suppresses STAT3 phosphorylation and has shown clinical benefits in myelofibrosis (Harrison *et al*, 2012). The potential for individual pro-inflammatory cytokines to decrease chemotherapeutic efficacy suggests that it may be a candidate for testing anti-inflammatory therapy in advanced PC patients. This study sought to characterise the impact of pro-inflammatory cytokines on the outcomes of systemic chemotherapy in patients with advanced PC.

MATERIALS AND METHODS

Patients. Treatment-naive patients with advanced PC and no obvious infections were eligible for enrolment in this study. Pathological confirmation was obtained from all the patients via either a fine-needle aspiration biopsy or a cytological examination. All the patients were scheduled to undergo chemotherapy at the National Cancer Center Hospital East. A serum sample was obtained on the morning before chemotherapy and was frozen at −70 °C until analysis. Clinical data were prospectively collected before chemotherapy, at 1 month after chemotherapy, and every 3 months after the start of chemotherapy. The tumour stage was evaluated according to the seventh criteria of the International Union Against Cancer (UICC) (Sobin *et al*, 2009). This study was approved by the National Cancer Center Ethics Committee, and patients who provided written informed consent were examined.

Systemic chemotherapy. Gemcitabine monotherapy (GEM) and GEM-based regimens were conducted according to previous reports (Ioka *et al*, 2011; Kindler *et al*, 2011). Most of the patients were scheduled to receive GEM as follows: 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 the occurrence of disease progression, unacceptable toxicity, or patient refusal. The dose intensity of GEM was calculated during the treatment interval between the date of the first administration and the date of the last administration. The planned dose intensity of GEM for a 28-day cycle was 750 mg m^{-2} per week.

Assessment of the anti-tumour effect. The anti-tumour effect of the systemic chemotherapy was evaluated using contrast computed tomography/magnetic resonance imaging images obtained every 4–8 weeks after treatment. The tumour response was determined as a complete response (CR), partial response (PR), stable disease (SD), progressive disease, or not evaluated according to the Response Evaluation Criteria in Solid Tumors (Therasse *et al*, 2000). The best overall response for each patient was recorded as the tumour response. The response rate was calculated as CR + PR/all evaluated patients. Disease control was defined as CR, PR, or SD. The disease control rate was calculated as CR + PR + SD/all evaluated patients.

Pro-inflammatory cytokine assays. The serum levels of cytokines were measured using multiplex assays manufactured by Meso Scale Discovery (Gaithersburg, MD, USA). On the bottom of each well of 96-well plate-based assays, antibodies for GM-CSF, IFN-γ, IL-1β, IL-2, IL-6, IL-8, IL-10, IL-12p40 (IL-12), and TNF-α were spotted by the manufacturer. Following the capture of the cytokines by the spotted antibodies, label detection antibodies were bound to the antigen. The detection antibodies were coupled to electrochemiluminescent labels that emitted light when electrochemically stimulated via carbon-coated electrodes located in the bottom of the array wells. The resulting signal was read using a charge-coupled device. The MSD Multi-Spot Array assay was performed according to the manufacturer's instructions. The raw data were computed as the levels of electrochemiluminescent signals (light) measured using photodetectors and were analysed using Discovery Workbench 3.0 software (Meso Scale Discovery). A four-parameter logistic fit curve was generated for each analyte using the standards and the calculated concentration of each sample.

Statistical analyses. Progression-free survival (PFS) was defined as the time between the start of chemotherapy and either documented disease progression or death. Overall survival (OS) was defined as the interval between the initial administration of chemotherapy and either death or the last follow-up examination. Survival differences in the univariate analyses were calculated using the Cox's proportional hazards regression model. Factors that were strongly associated with a short survival period ($P<0.01$) were evaluated using a multivariate analysis of the Cox's proportional hazards regression model. Survival curves were drawn using the Kaplan–Meier method, and the difference between two survival curves was evaluated using the log-rank test. The frequency of patients in the two groups was compared using the Fisher's exact test. A comparison of non-categorical data was performed using the Mann–Whitney *U* test. The significance level was set at $P<0.05$. All the analyses were performed using the JMP 8 software, Windows version (SAS Institute, Cary, NC, USA).

RESULTS

Patient characteristics. Between 2008 and 2009, 110 patients were enrolled in the study. Six patients were excluded from the study analysis because of the presence of inflammation at the start of chemotherapy, as follows: cholecystitis in three patients, cholangitis in two patients, and thrombophlebitis in both lower extremities in one patient. Four patients with rapid systemic weakness because of tumour progression refused to participate in the data collection after registering in the study. One patient with massive ascites who required multiple large-volume paracentesis procedures was judged unable to undergo systemic chemotherapy and was not evaluated in this study. Sixteen patients receiving S-1 monotherapy and 23 patients receiving GEM doublets were excluded because our focus was on the relationship between cytokine levels and the efficacy of GEM. The GEM doublets regimens consisted of GEM plus S-1 in 12 patients, GEM plus a cancer vaccine in 6 patients, and GEM plus axitinib in 5 patients. The remaining 60 patients were treated with GEM alone and were analysed in this study. The starting dose of GEM was 1000 mg m^{-2} in all the 60 patients. Patient characteristics and the clinical data obtained before chemotherapy are summarised in Table 1.

Pro-inflammatory cytokine levels. Each cytokine was studied in the following numbers of patients: GM-CSF ($n=58$), IFN-γ ($n=60$), IL-1β ($n=60$), IL-2 ($n=60$), IL-6 ($n=60$), IL-8 ($n=60$), IL-10 ($n=60$), IL-12 ($n=59$), and TNF-α ($n=60$) (Supplementary Table S1). The number of patients from whom samples were assayed was dependent on the accuracy of the measurement using the diluted sample. The following rates of detectable concentrations were observed: GM-CSF (33.5%), IFN-γ (20.0%), IL-1β (33.4%), IL-2 (20.0%), IL-6 (96.7%), IL-8 (100%), IL-10 (88.3%), IL-12 (37.3%), and TNF-α (98.3%). Undetectable concentrations of any cytokine were recorded as zero. According to the median value of each cytokine in all patients (Table 1), patients with higher concentrations than the median value were defined as the high cytokine group.

Tumour response and survival in patients with GEM alone. The tumour response was evaluated in all the 60 patients. None of the patients (0%) achieved a CR, and two patients (3.3%) had a PR. Twenty-nine patients (48.3%) were characterised as having SD, and one patient was categorised as not evaluated. The disease control rate was 51.6%. One patient was able to receive a pancreaticoduodenectomy after tumour reduction because of a good chemotherapeutic effect. The radiological and symptomatic progression of PC

Table 1. Patient characteristics

Variables		N (%)
Patients		60 (100)
Age (years)	Median (range)	66 (35–85)
Sex	Female	32 (53)
ECOG PS	0	32 (53)
	1	26 (43)
	2 or 3	2 (4)
Biliary drainage	Present	13 (22)
Opioid	Present	19 (32)
UICC-Stage	III	22 (37)
	IV	38 (63)
Liver metastasis	Present	29 (48)
Ascites	Present	21 (35)
Primary site	Head	19 (32)
Size of primary tumour (cm)	Median (range)	3.8 (1.8–9.7)
Second-line therapy	Chemotherapy	21 (35)
	Surgery	1 (2)
	Best supportive care	38 (63)
C-reactive protein (mg dl^{-1}dl)	Median (range)	0.36 (0.01–25.0)
GM-CSF (pg ml^{-1})	Median (range)	0.00 (0.00–289)
IFN-γ (pg ml^{-1})	Median (range)	0.00 (0.00–16.1)
IL-1β (pg ml^{-1})	Median (range)	0.00 (0.00–1.65)
IL-2 (pg ml^{-1})	Median (range)	0.00 (0.00–26.7)
IL-6 (pg ml^{-1})	Median (range)	1.93 (0.00–34.3)
IL-8 (pg ml^{-1})	Median (range)	19.6 (2.31–206)
IL-10 (pg ml^{-1})	Median (range)	1.81 (0.00–383)
IL-12 (pg ml^{-1})	Median (range)	0.00 (0.00–1700)
TNF-α (pg ml^{-1})	Median (range)	7.69 (0.00–23.0)

Abbreviations: CI = confidence interval; ECOG PS = Eastern Cooperative Oncology Group Performance Status; GM-CSF = granulocyte macrophage colony-stimulating factor; HR = hazard ratio; IFN = interferon; IL = interleukin; TNF = tumour necrosis factor; UICC-Stage = stage based on the seventh criteria of the International Union Against Cancer (UICC).

were observed in 48 (80.0%) and 11 patients (18.4%), respectively. Twenty-one patients (35.0%) received second-line chemotherapy for advanced PC: S-1 ($n=18$) and S-1 + oxaliplatin ($n=2$). Fifty-four patients died from PC before the end of the observation period (August 2011). The median times for OS and PFS were 228 days (95% confidence interval (CI), 138–299 days) and 91 days (95% CI, 49–102 days), respectively.

