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ORIGINAL ARTICLE

Predictive Factors for Anastomotic Leakage after Simultaneous Resection of Synchronous Colorectal Liver Metastasis

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Abstract
Background The optimal surgical strategy for resectable, synchronous, colorectal liver metastases remains unclear. The objective of this study was to determine which patients could benefit from staged resections instead of simultaneous resection by identifying predictive factors for postoperative morbidity and anastomotic leakage after simultaneous resection of synchronous, colorectal liver metastases and the primary colorectal tumor.
Methods This study involved 86 patients with synchronous colorectal liver metastases who underwent simultaneous resection of the primary colorectal tumor and the hepatic tumor. Postoperative mortality, morbidity, and other surgical outcomes, including survival and hospitalization, were assessed. Predictive factors for postoperative morbidity and for anastomotic leakage were evaluated.
Results Postoperative morbidity and anastomotic leakage were found in 55 (64%) and 18 (21%) patients. Predictive factors for postoperative morbidity and for anastomotic leakage were intraoperative blood loss and operation time >8 h, respectively. The overall 5-year survival rate was 45%.
Conclusions The frequency of morbidity and that of anastomotic leakage seemed to be high after simultaneous resection for synchronous colorectal liver metastases, especially when intraoperative blood loss or operation time increased greatly. Staged resections should be considered in cases in which excessive surgical stress from simultaneous resection of synchronous colorectal liver metastases would be expected.

Keywords Colorectal cancer · Hepatic metastasis · Liver metastasis · Morbidity · Anastomotic leakage

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Introduction

For patients with synchronous colorectal liver metastases (SCLM), hepatic resection is considered the best treatment, with reported 5-year survival rates between 23% and 37%.[1–4] Resections of both the primary colorectal lesion and the hepatic metastases are needed for patients with SCLM when they are resectable. However, the optimal surgical strategy for resectable SCLM still remains controversial.

From the perspectives of less operation with less mental stress and simplifying perioperative treatment, simultaneous resection of the primary colorectal and liver tumors is a favorable strategy for patients with SCLM.[5–8] However, several papers reported that the morbidity rate after simultaneous resection of primary and liver tumors was high because of greater surgical stress and a longer

operation time than for single-organ surgery. Staged resection with initial operation for the primary lesion followed by resection of hepatic tumors is regarded as an alternative strategy to avoid excessive surgical stress for patients with SCLM, though the efficacy of this strategy and the patients who could benefit from this strategy are unknown.[4–6,9,10]

Thus, this study was conducted to determine which patients could benefit from staged resections instead of simultaneous resection by identifying predictive factors for postoperative morbidity and anastomotic leakage after simultaneous resection of SCLM.

Patients and Methods

Patient Population

The medical records of all consecutive patients who underwent liver resections for colorectal liver metastases from January 1992 to January 2004 at our institution were analyzed retrospectively, with institutional review board approval. Eighty-six patients had SCLM. During this period, all SCLM patients received simultaneous resection of primary colorectal and hepatic tumors irrespective of the patient's or the tumor's characteristics. Lateral lymph node dissection was routinely performed in patients with advanced lower rectal cancer. All 86 patients underwent contrast enhanced computed tomography (CT) of the chest, abdomen, and pelvis, as well as hepatic MRI, preoperatively.

As a control, the morbidity of 167 patients who underwent hepatectomy for metachronous liver metastasis from colorectal cancer from January 1992 to January 2004 and that of 1,728 patients who underwent only resection for colorectal cancer with colorectal reconstruction during the same period were also reviewed. Of the 1,728 colorectal cancer patients, 1,319 had colon cancer and 409 had rectal cancer.

Postoperative Morbidity

Incidences of the following postoperative complications were analyzed: anastomotic leak, rectovaginal fistula, intraperitoneal or pelvic abscess, wound infection, wound dehiscence, ileus, enteroparesis, postoperative delirium, urinary tract infection, dysuria, empyema thoracis, pleural effusion, atelectasis, cholecystitis, perihepatic or subphrenic abscess, bile leak, liver failure, and others. Anastomotic leakage was defined as follows: peritonitis and a dehiscence in the anastomosis, discharge of pus from the anus, vaginal fistula, or feces from the abdominal drain. Leakage was confirmed by CT scan, contrast enema, re-operation, or digital rectal examination. All complications were graded according to the classification proposed by Clavien et al.[11] Postoperative mortality was defined to include any death during postoperative hospitalization or within 30 days.

Assessment of Predictive Factors for Postoperative Morbidity

Correlations between postoperative morbidity and the following patient, tumor, and surgical factors were analyzed: age, sex, body mass index (BMI), preoperative comorbidity, site of primary tumor, intestinal obstruction by tumor, size of primary tumor, differentiation of tumor, distribution of hepatic tumors, number of hepatic tumors, hepatic tumor size, operative methods, operation time, intraoperative blood loss, and blood transfusion.

Survival

Patients were followed regularly at 3-month intervals with blood testing and CT. Survival and follow-up were calculated from the time of the operation to the date of death or last available follow-up. The survivors' median follow-up time after surgery was 73 months.

Statistical Analysis

Statistical comparisons of baseline data were performed using the chi-square test. Continuous variables were compared with the independent t test. Multivariate analyses to evaluate the independent predictive factors for postoperative complications or anastomotic leakage were done by multiple logistic regression analysis. The survival rate was calculated by the Kaplan–Meier method.[12] A difference was considered significant when p was less than 0.05.

Results

Patients and Operative Details

From 1992 to 2004, 86 patients were treated with simultaneous resection of primary and hepatic tumors for SCLM. There were 37 female and 49 male patients, with a median age of 59 years (range, 40 to 85 years). The site of the primary tumor was colon in 48 and rectum in 38. The primary tumor was staged as T3 in 54 (63%) and T4 in 32 (37%) according to the TNM classification. Metastatic lymph nodes were found in 65 patients (76%). The mean diameter of the primary tumor was 55 mm (range, 26–140 mm).

Liver metastases were solitary in 29 patients and multiple in 57 patients. In 47 patients (55%), the hepatic

tumor showed a unilobar distribution, while a bilobar tumor distribution was observed in 39 (45%). The mean diameter of the hepatic tumor was about 43 mm (range, 5–200 mm). The mean resected liver volume was 380 g (range, 10–1,660 g).

The operation for primary colorectal cancer was right (hemi) colectomy in 17 patients, transverse colectomy in 1, left (hemi) colectomy in 4, sigmoidectomy in 24, high anterior resection in 7, low anterior resection in 20, very low anterior resection in 6, inter-sphincteric resection in 2, Hartmann's operation in 1, and abdomino-perineal resection in 4 (Table 4). A diverting stoma to prevent anastomotic leakage was made in 22 (26%) patients at the surgeon's discretion, and lateral lymph node dissection was performed in 20 (23%). In terms of liver tumor resection, lobectomy was performed in 11 patients, segmentectomy in 22, bisegmentectomy in 1, trisegmentectomy in 2, subsegmentectomy in 3, and partial resection in 47.

Adjuvant therapy was given to only 17 patients (19.8%) because adjuvant chemotherapy for colorectal cancer in stage III or more was performed since January 2003. Neoadjuvant chemoradiation targeting for rectal cancer was given to three patients (3.5%).

Morbidity

No patients died within 30 days of the operation, but 55 (64%) patients developed complications (Table 1). Eighteen patients (21%) experienced leakage, of whom 6 needed urgent re-operation with ileostomy and drainage of an intra-abdominal collection caused by leakage. Postoperative bleeding, wound dehiscence, and ileus were the reasons for the three other re-operation cases. The most frequent complication was wound infection.

The morbidity rate of the 167 patients who underwent hepatectomy for metachronous colorectal liver metastasis during the same period was 19.8%, and that of 1,728 patients who underwent only resection for colorectal cancer was 32.1%. Anastomotic leakage occurred in 123 (7.1%) of the aforementioned 1,728 patients.

Factors Affecting Complications, Especially Anastomotic Leakage

Postoperative complications were significantly correlated with presence of diverting stoma ($p<0.01$), duration of operation greater than 8 h ($p<0.01$), amount of intraoperative blood loss ($p<0.01$), and intraoperative blood transfusion ($p<0.01$). The aforementioned factors were entered into multivariate analysis. Only a greater amount of blood loss had a predictive value for increased occurrence of postoperative complications.

