Clinical significance of CXCL-8/CXCR-2 network in esophageal squamous cell carcinoma

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Background. *CXCL-8, known as proinflammatory cytokine interleukin (IL)-8, and its receptor, CXCR-2, are expressed in various cancer cells. CXCL-8/CXCR-2 network is believed to be involved in angiogenesis, growth, and invasion of tumor cells. To date, the clinical significance of CXCL-8/CXCR-2 network in esophageal squamous cell carcinoma (ESCC) remains unclear. Here, we investigated the role of CXCL- 8/CXCR-2 network in ESCC.*

Methods. *The subjects included 78 patients with primary ESCC. We examined CXCL-8 and CXCR-2 expression in surgically resected specimens using immunohistochemistry. The association between CXCL-8/CXCR-2 expression and level of preoperative serum cytokines, C-reactive protein (CRP), coagulation factors, clinicopathologic background factors, and survival of ESCC patients was assessed.*

Results. *Thirty-seven (47%) and 36 (46%) patients were positive for CXCL-8 and CXCR-2 expression, respectively. Both CXCL-8 and CXCR-2 were expressed in 26 patients (33%). We compared the results of these 26 patients [CXCL-8(+)/CXCR-2(+) group] with those of the other group (*n = 52*). The depth of invasion (pT factor;* P < .001*), lymph node metastasis (pN factor;* P = .001*), pathologic stage (*P < .001*), lymphatic invasion (*P = .010*), and venous invasion (*P = .001*) were significantly more advanced in the CXCL-8(+)/CXCR-2(+) group compared with the other group. Preoperative IL-6, IL-8, CRP, fibrin/fibrinogen degradation product, and fibrinogen levels in the CXCL-8(+)/CXCR-2(+) group were also significantly higher than those in the other group (*P = .046, .009, .029, .010, and <.001*, respectively). The CXCL-8(+)/CXCR-2(+) group also showed a significantly lower recurrence-free survival (RFS;* P < .001*) and disease-specific survival (*P = .008*). As per Cox's hazards model, CXCL-8/CXCR-2 expression (hazard ratio, 2.89;* P = .008*) was independent predictive factor for RFS.*

Conclusion. *Increased expression of both CXCL-8 and CXCR-2 correlated with tumor progression, metastasis, higher preoperative levels of proinflammatory cytokines, CRP, activation of exogenous coagulation factors, and poor prognosis in ESCC patients. These results indicate that overexpression of both CXCL-8 and CXCR-2 may be a useful marker for predicting the outcome in ESCC patients, and more important, has potential in becoming a critical diagnostic marker for selection of appropriate treatments. (Surgery 2013;154:512-20.)*

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Esophageal squamous cell carcinoma (ESCC) is among the most common incident cancers, accounting for a significant number of cancer-related deaths worldwide.[1-4] Presently, in Japan, preoperative chemotherapy and definitive chemoradiotherapy are important components of therapeutic strategy to locally advanced ESCC.[1,5-7] The overall 5-year survival rate of ESCC patients remains unsatisfactory, although advancements have been made in diagnostic development and treatment.[1,5,8-10] Therefore, there is still an urgent need to identify novel, promising markers that could predict the survival outcome of ESCC patients.

Interleukin (IL)-8, alternatively known as chemokine CXCL-8, was originally classified as a

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neutrophil chemoattractant; however, it is now reported to combine with CXCR-2, a G-protein–coupled receptor, and consequently play an important role in tumor progression and metastasis in a variety of human cancers. The biologic activity of IL-8 in tumors and tumor microenvironment contributes to tumor progression through its potential function in the regulation of angiogenesis, cancer cell growth and survival, tumor cell migration, leukocyte infiltration, and modification of immune responses.[11,12] Moreover, infiltrating macrophages in tumor stroma stimulate cancer cell growth, enhance angiogenesis, promote metastasis, and have prognostic significance in several human cancers.[11,12] However, the relationship between ESCC and the CXCL-8/CXCR-2 network has not yet been reported.

To address this issue, in our present study, immunohistochemistry (IHC) was used to examine the expression of CXCL-8 and CXCR-2 in surgically resected specimens obtained from primary ESCC patients treated with radical esophagectomy. The correlation between CXCL-8 and CXCR-2 overexpression, level of preoperative serum cytokines, C-reactive protein (CRP), coagulation factors, and patients' clinicopathologic factors was evaluated to determine whether the expression of these chemokines could predict the survival prognosis of ESCC patients.

