Serum level of CCL2 predicts outcome of patients with pancreatic cancer

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Abstract

Background **: Pancreatic cancer is one of the most deadly cancers worldwide with a five-year survival rate of less than 5%. Chronic pancreatitis showed increased risk to develop pancreatic cancer, in which chronic inflammation of the pancreas may play a critical role. Cytokines play an indispensable role in inflammatory reaction and tumorigenesis. The purpose of this study was to determine whether cytokines were associated with survival and poor prognosis of pancreatic cancer.**

Methods **: In this study, we examined levels of some important cytokines in the serum of 68 patients with pancreatic cancer, including CCL2, CCL17, CXCL-1, CXCL-5, G-CSF, GM-CSF, TGF-β and IFN-γ.**

Results **: We found that high level of serum CCL2 was strongly associated with poor survival and prognosis, but no significant association with other clinicopathological features, including gender, age, location and TNM staging. For other cytokines, no significant correlation with poor survival and prognosis was found.**

Conclusion **: Our results suggest that serum level of CCL2 may serve as a potential marker for predicting the outcome of patients with pancreatic cancer.** (Acta gastroenterol. belg., **2020**, 83, **295-299**).

Keywords : Pancreatic cancer, cytokines, CCL2, prediction marker

Introduction

Pancreatic cancer is one of the most deadly cancers worldwide with a five-year survival rate of less than 5%. So far, surgical resection remains the treatment of choice for pancreatic cancer. However, less than 20% of patients with pancreatic cancer were deemed suitable for surgical treatment (1,2). In addition, chronic pancreatitis show increased risk to develop pancreatic cancer, in which chronic inflammation of the pancreas may play a critical role (3, 4).

Previous studies revealed that a high level of inflammatory cytokines in pancreatic tumor microenvironment facilitates the proliferation of malignant cells (5,6). Chemokines are a family of cytokines that attracts macrophages or lymphocytes into the infected sites and plays an essential role in inflammatory response. There is increasing evidence that many chemokines, such as CCL2, CCL17, CXCL-1 and CXCL-5, are involved in tumor development and metastasis. Furthermore, the level of these chemokines in plasma correlates with poor prognosis (7-13). Besides, G-CSF (Granulocyte-colony stimulating factor) and GM-CSF (Granulocyte-macrophage colony-stimulating factor) are two kinds of cytokines necessary for tumor cell proliferation, invasion, and transendothelial migration (14, 15).

To further understand the functional roles of cytokines in the development and metastasis of pancreatic cancer and explore whether cytokines can serve as a potential marker to predict the prognosis of pancreatic cancer, we determined serum levels of some important cytokines, including CCL2, CCL17, CXCL-1, CXCL-5, G-CSF and GM-CSF, from 68 patients with pancreatic cancer. Furthermore, we also measured the serum levels of another two of the cytokines that play a dual role in cancer progression, TGF-β (transforming growth factor) and IFN-γ (Interferon-γ) (16-19). Finally, we found only CCL2 was associated with poor prognosis of pancreatic cancer.

Materials and methods

Patients

Sixty-eight patients with pancreatic adenocarcinoma admitted to the Shanghai Cancer Center (FUSCC) of Fudan University were selected. Most of the patients were diagnosed by histology, while for the patients with locally advanced pancreatic cancer, cytological diagnosis was used. Tumor staging is determined according to the seventh criteria of the Union for International Cancer Control (UICC). All patients provided written informed consent before enrollment in the study. This study was approved by the ethics committee of FUSCC.

Cytokine assays

The serum levels of various cytokines were measured with Q-Plex multiplex array (Cat. No. 107749GR ; Quansys Biosciences, Logan, UT, USA) on a Q-View Imager (Quansys Biosciences) according to the manufacturer's protocol.