Univariate and multivariate analyses for OS and PFS using serum levels of cytokines. The univariate and multivariate analysis for OS identified high IL-1β (HR 1.88; $P=0.048$) and high IL-6 (HR 2.10, $P=0.011$) levels as independent predictors of a poor OS (Table 2). In the univariate and multivariate analysis for PFS, a high IL-6 level was an independent risk factor for a short PFS (HR 2.32, $P=0.003$), and a high IL-1β level tended to be an independent risk factor for a poor PFS (HR 1.81, $P=0.056$).

To obtain detailed information regarding the efficacy of chemotherapy and the patient's prognosis according to the IL-6 and IL-1β concentrations, we tested the prognostic values of classifications based on the serum levels of IL-6 and IL-1β using survival curves of OS and PFS as follows: IL-6^{Low}/IL-1β^{Low} ($n=21$), IL-6^{Low}/IL-1β^{High} ($n=5$), IL-6^{High}/IL-1β^{Low} ($n=15$), and IL-6^{High}/IL-1β^{High} ($n=15$) (Figure 1). The OS and PFS curves of the IL-6^{High}/IL-1β^{High} group revealed higher risks for death and tumour progression than those of the IL-6^{Low}/IL-1β^{Low} group ($P<0.001$ in OS and $P<0.001$ in PFS). The difference between the IL-6^{High}/IL-1β^{Low} and the IL-6^{Low}/IL-1β^{Low} groups was obvious for PFS ($P=0.013$) and tended to be present for OS ($P=0.053$).

Prognosis and disease control classified according to the IL-6 and IL-1β status in patients with GEM alone. To identify the prognostic values of the IL-6/IL-1β classification, we calculated the risk of death and progression according to the status of the IL-6 and IL-1β levels. The relative risk of death and progression to the IL-6^{Low}/IL-1β^{Low} group was increased in the IL-6^{High}/IL-1β^{High} group (HR 4.06; $P<0.001$, HR 4.26; $P<0.001$) and in the IL-6^{High}/IL-1β^{Low} group (HR 1.90; $P=0.074$, HR 2.24; $P=0.021$) but not in the IL-6^{Low}/IL-1β^{High} group (HR 1.48; $P=0.497$, HR 1.68; $P=0.323$; Table 3).

Tumour control rates (TCRs) according to the IL-6 and IL-1β classifications were evaluated and are shown in Table 4. The TCRs of the IL-6^{High}/IL-1β^{High} and the IL-6^{High}/IL-1β^{Low} groups (20.0% and 40.0%) were lower than that of the IL-6^{Low}/IL-1β^{Low} group (76.0%, $P<0.001$ and $P=0.042$). A significant difference in the TCR between the IL-6^{High}/IL-1β^{High} group and the IL-6^{High}/IL-1β^{Low} group was not identified, but the actual value of TCR in the IL-6^{High}/IL-1β^{High} group was half of that in the IL-6^{High}/IL-1β^{Low} group.

GEM exposure according to IL-1β and IL-6 status. The median value of GEM dose intensity within 90 days after the start of chemotherapy (GEM-DI) was 737 mg m^{-2} per week in patients with GEM alone. GEM-DI was compared among the groups assigned the IL-6/IL-1β classification (Supplementary Table S2). The GEM-DI medians were increased in the IL-6^{High}/IL-1β^{High} (814 mg m^{-2} per week, $P=0.003$) and the IL-6^{High}/IL-1β^{Low} (781 mg m^{-2} per week, $P=0.012$) groups compared with the IL-6^{Low}/IL-1β^{Low} group (698 mg m^{-2} per week).

CRP levels according to IL-1β and IL-6 status. IL-6 and IL-1β promote the synthesis of CRP from hepatocyte (Morrone *et al*, 1988; Young *et al*, 2008). The serum CRP level is considered to be a good index for the physiological effects of IL-6 and IL-1β. We compared the CRP levels among the groups assigned to the IL-6/IL-1β classifications. The CRP level of the IL-6^{High}/IL-1β^{High} group was the highest of the groups with IL-6/IL-1β classifications (Table 5). The IL-6^{High}/IL-1β^{Low} group showed a higher CRP level than the IL-6^{Low}/IL-1β^{Low} group ($P=0.001$).

DISCUSSION

IL-6 is a pleiotropic cytokine with a variety of effects on cells and tissues (Trikha *et al*, 2003) that is synthesised by many different cell types, including immune cells, fibroblasts, endothelial cells, myocytes, adipocytes, a variety of endocrine cells, and PC cells (Tracey and Cerami, 1993; Van Snick, 1996; Fried *et al*, 1998; Martignoni *et al*, 2005). IL-6 mRNA is found in 64% of PC cases, in which the IL-6 mRNA expression ratio in relation to normal pancreatic tissue is strongly upregulated by a median of 62.4-fold (Bellone *et al*, 2006). The immunohistochemical expression of IL-6 in PC tumours is strong in the cytoplasm of PC cells and weak in inflammatory cells (Martignoni *et al*, 2005). Furthermore, the serum IL-6 level in patients with PC is higher than in healthy individuals (Okada *et al*, 1998; Barber *et al*, 1999; Ebrahimi *et al*, 2004; Martignoni *et al*, 2005; Talar-Wojnarowska *et al*, 2009). A high IL-6 level is correlated with tumour aggressiveness, inflammatory response, and systemic weakness, such as large tumour size, hepatic metastasis, an elevated level of serum CRP, body weight loss, and poorer performance status (Okada *et al*, 1998;

Table 2. Univariate and multivariate analyses for overall survival and progression-free survival according to cytokine level in patients receiving gemcitabine monotherapy for advanced pancreatic cancer

			Univariate analysis		Multivariate analysis	
Tested factor		**N**	**HR (95% CI)**	***P*-value**	**HR (95% CI)**	***P*-value**
Overall survival						
GM-CSF	High	20	1.84 (1.02–3.21)	0.042		
IFN-γ	High	12	1.16 (0.53–2.29)	0.686		
IL-1β	High	20	2.33 (1.27–4.18)	0.007	1.88 (1.01–3.45)	0.048
IL-2	High	12	2.09 (1.01–4.00)	0.048		
IL-6	High	30	2.41 (1.39–4.20)	0.002	2.10 (1.19–3.74)	0.011
IL-8	High	29	1.49 (0.87–2.57)	0.149		
IL-10	High	30	1.22 (0.71–2.11)	0.465		
IL-12	High	22	2.06 (1.12–3.72)	0.020		
TNF-α	High	30	0.98 (0.57–1.68)	0.939		
Progression-free survival						
GM-CSF	High	20	1.61 (0.91–2.76)	0.098		
IFN-γ	High	12	1.27 (0.64–2.33)	0.481		
IL-1β	High	20	2.33 (1.30–4.08)	0.005	1.81 (0.98–3.27)	0.056
IL-2	High	12	2.08 (1.02–3.97)	0.043		
IL-6	High	30	2.67 (1.56–4.56)	<0.001	2.32 (1.33–4.07)	0.003
IL-8	High	29	1.27 (0.75–2.14)	0.362		
IL-10	High	30	1.46 (0.87–2.45)	0.148		
IL-12	High	22	2.13 (1.21–3.72)	0.010		
TNF-α	High	30	1.15 (0.68–1.93)	0.595		

Abbreviations: CI = confidence interval; GM-CSF = granulocyte macrophage colony-stimulating factor; HR = hazard ratio; IFN = interferon; IL = interleukin; TNF = tumour necrosis factor.

Barber *et al*, 1999; Ebrahimi *et al*, 2004; Martignoni *et al*, 2005; Talar-Wojnarowska *et al*, 2009). The prognostic impact of the circulating IL-6 level was demonstrated in a study by Ebrahimi *et al* (2004), in which patients underwent either pancreatic resection or chemotherapy. This study clearly highlights the independent prognostic value of a high IL-6 level on OS in patients receiving GEM for PC. The correlation between high IL-6 levels and a shortened PFS was observed in hepatocellular carcinoma patients receiving sunitinib monotherapy (Zhu *et al*, 2009) and in diffuse large-cell lymphoma patients receiving chemotherapy (Seymour *et al*, 1995). To the best of our knowledge, the association between serum IL-6 levels and PFS in patients undergoing systemic chemotherapy for PC has not been previously reported. This study clearly showed the impact of a high IL-6 level on a shortened PFS in patients undergoing GEM for PC.