Then, the correlations between anastomotic leakage and clinicopathological factors were examined to identify risk factors for anastomotic leakage after simultaneous resection for SCLM. Patients who underwent abdomino-perineal

Table 1 Postoperative complications after simultaneous resection for SCLM according to Clavien grade

Complications	No. of patients	Gr I	Gr II	Gr IIIa	Gr IIIb	Gr IVa
Colon and rectum						
Anastomotic leakage	18 (21%)		12		6	
Intrapelvic abscess	6 (7%)	1	4		1	
Intraperitoneal abscess	5 (6%)	1	0	3	1	
Rectovaginal fistula	4 (5%)		1		3	
Liver						
Bile leakage	7 (8%)	6	1			
Hepatic abscess	7 (8%)		5	1	1	
Liver failure	3 (3%)	1	1			1
Postoperative bleeding	1 (1%)				1	
Other organs						
Wound infection	25 (29%)	23	2			
Pleural effusion	12 (14%)	1		11		
Wound dehiscence	6 (7%)	3	2		1	
Enteroparesis	5 (6%)	5				
Postoperative delirium	4 (5%)	1	3			
Dysuria	4 (5%)		4			
Urinary tract infection	3 (3%)		3			
Pneumonia	2 (2%)		2			
Others	7 (8%)	1	4		2	

resection (n=4) or Hartmann's operation (n=1) were excluded from the analysis. Anastomotic leakage was significantly correlated with lateral lymph node dissection (p<0.01), primary site of rectum (p=0.01), duration of operation greater than 8 h (p<0.01), and amount of intraoperative blood loss (p=0.02). Neither serum levels of TP and ALB, steroid usage, nor neoadjuvant therapy showed correlation with occurrence of anastomotic leakage (data not shown). Multivariate analyses revealed operation time greater than 8 h (p<0.01) as the only independent predictive factor for anastomotic leakage after simultaneous resection of SCLM (Table 2). Extent of hepatectomy, timing of anastomosis and hepatectomy, and usage of Pringle maneuver did not correlate with occurrence of complication or anastomotic leakage.

Table 3 showed the rates of complication ≥ IIIa and anastomotic leakage according to operative procedures of the primary and hepatic resections which were performed in the same patient. Complication ≥ IIIa and anastomotic leakage were more frequently observed in patients with rectal resection; however, extent of hepatectomy did not seem to affect occurrence of complication ≥ IIIa or anastomotic leakage.

Hospitalization was significantly longer in the 55 patients with postoperative morbidity (32.2 days) than in the 31 patients without postoperative morbidity (17.6 days) (p<0.01). In addition, hospitalization was significantly longer in the 18 patients with anastomotic leakage (43.5 days) than in the 63 patients without anastomotic leakage (22.2 days) (p<0.01).

Survival

The overall survival rate after simultaneous resection for SCLM of the 86 patients was 61% at 3 years and 45% at 5 years, with MST of 47 months.

Discussion

For patients with resectable SCLM, both primary tumor resection and hepatectomy for liver metastasis could lead to long-term survival, with a 5-year survival rate of 23–37%. However, the optimal strategy, including surgical resection and perioperative treatment, remains controversial for resectable SCLM. In terms of surgical resection for SCLM, it has not been resolved whether simultaneous resection or staged resections would be preferable.

There are several rationales for simultaneous resection of SCLM. In simultaneous resection, the treatment strategy would become simpler. In the staged resections, a series of neoadjuvant chemotherapy or chemoradiotherapy, resection of primary tumor, chemotherapy between two operations, hepatectomy, and adjuvant chemotherapy could be the maximal total treatment for SCLM, while simultaneous resection could simplify and shorten the treatment schedule by eliminating one operation. Completion of the two resections and initiation of adjuvant chemotherapy occur earlier with simultaneous resection than with staged resections. Considering survival, comparable survival for simultaneous resection was shown in comparison with that for staged resections.[13] Furthermore, simultaneous resection could relieve patients from a considerable degree of mental and physical stress and decrease total treatment cost by preventing a second resection for hepatic metastases. Recent advances in colorectal and hepatic surgery have enabled simultaneous resection to be performed more safely. Martin et al. reported the safety and efficacy of simultaneous resection. By avoiding a second laparotomy, the overall complication rate was reduced, and length of hospital stay was shortened, with no change in operative mortality.[7,8]

However, at present, staged resections with initial resection of the primary tumor followed by hepatic resection have been frequently performed in patients with SCLM for several reasons.[4,5,9,10] First, the perioperative risk of staged resections has been thought to be less than that of simultaneous resection.[4,13,14] Sheele et al. reported 13 anastomotic leakages of 90 simultaneous procedures in their series, and two of them led to death.[4] Thelen et al. proposed the criteria for simultaneous liver resection according to the age and extent of liver resection, because death after simultaneous liver resection (n=4) occurred after major hepatectomies, and three of these four patients were 70 years of age or older.[15] Second, staged resections might offer a chance to evaluate liver or extrahepatic metastases between the two operations. Lambert et al. reported that staged resections of synchronous hepatic metastases with an interval of 3 to 6 months might allow occult disease to become clinically detectable and could potentially identify patients for whom a hepatic resection would offer no survival benefit.[10] Fujita recommended an interval resection to assess the metastatic status of the regional lymph nodes, because the presence of six or more lymph node metastases was an independent poor prognostic factor in patients with resected SCLM and a relative contraindication for hepatic resection.[9] Some authors proposed chemotherapy between primary tumor resection and liver resection to select patients that could benefit from hepatectomy.[13,16] Alternatively, a liver-first approach of doing liver resection first and primary resection second was newly proposed as a strategy for SCLM.[17,18] The liver-first approach might avoid needless radical colorectal surgery by confirming curability of hepatic metastases first and also might increase resectability compared with the ordinary staged resections especially in patients with progressive hepatic metastases.

Table 2 Correlation between anastomotic leakage and clinicopathological factors in patients who underwent simultaneous resection for SCLM

		Leakage (−) (n=63)	Leakage (+) (n=18)	Univariate analysis p value	Multivariate analysis p value, RR (95%CI)
Patient characteristics					
Median age (range) (years)		59 (40–85)	59 (41–73)	0.81	
Male/female		33/30	12/6	0.42	
BMI (mean±SD)		21.9±2.9	22.5±2.2	0.44	
Preoperative comorbidity					
Absent		44	12	0.78	
Present		19	6		
Primary colorectal tumor					
Site	Colon	42	6	0.01	N.S.
	Rectum	21	12		
Stenosis	Absent	56	0	0.34	
	Present	7	18		
Tumor size, mm		52.0	58.0	0.25	
pT stage	pT3	41	9	0.25	
	pT4	22	9		
pN stage	pN0	17	2	0.22	
	pN+	46	16		
Histology	Well, mod	60	15	0.12	
	Poor	3	3		
Liver metastasis					
Distribution	Unilobar	38	9	0.43	
	Bilobar	25	9		
Number of tumors (range)		2.3 (1–8)	2.6 (1–8)	0.57	
Tumor size, mm		47	33	0.06	
Operative factors					
Lateral lymph node dissection					
Absent		55	10	<0.01	N.S.
Present		8	8		
Diverting stoma					
Absent		48	11	0.24	
Present		15	7		
Liver resection					
Partial Hx, segmentectomy		51	16	0.72	
≥Lobectomy		12	2		
Timing of anastomosis					
Colectomy→anastomosis→Hx		20	4	0.20	
Colectomy→Hx→anastomosis		7	5		
Hx→colectomy→anastomosis		36	9		
Pringle maneuver					
Absent		10	1	0.44	
Present		53	17		
Operation time					
<8 h		53	8	<0.01	<0.01, 6.63 (2.09–20.9)
≥8 h		10	10		
Blood loss, g (range)		1,345 (162–6,000)	2,487 (430–6,560)	0.02	N.S.
Transfusion					
Absent		39	9	0.37	
Present		24	9		
Blood transfusion, ml		343	1,212	0.05	

RR relative risk, *CI* confidence interval, *Hx* hepatectomy, *N.S.* non-significant (p>0.05)

Table 3 Rates of complication ≥ Gr IIIa and anastomotic leakage according to the site of primary colorectal resection and extent of hepatectomy

Primary colorectal resection	Hepatectomy	Complication ≥ Gr IIIa	Anastomotic leakage
Colectomy	<Lobectomy	4/40 (10%)	5/39[a] (13%)
	≥Lobectomy	0/7 (0%)	1/7 (14%)
Rectal resection	<Lobectomy	11/32 (34%)	11/28[b] (39%)
	≥Lobectomy	2/7 (29%)	1/7 (14%)

[a] One patient who underwent Hartmann's operation was excluded from the analysis

[b] Four patients who underwent abdomino-perineal resection were excluded from the analysis