Statistical analyses

Statistical analyses were performed using SPSS 22 software (SPSS, Chicago, IL). Spearman's X^2 test was

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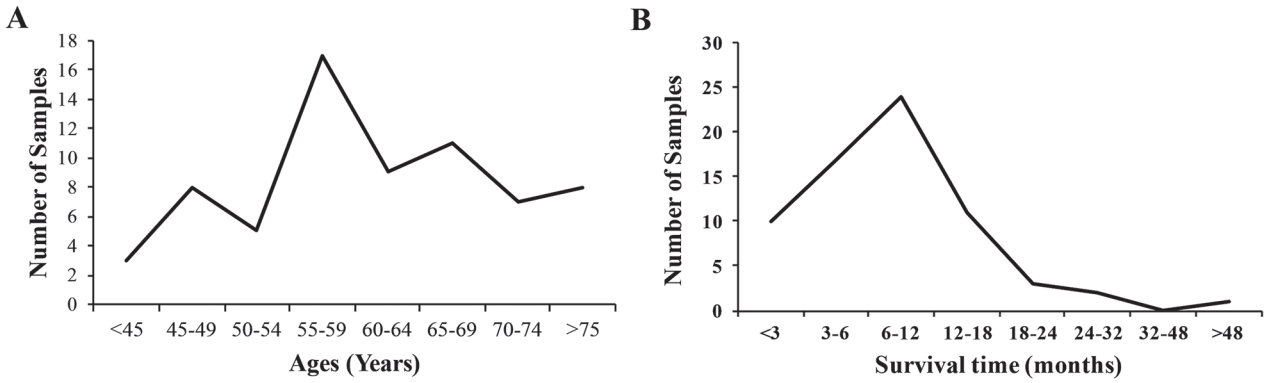

Figure 1. — The age and survival distributions of 68 patients with pancreatic cancer

applied to analyze the association of clinipathological parameters with expression of cytokines. The Kaplan–Meier method was employed to draw survival curves and the differences in survival curves was assessed by log-rank tests. Cox regression analysis was used to assess the factors that were strongly associated with survival.

Results

Patient characteristics

A total of 68 patients with pancreatic cancer were involved in this study. The age of patients were between 28 to 82 years old (Figure 1A). The survival time ranged from 38 days to 2427 days, and most patients survived less than one year (Figure 1B). The longest surviving patient was a male, who was diagnosed with TNM stage IV pancreatic cancer at the age of 67. The baseline clinical characteristics of all patients included in the study are shown in Table 1.

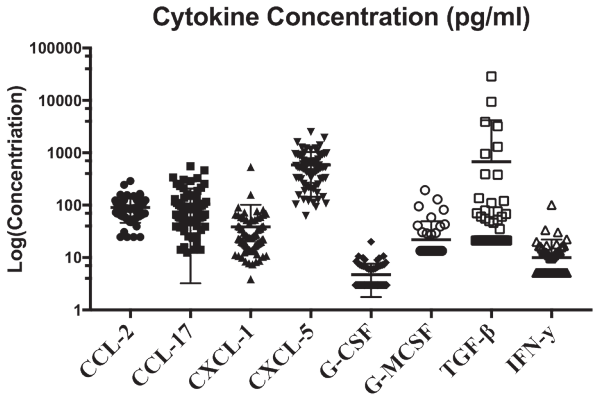

Figure 2. — Serum cytokine concentrations in patients with pancreatic cancer. The standard deviation (SD) is shown as error bars.

Table 1. — **Baseline clinical characteristics of the patients recruited (n=68)**

Characteristics	Mean ± SD or number (%)
Age (years)	60.9 ± 11.2
Sex	
Male	45 (63.2%)
Female	23 (36.8%)
TNM stage	
III	23 (36.8%)
IV	45 (63.2%)
Location	
Head & neck	29 (42.6%)
Body & tail	39 (57.4%)
CCL2 (pg/ml)	94.5 ± 56.9
CCL17 (pg/ml)	104.2 ± 106.2
CXCL-1 (pg/ml)	38.9 ± 66.2
CXCL-5 (pg/ml)	589.8 ± 445.8
G-CSF (pg/ml)	4.6 ± 2.4
GM-CSF (pg/ml)	22.3 ± 28.9
TGF-β (pg/ml)	819.3 ± 4296.7
IFN-γ (pg/ml)	9.0 ± 6.2

Serum levels of cytokines

Firstly, we determined the serum levels of of CCL2, CCL17, CXCL-1, CXCL-5, G-CSF, GM-CSF, TGF-β and IFN-γ in all 68 patients with pancreatic cancer (Table 1, Figure 2). Then, we explored the relationship between the serum levels of these cytokines and the survival time. Correlation analysis showed that only CCL2 was correlated significantly with survival time (Table 2). Furthermore, we also examined the relationship between serum level of CCL2 and clinicopathological features, including gender, age, location and TNM staging, but no significant association found (Table 3).

