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Serum interleukin-6 is associated with pancreatic ductal adenocarcinoma progression pattern

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Abstract

Several reports showed that interleukin-6 (IL-6) or -8 (IL-8) might be useful inflammatory biomarkers for pancreatic ductal adenocarcinoma (PDAC), although these clinical impact is still open to debate. The aim of this study was to elucidate whether serum levels of IL-6 and IL-8 at diagnosis could predict the tumor progression pattern of PDAC, especially in extensive hepatic metastasis.

According to the tumor burden of hepatic metastasis at the last follow-up, tumor progression pattern was defined as follows: no or limited (unilobar involvement and 5 or less in the within liver, limited group) and extensive hepatic metastasis (bilobar or more than 5, progressed group). Fifty-three PDAC patients with initially no or limited hepatic metastasis were enrolled retrospectively.

Around 42 (79.2%) were included in the limited and 11 (20.8%) in the progressed group. The median serum level of IL-6 in the progressed group was elevated significantly compared with the limited group. However, the median serum level of IL-8 was not. Furthermore, multivariate analysis revealed that the elevated serum level of IL-6 was an independent risk factor for progression to extensive hepatic metastasis (odds ratio 1.928, 95% confidence interval 1.131–3.365, $P = 0.019$), but IL-8 was not. However, higher IL-6 did not predict shorter survival.

High serum IL-6 can be an independent risk factor for progression to extensive hepatic metastasis in PDAC patients.

Abbreviations: CA19-9 = carbohydrate antigen 19-9, CI = confidence interval, CRP = C-reactive protein, ECOG = Eastern Cooperative Oncology Group, IL = interleukin, Ln = natural logarithm, NLR = neutrophil-lymphocyte ratio, PDAC = pancreatic ductal adenocarcinoma.

Keywords: hepatic metastasis, interleukin-6, pancreatic ductal adenocarcinoma, survival

1. Introduction

The crucial role of chronic inflammation in tumor development is particularly recognized in the context of pancreatic ductal adenocarcinoma (PDAC).[1,2] Although the roles of interleukin-6 (IL-6) and interleukin-8 (IL-8) in cancer biology are still unclear, it has been suggested that IL-6 or IL-8 promote cell proliferation, migration, invasion, and angiogenesis.[3–6] Chronic inflammation can lead to production of cytokines that upregulate proinflammatory cytokines, such as IL-6 and IL-8, and affects progression of PDAC.[1–6]

PDAC spreads extensively to various organs, especially the liver.[7] Several studies showed that the presence of hepatic metastasis is an independent adverse prognostic factor in patients with metastatic PDAC.[8–10] Recently, 1 autopsy study for patterns of failure in patients with PDAC suggested that it is classified to limited versus extensive metastasis according to the tumor burden in the metastatic setting.[11] Some studies showed that increased IL-6 or IL-8 serum level is associated with the presence of hepatic metastasis and shorter survival in patients with PDAC.[12–16]

We aimed to elucidate whether serum IL-6 and IL-8 could predict the tumor progression pattern of PDAC, especially regarding extensive hepatic metastasis and eventually long-term prognosis.

2. Patients and methods

2.1. Patients

From 2010 to 2011, a total of 82 patients with PDAC were identified in Seoul National University Bundang hospital. We retrospectively studied 53 consecutive PDAC patients with initially no or limited hepatic metastasis (unilobar involvement and 5 or less in the within liver), excluding 29 patients who already had initially extensive hepatic metastasis (bilobar or more than 5). According to the tumor burden of hepatic metastasis at the last follow-up, the tumor progression pattern was defined as follows: no or limited (unilobar involvement and 5 or less in the within liver, limited group) and extensive hepatic metastasis (bilobar or more than 5, progressed group). This study protocol was reviewed and approved by the institutional review board of Seoul National University Bundang Hospital (IRB No.: B-1003/096-005).

