of 2–6 months of induction therapy [26-28]. In the current study, the median duration

of primary chemotherapy was 7 months, which is longer than those used in these prospective trials. Because patients with rapidly progressing occult-metastatic disease were excluded from the present study, the tumors in our cohort might have deviated to relatively chemo-responsive tumors. Therefore, the duration of primary chemotherapy was not associated with survival after CRT in the current study. We could not draw any conclusion with regard to the optimum duration of induction chemotherapy from this retrospective cohort study.

In agreement with the current study, previous studies have shown that a highly-elevated CA 19–9 level is a poor prognostic factor for patients who had received CRT for LAPC [29,30]. A highly elevated serum CA19-9 level in patients prior to CRT suggests chemo-resistance of the tumor, as well as the existence of progressive occult metastasis. These patients might gain little benefit from the addition of salvage CRT.

Multivariate analysis revealed that the use of two regimens of primary chemotherapy was an unfavorable factor for survival after CRT. The MST of the patients who received two regimens of primary chemotherapy was 6.1 months from the start of salvage CRT, and no patient survived for 12 months or longer thereafter (Table 6). In all of the patients (n = 5) who underwent two regimens of primary chemotherapy before CRT, S-1 was used as a second-line chemotherapy. Of these patients, three received salvage CRT combined with 5-FU, and two received salvage CRT combined with S-1. Because both 5-FU and S-1 are fluorinated pyrimidine agents, failure of the tumor to respond to treatment with S-1 should cause resistance to salvage CRT combined with either 5-FU or S-1. If there are any signs of failure to respond to the primary chemotherapy, without distant metastasis, salvage CRT could be a treatment of choice as a second-line therapy.

Because of the retrospective nature of the current study, there were a number of limitations that affected the interpretation of our findings. The number of patients was very limited and the patient population was not homogeneous because of different clinical backgrounds, and they received CRT with salvage intent. Also, the patients were collected for over a period of 7 years, non-consecutively. The clinical response to primary chemotherapy was generally better than previously reported, possibly because of the exclusion of patients with chemo-resistant occult distant metastasis. Only patients who underwent primary chemotherapy and progressed locally without distant metastases were selected and included in the current analysis.

Whether or not the addition of chemotherapy prior to CRT will contribute to prolonging the survival of patients with LAPC has not been elucidated with sufficient statistical power in a prospective clinical trial. We are now investigating the value of induction chemotherapy with GEM versus no induction chemotherapy for LAPC in a multi-institutional randomized phase II study involving S-1 and concurrent radiotherapy (JCOG1106, UMIN000006811). A future phase III study will be conducted to compare GEM monotherapy and S-1 based CRT with or without induction GEM, depending on the results of the JCOG1106 study. Another phase III study, the GERCOR LAP 07 phase III trial (www.clinicaltrials.gov, identifier code NCT00634725) is also ongoing. This study was designed to elucidate the benefit of induction chemotherapy followed by CRT combined with capecitabine, with or without erlotinib during induction chemotherapy and a CRT phase. In future, results from these prospective clinical trials will become available to further define the role of chemotherapy followed by CRT for LAPC.

Conclusions

CRT combined with S-1 or 5-FU had moderate antitumor activity in patients with LAPC even after failure of GEM-based primary chemotherapy. If there are any signs of failure to primary chemotherapy without distant metastasis, salvage CRT could be a treatment of choice as a second-line therapy. Patients with a relatively low serum CA19-9 level after primary chemotherapy may obtain additional survival benefit from salvage CRT. The strategy of using chemotherapy alone as a primary treatment for LAPC, followed-by CRT with salvage intent should be further investigated in prospective clinical trials.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

HM, YI and JI participated in the design of the study, performed the statistical analysis, interpretation of data, and drafted the manuscript. HM, YI, NM, MM and MS carried out the chemoradiotherapy and analyzed tumor response. CM, HU, TO and SK carried out the chemotherapy and analyzed tumor response. All of the listed authors contributed to the writing of the manuscript. All authors read and approved the final manuscript.