This study evaluated morbidity, especially anastomotic leakage, after simultaneous resection for SCLM in order to assess the safety of simultaneous resection. Anastomotic leakage is sometimes fatal and can cause a difficult situation with physical and mental discomfort or pain. The morbidity rate of patients who underwent simultaneous resection for SCLM seemed to be higher than that of patients with resected metachronous colorectal hepatic metastasis or that of patients who underwent only resection for colorectal primary cancer. Predictive factors for postoperative morbidity and for anastomotic leakage were intraoperative blood loss and operation time greater than 8 h, respectively. The overall morbidity rate and the rate of anastomotic leakage were 91% and 50%, respectively, in patients with operation time greater than 8 h, and 54% and 13%, respectively, in patients with operation time less than or equal to 8 h. Blood loss and operation time usually represent the amount of surgical stress. Excessive surgical stress was possibly correlated with postoperative morbidity. Hospitalization of patients with complications was significantly longer than that of patients without complications. In particular, the average hospitalization of the 18 patients with anastomotic leakage was more than 43 days. Retrospective studies have also indicated that the occurrence of anastomotic leakage is associated with increased morbidity, mortality, and prolonged hospital stay. Additionally, anastomotic leakage may be associated with an increased risk of local recurrence.[19]

Various risk factors for anastomotic leakage have been analyzed by several investigators. Age, sex, obesity, level of anastomosis, smoking, blood transfusion, tumor diameter, preoperative (chemo) radiotherapy, physical status, obstruction, and coronary heart disease have been shown to be significant risk factors for leakage.[20–24] In simultaneous resection for SCLM, not only the factors related to the tumor, the patient, or the colorectal operation, but factors related to the hepatectomy could affect the occurrence of anastomotic leakage. However, the extent of hepatic resection, sequence of colectomy, hepatectomy, anastomosis, use of the Pringle maneuver, and total time of the Pringle maneuver were not predictive factors for anastomotic leakage or postoperative complications in patients with resected SCLM.

Recently, a diverting stoma has been often used to prevent anastomotic leakage in patients who undergo low anterior resection by diverting the fecal stream and keeping the anastomosis free of material.[19,25,26] In this study, the presence of a diverting stoma was not a predictive factor for absence of postoperative anastomotic leakage. However, the analysis estimating efficacy of a diverting stoma in this study was not accurate, because a diverting stoma was basically used in patients whose risk for anastomotic leakage was considered to be high by the surgeons. The site of primary tumor that has been reported as a strong predictive factor in previous studies was not a predictive factor for anastomotic leakage in this series. Use of diverting stoma might affect the result of analyses of predictive factors for anastomotic leakage. A randomized, controlled trial is needed to elucidate the efficacy of a temporary diverting stoma.

Although several rationales for the simultaneous resection for SCLM are clear, staged resections should be selected to prevent anastomotic leakage or serious complications when the scheduled operation would result in considerable surgical stress, i.e., predicted operation time greater than 8 h according to the results of the present study. Predicted operation time should be calculated by considering various factors, such as characteristics of the patient, primary and metastatic tumor, extent of operation, difficulty of the procedure, and so on. Based on the results of this study, we now select staged resections when operation time is expected to be greater than 8 h; otherwise, we select simultaneous resection. A prospective study of SCLM to evaluate the efficacy and safety of the operation time-based decision model is in progress.

Currently, adjuvant chemotherapy is one of the key factors which could affect prognosis. Then, comparison of ratio of patients who could receive adjuvant chemotherapy will be essential when comparing the efficacy of simultaneous resection and that of staged resections in a future study of SCLM. Furthermore, in staged resections, there is a risk that some patients could not undergo a second resection after the first resection due to tumor progression or complication of first surgery. Resection rate of patients who could undergo both primary and hepatic resections

should be assessed when comparing simultaneous resection and staged resections in SCLM.

The limitations of our study are its retrospective design and the relatively small number of patients studied.

Conclusion

The morbidity rate and the frequency of anastomotic leakage were high with simultaneous resection for SCLM, especially in patients with greater intraoperative blood loss or operation time greater than 8 h. For patients with SCLM, staged resections should be considered when simultaneous resection would involve excessive surgical stress.

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Original article

Treatment outcome for systemic chemotherapy for recurrent pancreatic cancer after postoperative adjuvant chemotherapy

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ABSTRACT

Objectives: A global consensus on how to treat recurrent pancreatic cancer after adjuvant chemotherapy with gemcitabine (ADJ-GEM) does not exist.
Methods: We retrospectively reviewed the clinical data of 41 patients with recurrences who were subsequently treated with chemotherapy.
Results: The patients were divided into two groups according to the time until recurrence after the completion of ADJ-GEM (ADJ-Rec): patients with an ADJ-Rec < 6 months ($n = 25$) and those with an ADJ-Rec ≥ 6 months ($n = 16$). The disease control rate, the progression-free survival after treatment for recurrence and the overall survival after recurrence for these two groups were 68 and 94% ($P = 0.066$), 5.5 and 8.2 months ($P = 0.186$), and 13.7 and 19.8 months ($P = 0.009$), respectively. Furthermore, we divided the patients with an ADJ-Rec < 6 months into two groups: patients treated with gemcitabine ($n = 6$) and those treated with alternative regimens including fluoropyrimidine-containing regimens ($n = 19$) for recurrent disease. Patients treated with the alternative regimens had a better outcome than those treated with gemcitabine.
Conclusions: Fluoropyrimidine-containing regimens may be a reasonable strategy for recurrent disease after ADJ-GEM and an ADJ-Rec < 6 months.

1. Introduction

Pancreatic cancer patients have an extremely poor prognosis. Although surgical resection is the only curative treatment, only 15%–20% of patients are candidates for resection. Even if a curative resection is performed, the 5-year-survival rate is only 10%–25%, and the median survival period is 11–20 months [1,2].

Various adjuvant chemotherapy or chemoradiotherapy regimens after surgical resection have been evaluated [2–6]. Recently, The Charite' Onkologie (CONKO)-001 trial was designed to determine the benefits of gemcitabine for patients with resected pancreatic cancer. Adjuvant chemotherapy with gemcitabine (ADJ-GEM) significantly improved the disease-free survival period, compared with surgery alone, in patients with resected pancreatic cancer. Although no significant difference in overall survival was seen at the time of publication, analysis after a longer follow-up period demonstrated a survival advantage for gemcitabine over observation-only (median progression-free survival, 22.8 months for ADJ-GEM vs. 20.2 months for observation-only; $P = 0.005$). At approximately the same time as the CONKO-001 trial, the Japanese Study Group of Adjuvant Therapy for Pancreatic Cancer (JSAP) conducted a randomized clinical trial evaluating adjuvant gemcitabine. Although no significant difference in overall survival was seen, the patients in the gemcitabine arm demonstrated a significantly longer disease-free survival period than the patients in the observation-only arm. These results were similar to those of the CONKO-001 trial and supported the concept that adjuvant chemotherapy using gemcitabine was effective in an Asian

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population [2,5]. Therefore, adjuvant therapy using gemcitabine for resected pancreatic cancer is now firmly established as a therapy that offers a modest but real improvement in overall survival [5,7].

In approximately 50% of patients, recurrent disease was reportedly seen within a year, even after receiving ADJ-GEM [5], and no global consensus exists regarding treatment strategies for recurrent disease after ADJ-GEM. If the length of time from the completion of adjuvant therapy until the detection of recurrence is less than 6 months, the NCCN guidelines recommend alternative chemotherapy using a fluoropyrimidine-based chemotherapy regimen. When this period is 6 months or greater, they recommend an alternative regimen or the same regimen as the previous therapy [8]. However, these recommendations have not been substantiated by actual clinical data.

In Japan, the oral fluoropyrimidine derivative S-1 is often used as an alternative regimen for gemcitabine-refractory cases. S-1 showed a non-inferiority to gemcitabine in terms of overall survival in a phase III trial and is considered an alternative to gemcitabine for chemonaïve patients with advanced pancreatic cancer [9]. Additionally, in gemcitabine-refractory metastatic cases, a recent phase II study of S-1 yielded results that demonstrated preferable activity, including a response rate of 9.5%–15% and a median overall survival time of 4.5–6.3 months [10,11]. Therefore, S-1 is widely used for the treatment of advanced pancreatic cancer in first-line and second-line settings in Japan.