2.2. Methods

The serum samples stored at −70°C until further evaluation. Four samples in each group were selected for target cytokines screening, and the level of 8 cytokines (granulocyte/macrophage colony-stimulating factor, interferon-γ, tumor necrosis factor-α, IL-2, IL-4, IL-6, IL-8, and IL-10) were measured using the Luminex Bead-based Multiplex Assay (R&D system, MN). Among 8 cytokines, the serum levels of the IL-6 and IL-8 were significant difference between limited and extensive hepatic metastasis (Supplementary Fig. 1, http://links.lww.com/MD/B528). After target cytokines validation, measurement of serum IL-6 level, IL-8 level, C-reactive protein (CRP), neutrophil-lymphocyte ratio (NLR), and tumor marker carbohydrate antigen 19–9 (CA19–9) were performed at the time of initial evaluation. Serum IL-6 and IL-8 level were normally log distributed within both groups; hence, values were transformed to the natural logarithm (Ln). High IL-6 and IL-8 were defined as more than the median values, respectively. The high CA19-9 level was defined as more than 1000 U/mL.[17,18] Overall survival was defined as the time interval from diagnosis to death.

2.3. Statistical analysis

Kaplan–Meier analysis was used to generate survival curves and calculate median survival times which were compared by the log-rank test. The analysis of the risk factors for death was performed by the univariate Cox proportional hazard regression model. Risk factors were expressed as the hazard ratio. The analysis of the risk factors for progression to extensive hepatic metastasis was performed by logistic regression analysis. Risk factors were

expressed as odds ratios. Among the clinical variables included in univariate analysis, those with a 2-sided P-value of less than 0.05 were chosen for multivariate analysis with stepwise selection. A comparison among the 2 subgroups was carried out using Student's t-test, the Mann–Whitney, or Pearson's correlation test for continuous variables, whereas the chi-square test was used to compare noncontinuous variables. A 2-sided P-value of less than 0.05 was considered to indicate a statistically significant difference. All statistical analyses were performed using SPSS statistics 21.0 software for Windows (IBM Corporation, Armonk, NY).

3. Results

3.1. Patient characteristics

A total of 53 consecutive patients with initially no or limited hepatic metastasis of PDAC were enrolled in this study. Baseline patient characteristics are shown in Table 1. According to the progression pattern, 42 (79.2%) patients were included in the limited group and 11 (20.8%) patients in the progressed group. The median patient age at diagnosis was 66 years, and 31 (58.5%) patients were men. Regarding the tumor progression pattern and the serum concentration of inflammatory cytokines, the median serum level of Ln IL-6 of the progressed group was significantly greater than that of the limited group (2.0 vs 1.4, $P =$ 0.025) (Fig. 1A). However, there was no significant correlation between the median serum level of Ln IL-8 and tumor progression pattern (3.2 vs 3.5, $P = 0.978$) (Fig. 1B). According to the progression pattern, the initial disease status was shown in Supplementary Fig. 2, http://links.lww.com/MD/B528. Median

Table 1

Patient characteristics.