Author details

[1]Division of Radiation Oncology, National Cancer Center Hospital, 5-1-1 Tsukiji, Chuo-ku, Tokyo 104-0045, Japan. [2]Divisions of Hepatobiliary and Pancreatic Oncology, National Cancer Center Hospital, 5-1-1 Tsukiji, Chuo-ku, Tokyo 104-0045, Japan.

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RESEARCH ARTICLE Open Access

Circulating endothelial cells and other angiogenesis factors in pancreatic carcinoma patients receiving gemcitabine chemotherapy

Shunsuke Kondo[1*], Hideki Ueno[1], Jun Hashimoto[1], Chigusa Morizane[1], Fumiaki Koizumi[2], Takuji Okusaka[1] and Kenji Tamura[3]

Abstract

Background: Pancreatic carcinoma is a significant cause of cancer-related death in developed countries. As the level of circulating endothelial cells (CECs) is known to increase in response to various cancers, we investigated the predictive potential of CEC levels and the association of these levels with the expression of proangiogenic factors in pancreatic carcinoma patients.

Methods: Pancreatic carcinoma patients receiving gemcitabine chemotherapy were prospectively assigned to this study. CEC levels were measured using the CellTracks system, and the plasma levels of several angiogenesis factors were measured using multiplex immunoassay. Associations between clinical outcomes and the levels of these factors were evaluated.

Results: Baseline CEC levels were markedly higher in pancreatic carcinoma patients (n = 37) than in healthy volunteers (n = 53). Moreover, these high CEC levels were associated with decreased overall survival (median, 297 days versus 143 days, $P < 0.001$) and progression-free survival (median, 150 days versus 64 days, $P = 0.008$), as well as with high vascular endothelial growth factor, interleukin (IL)-8, and IL-10 expression in the pancreatic carcinoma patients.

Conclusions: Several chemokines and proangiogenic factors correlate with the release of CECs, and the number of CECs detected may be a useful prognostic marker in pancreatic carcinoma patients undergoing gemcitabine chemotherapy.

Trial registration: UMIN000002323

Keywords: Pancreatic carcinoma, Circulating endothelial cells, Angiogenesis factors

Background

Pancreatic carcinoma is one of the most lethal tumors and is the fourth leading cause of cancer-related death in developed nations [1]. As pancreatic carcinoma has a high propensity for both local invasion and distant metastasis, surgery is precluded as a treatment for most patients who present with advanced-stage disease. These patients have a median survival of only 6 months and an overall 5-year survival of less than 5%. The prognosis for advanced pancreatic carcinoma patients is therefore extremely poor, and the impact of standard therapy is only modest, despite many advances that have improved the outcome of this disease.

Pancreatic carcinoma is not a grossly vascular tumor; however, it overexpresses multiple mitogenic growth factors that are also angiogenic, such as epidermal growth factor (EGF), hepatocyte growth factor (HGF), fibroblast growth factor (FGF), platelet-derived growth factor B chain (PDGF-BB), and vascular endothelial growth factor (VEGF). Angiogenesis often occurs in response to an imbalance in which proangiogenic factors predominate over antiangiogenic factors. For instance, VEGF expression has been shown to promote tumor growth in pancreatic carcinomas [2]. High VEGF expression is also

* Correspondence: shkondo@ncc.go.jp
[1]Hepatobiliary and Pancreatic Oncology Division, National Cancer Center Hospital, Tokyo, Japan
Full list of author information is available at the end of the article

associated with increased microvessel density [3] and is a predictor of poor outcomes and early tumor recurrence after curative resection [4]. Although agents that target the VEGF signaling pathway have been shown to inhibit tumor growth, metastasis, and angiogenesis [5], treating advanced pancreatic carcinoma patients with axitinib—a selective inhibitor of VEGF receptors 1, 2, and 3—in combination with gemcitabine was not found to improve overall survival in a phase 3 trial [6]. Despite this finding, proangiogenic factors remain an important therapeutic target for the treatment of pancreatic carcinoma.