MATERIALS AND METHODS

Patients. Seventy-eight patients who underwent transthoracic esophagectomy for ESCC at Keio University Hospital between August 2004 and August 2010 were investigated. Informed consent was obtained from each patient enrolled in this study. All patients underwent 2- or 3-field lymph node dissection through right thoracotomy, either video-assisted thoracic surgery or open thoracotomy. Physical examinations before surgery did not detect high-risk factors for transthoracic esophagectomy under general anesthesia in any of the patients.

Resected specimens and IHC. For this study, tissue was obtained from esophageal specimens after operative resection. For IHC analyses, slides were deparaffinized and rehydrated before proceeding with the staining protocol. Antigen retrieval was performed using a microwave for CXCL-8 staining and an enzyme solution (Proteinase K, DAKO, Glostrup, Denmark) for CXCR-2 staining. Monoclonal and polyclonal primary antibodies were used at the following dilutions: CXCL-8 mouse anti-human monoclonal antibody ab18672 (working dilution 7: 100, Abcam, Cambridge, UK) and CXCR-2 rabbit anti-human polyclonal antibody LS-A803 (working dilution 1:250, LifeSpan BioSciences, Seattle, WA). Prepared sections were incubated overnight at 4°C with the first-step antibody of choice. Slides were subsequently incubated with labeled secondary antibody for 30 (CXCR-2) or 60 (CXCL-8) minutes at room temperature. We used the ImmPRESS REAGENT KIT peroxidase Anti-MOUSE immunoglobulin (Vector Laboratories, Burlingame, CA) as the secondary antibody for IHC of CXCL-8. Biotinylated goat anti-rabbit IgG antibody BA-1000 (Vector Laboratories) was used as the secondary antibody for IHC of CXCR-2. Peroxidase activity was detected with the enzyme substrate 3, 3-diaminobenzidine tetrachloride solution. On the final section, the slide was lightly counterstained with hematoxylin and mounted. For each test case, a negative control was also included, which received the same treatment as the test slide, except that the primary antibody was omitted.[13] We also used esophageal squamous epithelial cells without inflammation that lacked CXCL-8 or CXCR-2 expression as negative controls. Positive controls (neutrophils and monocytes in lymph node for CXCL-8 antibody and those in the spleen for CXCR-2 antibody, according to Emadi et al[14]) were obtained to optimize the conditions. We evaluated CXCL-8 or CXCR-2 expression in viable ESCC cells on slides that contained the most invasive tumor lesion. As previously described,[15] immunoreactive score was calculated by the product of intensity of immunostaining (0, none; 1, weak; 2, moderate; and 3, strong) and percentage of positive tumor cells (0, none; 1, 10–25%; 2, 25–50%; and 3, ≥50%). Immunoreactive score was assessed by 2 investigators (M.O. and H.T.) who had no knowledge of clinicopathologic factors. For final statistical analysis, immunoreactive score value of 0 or 1 was ranked as negative expression, and immunoreactive score values of 2–9 were ranked as positive expression.

Blood sample measurements. Preoperatively, blood samples were collected, centrifuged, and stored at −80°C. The serum tumor necrosis factor (TNF)-α, IL-1β, IL-6, IL-8, IL-10, and high-mobility group box-1 (HMGB-1) concentrations were measured before surgery by enzyme-linked immunosorbent assay (Research and Diagnostics Systems, Minneapolis, MN, for TNF-α, IL-1β, IL-6, IL-8, and IL-10, and Central Institute, Shino-Test Corporation, Kanagawa, Japan for HMGB-1). CRP concentrations, fibrin/fibrinogen degradation product (FDP), and fibrinogen were measured as routine laboratory tests.

Statistical analysis. All data are presented as median values. Statistical significance was determined by a non-parametric Mann–Whitney U-test or Fisher's exact test. Cumulative survival rates for patient groups were calculated using the Kaplan–Meier method and were compared using the Mantel–Cox log-rank test. Cox proportional hazards models were used for multivariate analysis of variables for predicting postoperative survival. Statistical analyses were performed with SPSS v18.0 software (SPSS, Tokyo, Japan).