We studied the current status of treatments for recurrent pancreatic cancer after curative resection followed by ADJ-GEM. The objective of this study was to examine the adequacy of the

Table 1
Patient characteristics at resection ($n = 41$).

		n (%)			
	Variables	All patients $n = 41$	ADJ-Rec < 6 months $n = 25$	ADJ-Rec ≥ 6 months $n = 16$	*P* value
Age (years)	Median (range)	65 (38–78)	64 (38–78)	65 (50–77)	0.96
Gender	Male	27 (66)	16 (64)	11 (69)	1.00
	Female	14 (34)	9 (36)	5 (31)	
PS[a] at recurrence	0	30 (73)	20 (80)	10 (63)	0.34
	1	5 (12)	3 (12)	2 (12)	
	Unknown	6 (15)	2 (8)	4 (25)	
Primary site	Head	26 (63)	17 (68)	9 (56)	0.51
	Body or -tail	15 (37)	8 (32)	7 (44)	
Type of Resection	PD[b]	26 (64)	17 (68)	9 (56)	0.66
	DP[c]	12 (29)	6 (24)	6 (38)	
	TP[d]	3 (7)	2 (8)	1 (6)	
Resection status	R0	36 (88)	22 (88)	14 (88)	1.00
	R1	5 (12)	3 (12)	2 (12)	
Histology	Adenocarcinoma	39 (95)	23 (92)	16 (100)	0.51
	Adenosquamous carcinoma	2 (5)	2 (8)	0 (0)	
Stage[e] at resection	IIA	5 (12)	0 (0)	5 (31)	0.006
	IIB	36 (88)	25 (100)	11 (69)	
CEA[f] (ng/mL)	Median (range)	2.7 (0.7–51.8)	2.7 (0.7–21.0)	2.4 (1.2–51.8)	0.98
CA19-9[g] (U/mL)	Median (range)	202 (0.5–6450)	212 (0.5–6450)	138 (17–3203)	0.56
Histological grade	Well	5 (12)	3 (12)	2 (12.5)	0.83
	Moderately	28 (71)	17 (68)	12 (75)	
	Poorly	7 (17)	5 (20)	2 (12.5)	
Lymph node ratio[h]	0	5 (12)	0 (0)	5 (31)	0.008
	0.1–0.199	23 (56)	14 (56)	9 (57)	
	0.2–0.299	8 (20)	7 (28)	1 (6)	
	0.3–	4 (10)	4 (16)	0 (0)	
	Unknown	1 (2)	0 (0)	1 (6)	
Recurrent pattern[i]	Locoregional	21 (51)	10 (40)	11 (69)	0.15
	Liver	18 (44)	14 (56)	4 (25)	
	Peritoneum	4 (10)	4 (16)	0 (0)	
	Lungs	11 (27)	7 (28)	4 (25)	
	Bones	1 (2)	1 (4)	0 (0)	
Cycles of ADJ-GEM	Median (range)	6 (3–9)	6 (3–6)	6 (3–9)	0.88
ADJ-Rec[j] (months)	Median (range)	3.7(0.1–36.1)	1.3 (0.1–4.9)	11.5 (6.3–36.1)	
Chemotherapy[k]	GEM	21 (51)	6 (24)	15 (94)	0.00
	Alternatives[l]	20 (49)	19 (76)	1 (6)	
	(S1)	17 (41)	17 (68)	1 (6)	
	(GEM + S1)	1 (2)	0 (0)	0 (0)	
	(S1 + Radiation)	1 (2)	1 (4)	0 (0)	
	(S1 + oxaliplatin)	1 (2)	1 (4)	0 (0)	

[a] PS, performance status.
[b] PD, pancreaticoduodenectomy.
[c] DP, distal pancreatectomy.
[d] TP, total pancreatectomy.
[e] Stage, UICC 7th.
[f] CEA, carcinoembryonic antigen at resection.
[g] CA-19-9, carbohydrate antigen 19-9 at resection.
[h] Lymph node ratio, number of metastatic lymph nodes divided by number of examined nodes.
[i] Recurrent pattern, numbers of locoregional, extra-pancreatic, and combined recurrences were 11, 20, and 10 patients.
[j] ADJ-Rec, period between the last date of ADJ-GEM and recurrence.
[k] Chemotherapy, chemotherapy for recurrent disease after adjuvant chemotherapy.
[l] Alternatives, all alternative regimens consisted of fluoropyrimidine-containing regimens.

NCCN guidelines for recurrent pancreatic cancer after adjuvant chemotherapy, which recommend that the treatment options should be determined by the period between the last date of ADJ-GEM and recurrence (ADJ-Rec), with a threshold of 6 months.

2. Patients and methods

2.1. Patients

A retrospective review was conducted for 113 pancreatic cancer patients who underwent curative resection followed by ADJ-GEM at the National Cancer Center Hospital (NCCH) and NCCH East in Japan between April 2002 and October 2010. Forty-two patients with no recurrence after ADJ-GEM, 10 patients with withdrawal from ADJ-GEM within 2 cycles, 6 patients with recurrence during ADJ-GEM, and 14 patients who changed hospitals after recurrence were excluded. We finally retrieved the clinical data of 41 patients with recurrences who were subsequently treated with chemotherapy at our hospitals.

2.2. Treatment

After resection, we started ADJ-GEM within 10 weeks. An initial gemcitabine dose of 1000 mg/m^2 was administrated intravenously for 30 min on days 1, 8 and 15 every 4 weeks for 3 to 6 cycles, in principle. A computed tomography examination was performed every 3–6 months. Once evidence of recurrence was revealed, treatment for recurrent disease was initiated.

2.3. Data collection and evaluation of tumor response

The following data were collected from the medical records: patient characteristics at resection, the resection status, the ADJ-Rec, the treatment regimen, and the outcome of treatment after the recurrence. We also compared the treatment outcomes according to the length of the ADJ-Rec and the treatment regimens. Tumor responses were evaluated according to the RECIST criteria, Ver.1.1. We evaluated the best overall response and the disease control rate (DCR). The DCR was defined as the rate of complete response + partial response + stable disease. When the disease status was stably maintained for more than 8 weeks, the patient was considered to have stable disease.

2.4. Statistical analysis

The Fisher exact test was used to assess the hypothesis of independence between categorical variables. For quantitative data such as age and the carcinoembryonic antigen (CEA) and carbohydrate antigen 19-9 (CA19-9) levels, we used the Mann–Whiney test. ADJ-Rec was defined as the period between the last date of the administration of ADJ-GEM and the date on which local or distant recurrence was noted. The date of recurrence was defined as the date of documentation of recurrent disease using diagnostic imaging techniques. Progression-free survival (PFS) was defined as the period between the start of treatment for recurrent disease and the date of progression, the last follow-up visit, or death from any cause. Overall survival after recurrence (r-OS) was defined as the period between the start of treatment for recurrent disease and death from any cause or the last follow-up. Patients who were lost to follow-up were treated as censored cases. Survival curves were estimated using the Kaplan–Meier method, and the significances were evaluated using a log-rank test. All the analyses were performed using Stata/SE, Version 11.1 (StataCorp, USA).

3. Results

3.1. Patient characteristics

The characteristics at resection of the 41 eligible patients are listed in Table 1. R0 resection (complete resection with no microscopic residual tumor) was performed in 36 patients (88%). Concerning the pathological stage, 5 (12%) of the patients had stage IIA disease and 36 (88%) had stage IIB. The sites of recurrence were locoregional (21 patients), the liver (18 patients), and the lung (11 patients). Patients with an ADJ-Rec $\geq$ 6 months (16 patients) had a significantly better status than patients with an ADJ-Rec < 6 months (25 patients) with regard to disease stage ($P = 0.006$) and the lymph node ratio (the number of metastatic lymph nodes divided by the number of examined nodes) ($P = 0.0075$). As for the treatments for recurrent disease, 21 patients were treated with gemcitabine monotherapy and 20 patients were treated with alternative regimens. All the alternative regimens were fluoropyrimidine-containing regimens (17 patients received S-1 and 1 patient each received GEM + S-1, S-1 + radiation, and S-1 + oxaliplatin). The treatment strategy after recurrence depended on each oncologist's plan, without a unified policy. Among the 25 patients with an ADJ-Rec < 6 months, 6 were treated with gemcitabine monotherapy and 19 were treated with alternative regimens. Among the 16 patients with an ADJ-Rec $\geq$ 6 months, 15 were treated with gemcitabine monotherapy and 1 was treated with an alternative regimen.

3.2. Treatment efficacy and survival analysis of treatments for recurrence

Overall, 2 of the 41 patients responded to the treatments for recurrent disease (4.9%; 2 partial responses; 95% confidence interval (95% CI), 0.60%–16.53%). The DCR was 78% (32 of the 41 patients; 95% CI, 62.39%–89.44%). The median PFS and median r-OS were 5.5 months (95% CI, 3.7–8.1 months) and 18.3 months (95% CI, 13–19.8 months), respectively (Fig. 1).