Circulating endothelial cells (CECs) are mature cells that are not associated with vessel walls but are detached from the endothelium and circulate within peripheral blood. The number of CECs present in the blood has been found to increase in response to cardiovascular disease, vasculitis, infectious disease, and various cancers [7,8]. Indeed, the level of CECs has been recognized as a useful biomarker for vascular damage. It has also been reported that the number of CECs found in non-small cell lung cancer patients treated with carboplatin plus paclitaxel is a promising predictive marker of the clinical efficacy of these drugs [9]. We believe that CEC levels may also be a potential biomarker for pancreatic carcinoma; therefore, we investigated the levels of CECs found in patients with different severities of pancreatic carcinoma, as well as the effects of gemcitabine treatment on CEC levels. Furthermore, the associations between CEC levels and the expression levels of several factors involved in angiogenesis and neovascularization were also examined in this study.

Methods

Study approval

This prospective study was approved by the Institutional Review Board of the National Cancer Center, and written informed consent was obtained from all patients. This study is registered with the University Hospital Medical Information Network in Japan (UMIN; number UMIN000002323) and has been completed.

Patients and blood sample collection

A total of 37 chemotherapy-naïve patients with histologically or cytologically confirmed invasive ductal pancreatic carcinoma were prospectively enrolled in this study between April 2009 and March 2010 and received gemcitabine chemotherapy. Patients with coexisting infections and/or cardiovascular illness were excluded. The detailed history of all the patients was obtained and a physical examination was performed before beginning gemcitabine treatment. Pretreatment baseline laboratory parameters were also assessed for all patients. The baseline tumor status of each patient was evaluated using computed tomography (CT) scans of the chest, abdomen, and pelvis, while peripheral blood sampling was performed both prior to treatment initiation (baseline) and at day 28 ± 7 after starting chemotherapy. A dose of 1000 mg/m^2 gemcitabine was administered intravenously for 30 min on days 1, 8, and 15 of a 28-day cycle until disease progression, unacceptable toxicity, or patient refusal occurred. The data collected included those pertaining to standard demographics and disease characteristics, the date of initial treatment, the best response to treatment, date of progression, and the date of death or last follow-up. The tumors were evaluated every 6–8 weeks after starting each course of gemcitabine, and best responses were documented according to the Response Evaluation Criteria in Solid Tumors (RECIST).

CEC enumeration

Blood samples from advanced pancreatic carcinoma patients were drawn into 10 mL CellSave Preservative Tubes (Immunicon Corp. Huntingdon Valley, PA) for CEC enumeration. Samples were obtained both before starting chemotherapy (baseline) and at 28 ± 7 days after starting chemotherapy. Samples were kept at room temperature and processed within 42 h of collection. All of the evaluations were performed without knowledge of the clinical status of the patients. The CellTracks system (Veridex, LLC), which consists of the CellTracks AutoPrep system and the CellSpotter Analyzer system, was used for endothelial cell enumeration. In this system, CECs are defined as CD146$^+$/DAPI$^+$/CD105-PE$^+$/CD45APC$^-$ cells. Briefly, CD146$^+$ cells were captured immunomagnetically by using ferrofluids coated with CD146 antibodies. The enriched cells were then labeled with the nuclear dye 4 V, 6-diamidino-2-phenylindole (DAPI), CD105 antibodies were conjugated to phycoerythrin (CD105-PE), and the pan-leukocyte antibody CD45 was conjugated to allophycocyanin (CD45-APC). Cells with the DAPI$^+$/CD105$^+$/CD45$^-$ phenotype were enumerated. We evaluated morphological cell viability and excluded dead cells from the cell count. The number of CECs in each sample was determined twice, and the mean value was calculated.