RESULTS

Background of all patients is shown in Table I. The median age of patients was 62 years (interquartile range, 56–67) with a male/female ratio of 71/7. Pathologic stage, as determined using the TNM/UICC classification 6th edition of I:II_A:II_B:III, was 16:10:24:28. About half of the 78 patients received preoperative chemotherapy or chemoradiotherapy. As shown in Figs 1 and 2, and Table II, 37 patients (47%) were positive for CXCL-8 and 36 patients (46%) were positive for CXCR-2. In vitro, CXCR-2 protein overexpression was confirmed in an ESCC cell line TE4 by western blot (data not shown). We also confirmed CXCR-2 mRN expression in TE4 by reverse transcriptase polymerase chain reaction (data not shown). IHC results also showed esophageal squamous epithelial cells without inflammation lacked CXCL-8 or CXCR-2 expression. We divided these 78 patients into 4 groups (Table II): (1) CXCL-8- and CXCR-2-positive group [CXCL-8(+)/CXCR-2(+) group], n = 26 (33%); (2) only CXCL-8-positive group [CXCL-8(+)/CXCR-2(−) group], n = 11 (14%); (3) only CXCR-2-positive group [CXCL-8(−)/CXCR-2(+) group], n = 10 (13%); and (4) both CXCL-8- and CXCR-2-negative group [CXCL-8(−)/CXCR-2(−) group], n = 31 (40%).

During the mean follow-up time of 26 months, 40 (51%) of 78 patients experienced tumor recurrence. Fig 3, *A* shows the Kaplan–Meier curve of recurrence-free survival (RFS) according to the 3 divided groups. Patients in the CXCL-8(+)/CXCR-2(+) group were predicted to have a higher risk of subsequent tumor recurrence than those in the CXCL-8(+)/CXCR-2(−) and CXCL-8(−)/CXCR-2(+) group, and CXCL-8(−)/CXCR-2(−) group (Fig 3, *A*; P = .002 and P < .001, respectively). There was no significant difference between CXCL-8(+)/CXCR-2(−) and CXCL-8(−)/CXCR-2(+) group, and CXCL-8(−)/CXCR-2(−) group (Fig 3, *A*; P = .58). Because the CXCL-8(+)/CXCR-2(+) group had the highest risk of tumor recurrence, we assumed that clinicopathologic differences existed between the CXCL-8(+)/CXCR-2(+) group and the others. Therefore, we divided the 78 patients into 2 groups (CXCL-8(+)/CXCR-2(+) group and the other group). Fig 3, *B* shows that the CXCL-8(+)/CXCR-2(+) group had a significantly poorer prognosis than the other group (P < .001). Clinical background of the 2 groups is provided in Table III. Fourteen patients (54%) in the CXCL-8(+)/CXCR-2(+) group received preoperative chemotherapy or chemoradiotherapy, and 29 patients (56%) in the other group received preoperative therapy (Table III). However, there were no ESCC patients showing a complete response after preoperative therapy.

Compared with the other group, the CXCL-8(+)/CXCR-2(+) group was significantly more advanced with regard to the depth of invasion (pT factor, P < .001), lymph node metastasis (pN factor, P = .001), pathologic stage (P < .001), lymphatic invasion (P = .010), and venous invasion (P = .001; Table IV).

Preoperative levels of serum cytokines such as TNF-α, IL-1β, IL-10, and HMGB-1 showed no significant difference between the 2 groups (Table V). However, preoperative concentrations of serum IL-6 and IL-8 were significantly higher in the CXCL-8(+)/CXCR-2(+) group than those in the other group (P = .046 and .009, respectively;

Table I. Patient background (n = 78)

Characteristic	*Value*
n	78
Age (y)	
Median	62
Interquartile range	56–67
Gender (male/female)	71/7
Tumor site	
Thoracic upper third	8
Thoracic middle third	45
Thoracic lower third	23
Abdominal	2
Depth of tumor invasion	
pT1a	6
pT1b	24
pT2	14
pT3	30
pT4	4
Lymph node metastases (+)	52 (66.7%)
UICC pathologic stage	
I	16
II_A	10
II_B	24
III	28
Neoadjuvant chemotherapy (+)	32 (41.0%)
Neoadjuvant chemoradiotherapy (+)	11 (14.1%)