IL-1β is a pro-inflammatory cytokine that is synthesised by many cell types, including monocytes, tissue macrophages, and PC cells (Bellone *et al*, 2006; Angst *et al*, 2008). IL-1β mRNA can be identified in >80% of PC tumour tissues, and the IL-1β mRNA expression ratio in relation to normal pancreatic tissue in resected PC specimens is, on average, strongly upregulated by 28.5-fold (Ebrahimi *et al*, 2004; Bellone *et al*, 2006). IL-1β from tumour cells and monocytes contributes to the chemoresistance of PC cells (Arlt *et al*, 2002; Angst *et al*, 2008). The serum levels of IL-1β are rarely measured in healthy tissues. In fact, the total daily production of IL-1β was calculated to be approximately 6 ng day^{-1} in a study using a specific antibody to human IL-1β (Lachmann *et al*, 2009), whereas in humans injected with an endotoxin, the levels of IL-1β were below the detection limit (<2 pg ml^{-1}) at baseline and were elevated for approximately 2 h, reaching maximal concentrations of 50–60 pg ml^{-1} (Granowitz *et al*, 1991). No relationship has been reported between the serum IL-1β level and its clinical significance in PC patients because the serum IL-1β levels are usually below the lower measurable limit of detection (LOD). The LOD for IL-1β was previously found to be 0.3 pg ml^{-1} using an enzyme-linked immunosorbent assay (Ebrahimi *et al*, 2004). In this study, the LOD of IL-1β was 0.19 pg ml^{-1} ml^{-1}, and the detectable rate of serum IL-1β was 33.4%. Our assay for the detection of pro-inflammatory cytokines was based on electrochemiluminescence, which is a superior detection method compared with enzyme-linked immunosorbent assay; hence, our LOD was lower. Recent progress in assay methods has improved the detection of serum IL-1β, enabling the use of the serum IL-1β concentration for predicting the efficacy of chemotherapy and the identification of a patient's prognosis in this study. A high IL-1β serum level was an independent prognostic factor that, in this study, showed a tendency toward an association with a shortened PFS. IL-1β promotes metastasis and angiogenesis because of the upregulation of pro-metastatic genes and molecules, including matrix metalloproteinases and endothelial adhesion molecules, along with vascular endothelial cell growth factor, chemokines, growth factors, and TGFβ (Dinarello, 2010). A high IL-1β level may be related to an aggressive tumour status and may be correlated with a poor prognosis.

The IL-6^{High}/IL-1β^{High} group had shortened PFS and OS compared with the IL-6^{Low}/IL-1β^{Low} group. The disease control rate in the IL-6^{High}/IL-1β^{High} group was decreased by one-fourth compared with that of the IL-6^{Low}/IL-1β^{Low} group. Interestingly, GEM-DI in the IL-6^{High}/IL-1β^{High} was higher than in the IL-6^{Low}/IL-1β^{Low} group. The CRP serum level, a good index of the IL-6 and IL-1β effects via STAT3 and NF-κB, was higher in the IL-6^{High}/IL-1β^{High} group. These results may indicate that the resistance of PC tumour cells against GEM was dependent on the effects of IL-6 and IL-1β via STAT3 and NF-κB. GEM leads to DNA damage in PC cells, which results in GEM-induced apoptosis (Arlt *et al*, 2010). The resistance of PC cells to chemotherapeutic agents is due to an altered balance between pro- and anti-apoptotic proteins, resulting in reduced apoptotic responsiveness

(Grivennikov and Karin, 2010). Bcl-2 and Bcl-xL are anti-apoptotic proteins that are activated by STAT3 and NF-κB, whereas Mcl-1, another of the anti-apoptotic proteins, is primarily STAT3-dependent (Arlt *et al*, 2010). IL-6 and IL-1β can activate STAT3 and NF-κB (Nishikawa *et al*, 2008), possibly resulting in an increase of anti-apoptotic proteins in PC cells. Based on the above context, the inhibition of STAT3 and NF-κB was expected to resolve the chemoresistance of PC cells.

The IL-6^{High}/IL-$1\beta^{Low}$ group had poor outcomes for OS and PFS compared with the IL-6^{Low}/IL-$1\beta^{Low}$ group. The disease control rate in the IL-6^{High}/IL-$1\beta^{Low}$ group was reduced to half of that in the IL-6^{Low}/IL-$1\beta^{Low}$ group, though GEM-DI in the IL-6^{High}/IL-$1\beta^{Low}$ was higher than in the IL-6^{Low}/IL-$1\beta^{Low}$ group. CRP was able to be synthesised by the effect of IL-6 alone, and the CRP concentration was elevated in the IL-6^{High}/IL-$1\beta^{Low}$ group compared with the IL-6^{Low}/IL-$1\beta^{Low}$ group. These results imply that the PC tumour cells were resistant to GEM via IL-6 only.

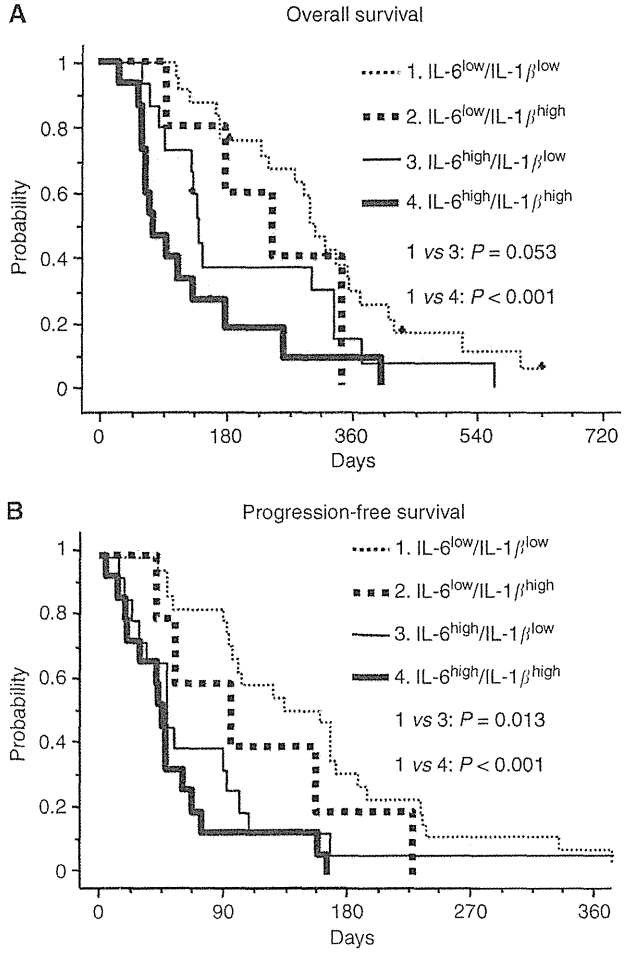

Figure 1. The OS and PFS curves according to the status of IL-6 and IL-1β. (A) OS and (B) PFS curves in the IL-6^{Low}/IL-$1\beta^{Low}$ (dotted line), the IL-6^{Low}/IL-$1\beta^{High}$ (bold dotted line), the IL-6^{High}/IL-$1\beta^{Low}$ (solid line), and the IL-6^{High}/IL-$1\beta^{High}$ groups (bold line).

Table 4. Tumour control rates according to serum levels of IL-6 and IL-1β in patients with gemcitabine monotherapy for advanced pancreatic cancer

IL-6/IL-1β classification	N	Median (95% CI) (%)	*P*-value
IL-6^{Low}/IL-$1\beta^{Low}$	25	76.0 (56.6–88.5)	Ref.
IL-6^{Low}/IL-$1\beta^{High}$	5	60.0 (23.1–88.2)	0.589
IL-6^{High}/IL-$1\beta^{Low}$	15	40.0 (19.8–64.3)	0.042
IL-6^{High}/IL-$1\beta^{High}$	15	20.0 (7.0–45.2)	<0.001

Abbreviations: CI = confidence interval; IL = interleukin.

Table 5. CRP level according to serum levels of IL-6 and IL-1β in patients with gemcitabine monotherapy for advanced pancreatic cancer

IL-6/IL-1β classification	N	Median (95% CI) (mg dl^{-1})	*P*-value
IL-6^{Low}/IL-$1\beta^{Low}$	25	0.13 (0.06–0.25)	Ref.
IL-6^{Low}/IL-$1\beta^{High}$	5	0.08 (NA)	0.140
IL-6^{High}/IL-$1\beta^{Low}$	15	1.19 (0.17–2.79)	0.001
IL-6^{High}/IL-$1\beta^{High}$	15	5.61 (2.83–10.09)	<0.001

Abbreviations: CI = confidence interval; CRP = C-reactive protein; HR = hazard ratio; IL = interleukin; NA = not applicable; OS = overall survival; PFS = progression-free survival.

Table 3. Impacts of the classification using IL-6 and IL-1β levels on overall survival and progression-free survival in patients with gemcitabine monotherapy for advanced pancreatic cancer

Overall survival

IL-6/IL-1β classification	N	Median OS (95%CI) (days)	HR (95% CI)	*P*-value
IL-6^{Low}/IL-$1\beta^{Low}$	25	306 (228–355)	1	Ref.
IL-6^{Low}/IL-$1\beta^{High}$	5	246 (97–346)	1.48 (0.43–3.97)	0.497
IL-6^{High}/IL-$1\beta^{Low}$	15	140 (83–334)	1.90 (0.94–3.72)	0.074
IL-6^{High}/IL-$1\beta^{High}$	15	79 (61–134)	4.06 (1.96–8.18)	<0.001

Progression-free survival

IL-6/IL-1β classification	N	Median PFS (95%CI) (days)	HR (95% CI)	*P*-value
IL-6^{Low}/IL-$1\beta^{Low}$	25	158 (96–187)	1	ref
IL-6^{Low}/IL-$1\beta^{High}$	5	96 (42–229)	1.68 (0.56–4.11)	0.323
IL-6^{High}/IL-$1\beta^{Low}$	15	48 (23–92)	2.24 (1.14–4.29)	0.021
IL-6^{High}/IL-$1\beta^{High}$	15	46 (19–61)	4.26 (2.08–8.55)	<0.001

Abbreviations: CI = confidence interval; HR = hazard ratio; IL = interleukin; OS = overall survival; PFS = progression-free survival.