Antibody suspension bead array system

Peripheral blood was drawn into prechilled tubes containing ethylenediaminetetraacetic acid; was immediately subjected to centrifugation at 1000 g and 4°C for 15 min, plasma was transferred to microtubes and subjected to further centrifugation at 10,000 g and 4°C for 10 min to remove contaminating platelets. Plasma samples were collected from patients before gemcitabine treatment was initiated and were stored at −80°C until they were used for testing. The plasma concentrations of 7 biological markers (interleukin [IL]-6, IL-8, IL-10,

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.

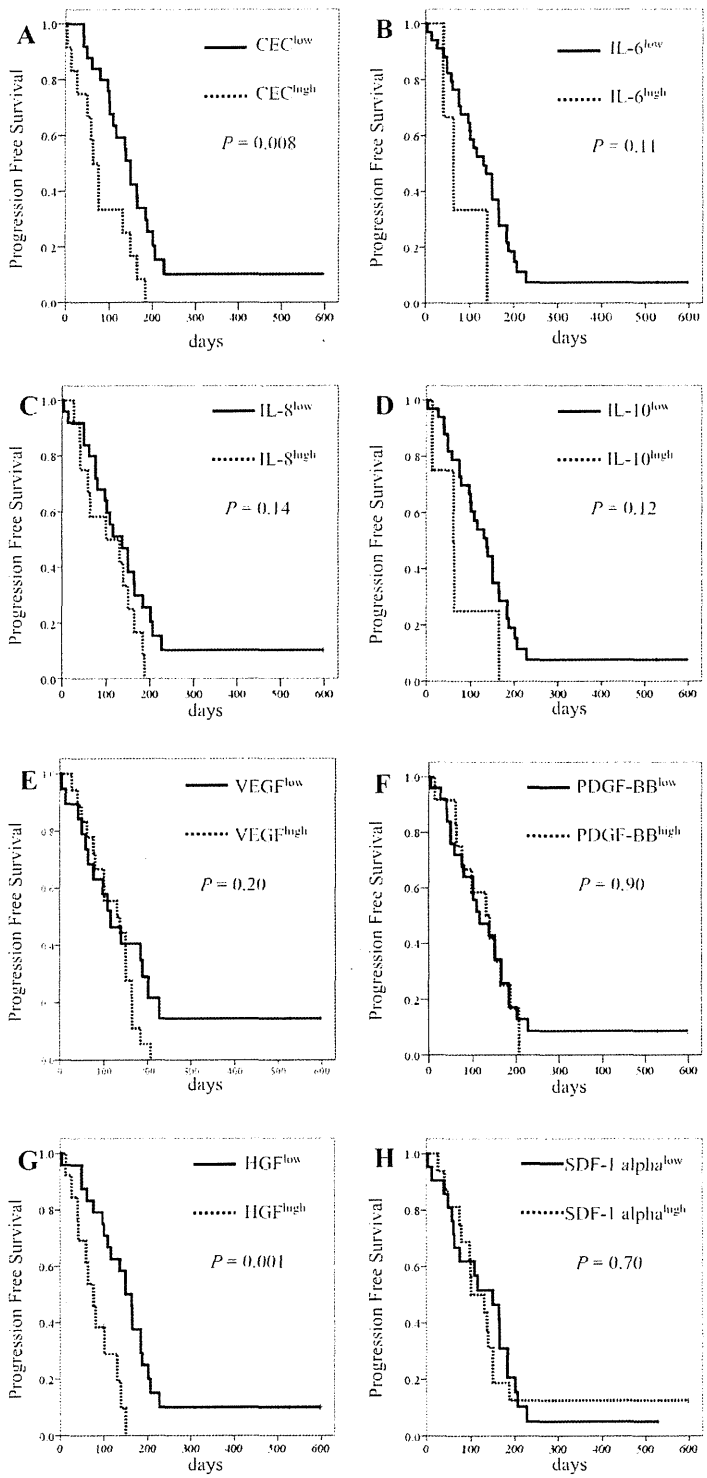

Figure 1 Kaplan-Meier curves for (A) progression-free survival with CEC counts, (B) progression-free survival with IL-6 levels, (C) progression-free survival with IL-8 levels, (D) progression-free survival with IL-10 levels, (E) progression-free survival with VEGF levels, (F) progression-free survival 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 1 G). 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 2 G).