Table 2. — **The relationship between the expression of cytokines and survival time**

		CXCL-5	GCSF	GM-CSF	CXCL-1	IFNγ	CCL2	CCL17	TGF-β
Survival time	Correlation Coefficient	-0.147	-0.001	-0.226	-0.112	-0.026	-0.334**	-0.127	0.092
	Sig.	0.244	0.993	0.064	0.364	0.835	0.005	0.301	0.456

Table 3. — **Relationship between the expression of CCL2 and clinicopathological features**

		Survival time	TNM stage	Gender	Age	Location
CCL2	Correlation Coefficient	-0.334**	0.101	0.012	-0.147	0.08
	Sig.	0.005	0.411	0.925	0.231	0.519

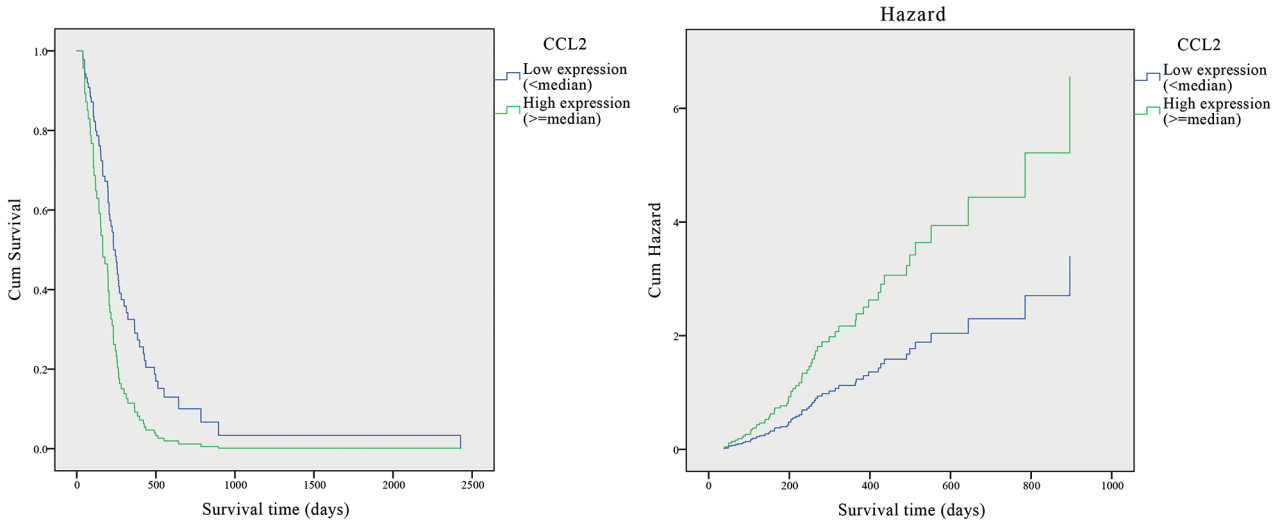

Figure 3. — The association between CCL2 and overall survival (OS) in patients with pancreatic cancer. A : Kaplan-Meier curves for OS for all of the cases. B : Hazard ratios (HRs) of CCL2 for OS. The median CCL2 level was selected as the cut-off between low and high CCL2 levels.

Relationship between CCL2 and patient's outcomes

To further investigate the correlation between CCL2 level and pancreatic cancer patient survival, Univariate Cox analysis and Kaplan-Meier curves with log-rank analysis of overall survival were performed. Patients with high CCL2 expression (greater than the median expression) tended to have a significantly shorter survival time than that with low concentration (less than the median expression) ($p<0.05$) (Figure 3). Univariate Cox analysis revealed that a high serum level of CCL2 was positively correlated with poor survival (HR = 0.518, 95% CI : 0.307-0.874, $p < 0.05$) (Table 3, Figure 3).

Discussion

Chemokines are small proteins with crucial roles in immune and inflammatory responses (20). It is well known that differential expression of chemokine system components is involved in microbial pathogens, tumor development, immune regulation, tissue repair and remodeling (21). The chemokines, CCL2, CCL17, CXCL-1, CXCL-5, also known as MCP-1, TARC, GROα and ENA-78 respectively, have been reported to play an important role in tumor development and metastasis. The increased secretion of CCL2 can recruit prostate cancer epithelial cells to the bone microenvironment and regulate their proliferation (7). Consistent with previous studies (22), we found that high level of CCL2 was correlated with shorter survival time and poor prognosis of pancreatic cancer. Furthermore, CCL17 plays important roles in Th2-type immune response and promotes the proliferation of cancer cell (23) ; CXCL1 expression contributes to prostate and breast cancer progression (10, 11) ; CXCL-5 increases migration and invasion of liver cancer and can be used as a potential prognostic factor in early stage of lung cancer (12, 13). However, serum levels of CCL17, CXCL-1 and CXCL-5 did not show a correlation with the survival rate of pancreatic cancer in our study.