IL-6 binds a nonsignalling α-receptor (IL-6 receptor), and the dimerisation of gp130 (a signalling β-receptor) and the binding of IL-6 to its receptor lead to the activation of receptor-associated kinases within the cell. These lead to the phosphorylation of proximal tyrosine residues within the intracellular portion of gp130 and the subsequent control of STAT1 and STAT3 activity (Jones *et al*, 2011). Inhibition of the above IL-6 pathway would improve the resistance against GEM in PC tumour cells.

In conclusion, this study demonstrated that the serum levels of IL-6 and IL-1β were predictive of both the efficacy of GEM and the prognosis of patients with advanced PC. Inhibition of the IL-6 and IL-1β pathways may be a candidate target for novel therapies for advanced PC.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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ORIGINAL ARTICLE

Associations of interleukin-6 with vegetative but not affective depressive symptoms in terminally ill cancer patients

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Abstract
Purpose Previous studies have reported associations of depressive symptoms with pro-inflammatory cytokines, especially with interleukin-6 (IL-6) in noncancer subjects and cancer patients. Meanwhile, symptoms such as tiredness and appetite loss may be vegetative symptoms of depression when associated with other diagnostic criteria of depression. Such vegetative-type symptoms worsen during the last 6 months of life in cancer patients and may not be associated with affective depressive symptoms such as sadness and nervousness. This study explored associations between depressive symptoms and plasma IL-6 in terminally ill cancer patients whose survival period was confirmed to be less than 6 months by follow-up, with attention to differences in vegetative and affective depressive symptoms.
Methods Data from 112 consecutively recruited terminally ill cancer patients who registered at a palliative care unit without any active anticancer treatment were used. Plasma IL-6 levels were measured using an electrochemilumi nescence assay. Depressive symptoms included in the DSM-IV and Cavanaugh criteria were assessed by structured interviews and were categorized into affective symptoms and vegetative symptoms. Affective symptoms were also measured with the depression subscale of the Hospital

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Anxiety and Depression Scale, which does not include vegetative symptoms.
Results Vegetative symptoms, such as appetite loss, insomnia, and fatigue, were significantly associated with IL-6 levels. However, neither of the affective symptoms nor their severity was associated with IL-6 levels.
Conclusions IL-6 was associated with vegetative depressive symptoms in terminally ill cancer patients but not with affective depressive symptoms, suggesting possible differences in the pathophysiological mechanisms between these sets of symptoms.

Keywords Depression · Fatigue · Terminal illness · Cytokine · Sickness behavior · Cancer

Introduction

Terminally ill cancer patients frequently suffer from many distressing depressive symptoms [1]. These symptoms are frequently observed and comorbid with each other. These depressive symptoms deteriorate a patient's quality of life [1–6] and sometimes cause suicidal ideation and suicide attempts [7–12]. Thus, the development of new treatments for these distressing depressive symptoms is an urgent issue to be tackled [13].

We will first summarize the findings with another pro-inflammatory cytokine, interferon-α (IFN-α), to provide additional background for the association of this class of compounds with depressive symptoms. IFN-α has been known to induce depressive symptoms [14–16]. It has been known that the manifestation of each symptom has specific characteristics as follows: Severity of feelings of guilt was milder and severity of retardation and loss of weight were more severe in IFN-α-treated depressed patients compared with medically healthy depressed patients [16, 17]. Thus, the influence of IFN-α on depressive symptoms may be different depending on the symptom. Other studies have reported that the order of manifestation of symptoms was different between vegetative symptoms and affective symptoms in nonterminally ill patients with resected malignant melanoma who were treated by IFN-α [14, 18]. Vegetative symptoms occurred earlier than mood disturbance [14, 18]. In addition, in patients with melanoma treated with IFN-α, vegetative symptoms developed nonspecifically in a large proportion of IFN-α-treated patients, whereas mood and cognitive effects were more apparent in specific patients [16], suggesting that vegetative symptoms overlapped with depression (hereinafter referred to as "vegetative depressive symptoms") and affective depressive symptoms are thought to occur via distinct mechanisms [14, 18].

A previous study has observed the trajectory of symptoms during the last 6 months of life for terminally ill cancer patients [19]. In that study, severity of appetite loss and tiredness (lack of energy) measured by the Edmonton Symptom Assessment System (ESAS) increased over time, but depression (feeling sad) and anxiety (feeling nervous) scores of the ESAS remained relatively stable, showing a different pattern of manifestation of symptoms between vegetative depressive symptoms and affective depressive symptoms [19]. Thus, vegetative depressive symptoms (i.e., appetite loss, insomnia, and fatigue) and affective depressive symptoms (i.e., depressed mood, loss of interest, diminished ability to concentrate, psychomotor retardation, worthlessness, and suicidal ideation) may occur via distinct mechanisms in terminally ill cancer patients.

Previous studies have reported associations between depression and interleukin-6 (IL-6) levels, which is one of the important pro-inflammatory cytokines, in noncancer subjects [20, 21] and in cancer patients [22–25]. Proinflammatory cytokines including IL-6 have profound effects on the central nervous system, inducing a syndrome of "sickness behaviors" characterized by anhedonia and vegetative symptoms such as fatigue, appetite loss, and insomnia [14, 16]. A study in patients with ovarian cancer has reported a significant association of IL-6 levels with vegetative depressive symptoms (the items of "bothered," "appetite," "mind," "effort," "sleep," "talk," and "going" in the Center for Epidemiologic Studies Depression Scale (CES-D)) but neither with depressed affect ("blues," "depressed," "failure," "fearful," "lonely," "crying," and "sad"), positive affect ("good," "hopeful," "happy," and "enjoy"), nor interpersonal relations ("unfriendly" and "dislike") measured by the CES-D [26]. Regarding physical, affective, and cognitive dimensions of fatigue, we reported significant associations of IL-6 levels with the physical dimension of fatigue, but not with the affective and cognitive dimensions of fatigue in terminally ill cancer patients [27], although there were inconsistent findings in another study [28]. Based on these findings, we newly hypothesized that IL-6 levels might be associated with vegetative depressive symptoms especially in terminally ill cancer patients, but that the associations with affective depressive symptoms would be weak.

Methods

The data of the present study were obtained from a cohort study to research distress symptoms and psychiatric disorders in terminally ill cancer patients.

Participants

This study was approved by the Ethics Committee of the National Cancer Center (NCC), Japan and was performed after obtaining written informed consent from participants. Cancer patients who registered with the palliative care unit

(PCU) of the NCC Hospital East, Japan, between October 1997 and November 1999 were prospectively and consecutively recruited to participate in the cohort study. Several reports based on information contained in this database have already been published [7, 27, 29–31].

The eligibility criteria for enrollment in the cohort study were (1) age 18 years or older, (2) newly registered with the PCU and having visited our outpatient clinic at least once after registration with the PCU, (3) not receiving or currently undergoing curative anticancer treatment, (4) informed of their cancer diagnosis, (5) not too ill to complete questionnaires and to participate in an interview, (6) not exhibiting cognitive impairment judged by a score of more than 23 on the Mini-Mental State Examination [32, 33], and (7) able to verbally communicate in Japanese.

In addition to the enrollment criteria, we added the following inclusion criterion to the present study: a terminally ill patient was defined as a patient whose survival period was less than 6 months. Thus, the survival status of participants was checked at least 6 months after the assessment, and only data for patients whose deaths were confirmed to have occurred within 6 months of our assessment were analyzed. We excluded patients who were prescribed steroids from the present study because of their potential to influence both IL-6 levels and depressive symptoms.

Procedure

The procedure of the cohort study for enrolling participants was as follows: After obtaining the participants' written informed consent, eligible patients participated in an interview, including the Structured Clinical Interview for DSM-IV Axis I Disorders, Clinician Version (SCID-I) [34], and completed questionnaires, including the Hospital Anxiety and Depression Scale (HADS) [35–37] as described below, while in the outpatient clinic on their next visit after their registration with the PCU. Blood collection was performed by venipuncture into blood collection tubes between 10:30 a.m. and 5:30 p.m. on the same day as the interview was conducted and the questionnaires were administered.