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

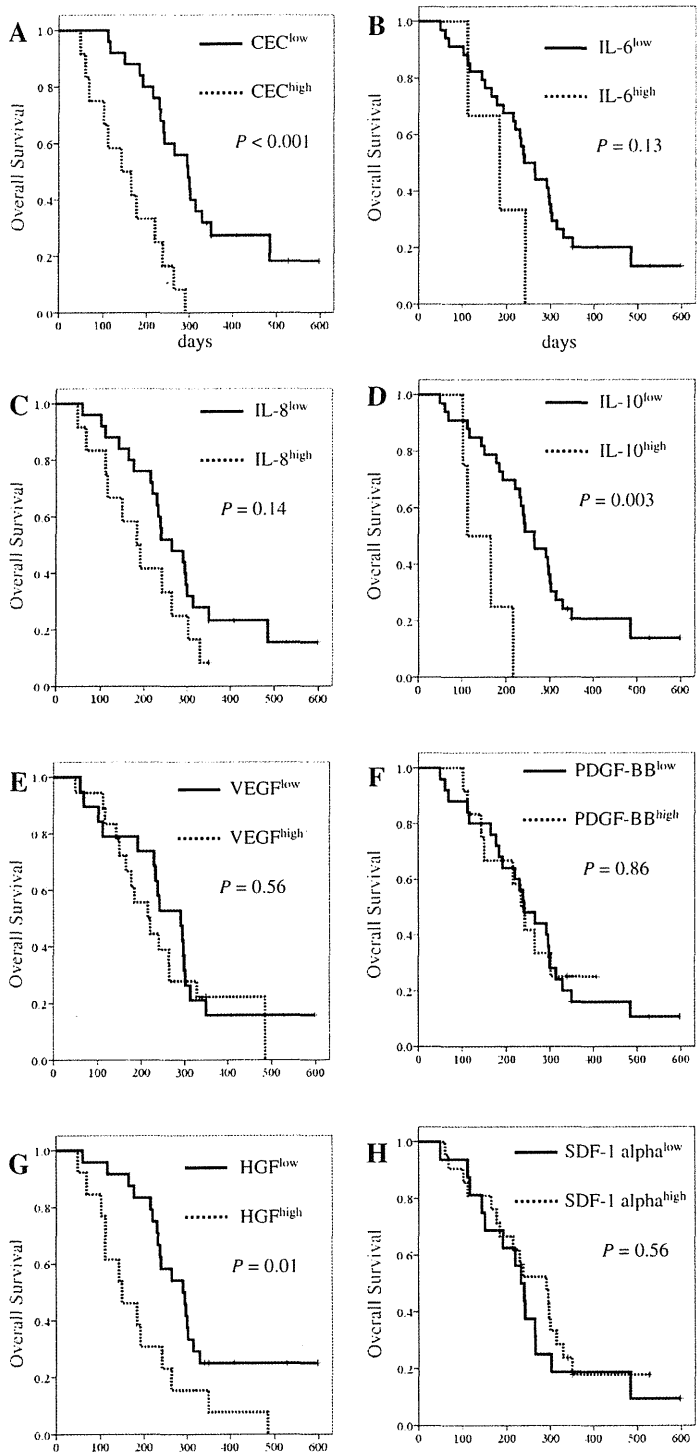

Figure 2 Kaplan-Meier curves for (A) overall survival with CEC counts, (B) overall survival with IL-6 levels, (C) overall survival with IL-8 levels, (D) overall survival with IL-10 levels, (E) overall survival with VEGF levels, (F) overall survival with PDGF-BB levels, (G) overall survival with HGF levels, 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.