We divided the patients into two groups according to the length of the ADJ-Rec: patients with an ADJ-Rec < 6 months ($n = 25$), and patients with an ADJ-Rec $\geq$ 6 months ($n = 16$). The DCRs were 68% and 94% ($P = 0.066$), and the median PFS periods were 5.5 and 8.2 months ($P = 0.186$; Fig. 2A), respectively. The median r-OS of the patients with an ADJ-Rec < 6 months was significantly shorter than

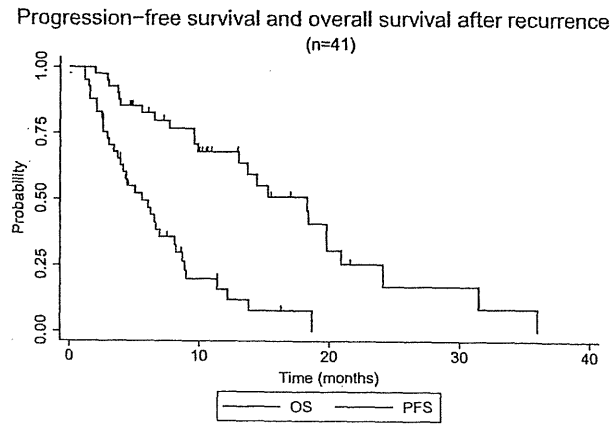

Fig. 1. Progression-free survival (PFS) and overall survival after recurrence (r-OS) in all patients ($n = 41$). The median PFS and r-OS were 5.5 and 18.3 months, respectively.

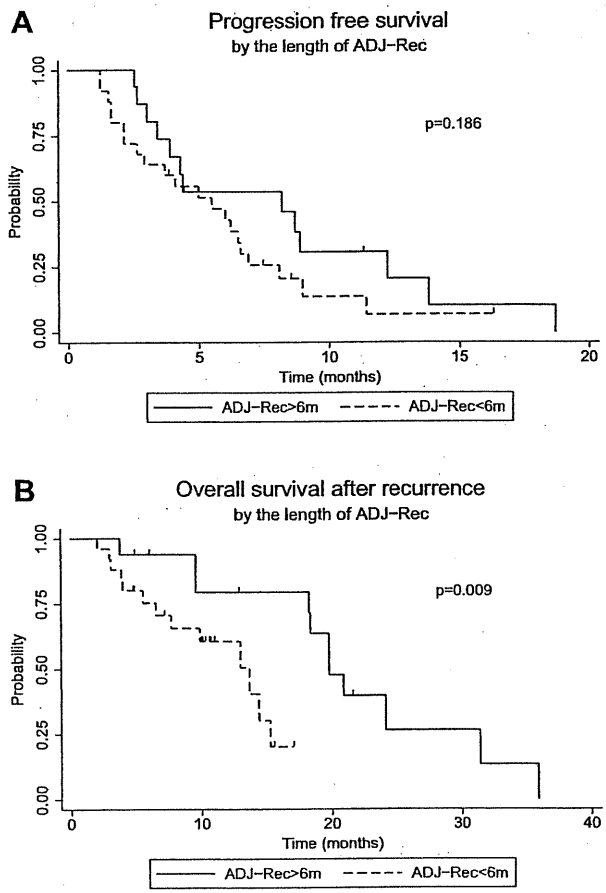

Fig. 2. Progression-free survival (PFS) and overall survival after recurrence (r-OS) according to the length of the ADJ-Rec: patients with an ADJ-Rec < 6 months ($n = 25$), and patients with an ADJ-Rec ≥ 6 months ($n = 16$). (A) The median PFS for each group was 5.5 and 8.2 months ($P = 0.186$), respectively. (B) The median r-OS was 13.7 and 19.8 months ($P = 0.009$), respectively.

that of the patients with an ADJ-Rec ≥ 6 months (13.7 and 19.8 months, $P = 0.009$; Fig. 2B).

Additionally, we divided the patients with an ADJ-Rec < 6 months into two groups according to the treatment regimens for recurrent disease: patients treated with gemcitabine ($n = 6$) and patients treated with alternative regimens ($n = 19$). The outcomes are shown in Table 2 and Fig. 3. For the patients treated with gemcitabine and those treated with alternative regimens, the DCR, median PFS and median r-OS were 67% and 68% ($P = 0.651$), 2.9 and 6.5 months ($P = 0.065$; Fig. 3A), and 7.7 and 13.0 months ($P = 0.242$; Fig. 3B), respectively.

4. Discussion

In this study, at first we examined the current status of the treatment strategy for pancreatic cancer patients with recurrence after adjuvant chemotherapy. Most patients with ADJ-Rec ≥ 6 months were placed on gemcitabine. Even for patients with an ADJ-Rec < 6 months, gemcitabine was resumed in 24% of these patients. Generally, patients who relapse within a short period after receiving adjuvant chemotherapy should be considered as being resistant to those drugs. The NCCN guidelines also recommend that the options for recurrent disease after adjuvant therapy should be assessed according to the ADJ-Rec. However, these guidelines are only the recommendation of the panel, and these strategies have not yet been substantiated by actual clinical data. In the case of ovarian cancer, a consensus based on actual clinical data exists with regard to the treatment strategy for relapsed disease. Patients who have relapsed within an interval of less than 6 months since the previous paclitaxel-plus-platinum chemotherapy should be considered as platinum resistant [12,13]. However, the chemosensitivity and the key drugs are quite different between pancreatic cancer and ovarian cancer. Therefore, actual clinical data for pancreatic cancer is needed.

The outcome of patients with a short ADJ-Rec was worse than that of the patients with a long ADJ-Rec. This finding suggests that patients with a long ADJ-Rec may owe their period of prolonged sensitivity to the adjuvant gemcitabine treatment, slow tumor growth, and a smaller quantity of residual tumor. Concerning advanced pancreatic cancer, similar findings have been reported in a previous study, which indicated that the progression-free survival period after first-line chemotherapy was an independent prognostic factor [14]. Additionally, patients with pathological stage IIA or a lymph node ratio of 0 had a long ADJ-Rec in the present study, possibly influencing the outcome. However, our results should be interpreted with caution because biases introduced by the different selection of treatment regimens between the two groups may exist.

Among the patients with an ADJ-Rec ≥ 6 months, we were unable to compare the treatment outcome according to regimens, since most of them (15 out of 16) received gemcitabine monotherapy and seldom received alternative options such a fluoropyrimidine-based regimens. In the present study, the patients treated with gemcitabine had a better DCR, PFS and r-OS than the metastatic or recurrent pancreatic cancer patients treated with gemcitabine in past studies [15,16]. Even after considering the possibility that an ADJ-Rec ≥ 6 months may be a good prognostic factor, these preferable outcomes suggest the appropriateness of a re-challenge with gemcitabine.

Among the patients with an ADJ-Rec < 6 months, patients receiving alternative regimens tended to have a better DCR, PFS,

Table 2
Outcomes of patients according to ADJ-Rec and treatment regimens.

	<6 months				≥6 months			
ADJ-Rec	All	GEM	Alternative	*P* value	All	GEM	Alternative	*P* value
n	25	6	19		16	15	1	
DCR (%)	68	67	68	1.00	94	93	(100)	1.00
95% CI	62.4–89.4	22.3–95.7	43.5–87.4		69.8–99.8	68.1–99.8	2.5–100	
Median PFS (m)	5.5	2.9	6.5	0.06	8.2	8.2	(12.2)	0.69
95% CI	2.6–6.6	1.5–	2.1–8.1		3.4–12.2	3.0–13.8		
Median r-OS(m)	13.7	7.7	13.0	0.24	19.8	20.9	(19.8)	0.67
95% CI	6.5–15.3	2.9–	6.5–		9.6–31.4	9.6–31.4		

ADJ-Rec, period between the last date of ADJ-GEM and recurrence; DCR, disease control rate; PFS, progression-free survival time; r-OS, survival time from recurrence; Alternative*, including S-1, GEM + S-1, S-1 + radiation, and S-1 + oxaliplatin.

and r-OS than those receiving gemcitabine monotherapy. Although the optimal ADJ-Rec threshold was not clarified, the present results support the recommendations of the NCCN guidelines, which recommend alternative regimens for patients with an ADJ-Rec < 6 months after previous treatment with gemcitabine. These findings suggest that a certain proportion of patients with a short ADJ-Rec may already have a gemcitabine-refractory status at the time of ADJ-GEM.

This study had some limitations. This study was a retrospective analysis with an insufficient sample size, and the treatment strategy after recurrence depended on each oncologist's plan, with no unified policy. Another limitation concerns the alternative treatment options after recurrence. The NCCN guidelines recommend alternative regimens as second-line therapies for metastatic disease. The recommended regimens consist of fluoropyrimidine-based therapies, such as 5-FU/leucovorin (LV)/oxaliplatin (Oxal) [17] or capecitabine/Oxal [18]. The CONKO-003 study revealed the survival advantage of 5-FU + LV + Oxal for gemcitabine-refractory pancreatic cancer. In Japan, these drugs have not yet been approved under the Japanese medical insurance system for the treatment of pancreatic cancer. S-1 monotherapy was mainly used as the alternative option in our study. Although S-1 demonstrated a non-inferiority to gemcitabine as a first-line treatment [8,9] and had a marginal activity as a second-line regimen for gemcitabine-refractory pancreatic cancer [10,11], it has not been accepted as a global standard therapy for gemcitabine-refractory pancreatic cancer.