We recruited patients at the time of their registration with the PCU based on the eligibility criteria and they completed the questionnaires and interview described below in the outpatient clinic at their first visit after their registration with the PCU. Blood specimens were immediately centrifuged (4 °C, 300 rpm, 10 min) to separate the plasma from the whole blood. The plasma was stored at −80 °C until the IL-6 assay.

Background and clinical factors

We conducted structured interviews to obtain demographic information. Clinical information was obtained by chart review.

Vegetative and affective depressive symptoms included in DSM-IV and the Cavanaugh criteria

A trained psychiatrist (T.O.) conducted the SCID-I [34] and the Cavanaugh criteria interview [38] to evaluate depressive symptoms. One nonsomatic item in the Cavanaugh criteria (*not participating in medical care despite being able to do so, not progressing despite an improved medical condition, and/or functioning at a lower level than the medical condition warrants*) was added to the nine items of the DSM-IV (Table 2). If any of the symptoms in the Cavanaugh item was present, we coded the symptoms in the item as "presence." The presence, subthreshold level, and absence of each depressive symptom were coded as 2, 1, and 0, respectively. For each symptom, the criterion "The symptoms are not due to the direct physiological effects of a substance (e.g., a drug of abuse, a medication) or a general medical condition (e.g., hypothyroidism)" was strictly adopted. In addition, the interviewer evaluated whether each symptom met the criteria for Mood Disorder Due to a General Medical Condition (criteria B: There is evidence from the history, physical examination, or laboratory findings that the disturbance is the direct physiological consequence of a general medical condition; and criteria C: The disturbance is not better accounted for by another mental disorder [e.g., Adjustment Disorder With Depressed Mood in response to the stress of having a general medical condition]). If a symptom met the criteria of the Mood Disorder Due to a General Medical Condition, the symptom was not judged as a vegetative or affective depressive symptom in the present study.

The reliability (kappa coefficient) of the interview ratings was investigated by having another trained psychiatrist attend the first 29 consecutive interviews as a second rater. The score was 0.87 for major depression diagnosed by the DSM-IV criteria.

We categorized depressed mood, loss of interest or pleasure, diminished ability to concentrate, psychomotor retardation, worthlessness, and suicidal ideation in the DSM-IV criteria and not participating in medical care in the Cavanaugh criteria as affective/cognitive depressive symptoms. Fatigue, appetite loss, and insomnia in the DSM-IV criteria were categorized as vegetative depressive symptoms.

In addition to each depressive symptom, we examined associations of depression diagnosis with IL-6 levels. Major depression was defined as having five or more symptoms including either depressed mood or loss of interest/pleasure. Minor depression was defined as having two or more symptoms including either depressed mood or loss of interest/pleasure. We defined depression as including both major and minor depression.

Perceived distress of mood and fatigue and other symptoms

In addition to these symptoms, perceived distressing mood and subjective distress from fatigue (What level is your distress caused by fatigue?) were assessed by asking the patient to describe the severity of each on a scale of 1, absent, to 5, extreme. We hypothesized that perceived distress from fatigue reflected the severity of a vegetative depressive symptom and that perceived distressing mood reflected severity of affective depressive symptoms. In addition to depressive symptoms, other frequent and distressing physical symptoms, such as pain and dyspnea, were asked about in the same way.

Severity of affective depressive symptoms

To assess severity of nonphysical depressive symptoms, we used the HADS depression subscale which consists of seven self-administered questions [35–37] in which patients rate how they felt during the previous week on a four-point Likert scale. Total scores range from 0 to 21, and a higher score indicates greater depression. The questionnaire does not include physical symptoms that can be caused by physical illness. The Japanese version of HADS was validated as having a good correlation of scores between test and retest (r=0.82) with Japanese cancer patients at the National Cancer Center Hospital East, Japan [36]. The sensitivity and specificity for the major depression were 82.4 and 95.1 %, respectively [36].

IL-6, other cytokines, and laboratory data

Laboratory data, including hematological and biochemical data (Table 1), were also measured as part of the routine clinical examinations conducted. IL-6 levels were measured with a multiplex electrochemiluminescence assay using the Human Pro-Inflammatory 9-plex Ultra-Sensitive Kit (Meso Scale Discovery, MD, USA). Each well of the 96-well plate-based assay contains antibodies to IL-6. We assayed samples in duplicate according to the manufacturer's protocol and obtained results using a SectorTM Imager 6000 (Meso Scale Discovery). Sample cytokine concentrations were determined using standard curves obtained with recombinant IL-6. The mean of two measurements was recorded as the sample concentration. The lower limit of detection (LOD) of IL-6 was 0.23 pg/mL. The lower limit of quantitation (LOQ) of the assay was defined as the value for which the coefficient of variation and relative error were less than 20 % in a preparative examination using triplicate measurements of seven standard concentrations (from 0.61 to 39.1 pg/mL) [39]. The LOQ of IL-6 was 2.44 pg/mL. We confirmed that IL-6 levels were above the LOQ in more than 80 % of participants. The median concentration of plasma IL-6 was 7.5 pg/mL (range, 0.61–99.0 pg/mL), and three had outlier IL-6 levels [1,400, 697, and 656 pg/mL]. All participants had IL-6 levels above the LOD and more than 80 % had levels above the LOQ.

In addition to IL-6, the assay included antibodies to granulocyte macrophage colony-stimulating factor, interferon-γ, IL-1β, IL-2, IL-8, IL-10, IL-12p70, and TNF-α. Among these cytokines, IL-8, Il-10, and TNF-α were above their respective LOQs in more than 80 % of participants (data not shown). Accordingly, associations between symptoms and levels of IL-8, TNF-α, and IL-10 were shown as supplements.

Statistical analysis

The primary outcomes of this explorative examination were associations between IL-6 levels and vegetative depressive symptoms (appetite loss, insomnia, and fatigue) and affective depressive symptoms (depressed mood, loss of interest or pleasure, diminished ability to concentrate, psychomotor retardation, worthlessness, suicidal ideation, and not participating in medical care) evaluated by the SCID using the DSM-IV and Cavanaugh criteria. We examined associations between IL-6 levels and the ten depressive symptoms (presence, subthreshold level, and absence coded as 2, 1, and 0, respectively) using Spearman's rank correlation tests, because the IL-6 levels did not normally distribute.

We supplemented the exploratory correlation analyses with the additional association between IL-6 levels and the HADS depression subscale score and distress of mood, fatigue, and other symptoms using Spearman's rank correlation test. In addition to the Spearman's rank correlation tests, we performed regression analyses with logarithmic-transformed IL-6 as an independent variable and each depressive symptom as a dependent variable with age, sex, height, and body weight as covariates.

As supplemental information, we performed subgroup analyses in a group of participants with lung cancer, which was the largest group of the present sampling set, to confirm whether the findings were reproduced (n=38). As supplemental information, we performed subgroup analyses in a group of participants who had blood drawn in a narrow time window to check that the findings were not biased by the timing of blood sampling. The time frame between 14:00 p.m. and 16:00 p.m. was chosen in order to include as many participants in the subanalysis as possible in a narrow time windows (2 h). All statistical analyses were performed with SPSS 17.0 J for Windows (SPSS, Tokyo, Japan).

Results

The sampling process of the study is shown in Fig. 1. A total of 112 participants were included and their IL-6 levels were

Table 1 Background and clinical factors of participants (n=112)

	Mean ± standard deviation	No.	Percent	Median	Range
Age (years)	60.0±10.0				
Gender: female		36	33.0		
Height (cm)	160.9±7.5				
Weight (kg)	52.9±10.0				
Body mass index	20.4±3.0				
Education year	11.9±2.9				
Cancer site					
Lung		38	33.9		
Colon		17	15.2		
Pancreas		12	10.7		
Gastric		9	8.0		
Head and neck		8	7.1		
Breast		7	6.3		
Other		21	18.8		
Cancer metastasis		102	91.1		
Previous treatment					
Surgery		52	46.4		
Chemotherapy		59	52.7		
Radiation		35	31.3		
Immunological		2	1.8		
Blood laboratory data					
WBC (10^2/dL)				67.5	3.9–239.0
RBC (10^4/μL)				378	104–596
Hemoglobin (%)				11.2	5.7–16.5
Cholesterol (mg/dL)				183	11–523
Albumin (g/dL)				3.6	2.0–4.5
ECOG PS				2	0–4
Survival time (days)				71	2–176
Depression		23	20.5		
Major depressive disorder		11	9.8		
Minor depressive disorder		12	10.7		
Drug					
Opiate		43	38.4		
NSAID		61	54.5		
Hormone		6	5.4		
Hypnotic		19	17.0		
Anxiolytic		15	13.4		
Antidepressant		3	2.7		
Antibiotics		5	4.5		

Other cancer sites included the liver, prostate, uterus, kidney, esophagus, bladder, ovary, and unknown. Sites of cancer metastasis included the brain, bone, liver, lung, peritoneum, and lymph node

PS performance status, *ECOG* the Eastern Cooperative Oncology Group, *WBC* white blood cell, *RBC* red blood cell

measured in the present study. Backgrounds and clinical factors for the 112 participants are shown in Table 1.