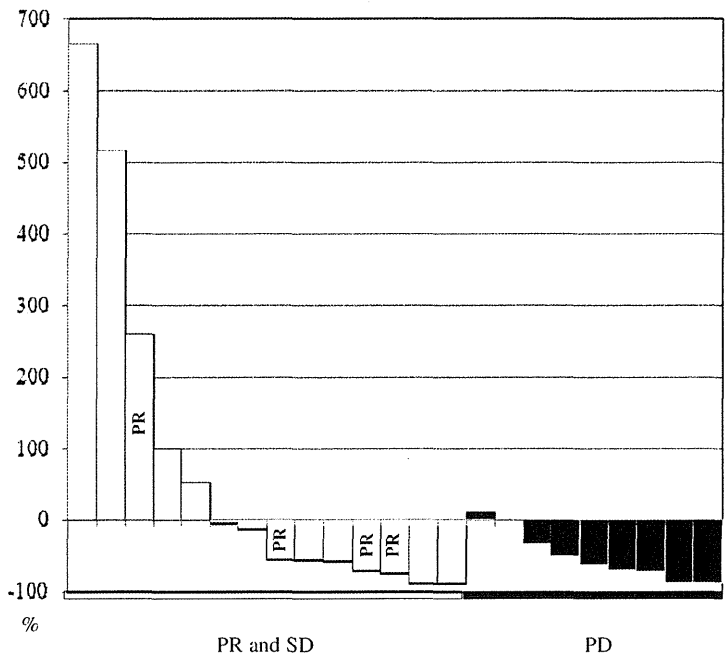

Figure 3 Waterfall plot showing the changes in CEC counts and tumor response in patients without progressive disease (PD) (those with partial response [PR] or stable disease [SD]) and patients with PD, after 28 ± 7 days of gemcitabine treatment.

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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Author details
[1]Hepatobiliary and Pancreatic Oncology Division, National Cancer Center Hospital, Tokyo, Japan. [2]Shien-Lab, National Cancer Center Hospital, Tokyo, Japan. [3]Breast and Medical Oncology Division, National Cancer Center Hospital, Tokyo, Japan.

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Case Reports

Gemcitabine-induced Pleuropericardial Effusion in a Patient with Pancreatic Cancer

Hidenori Kido[1], Chigusa Morizane[1,*], Tetsutaro Tamura[2], Atsushi Hagihara[1], Shunsuke Kondo[1], Hideki Ueno[1] and Takuji Okusaka[1]

[1]Hepatobiliary and Pancreatic Oncology Division, National Cancer Center Hospital and [2]Cardioangiology Division, National Cancer Center Hospital, Tokyo, Japan

*For reprints and all correspondence: Chigusa Morizane, Hepatobiliary and Pancreatic Oncology Division, National Cancer Center Hospital, 5-1-1 Tsukiji, Chuo-ku, Tokyo 104-0045, Japan. E-mail: cmorizan@ncc.go.jp

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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	—	

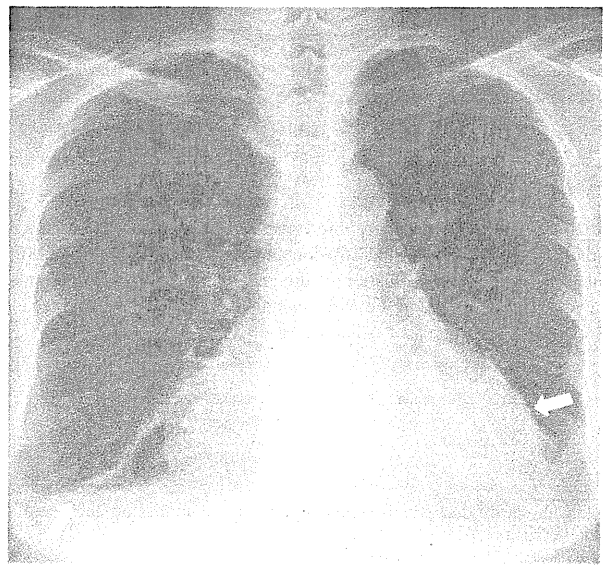

Figure 1. Chest X-ray obtained upon admission shows cardiac enlargement (60%), increased pulmonary markings and bilateral pleural effusion.