In conclusion, patients with an ADJ-Rec ≥ 6 months had a relatively favorable outcome when treated with a gemcitabine rechallenge. Among the patients with an ADJ-Rec < 6 months, those patients receiving alternative regimens tended to have a better DCR, PFS, and r-OS, compared with those receiving gemcitabine. As a result, our results did not deny the appropriateness of strategies outline in the NCCN guidelines. A well-designed prospective study with a sufficient sample size is needed to identify the optimal regimen for the treatment of recurrent pancreatic cancer after postoperative adjuvant chemotherapy.

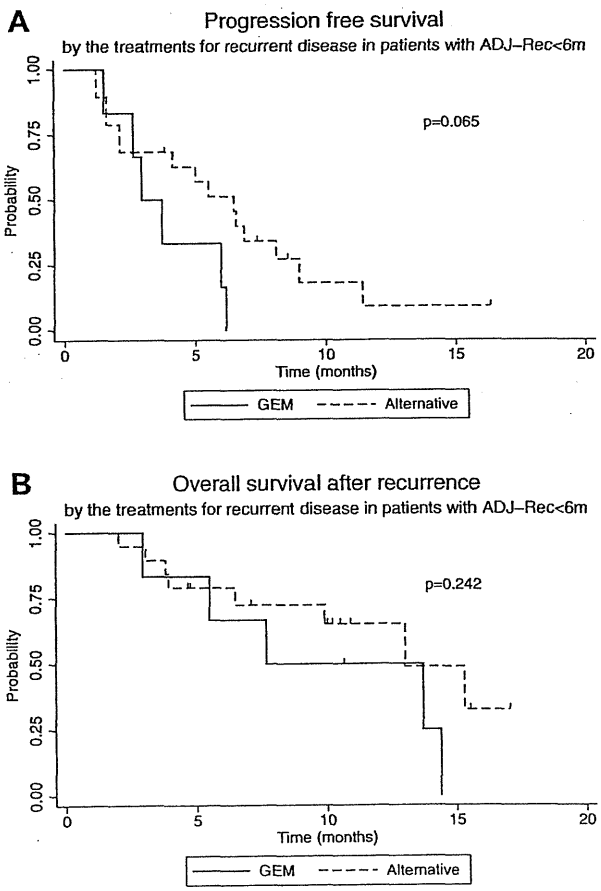

Fig. 3. Progression-free survival (PFS) and overall survival after recurrence (r-OS) according to treatments for recurrent disease in patients with an ADJ-Rec < 6 months: patients treated with gemcitabine ($n = 6$), and patients treated with alternative regimens ($n = 19$). (A) The median PFS for each group was 2.9 and 6.5 months ($P = 0.065$), respectively. (B) The median r-OS was 7.7 and 13.0 months ($P = 0.242$), respectively.

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None declared.

Conflict of interest

Takuji Okusaka had research findings and honoraria to disclose from Taiho pharmaceutical co. and Eli Lilly Japan.

Hideki Ueno had honoraria to disclose from Taiho pharmaceutical co. and Eli Lilly Japan, and had a consultation or advisory relationship to disclose from Taiho phatmaceutical co.

Tomoo Kosuge had honoraria to disclose from Taiho pharmaceutical co. and Eli Lilly Japan.

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Impact of tumor-associated macrophages on invasive ductal carcinoma of the pancreas head

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Tumor-associated macrophages (TAMs) are candidate histological factors in invasive ductal carcinoma (IDC) of the pancreas. Tumor-associated macrophages can be affected by cancer-related inflammation and pancreatitis and interact with important invasive behavior in a recurrent manner in pancreatic IDC. These features may help elucidate the aggressiveness of pancreatic IDC. The aim of this study was to characterize TAMs in pancreatic IDC in comparison with chronic pancreatitis (CP) and to reveal TAM-related factors and the clinical impact of TAMs. CD68 (a pan-macrophage marker) and CD204 (an M2 macrophage marker) immunohistochemistry was carried out in pancreas head specimens from 107 IDC cases and 11 CP cases. Immunopositive cell areas were calculated at the periphery and center of the tumor. The distributions of macrophages in IDC and CP and the relationship between TAMs and histological tumor factors, survival, and recurrence were evaluated. Macrophages were more frequently observed in the lesion periphery than the center in IDC and CP. The density of macrophages was elevated in IDC compared to CP. Dense M2 macrophages at the tumor periphery were frequently seen in large tumors and showed an independent impact on overall survival and disease-free time. Early recurrence in the liver or the local manipulated area was associated with high accumulation of peripheral M2 macrophages. More M2 macrophages were seen in IDC than in CP in both the periphery and the center. High numbers of peripheral M2 macrophages were associated with large tumor size, early recurrence in the liver, local recurrence, and shortened survival time in patients with pancreatic IDC. (*Cancer Sci* 2012; 103: 2012–2020)

The prognosis of patients undergoing resection for pancreatic invasive ductal carcinoma (IDC) remains poor.[1–5] Histological studies have been carried out to elucidate the aggressiveness of pancreatic IDC and have revealed prognostic factors including tumor size, lymph node involvement, nerve plexus invasion, positive resected margin, and low tumor grade.[1–8] Tumor-associated macrophages (TAMs) have recently been reported as a candidate factor in poor prognosis.[9]

Macrophages are the most abundant cancer stromal cells involved in the host immune system,[10] and TAMs have been found to play important roles in tumorigenesis, angiogenesis, matrix remodeling, and metastasis.[11–13] Tumor-associated macrophages have a prognostic impact in prostate, breast, and lung cancers, as well as pancreatic IDC.[9,14–16] The heterogeneity of macrophages has been discussed with regard to their different responses to various microenvironmental stimuli. Macrophages are classically activated towards the M1 phenotype by lipopolysaccharide and interferon-γ. M1 macrophages are characterized by high expression of pro-inflammatory cytokines, such as interleukin (IL)-1, IL-6, IL-12, and tumor necrosis factor. Alternatively, macrophages are activated towards the M2 phenotype by IL-4, IL-13, and IL-10. M2 macrophages are characterized by high expression of IL-4 and IL-10 and low expression of IL-12.[12] Recent studies have revealed high CD204 expression in M2 macrophages and have shown that TAMs are polarized to the M2 phenotype.[12,17,18]

The distribution of TAMs was recently evaluated as a prognostic index in various cancers. A high number of TAMs in the peripheral area of the tumor is correlated with poor prognosis in gastric cancer,[19] hepatocellular carcinoma,[20] and non-small-cell lung cancer,[21] although an increased number of TAMs in the invasive front of colon cancer is associated with favorable prognosis.[22] Increased numbers of TAMs in many cancers are linked to reduced patient survival. In pancreatic IDC, high accumulation of TAMs in the periphery of the tumor is correlated with extrapancreatic invasion, lymph vessel invasion, lymph node involvement, and shortened survival time.[9] Tumor-associated macrophages may be a key to elucidating the aggressiveness of pancreatic IDC. Detailed clinicopathological studies should be carried out to estimate the role of TAMs. First, the distribution of macrophages should be compared between mass-forming chronic pancreatitis (CP) and pancreatic IDC. Macrophages accumulate at the inflammatory site and play crucial roles in the diverse phase.[23,24] Pancreatitis is prevalent in pancreatic IDC and CP due to obstruction of the main pancreatic duct.[25] Tumor-associated macrophages in pancreatic IDC can be affected by both pancreatitis and inflammatory mediators from tumor cells; macrophages in CP are affected by pancreatitis only. The comparison of macrophages between pancreatic IDC and CP may provide evidence that tumor cells mainly lead to TAM accumulation in pancreatic IDC. Second, TAM-related tumor factors should be examined in detail. Tumor-associated macrophages are attracted to and retained in avascular and necrotic areas where they are exposed to tumor hypoxia.[26,27] Our previous clinicopathological study showed that tumor necrosis is frequent in large tumors.[7] Tumor size may be associated with TAM accumulation. Identification of the precise TAM-related tumor factors is useful for estimating microenvironmental interactions between TAMs and pancreatic IDC. Third, the impact of TAMs on tumor relapse should be evaluated. The prognostic value of TAMs may indicate that TAMs are predictive markers of recurrence. The impact of TAMs on recurrence will reinforce the clinical significance of TAMs. Finally, multivariate analysis should be carried out to confirm the impact of TAMs on prognosis. The prognostic value of TAMs has only been tested with univariate analysis. Establishment of the prognostic importance needs to show independence among various tumor factors with multivariate analysis.