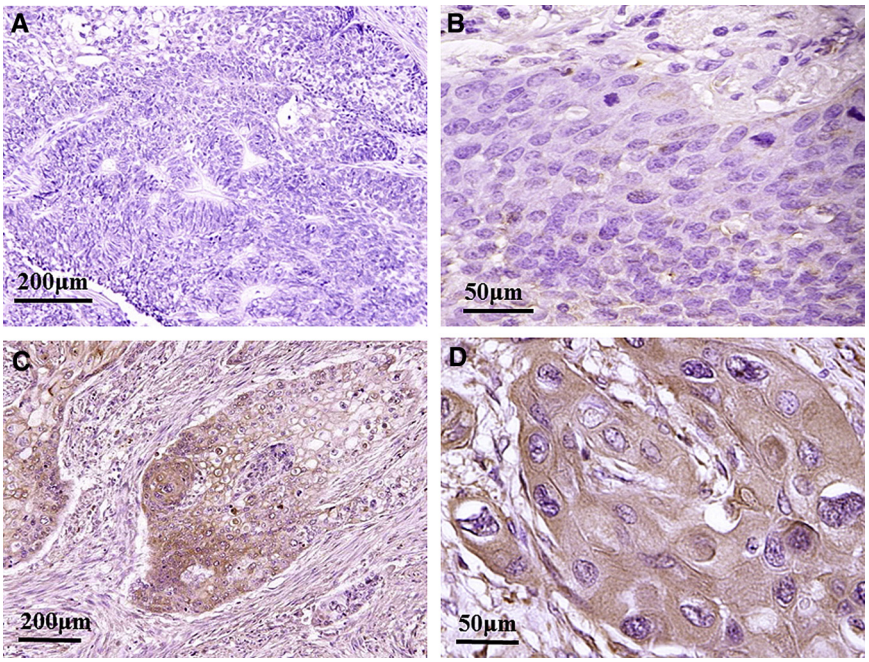

Fig 1. (*A, B*) Immunohistochemistry of CXCL-8. Esophageal cancer cells were negative for CXCL-8 expression. (*C, D*) CXCL-8–positive staining was observed in cytoplasm and membrane of esophageal cancer cells. Scale bars, 200 μm and 50 μm. (Color version of figure is available online.)

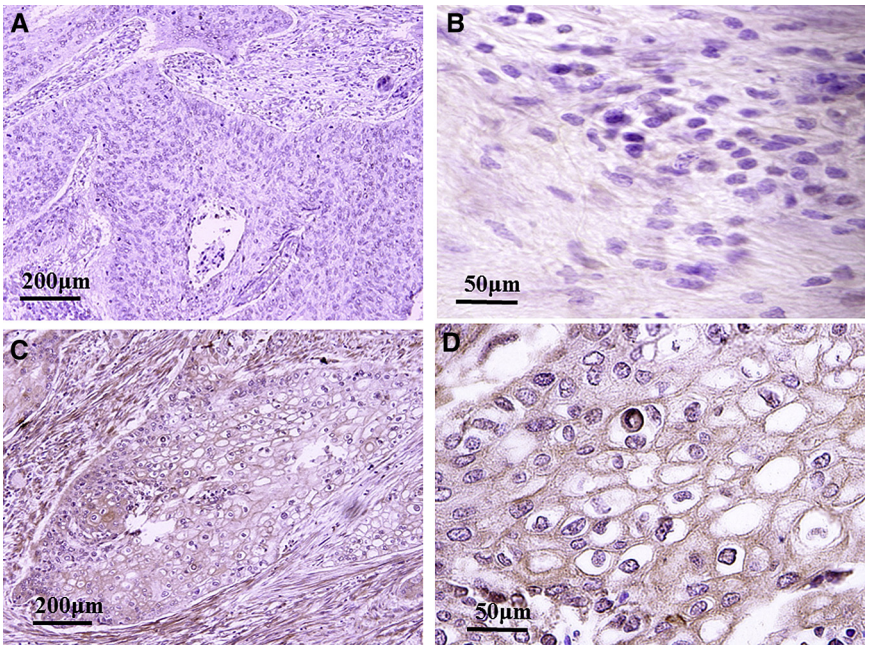

Fig 2. Immunohistochemistry of CXCR-2. (*A, B*) Esophageal cancer cells were negative for CXCR-2 expression. (*C, D*) CXCR-2–positive staining was observed on the surface and in the cytoplasm of esophageal cancer cells. Scale bars, 200 μm and 50 μm. (Color version of figure is available online.)