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

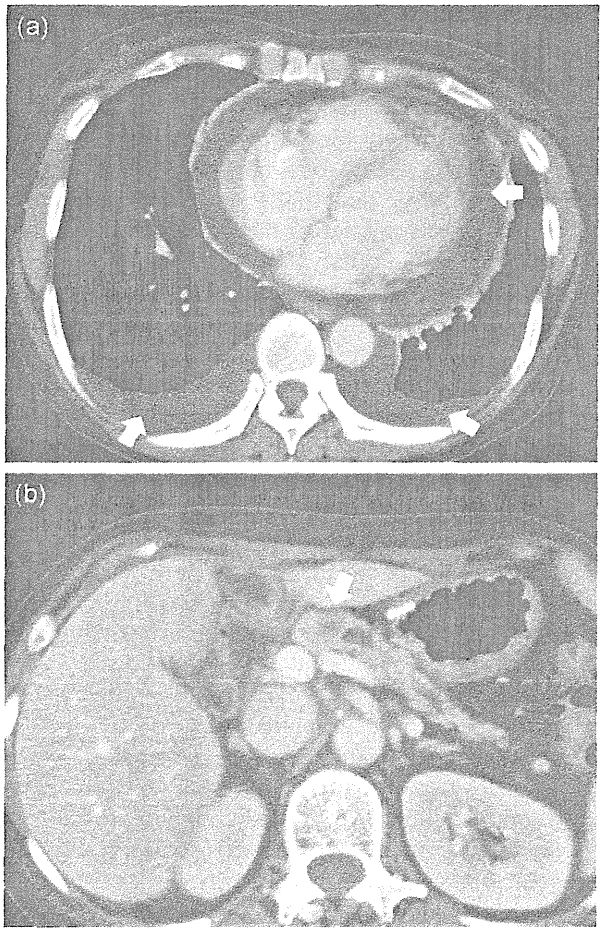

Figure 2. (a) Computed tomographic examination of the chest obtained upon admission shows pericardial effusion and bilateral pleural effusion. (b) Computed tomographic examination of the abdomen obtained upon admission shows local recurrence in the remnant stump of the pancreas; the recurrence was almost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

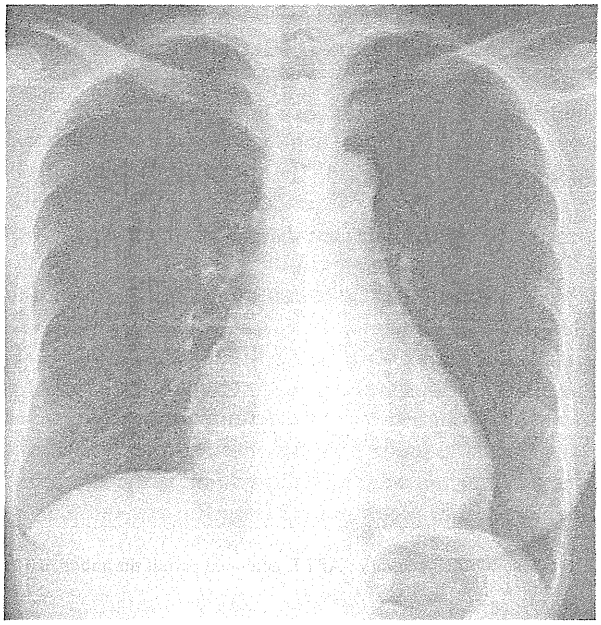

Figure 3. Chest X-ray obtained 2 months after discharge showing a normal cardiac shadow.

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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