The aim of this study was to characterize TAMs in pancreatic IDC in comparison with CP and to reveal TAM-related

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Table 1. Characteristics of patients who underwent pancreaticoduodenectomy with curative intent for a pancreatic head tumor

Parameter	Invasive ductal carcinoma	Chronic pancreatitis
Number	107	11
Age (years), median (range)	64.0 (37–82)	52.0 (38–72)
Gender (male/female)	64/44	10/1
CEA (ng/mL), median (range)	3.5 (0.8–60.3)	3 (0.9–15.7)
CA19-9 (U/mL) (median, range)	109.0 (1.0–21400.0)	14.0 (5.0–245.8)
Combined resection (portal vein/inferior vena cava/colon/liver)	51/2/2/2	0
Intraoperative radiotherapy	30	0
Adjuvant chemotherapy	10 (GEM:8, S-1:2)	0
Stage (UICC 6th) (IA/IB/IIA/IIB/III/IV)	0/0/19/79/1/8	

S-1, an oral anti-cancer drug that combines tegafur, a prodrug of fluorouracil, with 5-chloro-2,4-dihydropyrimidine and potassium oxonate in a molar ratio of 1.0:0.4:1.0 (Taiho Pharmaceutical, Tokyo, Japan). CA19-9, carbohydrate antigen 19-9; CEA, carcinoembryonic antigen; GEM, gemcitabine.

factors and the clinical impact of TAMs on tumor relapse and prognosis.

Materials and Methods

Patients. Between September 1992 and December 2007, 116 patients with a pathological diagnosis of pancreatic IDC who underwent a pancreaticoduodenectomy with curative intent at our institution were investigated, because pancreatitis due to obstruction of the main pancreatic duct is evident in the pancreatic head lesions of IDC and CP cases. Three in-hospital deaths, two patients with incomplete follow-up data, two patients who died of non-cancerous causes within 5 years of the pancreaticoduodenectomy (one due to liver cirrhosis and one due to brain infarction), and two patients whose surgical specimens were of poor quality were excluded from the study. The remaining 107 patients were investigated. For the CP cases in this study, 11 patients who underwent pancreaticoduodenectomy during the same period and were pathologically diagnosed with CP were assessed. Chronic pancreatitis was diagnosed according to The Revised Japanese Clinical Diagnostic Criteria for Chronic Pancreatitis.[28] All CP cases showed fibrosis that was distributed primarily in the interlobular spaces, showing a nodular pattern of lobules called cirrhosis due to the disruption of dense interlobular fibrosis or the loss of exocrine parenchyma with irregular fibrosis. All patients signed an institutional review board-approved informed consent form. The median age of the IDC patients was 64.0 years (range, 37–82 years), and 44 were women (41.1%). The median age of the CP patients was 52.0 years (range, 38–72 years), and 1 (9.1%) was a woman (Table 1). None of the 107 IDC patients received neoadjuvant chemotherapy or radiotherapy; 30 received intraoperative radiotherapy,[29] and 10 received adjuvant chemotherapy. Extended lymphadenectomy including regional and peripancreatic lymph node dissection was carried out with pancreaticoduodenectomy, according to the Japanese Classification of Pancreatic Cancer.[30] Combined resection of the portal vein, inferior vena cava, colon, and para-aortic lymph node was carried out for macroscopically curative resection.

To assess initial recurrence of the tumor, follow-up contrast computed tomography was done every 3 months after surgery or earlier if clinically indicated by examination, symptoms, or a rise in tumor markers, such as serum carcinoembryonic antigen and serum carbohydrate antigen 19-9, which were checked every month. If necessary, further examination such as cytology was carried out to diagnose peritoneal dissemination.

Evaluation of clinicopathological features. Clinical characteristics and pathological examination results were retrieved from the clinical records. Lymphatic (ly), venous (v), and intrapancreatic nerve invasion (ne) were classified into four groups according to the definition of the Japan Pancreas Society and were based on the most extensively involved area observed

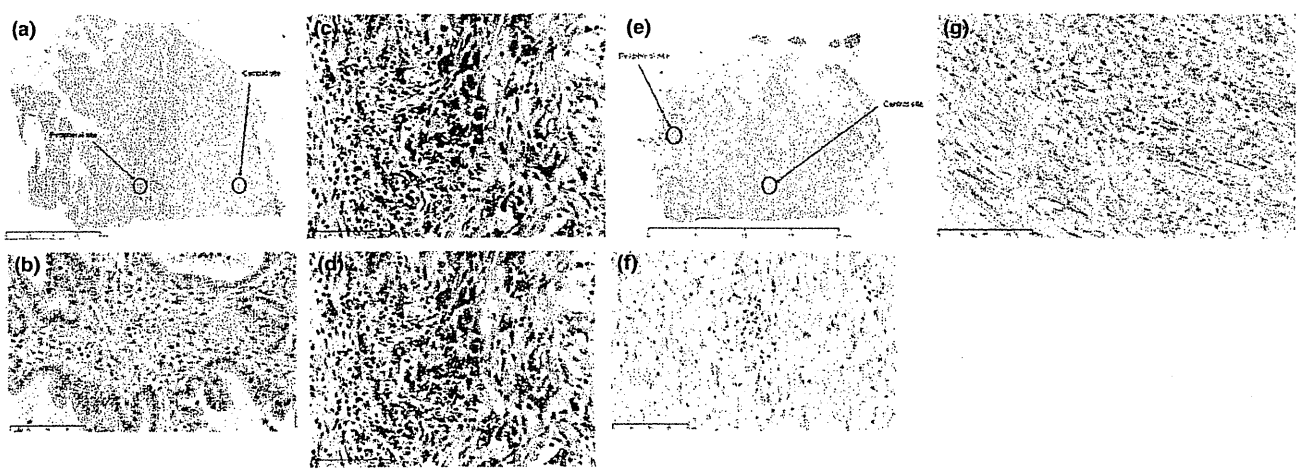

Fig. 1. Objective measurement of the area ratio of immunopositive cells. (a) Using the section showing the maximum diameter of the invasive ductal carcinoma tumor that was stained with anti-CD204, hot spots in the center and the periphery of the tumor were observed at a magnification of ×40. Center (b) and periphery (c) of invasive ductal carcinoma of the pancreas (magnification, ×400). We measured the area of immunopositive cell bodies at this magnification using the Automeasure function of Axio Vision 4.7.1. Axio Vision software visualized the CD204-positive area as red-colored areas (d) and objectively calculated the positive area ratio (summed area of immunopositive cells/measured area). CD204 expression in chronic pancreatitis (CP) tumors was measured using the section with the maximum diameter of the CP tumor (e–g). In the entire image of the CD204-stained CP section (E; magnification, ×40), hot spots of CD204 expression at the central and the peripheral sites of the CP (f, g; magnification ×400) were selected. The positive area ratio of CD204 in the selected image was objectively calculated with Axio Vision software.