G-CSF and GM-CSF are two important colony-stimulating factors. G-CSF is highly expressed in gastric and colon cancers, and promotes cancer cell proliferation and migration (15). While GM-CSF is required for tumor cell proliferation, invasion, and transendothelial migration. Depletion of GM-CSF in cancer-associated mesenchymal stem cells will inhibit the ability of these cells to promote tumor cell growth and metastasis in pancreatic ductal adenocarcinoma (14). However, in this study, we did not found a significant association between G-CSF or GM-CSF and poor survival of pancreatic cancer patients.

TGF-β and IFN-γ are ubiquitously expressed cytokines that mediate intercellular communication during innate and acquired immune responses, maintenance of cellular homeostasis, angiogenesis, as well as tumor surveillance

(18,20,24). Previous studies have shown that TGF-β and IFN-γ both have dual roles in the tumor development and metastasis. They can act as anti- and pro-tumorigenic factors, which may be dependent on the contexts of tumor specificity, microenvironmental factors, and signaling intensity (16,19). In this study, we also did not find a significant association between TGF-β or IFN-γ and poor survival of pancreatic cancer patients.

Taken together, we analyzed the relationship between eight cytokines and patient's outcome, and found only CCL2 level was positively correlated with shorter survival time and poor prognosis in the patients with pancreatic cancer. CCL2 was first identified in 1989 (25) and can be produced by a variety of activated cells, such as fibroblasts, endothelial cells, lymphocytes and macrophages (26,27). CCL2 mediates the serine/threonine phosphorylation of the C terminal of receptor protein through binding to the N terminal of CCR2 on target cell membrane, and participates in many physiological and pathological activities. It is highly expressed in a variety of tumor diseases such as prostate cancer, breast cancer, liver cancer, kidney cancer, multiple myeloma and leukemia (28). Previous study has reported that overexpression of CCL2 in metastatic breast cancer inhibited macrophage invasion to cancer tissue as well as tumor growth and metastasis (29). In pancreatic cancer, CCL2 can activate PI3K/AKT pathway to promote the proliferation of cancer cells, and it can also increase the expression of survivin against the autophagic death of prostate cancer cells (30-32). In addition, CCL2 could be a positive regulator of pancreatic cancer progression, which can support tumor proliferation and neovascularization after radiotherapy (33). Given the important role of CCL2 in cancer development, Carlumab (CNTO 888), a CCL2 specific antibody, has been found to enhance taxol sensitivity and reduce tumor burden. Carlumab have begun clinical trials in many solid tumors including pancreatic cancer (34-36). Controversially, another study conducted by Monti et al (2003) reported that patients with high serum level of CCL2 had a positive association with survival in pancreatic cancer patients (37). In this study, 212 patients with suspected pancreatic neoplasm were involved, with no available information on TNM stages. And the mean follow-up of the survivors was 476 ± 256 days, which is much longer than that in our study. Different pathological stages of patients may be the cause of contradiction between previous study and ours. Considering the important role of CCL2 in cancer metastasis (38), our results supported that serum CCL2 was inversely correlated with survival in pancreatic cancer patients.

Our result that patients with high level of serum CCL2 has a poor survival outcome may be influenced by the time bias of diagnosis regarding the survival time. Pancreatic cancer is a highly malignant tumor with a relatively short course. The patients involved in this study were in TNM stage III to IV, and most of them survived less than one year. Rapid progression and deterioration of the pancreatic cancer can reduce the time bias of diagnosis. Therefore, our results indicated that, at least in part, high level of serum CCL2 was indeed correlated with survival. However, further study with a much larger cohort will be also required to confirm the role of CCL2 in the diagnosis of pancreatic cancer.

In summary, our results suggested that high level of serum CCL2 were strongly associated with poor survival and prognosis, which can serve as a useful marker to predict outcome of patient with pancreatic cancer.

Acknowledgements

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Conflicts of interest

The authors declare no conflict of interest.

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