Table V). In the CXCL-8(+)/CXCR-2(+) group, concentrations of CRP, FDP, and fibrinogen were also significantly higher than those of the other group (P = .029, .010, and <.001, respectively; Table V). CRP and activation of exogenous coagulation factors strongly correlated with the expression of CXCL-8/CXCR-2.

Univariate and multivariate analyses were performed to determine the predictors of subsequent tumor recurrence. We evaluated 9 variables for

Table II. Immunoreactive score (IRS) by histology

	CXCR-2 (−); IRS = 0 or 1, n *(%)*	*CXCR-2 (+); IRS = 2–9,* n *(%)*	*Total,* n *(%)*
CXCL-8 (−); IRS = 0 or 1	31 (39.7)	10 (12.8)	41 (52.6)
CXCL-8 (+); IRS = 2–9	11 (14.1)	26 (33.3)	37 (47.4)
Total	42 (53.8)	36 (46.2)	78 (100)

The number of patients expressing both CXCL-8 and CXCR-2 was 26. These patients were assigned to the CXCL-8(+)/CXCR-2(+) group, whereas the remaining 52 patients were assigned to the other group.

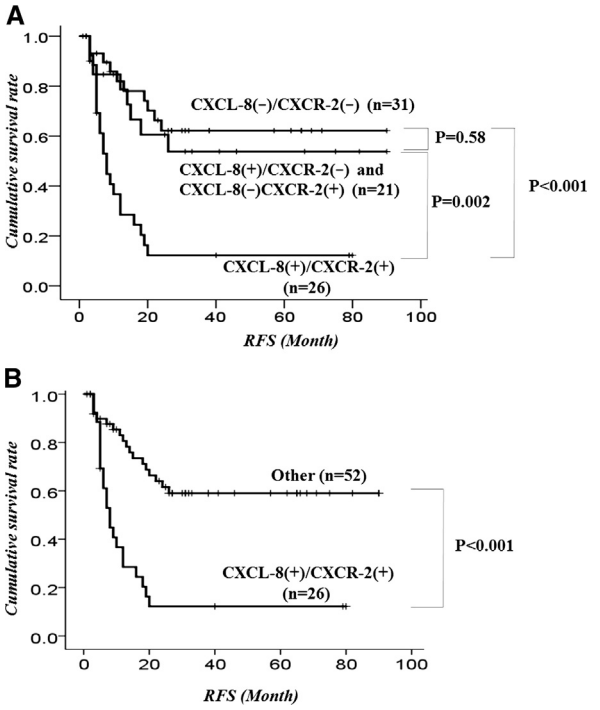

Fig 3. (*A*, *B*) Recurrence-free survival (RFS) after esophagectomy between the 3 groups or between the 2 groups.

survival prognosis by univariate analysis (Table VI). We detected depth of tumor invation of pT3 or greater, the number of lymph node metastases ≥3, preoperative FDP level ≥3.1, and CXCL-8(+)/CXCR-2(+) as predictive markers of RFS. We performed the multivariate analysis for these 4 prognostic factors. CXCL-8(+)/CXCR-2(+) was the independent risk factor for subsequent tumor recurrence, showing the highest hazard ratio compared with other preoperative variables and each pathologic feature.

A total of 19 subjects (24%) died from ESCC recurrence during follow-up. Univariate analysis was performed to investigate whether CXCL-8(+)/CXCR-2(+) could be a biomarker for predicting disease-specific survival. No difference was found among preoperative and pathologic variables except CXCL-8(+)/CXCR-2(+) by univariate analysis. The disease-specific survival rate of the patients with CXCL-8(+)/CXCR-2(+) expression was significantly lower than that of the other patients ($P = .008$; Fig 4).

DISCUSSION

Many studies on the CXCL-8/CXCR-2 signaling system have been conducted, mainly in the inflammatory fields such as lymphocyte homing and infection. More recently, the relationship between the CXCL-8/CXCR-2 signaling system and tumor progression is beginning to be elucidated. In 2003, Heidemann et al[16] showed that IL-8 provoked the migration of neutrophils by interaction with CXCR-2, and its angiogenic activity was predominantly mediated by CXCR-2 in colonic adenocarcinoma.