| | Tumor progression pattern at last follow-up | | | |
	Limited group (n = 42)	Progressed group (n = 11)	Overall (N = 53)	P
Median age, year, IQR	66 (58–72)	64 (55–68)	66 (57–72)	0.389
Male sex, no., %	24 (57.1)	7 (63.6)	31 (58.5)	0.697
ECOG score, no., %				
<2	38 (90.5)	10 (90.9)	48 (90.6)	0.965
≥2	4 (9.5)	1 (9.1)	5 (9.4)	
Smokers, no., %	15 (35.7)	3 (27.3)	18 (34.0)	0.730
Diabetes, no., %	28 (66.7)	5 (45.5)	33 (62.3)	0.196
Median CRP, mg/L, IQR	4.5 (3.0–12.1)	10.5 (3.3–22.4)	4.9 (3.0–13.2)	0.366
Median NLR, IQR	1.9 (1.6–2.8)	2.6 (1.5–4.8)	2.1 (1.6–3.2)	0.430
CA19-9 > 1000 U/mL, no., %	12 (28.6)	2 (18.2)	14 (26.4)	0.706
Median Ln IL-6, IQR	1.4 (0.9–2.2)	2.0 (1.2–3.7)	1.6 (1.0–2.2)	0.025
Median Ln IL-8, IQR	3.5 (2.6–4.1)	3.2 (3.0–3.7)	3.4 (2.6–4.0)	0.978
Longest dimension of primary tumor, cm, IQR	3.4 (2.5–4.6)	2.4 (2.0–4.0)	3.0 (2.3–4.6)	0.286
Primary tumor location, no., %				
Head/body	30 (71.4)	6 (54.5)	36 (67.9)	0.286
Tail	12 (28.6)	5 (45.5)	17 (32.1)	
Initial disease status, no., %				0.315
Resectable	19 (45.2)	7 (63.6)	26 (49.1)	
Locally advanced	11 (26.2)	2 (18.2)	13 (24.5)	
Metastatic	12 (28.6)	2 (18.2)	14 (26.4)	
Deaths, no., %	32 (76.2)	11 (100)	43 (81.1)	0.072
Cause of death, no, %				
Disease progression	22 (52.4)	9 (81.8)	31 (58.5)	
Infection	8 (19.0)	1 (9.1)	9 (17.0)	
Tumor bleeding	1 (2.4)	0 (0.0)	1 (1.9)	
Cardiovascular event	1 (2.4)	1 (9.1)	2 (3.8)	
Median overall survival, mo, 95% CI	9.9 (4.0–16.0)	10.0 (8.7–11.4)	10.0 (6.5–13.5)	0.214

CA19-9 = carbohydrate antigen 19-9, CI = confidence interval, CRP = C-reactive protein, ECOG = Eastern Cooperative Oncology Group, IL = interleukin, IQR = interquartile range, Ln = natural logarithm, NLR = neutrophil-lymphocyte ratio.
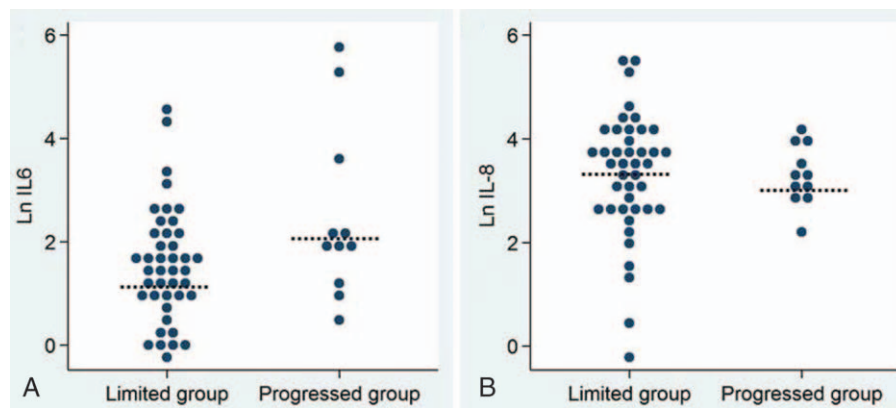
Figure 1. (A) The serum level of the Ln IL-6 in the limited and progressed group. The median serum level of Ln IL-6 of the progressed group was significantly greater than that of the limited group (2.0 vs 1.4, $P=0.025$). (B) The serum level of the Ln IL-8 in the limited and progressed group. There was no significant correlation between the median serum level of Ln IL-8 and the tumor progression pattern (3.2 vs 3.5, $P=0.978$). Ln = natural logarithm.

follow-up of survivors showed 43 of 53 patients died (32 of 42 patients with the limited group, and 11 of 11 progressed groups). The causes of death in the limited group patients were disease progression in 22 (52.4%), infection in 8 (19.0%), and 1 each (2.4%) due to tumor bleeding and cardiovascular event. Most of the patients in the progressed group died because of disease progression (9, 81.8%), and there was 1 case (9.1%) each due to infection and cardiovascular event.