IL-6 levels and depressive symptoms

Among depressive symptoms, appetite loss, insomnia, and fatigue, which were physical symptoms, were significantly associated with IL-6 levels (Table 2). None of the affective symptoms were associated with IL-6 levels (Tables 2 and 3).

Regression analyses with logarithmic-transformed IL-6 as an independent variable and each depressive symptom as a dependent variable with age, sex, height, and body weight as covariates showed significant associations between IL-6 levels and appetite loss (standardized beta=0.17, p=0.08), insomnia (standardized beta=0.30, p<0.01), and fatigue (standardized beta=0.20, p=0.04). However, IL-6 levels were not associated with affective depressive symptoms (data not shown).

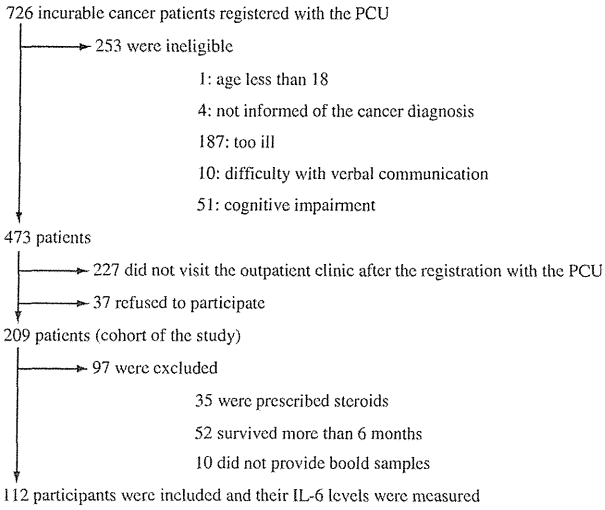

Fig. 1 Sampling process

IL-6 levels and severity of affective depressive symptoms

The median (range) score of the HADS was 6.5 (0–18). The HADS depression subscale score which did not contain physical symptoms was not significantly correlated with IL-6 levels (Spearman's r=0.16, p=0.09).

Subgroup analysis using participants with lung cancer

In subanalyses using participants with lung cancer (n=38) to reproduce the results, insomnia (r=0.51, p<0.01) and perceived fatigue (r=0.33, p=0.04) were associated with IL-6 levels. Also, dyspnea (r=0.36, p=0.03) was associated with IL-6 levels.

Subgroup analysis using participants who had blood drawn between 14:00 and 16:00

In another subanalyses using participants who had blood drawn between 14:00 p.m. and 16:00 p.m. (n=22), physical symptoms, such as insomnia (r=0.53, p=0.01) and fatigue (r=0.43, p<0.05), pain (r=0.45, p=0.04), but not affective depressive symptoms (data not shown), were associated with IL-6 levels.

IL-6 levels and depression diagnosis

Twenty-three participants (21.1 %) were diagnosed as having depression (major depression, 11 [10.1 %]; minor depression, 12 [11.0 %]) using DSM-IV criteria and 86 were not. There was no significant difference in plasma IL-6 levels between participants with and without a depression diagnosis (Mann–Whitney U=859.5, p=0.34). Using Cavanaugh criteria (seven items: excluding vegetative symptoms which are appetite loss, insomnia, and fatigue from the DSM-IV criteria, and adding one nonphysical symptom, not participating in medical care), 19 participants (17.4 %) were depressed (major depression, 3 [2.8 %]; minor depression, 16 [14.7 %]) and 90 were not. There was no significant difference in plasma IL-6 levels between participants with and without a depression diagnosis (Mann–Whitney U=801.0, p=0.67).

Table 2 Associations between IL-6 levels and depressive symptoms, fatigue, and other symptoms (n=112)

	% of participants			Correlation coefficient		
	0	1	2	r	95 % CI	p
Affective depressive symptoms						
Depressed mood	40.2	46.4	13.4	0.01	−0.18, 0.20	0.91
Loss of interest or pleasure	45.5	37.5	17.0	0.09	−0.10, 0.27	0.34
Diminished ability to concentrate	44.6	27.7	27.7	0.14	−0.04, 0.32	0.14
Psychomotor retardation	71.4	17.9	10.7	−0.08	−0.26, 0.11	0.41
Worthlessness	78.6	14.3	7.1	−0.00	−0.19, 0.18	0.97
Suicidal ideation	63.4	26.8	9.8	0.07	−0.12, 0.25	0.48
Not participating in medical care despite being able to do so	92.0	0	9	0.03	−0.16, 0.22	0.75
Vegetative depressive symptoms						
Appetite loss	36.7	26.8	36.7	0.19	0.01, 0.37	0.04
Insomnia	55.4	27.7	17.0	0.37	0.20, 0.52	<0.001
Fatigue	29.5	38.4	32.1	0.19	0.01, 0.37	0.04

Depressed symptoms were assessed as present, subthreshold, or absent (coded as 2, 1, and 0, respectively) using items in DSM-IV and the Cavanaugh criteria. r (correlation coefficient) and p values were calculated using Spearman's rank correlation test. 95 % CI indicates 95 % confidential interval of r (correlation coefficient)

Among the 23 participants who were diagnosed as having depression, all patients had loss of interest or pleasure (n =23, 100 %), and most patients had fatigue (n=22, 95.7 %) and diminished ability to concentrate (n=21, 91.3 %). Thus, associations between these three symptoms and IL-6 levels were not analyzed. Among other symptoms, appetite loss had a trend toward association with IL-6 levels (r=0.38, p=0.07), but no symptoms were significantly associated with IL-6 levels (data not shown). Among the remaining 89 participants without depression, IL-6 levels were significantly associated with insomnia (r=0.40, p<0.001), perceived distress from fatigue (r=0.36, p<0.001), and dyspnea (r=0.27, p=0.01), but not with other symptoms.

Other cytokines

Other cytokines, such as IL-8, IL-10, and TNF-α, were not associated with any of the depressive symptoms among the DMS-IV or Cavanaugh criteria (data not shown) or HADS depression scores (data not shown).

Discussion

In this study, we examined associations between IL-6 levels and frequently observed depressive symptoms in terminally ill cancer patients. Affective depressive symptoms, such as depressed mood, loss of interest or pleasure, and suicidal ideation, were not associated with IL-6 levels. Only vegetative depressive symptoms, such as fatigue, appetite loss, and insomnia, were associated with IL-6 levels. The severity of affective depressive symptoms measured by HADS, which does not include vegetative depressive symptoms, was not associated with IL-6 levels. Furthermore, perceived distress from fatigue, but not distress from mood, was associated with IL-6 levels. These results suggest that, at least in terminally ill cancer patients, the manifestation of affective depressive symptoms might not be attributed to IL-6.

This result confirmed the notion that IL-6 levels are associated only with vegetative symptoms of depression in terminally ill cancer patients. Our results are consistent with those of a previous study [26] in ovarian cancer patients, which showed that IL-6 levels were associated only with the vegetative depression subscale score of CES-D, but not with subscale scores of depressed affect, positive affect, and interpersonal relations. The present study extends these findings to terminally ill cancer patients.

A previous study reported a significant correlation between IL-6 levels and depression severity as measured by the Hamilton Rating Scale for Depression (HRSD) [40]. While the HADS was constructed to exclude physical symptoms, the HRSD includes several vegetative depressive symptoms [37]. This implies that previous studies showing an association between IL-6 levels and severity of depressive symptoms may reflect, in part, the contribution of the associations between IL-6 levels and vegetative depressive symptoms.

The results of the present study showed associations between IL-6 levels and fatigue and insomnia, although the causality of these associations is unknown. Several recent studies reported on the effects and safety of an anti-IL-6 receptor antibody on fatigue and insomnia in patients with rheumatoid arthritis and Castleman's disease [41–43]. We hypothesized that a drug to block the IL-6 signal, like this antibody, would be useful not only to investigate causality but also to palliate vegetative depressive symptoms. In addition, control of systemic inflammation would be useful in terminally cancer patients who were distressed by vegetative depressive symptoms. Further studies are needed to develop a new intervention for vegetative depressive symptoms in terminally ill cancer patients.

A recent study reported no significant associations between IL-6 level and fatigue as measured by the Brief Fatigue Inventory (BFI) in terminally ill cancer patients [28], which is inconsistent with the results of the present study. The inconsistency may be caused by differences in measurements used, demographic characteristics of subjects

Table 3 Associations between IL-6 levels and perceived distress from mood, perceived distress from fatigue, and other symptoms (n=112)

	% of participants					Correlation coefficient		
	1	2	3	4	5	r	95 % CI	p
Perceived distressing mood	30.4	35.7	14.3	15.2	4.5	−0.01	−0.19, 0.18	0.95
Perceived distress from fatigue	33.9	25.9	24.1	16.1	0	0.33	0.15, 0.48	<0.001
Dyspnea	40.2	38.4	12.5	8.9	0	0.27	0.09, 0.43	0.004
Pain	36.6	33.0	18.8	11.6	0	0.08	−0.11, 0.26	0.44

Severity of perceived distressing mood and other distressing symptoms were assessed as 1, absent, to 5, extreme. r (correlation coefficient) and p values were calculated using Spearman's rank correlation test. 95 % CI indicates 95 % confidential interval of r (correlation coefficient)

(cancer site, survival time), and current medication used (steroid treatment). The BFI contained some questions regarding interference with mood and enjoyment of life, which may be associated with the affective dimension of fatigue. We previously reported no significant associations of IL-6 with affective and cognitive dimensions of fatigue in terminally ill cancer patients [27]. In the present study, survival time (median 72 days) was longer than that of the previous study (27 days). The ratio of patients with lung cancer (34.9 %) was larger than that of the previous study (19 %).