Referring to esophageal cancer, CXCL-8 expression was markedly elevated in Barrett's esophageal adenocarcinoma.[17] Indeed, a recent study demonstrated that an increased CXCL-8 expression relates to poorer prognosis, thereby confirming its protumorigenic role in esophageal adenocarcinoma and Barrett's-associated adenocarcinoma.[18,19] In ESCC, elevated levels of circulating IL-8 were detected in comparison with healthy controls, and the increase in serum IL-8 levels correlated with tumor size, lymph node involvement, and distant metastases.[20] However, the expression and role of IL-8 (CXCL-8) and its receptor, CXCR-2, in ESCC are poorly understood. In the present study, we investigated the relationship between CXCL-8/CXCR-2 expression and clinicopathologic factors of 78 patients who underwent radical esophagectomy for ESCC. We successfully detected CXCL-8/CXCR-2 overexpression in a subset of ESCC specimens, similar to previous reports.[21,22] Cytoplasmic and membrane CXCL-8/CXCR-2 staining were observed in the tumor cells, as previously reported.[23,24] The current study indicates a positive relationship between CXCL-8/CXCR-2 overexpression and tumor malignancy. Depth of tumor invasion, lymph node metastasis, and pathologic stages were further advanced in esophageal specimens with CXCL-8/CXCR-2 overexpression in comparison with those in the other group. Additionally, the lymphatic invasion and venous invasion were highly positive in the CXCL-8(+)/CXCR-2(+) group.

Secretion of IL-8 from cancer cells and tumor-associated macrophages combined with CXCR-2 can enhance the proliferation and survival of cancer

Table III. Clinical factors between the 2 groups

Clinical parameter	*CXCL-8(+)/CXCR-2(+) group* (n = 26), n (%)	*Other* (n = 52), n (%)	P *value*
Age (y)			
Median	61	63	
Interquartile range	56.8–65	56.8–69	.29
Gender			
Male	24 (92.3)	47 (90.4)	
Female	2 (7.7)	5 (9.6)	.57
Preoperative chemotherapy or chemoradiotherapy	14 (53.8)	29 (55.8)	.53
Location of main tumor			
Thoracic upper third	3 (11.5)	5 (9.6)	
Thoracic middle third	12 (46.2)	33 (63.5)	
Thoracic lower third	9 (34.6)	14 (26.9)	
Abdominal	2 (7.7)	0 (0.0)	.18

Table IV. Pathologic factors between the 2 groups

Pathologic parameter	*CXCL-8(+)/CXCR-2(+) group* (n = 26), n (%)	*Other group* (n = 52), n (%)	P *value*
pT			
T1a	1 (1.3)	5 (6.4)	
T1b	1 (1.3)	23 (29.5)	
T2	4 (5.1)	10 (12.8)	
T3	17 (21.8)	13 (16.7)	
T4	3 (3.8)	1 (1.3)	<.001
pN			
N (−)	2 (2.6)	24 (30.8)	
N (+)	24 (30.8)	28 (35.9)	.001
pStage			
I	1 (1.3)	15 (19.2)	
II_A	1 (1.3)	9 (11.5)	
II_B	5 (6.4)	19 (24.4)	
III	19 (24.4)	9 (11.5)	<.001
Lymphatic invasion*			
0	3 (3.8)	25 (32.1)	
1	11 (14.1)	13 (16.7)	
2	8 (10.3)	10 (12.8)	
3	3 (3.8)	4 (5.1)	.010
Venous invasion†			
0	3 (3.8)	28 (35.9)	
1	14 (17.9)	12 (15.4)	
2	6 (7.7)	10 (12.8)	
3	1 (1.3)	2 (2.6)	.001

*Lymphatic invasion: 0, absence of cancer cells in any lymphatic vessels; 1, existence of cancer cells in several lymphatic vessels; 2, middle of lymphatic invasion 1 and 3; 3, existence of cancer cells in many lymphatic vessels.
†Venous invasion: 0, absence of cancer cells in any venules; 1, existence of cancer cells in 0 or 1 venules; 2, middle of venous invasion 1 and 3; 3, existence of cancer cells in many venules.