3.2. Correlation of inflammatory cytokines with CRP and NLR

The serum level of Ln IL-6 showed a significant direct correlation with the serum level of CRP and NLR ($R=0.395$,

$P=0.007$; $R=0.326$, $P=0.017$), but there was no significant correlation between the serum level of Ln IL-8 and the serum level of CRP and NLR ($R=0.206$, $P=0.203$; $R=0.022$, $P=0.881$) (Fig. 2).

3.3. Risk factors for progression to extensive hepatic metastasis

By univariate logistic regression analysis, location of the primary tumor in the tail of the pancreas and serum Ln IL-6 level were significantly associated with the tumor burden of hepatic metastasis at the last follow-up. Among these, a higher serum Ln IL-6 level was significantly associated with a large tumor
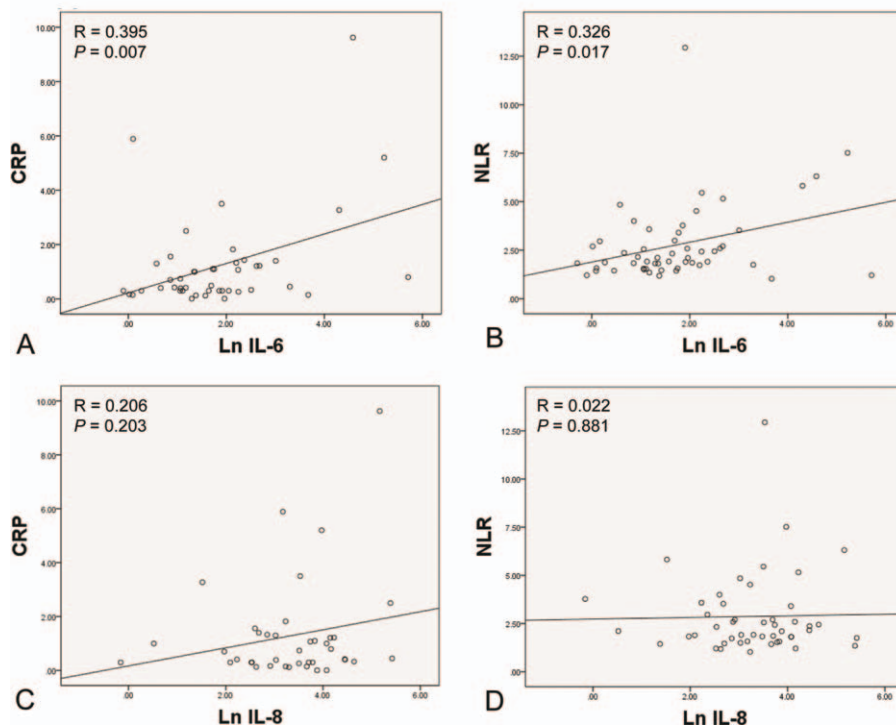

Figure 2. (A) The correlation between the serum level of Ln IL-6 and CRP. (B) The correlation between the serum level of Ln IL-6 and NLR. (C) The correlation between the serum level of Ln IL-8 and CRP. (D) The correlation between the serum level of Ln IL-8 and NLR. Ln = natural logarithm, IL = interleukin, CRP = C-reactive protein, NLR = neutrophil-lymphocyte ratio.

Table 2

Risk factors for progression to extensive hepatic metastasis.