The pathway by which IL-6 has influence on vegetative depressive symptoms is yet unclear. On the other hand, previous studies showed that vegetative depressive symptoms induced by another pro-inflammatory cytokine IFN-α were nonresponsive to antidepressant treatment, whereas affective depressive symptoms were responsive to the treatment [18]. In addition, impaired serotonin metabolism was found to be involved in the development of IFN-α-induced affective depressive symptoms. Also, vegetative depressive symptoms were found to correlate with changes in basal ganglia activity, possible related to alteration in dopamine metabolism [44]. These previous findings suggest a hypothesis that vegetative depressive symptoms related to a pro-inflammatory cytokine IL-6 in terminally ill cancer patients may be related to dopaminergic dysregulation.

The present study has some strengths. First, we analyzed data from patients whose survival period was confirmed to be less than 6 months. This was based on our intention to selectively isolate depressive symptoms specific to patients with terminal cancer. Second, because we included only patients who were not receiving any active anticancer treatment, we excluded any influences of ongoing anticancer treatment on the associations between IL-6 and observed symptoms. Also, we analyzed data only from patients who did not receive any steroids.

There are some limitations of the study. First, the participation rate was small to avoid potential bias (among 726 incurable cancer patients registered with the PCU, 187 were excluded because they were too ill, 227 did not visit the clinic after the registration, and 47 refused to participate). Severely ill patients, such as those whom we excluded, might demonstrate more severe depressive symptoms. Second, we performed multiple comparisons without any corrections because the present study was explorative. In addition, we did not have corrections because each symptom may not be independent from other symptoms [13, 45] and depressive symptoms may have common pathophysiology [46]. Third, although a subanalysis focused solely on participants with lung cancer partially reproduced the results, the present study included participants with various types of tumors and did not control for disease extent or stage. This would complicate any inferences from the results to identify the involvement of cytokines in cancer site-specific symptoms. Fourth, the time of the blood sampling and the time of the interview were not controlled. Controlling the timing of the blood sampling may produce more conclusive findings. The results of the present study should be confirmed in further studies. Fifth, we did not control some factors potentially influencing IL-6 and distressing symptoms, such as the body mass index, diet, and physical activity [47–49]. Further studies are needed to elucidate the complex associations.

In conclusion, loss of appetite, insomnia, and fatigue (often seen as well in major depression) in the setting of terminal cancer are associated with elevated IL-6 levels, but other typical depressive symptoms, affective and/or cognitive, were not associated with increased IL-6 levels. Since elevated IL-6 levels may also be associated with depression without other medical conditions, some exploration of the possible causal relationships and pathways would be of interest. The findings of the present study may help to introduce specific treatment, such as IL-6 antibodies, while the effect of such interventions may be limited to vegetative depressive symptoms in terminally ill cancer patients.

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Conflict of interest Kimio Terao is an employee of Chugai Pharmaceutical Co., Ltd., Tokyo, Japan. Other authors have no conflict of interest.

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COPD

Clinical Usefulness of the Two-Question Assessment Tool for Depressive Symptoms in Japanese Patients with Chronic Obstructive Pulmonary Disease

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Abstract
Purpose Depressive symptoms are highly prevalent in patients with chronic obstructive pulmonary disease (COPD) and have been associated with poor outcomes. Developing a concise questionnaire to measure depressive symptoms in COPD patients is needed in outpatient settings. We evaluated the clinical usefulness of a concise two-question instrument to assess depressive symptoms in patients with COPD.
Methods The study was conducted as a cross-sectional analysis in patients with COPD. All patients completed a self-reported questionnaire consisting of the two-question instrument, as well as a shortened version of the Center for Epidemiologic Studies Depression Scale (CESD-10) to measure depressive symptoms. Performance of the two-question instrument was evaluated using the results for CESD-10 as standard. We also measured patients' health-related quality of life using the Medical Outcomes Study 8-Item Short-Form Health Survey (SF-8) to determine whether the instrument was related to SF-8.
Results Sensitivity of the two-question instrument in the detection of depressive symptoms was 73.3 % (95 % confidence interval [CI] 51–95.7), specificity was 73 % (95 % CI 58.7–87.3), and area under the receiver operating characteristics curve was 0.73 (95 % CI 0.59–0.87). When study patients were divided into two groups with a cutoff of 1 point on the two-question instrument, scores for all subscales of the SF-8 except "bodily pain" were significantly lower in patients with than without depressive symptoms.
Conclusions This concise two-question instrument is useful as assessment of depressive symptom in patients with COPD in busy outpatient settings.

Keywords Chronic obstructive pulmonary disease · Cross-sectional study · Depression · Quality of life · Two-question instrument

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Introduction

Chronic obstructive pulmonary disease (COPD) is a progressive disease characterized by exacerbations of symptoms that impair quality of life (QOL) [1]. Depressive symptoms are a highly prevalent yet underdiagnosed comorbidity in these patients [2], significantly more prevalent than in healthy controls and even in patients with other chronic illnesses [3–6]. A recent, large, observational study reported further evidence for the increased risk of depression in patients with COPD [7]. Several studies reported a prevalence of depressive symptoms in COPD patients of between 6 and 56 % [2, 8–11]. Depressive symptoms in these patients were associated with impaired QOL, longer hospitalization,

increased symptom burden, higher readmission rates, and even mortality [3, 7–9, 12–14]. Given the clinical importance of improving health-related quality of life (HRQOL) in these patients, physicians treating them should ensure that this variable is evaluated. Depression is frequently evaluated using questionnaires, such as the Center for Epidemiologic Studies Depression Scale (CESD), Hospital Anxiety and Depression Scale, and Beck Depression Inventory [2, 10, 11], whereas the Medical Outcomes Study 36-Item Short-Form Health Survey (SF-36) is used as a measure of general HRQOL [15]. Ensuring complete compliance with these questionnaires in busy outpatient settings is difficult, however, suggesting the need for a simple instrument able to assess depressive symptoms and easily evaluate HRQOL.

One candidate is the two-question screening method (two-question instrument), a brief questionnaire that can be used to screen for depressive symptoms in primary care [16] and work place settings [17]. To our knowledge, however, the clinical usefulness of this tool for COPD patients has not been investigated.

We evaluated the clinical usefulness of the two-question instrument in Japanese patients with COPD by assessing its performance in detecting depressive symptoms using the shortened version of the CESD (CESD-10) as reference standard. We also examined whether the two-question instrument score was related with HRQOL as scored by the Medical Outcomes Study 8-Item Short-Form Health Survey (SF-8).

Methods

Study Population

The study was conducted at the pulmonary departments of five hospitals in Miyazaki Prefecture, Japan. A consecutive sample of clinically stable COPD patients visiting the department between February and April 2008 was invited to participate. The diagnosis of COPD was made or confirmed by a pulmonologist based on the guidelines of the Global Initiative for Chronic Obstructive Lung Disease (GOLD) [1]. All patients had to be clinically stable for at least 4 weeks before the time of visit. Subjects were excluded if they had concurrent diagnosis of asthma, active mycobacterium infection, interstitial pneumonia, cardiovascular disease, renal failure, malignant tumor, dementia, neurological conditions, or age younger than 40 years.

Study Design

The study was conducted under a cross-sectional design. All patients who visited during the study period were given a verbal and written explanation of the study by the attending physician, and those who provided consent to participate were enrolled. Eligible subjects completed a self-reported questionnaire consisting of the two-question instrument, CESD-10, SF-8, and questions concerning characteristics, such as sex, smoking status, body mass index (BMI), and assessments of the Medical Research Council (MRC) dyspnea scale. This survey form was collected by a clinical research coordinator, who asked the patients to complete any omissions. Patient characteristics, such as complications, assessments of pulmonary function tests, and treatment, were obtained from the medical record by the attending physician. The study protocol was approved by the Ethics Committees of Faculty of Medicine, University of Miyazaki.

Measurements

Two-Question Instrument

The two-question instrument, developed as a screening tool for the detection of depression in primary care settings, consists of a brief questionnaire that can be used to screen for depressive symptoms. The questions are related to depressed mood and anhedonia [16]. One study of this instrument reported a sensitivity of 96 % and specificity of 57 % [16]. We used validated Japanese translations of the following two-question instrument: (i) "During the past month, have you often been bothered by feeling down, depressed, or hopeless?"; and (ii) "During the past month, have you often been bothered by little interest or pleasure in doing things?" [17]. The yes/no responses to the two questions were added to give possible scores of 0, 1, or 2. A score $\geq$ 1 was counted as a positive response.