Table V. Preoperative serum levels of each parameter between the 2 groups

	CXCL-8(+)/CXCR-2(+) group (n = 26)	*Other group* (n = 52)	P *value*
TNF-α (pg/mL)			
Median	0.64	0.21	.67
IQR	0.00–2.36	0.00–1.58	
IL-1β (pg/mL)			
Median	0.15	0.19	.39
IQR	0.08–0.27	0.00–0.29	
IL-6 (pg/mL)			
Median	5.36	2.42	.046
IQR	1.69–10.47	1.33–5.16	
IL-8 (pg/mL)			
Median	11.4	6.11	.009
IQR	6.00–15.97	4.77–10.05	
IL-10 (pg/mL)			
Median	0.75	0.65	.32
IQR	0.42–1.38	0.40–0.91	
HMGB-1 (ng/mL)			
Median	4.00	5.30	.72
IQR	3.03–6.65	3.50–8.23	
CRP (mg/dL)			
Median	0.32	0.11	.029
IQR	0.10–0.26	0.06–0.15	
FDP (μg/dL)			
Median	3.7	2.9	.010
IQR	3.13–4.93	2.57–3.63	
Fibrinogen (mg/dL)			
Median	385	318	<.001
IQR	352–457	273–356	

CRP, C-reactive protein; *FDP*, fibrin/fibrinogen degradation product; *HMGB-1*, high-mobility group box-1; *IL*, interleukin; *IQR*, interquartile range; *TNF*, tumor necrosis factor.

cells through autocrine or paracrine signaling pathways. In addition, tumor-derived IL-8 activates endothelial cells in the tumor vasculature to promote angiogenesis and induces a chemotactic gradient. Consequently, IL-8 induces coagulopathy, similar to serum IL-6.[25,26]

IL-6 augments coagulation and inhibits fibrinolysis, whereas IL-8 augments synergistic inflammation

Table VI. Multivariate analysis of variables predicting recurrence free survival

	Recurrence-free survival		
		Multivariate	
Characteristic	*Univariate* P *value*	*HR (95% CI)*	P *value*
pT (<pT3 vs ≥pT3)	.003	1.32 (0.63–2.75)	.47
the number of lymph node metastases (<3 vs ≥3)	.001	1.73 (0.85–3.51)	.13
Preoperative therapy or not	.35		
CXCL-8(+)/CXCR-2(+) or not	<.001	2.89 (1.32–6.34)	.008
CRP (<0.10 vs ≥0.10 mg/dL)	.18		
FDP (<3.1 vs ≥3.1 μg/dL)	.047	1.12 (0.56–2.24)	.76
Fibrinogen (<340 vs ≥340 mg/dL)	.066		
IL-6 (<3 vs ≥3 pg/mL)	.071		
IL-8 (<9 vs ≥9 pg/mL)	.11		

CI, Confidence interval; *HR*, hazard ratio.

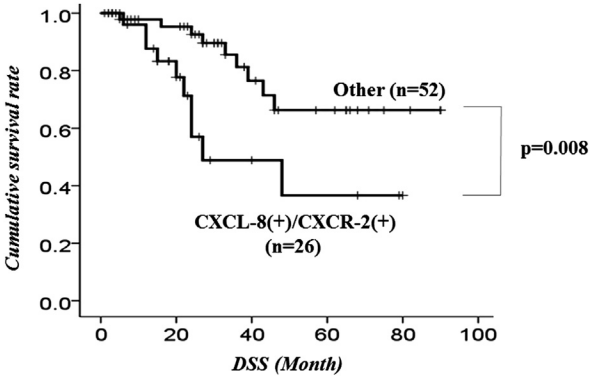

Fig 4. Disease-specific survival (DSS) after esophagectomy between the 2 groups.

and coagulation.[27] Coagulation pathways as well as chronic inflammation are often activated in cancer.[18] It is increasingly recognized that hemostatic variables, particularly plasma fibrinogen levels, have prognostic significance in patients with cancer.[3,28] Elevated plasma fibrinogen levels may be caused by the state of hyperfibrinogenemia and hypoxia induced by tumor growth[29,30] or by inflammatory-mediated cells, such as macrophages and epithelial cells.[31]

Our study also demonstrates that serum IL-6, IL-8, CRP, FDP, and fibrinogen levels were more markedly elevated preoperatively in patients with ESCC in which CXCL-8/CXCR-2 were overexpressed. In cytokine profiles, above all, preoperative serum levels of IL-8 most strongly correlated with CXCL-8/CXCR-2 overexpression in ESCC specimens. To the best of our knowledge, this relationship between CXCL-8/CXCR-2 overexpression and serum IL-8 levels in ESCC has not been previously reported. Our results suggest that CXCL-8 secreted from ESCC is closely connected with high concentrations of serum IL-8.