Variables	Univariate analysis		Multivariate analysis	
	OR (95%CI)	P	OR (95%CI)	P
Age	0.961 (0.854–1.081)	0.505		
Male sex	1.530 (0.149–15.748)	0.721		
Smokers	0.540 (0.050–5.802)	0.611		
Diabetes	0.539 (0.062–4.682)	0.575		
CA19-9 > 1000 U/mL	1.477 (0.042–52.527)	0.830		
Ln IL-6	2.294 (1.074–4.899)	0.032	1.928 (1.131–3.365)	0.019
Ln IL-8	0.746 (0.358–1.554)	0.434		
Longest dimension of primary tumor	0.611 (0.271–1.380)	0.236		
Primary tumor location				
Head /body	1 (ref)			
Tail	1.654 (1.170–2.768)	0.043	1.341 (0.719–2.269)	0.090
Initial disease status				
Resectable	1 (ref)			
Locally advanced	0.567 (0.040–8.034)	0.675		
Metastatic	0.242 (0.013–4.625)	0.346		

CA19-9 = carbohydrate antigen 19-9, CI = confidence interval, ECOG = Eastern Cooperative Oncology Group, IL = interleukin, Ln = natural logarithm, OR = odds ratio, ref = reference.

burden of hepatic metastasis by multivariate logistic regression analysis (odds ratio 1.928, 95% confidence interval [CI] 1.131–3.365, $P = 0.019$). However, the serum Ln IL-8 level was not significantly associated with the tumor burden of hepatic metastasis (odds ratio 0.746, 95% CI 0.358–1.554, $P = 0.434$) (Table 2).

3.4. Risk factors for overall survival

The median overall survival for this study population was 10.0 months (95% CI, 6.5–13.5). According to the tumor progression pattern, the median overall survival of patients in the limited group and progressed group were 9.9 months (95% CI, 4.0–16.0) and 10.0 months (95% CI, 8.7–11.4), respectively. Analysis of the overall survival by univariate Cox regression

Table 3

Risk factors for overall survival.

Variables	Univariate analysis	
	HR (95%CI)	P
Age	1.039 (1.005–1.074)	0.025
Male sex	1.116 (0.608–2.048)	0.724
ECOG score ≥ 2	2.988 (1.110–8.044)	0.030
Smokers	1.291 (0.687–2.426)	0.428
Diabetes	1.892 (0.939–3.218)	0.119
CA19-9 > 1000 U/mL	2.203 (1.041–3.933)	0.038
Ln IL-6	1.070 (0.830–1.378)	0.602
Ln IL-8	0.903 (0.683–1.194)	0.475
Longest dimension of primary tumor	1.338 (1.123–1.594)	0.001
Primary tumor location		
Head/body	1 (ref)	
Tail	1.321 (0.700–2.495)	0.391
Initial disease status		
Resectable	1 (ref)	
Locally advanced	3.114 (1.448–6.694)	0.004
Metastatic	5.803 (2.608–12.916)	<0.001
Tumor progression pattern		
Limited group	1 (ref)	
Progressed group	1.546 (0.773–3.093)	0.218

CA19-9 = carbohydrate antigen 19-9, CI = confidence interval, ECOG = Eastern Cooperative Oncology Group, HR = hazard ratio, IL = interleukin, Ln = natural logarithm, ref = reference.

analysis showed higher age, poor ECOG performance status, high CA19-9, higher longest dimension of the primary tumor, and initially locally advanced or metastatic cancer were significantly associated with shorter survival (Table 3). However, the survival curve according to the median serum values of IL-6 and IL-8 showed no significant difference in relationship to overall survival, respectively ($P = 0.660$, $P = 0.915$; Fig. 3).

4. Discussion

The role of chronic pancreatic inflammation in tumor progression has been demonstrated to be major.[2,19] It has been known that IL-6 or IL-8, proinflammatory cytokines, are involved with tumor progression in PDAC patients.[3–6] This study defined the tumor progression pattern according to the hepatic metastatic burden of PDAC at the last follow-up. We attempted to investigate whether serum IL-6 and IL-8 could predict tumor progression pattern and overall survival. The serum level of IL-6 of the progressed group was significantly greater than the limited group, and higher IL-6 was the only independent risk factor for progression to extensive hepatic metastasis. Therefore, it would be suggested that more aggressive treatment from the beginning might be considered in PDAC patients with high IL-6.