Short Form of the Center for Epidemiologic Studies Depression Scale (CESD-10)

The CESD consists of 20 items that ask about the symptoms of depression in older adults and has been widely used in community- and population-based studies [18]. The reliability and validity of the CESD have been verified, including the Japanese version [19]. The reliability of a shortened, more convenient 10-item version (CESD-10) also has been verified [20]. We used the Japanese version of the CESD-10 to define the depression symptoms. Scores for this scale range from 0 to 30; a higher score represents a greater degree of depressed mood, and a score $\geq$ 10 is considered to indicate depressive symptoms [20].

Medical Outcomes Study 8-Item Short-Form Health Survey (SF-8)

We used a Japanese version of the SF-8, a shortened and more convenient 8-item version of the SF-36, which

consists of eight subscales: General health, Physical functioning, Role limitation due to physical problems, Bodily pain, Vitality, Social functioning, Mental health, and Role limitation due to emotional problems. The Japanese versions of both instruments have been validated [21, 22]. In the present study, the score of each of the eight subscales, as well as the physical health component summary score and mental health component summary score, were measured using the Norm-based scoring method, based on a large-scale population study conducted in Japan [22]. HRQOL score is shown as the mean score with standard deviation, with a higher score representing a better QOL.

Statistical Analysis

First, the prevalence of depressive symptom was determined by calculating the percentage of patients with a score of 10 or higher on the CESD-10. Next, we computed the sensitivity, specificity, positive predictive values, negative predictive values, positive likelihood ratio, negative likelihood ratio, and area under the receiver operating characteristics curve (ROC) for the two-question instrument scores of ≥ 1 and 2 using the CESD-10 as standard, and the stratified likelihood ratios of each of the two-question instrument scores. The stratified likelihood ratios were then used to calculate the positive likelihood ratios for each of the two-question instrument scores. This was done by taking the proportion of positive two-question instrument results among COPD patients with a CESD-10 score $\geq$ 10 as the numerator, and the proportion of positive two-question instrument results among COPD patients with a CESD-10 score $<$ 10 as the denominator.

To determine whether the instrument was related with QOL, we also compared SF-8 scores on the categorization of patients into two groups by the cutoff value of 1 point using the *t* test. Differences in scores of each of the eight subscales, and of the physical and mental health component summary score by the two-question instrument score were analyzed by using analysis of variance, and if positive, post hoc comparisons were carried out by Scheffe's procedure.

All analyses were performed by using a commercially available statistical software package (STATA SE 11.0, Stata Corp., College Station, TX). *P* values < 0.05 were considered to be statistically significant.

Results

Fifty-two consecutive COPD patients were studied. All 52 patients completed the self-reported questionnaire. General characteristics and CESD-10 scores of these patients are shown in Table 1. Mean age was 72.7 ± 7.5 years, and most were men ($n = 49$). Mean BMI was 21.0 ± 3.4 kg/m^2. All patients were current or former smokers with an average of 60.8 ± 38.2 pack years. The majority of patients had mild to moderate COPD, but 56 % of cases were grade 3 or higher by the MRC dyspnea scale. Forty-five patients (87 %) were treated with inhaled agents. Of these patients, 34 patients (65 %) inhaled long-acting antimuscarinic agent (LAMA). Among reminder patients, six patients (12 %) were treated with inhaled corticosteroid plus long-acting β-agonist (ICS/LABA) only, three patients (6 %) were treated with LABA only, and two patients

Table 1 Subject characteristics

Characteristic	*n* (%), 52 (100 %)
Age (years)	
40–60	3 (6 %)
60–69	15 (29 %)
70–79	25 (48 %)
≥80	9 (17 %)
Sex	
Male	49 (94 %)
BMI (kg/m^2)	
>25.4	5 (10 %)
22.0–25.4	17 (33 %)
18.0–22.0	17 (33 %)
<18.0	13 (25 %)
Smoking status	
Current	6 (12 %)
Former	46 (88 %)
Pack year	
0–14	2 (4 %)
15–24	4 (8 %)
25–49	16 (30 %)
≥50	30 (58 %)
COPD stage defined by GOLD	
I	12 (23 %)
II	21 (40 %)
III	13 (25 %)
IV	6 (12 %)
MRC scale	
Grade 3 or over	29 (56 %)
Pulmonary function	
FEV_1, L	1.3 ± 0.6
FEV_1/FVC, %	50.2 ± 14.6
FEV_1, % predicted	62.5 ± 25.7
Long-term oxygen therapy	11 (21 %)
Use of antianxiety drugs or sleep drugs	9 (17 %)
CESD-10 score (points)	8.3 ± 5.7
Depressive symptoms (CESD-10 score ≥ 10)	15 (29 %)

Data are presented as number (%) or mean ± standard deviation

FEV_1 forced expiratory volume in one second, *FVC* forced vital capacity

Table 2 Association of subject characteristics with CESD-10 or the two-question instrument

Characteristic	CESD-10		Two-question instrument score 1 or 2
	Score (points)	score ≥ 10	
Age (years)			
40–69	8.4 ± 4.0	4 (22 %)	7 (39 %)
≥70	8.3 ± 6.4	11 (32 %)	14 (41 %)
BMI (kg/m^2)			
≥18.0	8.1 ± 5.7	10 (26 %)	13 (33 %)
<18.0	9.2 ± 5.8	5 (38 %)	8 (62 %)
Smoking status			
Current	7.3 ± 5.0	1 (17 %)	2 (33 %)
Former	8.5 ± 5.8	14 (30 %)	19 (41 %)
COPD stage defined by GOLD			
I	7.2 ± 6.3	3 (25 %)	5 (42 %)
II	8.1 ± 6.6	6 (29 %)	7 (33 %)
III	8.2 ± 3.4	2 (15 %)	6 (46 %)
IV	11.8 ± 4.4	4 (67 %)	3 (50 %)
MRC scale			
Grade 3 or over	9.1 ± 5.7	11 (38 %)	15 (52 %)
Grade 2 or less	7.4 ± 5.6	4 (17 %)	6 (26 %)
Long-term oxygen therapy			
Use	10.0 ± 6.6	5 (45 %)	6 (55 %)
None	7.9 ± 5.4	10 (24 %)	15 (37 %)

Data are presented as mean ± standard deviation or number (%)

(4 %) were treated with ICS only. Twenty patients (38 %) who were treated with LAMA were combined with the other agent (eight patients with LABA, eight patients with ICS/LABA, and four patients with ICS). In addition to inhaled agents, 21 patients (40 %) received oral theophylline and 2 (4 %) received oral prednisolone. The reasons of the use of oral prednisolone were unknown. Eleven patients (21 %) were managed with long-term oxygen therapy (LTOT), and four received no medication. The prevalence of depressive symptoms as judged by the CESD-10 among patients was 28.9 %. None had been diagnosed with depression, whereas four patients had been diagnosed with anxiety or insomnia. Five patients with depressive symptoms as judged by the CESD-10 were subsequently prescribed antianxiety drugs or sleeping drugs.

Table 2 shows the association of several characteristics of subjects with CESD-10 or the two-question instrument. It has reported that these characteristics were associated with depression in patients with COPD [2, 7]. The prevalence of depressive symptom using CESD-10 or the two-question instrument tended to be higher as elderly, low BMI, severe stage defined by GOLD, severe dyspnea, and use of LTOT.

A 2 × 2 table of the two-question instrument using cutoffs of 1 or 2 points is shown in Table 3. Twenty-one patients with COPD (40 %) scored at least 1 on the two-question instrument and 13 (25 %) scored 2. Table 4 shows test performance of the 1- or 2-point cutoffs of the two-question instrument using the CESD-10 as standard. Stratified likelihood ratio of the two-question instrument was 0.37 (95 % confidence interval [CI] 0.25–0.54) for 0-point responses, 0.82 (95 % CI 0.27–2.52) for 1-point responses, and 5.55 (95 % CI 3.29–9.37) for 2-points responses.

Table 5 shows the SF-8 score. Except for bodily pain, mental health, and mental health component summary score, scores on the eight subscales of the SF-8 in patients were <50 of the national reference values. When patients were divided into two groups using a cutoff score of 1 point by the two-question instrument, scores for all subscales except bodily pain were significantly lower in patients with than without depressive symptoms. Scores of each of the eight subscales, and physical and mental health component summary score by scores of the two-question instrument are shown in Fig. 1. Except for Bodily pain, HRQOL scores tended to decline as the score of the two-question instrument rose. In particular, HRQOL scores in patients with a score of 2 points were significantly lower than those in patients with 0 points, again except for bodily pain.

Discussion

In this study, we investigated the test performance of the two-question instrument in assessment of depressive symptoms