Moreover, Kaplan–Meier analysis indicated that patients with CXCL-8/CXCR-2 expression were predicted to have a higher risk of subsequent tumor recurrence. ESCC patients with CXCL-8/CXCR-2 overexpression had a significantly lower RFS than other patients. Multivariate analysis demonstrated that CXCL-8/CXCR-2 expression was an independent predictor of RFS. Expression of CXCL-8/CXCR-2 could predict the prognosis, and this was true for RFS in patients with ESCC. From our results, the relationship between CXCL-8/CXCR-2 expression and esophageal carcinogenesis remains unclear. However, the data in the present study suggests that tumor cells positive for CXCL-8/CXCR-2 may induce chronic inflammation. The inflammation caused by secreted cytokines, such as IL-6 and IL-8, may activate the coagulation pathways and produce fibrinogen and FDP, thereby resulting in tumor growth, invasion, metastasis, and poor prognosis by promoting tumor neovascularization and supporting sustained adhesion of tumor cells.[3,28,32] It was recently reported that transfection of CXCL-8 resulted in increased angiogenesis and carcinogenesis of human gastric carcinoma cells in mice.[33] Asfaha et al[34] also reported that IL-8 contributes to gastrointestinal carcinogenesis by mobilizing immature CD11b(+) Gr-1(+) myeloid cells. Dutta et al[35] also show that an inflammatory-based prognostic score, such as the Glasgow Prognostic Score, predicts survival in patients with the esophagogastric cancer using CRP and albumin. Therefore, CXCL-8(+)/CXCR-2(+) likely results in promotion of stronger inflammation in the microenvironment around the cancer cell. This inflammatory state around the cancer cells may cause systemic inflammation, leading to a poor prognosis in ESCC patients. CXCL-8(+)/CXCR-2(+) correlated with T and N

factors and might be accompanied by growth and invasion of cancers. However, by multivariate analysis, CXCL-8(+)/CXCR-2(+) was the only independent prognostic factor for RFS. In disease-specific survival, CXCL-8(+)/CXCR-2(+) was the predictive factor, whereas T and N factors were not through univariate analysis. Our results suggest that the prognosis of ESCC patients that expressed CXCL-8/CXCR-2 may be not affected by chemotherapy or chemoradiotherapy.

We propose to measure easy and simple markers to predict outcome in this complex disease, which incorporates inflammation and carcinoma. Expression of CXCL-8/CXCR-2 in a cancer cell is an important predictive factor for survival prognosis. However, as a "more simple" inflammatory marker, we prefer to use preoperative serum CRP, FDP, and fibrinogen levels that were measured at the routine blood examination. If preoperative serum CRP, FDP, and fibrinogen levels are high, anti-inflammatory treatments may benefit the patient before surgery.

ESCC that expressed CXCL-8/CXCR-2 was not affected by chemotherapy or chemoradiotherapy. Therefore, if preoperative CRP, FDP, and fibrinogen levels are elevated, for instance, preoperative immunonutrition therapy or preoperative steroid administration may be effective therapies, in addition to preoperative chemotherapy or chemoradiotherapy. Spite et al[36] reported that resolvin (omega-3 fatty acids), a component of immunonutrition, normalized the overproduction of proinflammatory cytokines and hyperactivation of neutrophils. It might be possible that preoperative immunonutrition therapy was useful for these complex patients. It was also reported that preoperative steroid administration was safe and effective for inhibition of inflammatory mediators of patients with esophageal cancer.[37] Furthermore, we need to further investigate whether CXCL-8/CXCR-2 was expressed on the esophageal specimens. If the chemokines were expressed, a follow-up study on ESCC patients with expression of CXCL-8/CXCR-2 strictly after esophagectomy, or additional postoperative chemotherapy or chemoradiotherapy may be currently conducted. However, in the future, molecular targeted therapy with blockade of the CXCL-8-CXCR-2 network might be effective in reducing tumor growth and improving the prognosis of ESCC.

Overexpression of both CXCL-8 and CXCR-2 may be a useful marker for predicting the outcome in ESCC patients and, more important, has potential in becoming a critical diagnostic marker for selection of appropriate treatments.

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