IL-6 is a proinflammatory cytokine that is synthesized by many cell types, including macrophages, fibroblasts, endothelial cells, and myeloid cells, and it promotes synthesis of CRP from hepatocyte.[20,21] This study revealed that IL-6 is correlated with inflammatory markers such as CRP and NLR, and that it was an independent risk factor for progression to extensive hepatic metastasis.

IL-8 is a proinflammatory cytokine and a member of the CXC chemokine family.[22] Several studies showed that IL-8 plays a crucial role in tumor progression including cancer invasion and angiogenesis.[5,6,15] However, this study did not show that IL-8 is correlated with inflammatory markers, and that it was shown to be able to predict the tumor progression pattern.

Although IL-6 was an independent risk factor for progression to extensive hepatic metastasis, higher IL-6 and IL-8 were not associated with shorter survival in this study. The authors cannot explain exactly why high IL-6 indicating extensive hepatic metastasis in the future, does not project onto lower survival.
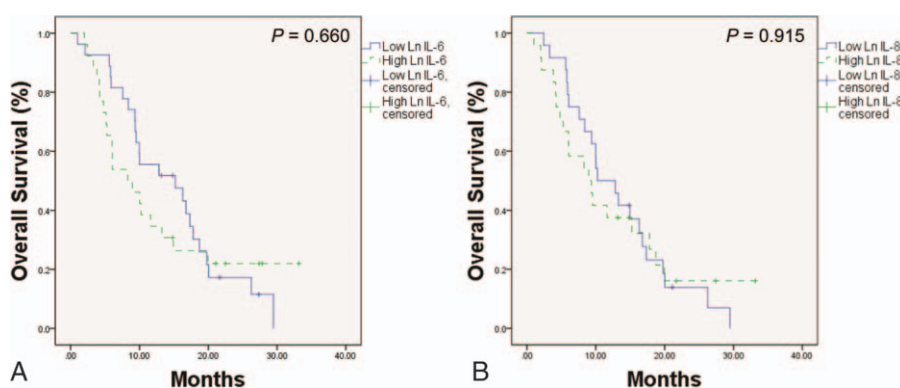
Figure 3. (A) Kaplan–Meier analysis of overall survival according to the median serum values of Ln IL-6. (B) Kaplan–Meier analysis of overall survival according to the median serum values of Ln IL-8. Ln = natural logarithm, IL = interleukin.

Small sample size might be influence the outcome. On the contrary to this, this study showed that a higher CA19-9, larger primary tumor size, or advanced initial disease status was associated with shorter survival, but not risk factors for progression to extensive hepatic metastasis.

Previous autopsy studies showed that most PDAC patients die with a broad range of metastatic burden, from limited to extensive.[11,23] Furthermore, 10 to 20% of PDAC patients die of locally advanced disease, without metastatic disease.[11,24] This study revealed that mortality due to disease progression account for almost all causes of death in the progressed group patients. However, in limited group patients, localized infection and tumor bleeding in relation to the primary tumor, in addition to disease progression, accounted for 25% of overall death. Findings of this study were consistent with a previous autopsy study.

The present study has limitations. First, it is retrospective design. Second, the tumor progression pattern was assessed by the abdomen computed tomography not autopsy, and by using measurable lesion of the hepatic metastatic burden except for nonmeasurable lesion such as peritoneal seeding, lymphatic pulmonary metastasis, and bone destructive metastasis. To the best of our knowledge, we investigate of the relationship between proinflammatory cytokines and tumor progression pattern for the first time. In conclusion, higher serum IL-6 was an independent risk factor for progression to extensive hepatic metastasis of PDAC, even though it did not correlate with survival. Therefore, more aggressive chemotherapy from the beginning should be considered in PDAC patients with high IL-6.

Acknowledgments

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