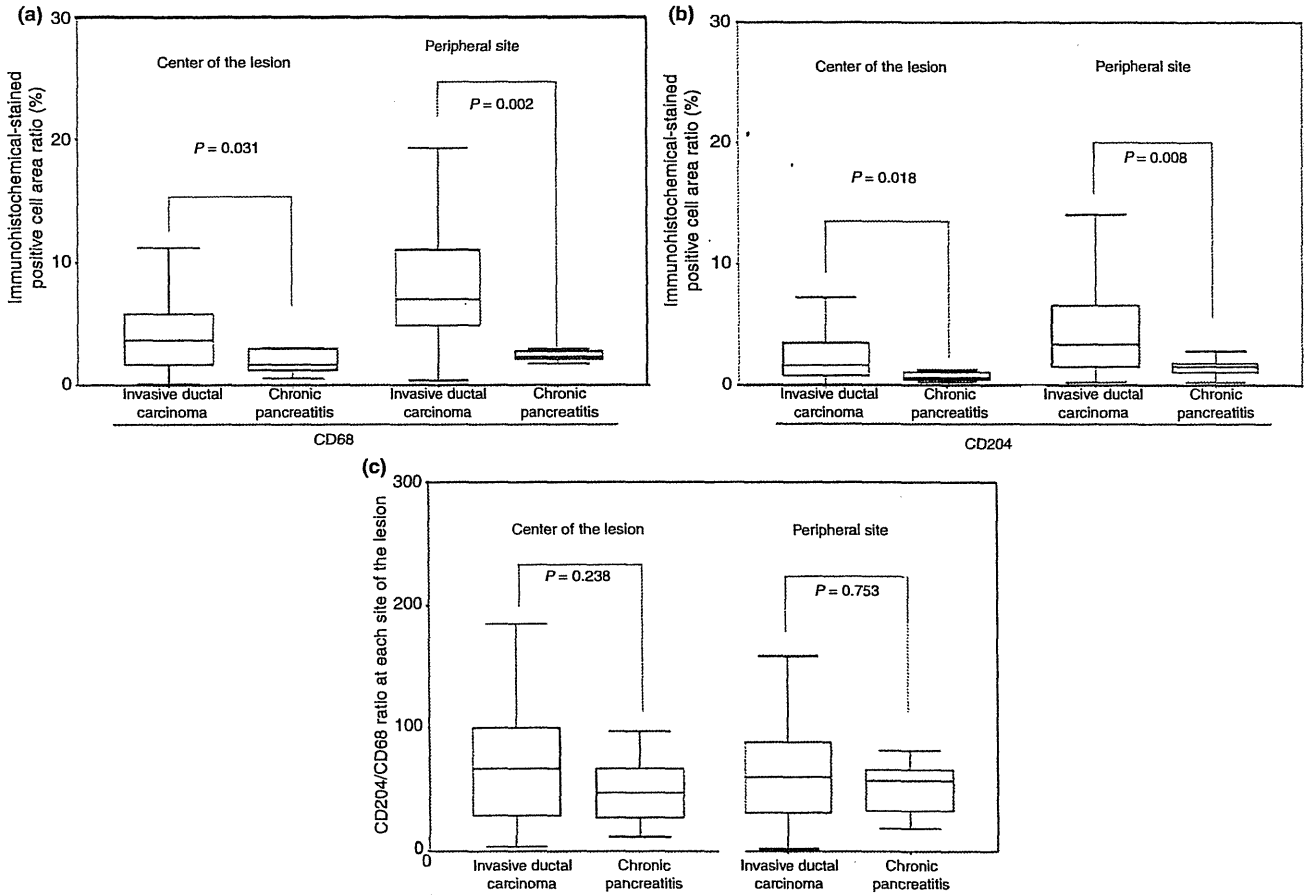

Fig. 2. Distribution of CD68- and CD204-positive cells at the center and periphery of the lesion in invasive ductal carcinoma and chronic pancreatitis. In each site of the lesion and for each immunohistochemical stain, significantly more immunopositive cells were observed in invasive ductal carcinoma than in chronic pancreatitis. (a,b) The CD204/CD68 ratio does not show a significant difference between these two types of lesions (c).

under low-power magnification (×100): 0, no invasion of cancer cells; (i) invasion of a few cancer cells (1–3 points); (ii) moderate invasion of cancer cells (4–8 points); and (iii) marked invasion of multiple cancer cells (>8 points).[30]

The following clinicopathological factors were investigated retrospectively to assess their impact on survival: age (≤64 years *vs* >64 years); sex; serum carcinoembryonic antigen (≤3.5 ng/mL *vs* >3.5 ng/mL), serum carbohydrate antigen 19-9 (≤109 U/mL *vs* >109 U/mL); grade of tumor differentiation (well *vs* moderate or poor); tumor size (≤3 cm *vs* >3 cm); serosal invasion (absent *vs* present); retropancreatic tissue invasion (absent *vs* present); portal vein invasion (absent *vs* present); lymphatic invasion (ly0, 1 *vs* ly2, 3); venous invasion (v0, 1 *vs* v2, 3); intrapancreatic nerve invasion (ne0, 1 *vs* ne2, 3); extrapancreatic nerve plexus invasion (absent *vs* present); and lymph node involvement (absent *vs* present).

Antibodies and immunohistochemistry. Paraffin-embedded blocks of tumor at the maximum diameter were cut into 3-μm serial sections. The sections were deparaffinized in xylene, dehydrated in a graded ethanol series, and immersed in 0.3% hydrogen peroxide in methanol for 15 min to inhibit endogenous peroxidase activity. For antigen retrieval, the slides were heated at 95°C for 15 min in a microwave oven (H2800 Microwave Processor; Energy Beam Sciences, East Granby, CT, USA) in 0.1 M citric acid buffer then allowed to cool for 1 h at room temperature. After washing the slides three times in PBS, non-specific binding was blocked by pre-incubating in 2% normal swine serum in PBS (blocking buffer) for 30 min at room temperature. Individual slides were then incubated overnight at 4°C in mouse anti-human CD68 antibody (1:400 in blocking buffer; Dako, Glostrup, Denmark) or mouse anti-human CD204 antibody (Scavenger Receptor class A-E5, 1:400 in blocking buffer; Transgenic, Kumamoto, Japan). The slides were again washed three times with PBS and incubated with EnVision (Dako) for 1 h at room temperature. After extensive washing with PBS, the color reaction was developed with 2% 3, 3'-diaminobenzidine in 50 mM Tris-buffer (pH 7.6) containing 0.3% hydrogen peroxide. The sections were then counterstained with Mayer's hematoxylin, dehydrated, and mounted.

Definition of center of lesion and peripheral site. To identify the center of the lesion, H&E stained sections were scanned at a magnification of ×40, and the margin of the tumor was marked on each slide. The intersection of the major and minor axes was defined as the center of the lesion, and four fields including the center at a magnification of ×100 were defined as the center of the lesion. Peripheral sites were defined as fields that included cancer cells and adjacent non-cancerous cells at a magnification of ×100. In the pancreatitis cases, the same procedure was used to identify the center and the margin of the dense fibrosing area.

Evaluation of immunohistochemistry (IHC). The IHC-positive cells were quantified by determining the percentage of IHC-positive cells in an area (IHC%) and the IHC-positive cell

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Table 2. Distribution of the percentage of the CD68-positive cell area at the center and periphery of lesions in pancreatic tumors according to clinicopathological features

Parameter	Category	*n*	Central CD68%, median (range)	*P*	Peripheral CD68%, median (range)	*P*
Age (years)	≤64	58	3.75 (0.22–18.60)	0.574	6.25 (0.47–18.70)	0.422
	>64	49	3.63 (0.22–16.40)		7.58 (0.37–25.10)	
Gender	Male	63	3.47 (0.22–18.60)	0.582	6.54 (0.47–25.10)	0.695
	Female	44	3.96 (0.22–17.80)		7.26 (0.37–18.40)	
CEA (ng/mL)	≤3.5	57	3.64 (0.22–11.20)	0.980	6.92 (0.86–25.10)	0.450
	>3.5	50	3.82 (0.22–18.60)		6.76 (0.37–23.20)	
CA19-9 (U/mL)	≤109	53	3.86 (0.22–18.60)	0.815	6.19 (0.47–23.20)	0.108
	>109	54	3.64 (0.42–17.80)		7.50 (0.37–25.20)	
Differentiation	Well	31	3.88 (0.22–0.42)	0.752	7.97 (0.86–23.20)	0.374
	Moderate/Poor	76	3.64 (0.42–18.60)		6.34 (0.37–25.10)	
Tumor size (cm)	≤3.0	66	3.72 (0.22–18.60)	0.414	6.20 (0.69–25.10)	0.526
	>3.0	41	3.25 (0.42–17.80)		7.42 (0.37–18.70)	
Serosal invasion	Absent	84	3.65 (0.22–18.60)	0.554	6.49 (0.37–25.10)	0.451
	Present	23	3.86 (0.22–17.80)		7.42 (0.93–18.40)	
Retroperitoneal invasion	Absent	9	3.86 (0.47–12.10)	0.556	8.39 (0.37–14.30)	0.827
	Present	98	3.65 (0.22–18.60)		6.54 (0.47–25.10)	
Lymphatic invasion	ly0/1	60	3.80 (0.22–18.60)	0.660	6.25 (0.47–23.20)	0.332
	ly2/3	47	3.47 (0.44–17.80)		7.59 (0.37–25.10)	
Vessel invasion	v0/1	10	3.49 (0.64–12.10)	0.822	6.75 (1.56–16.60)	0.756
	v2/3	97	3.65 (0.22–18.60)		6.92 (0.37–25.10)	
Intrapancreatic nerve invasion	ne0/1	27	3.78 (0.22–16.40)	0.917	7.09 (0.93–25.20)	0.346
	ne2/3	80	3.65 (0.22–18.60)		6.92 (0.37–25.10)	
Extrapancreatic nerve	Absent	48	3.72 (0.22–18.60)	0.975	7.06 (0.69–25.10)	0.643
Plexus invasion	Present	59	3.64 (0.22–17.80)		6.22 (0.37–23.20)	
Portal vein invasion	Absent	81	3.56 (0.22–18.60)	0.437	7.37 (0.47–25.10)	0.079
	Present	26	3.92 (0.42–11.00)		5.92 (0.37–15.90)	
Lymph node involvement	Absent	22	1.82 (0.22–11.20)	0.091	5.45 (0.69–19.30)	0.045*
	Present	85	3.78 (0.22–18.60)		7.42 (0.37–25.10)	

*$P < 0.05$. Differences between the two groups were evaluated using the Mann–Whitney *U*-test. CA19-9, carbohydrate antigen 19-9; CEA, carcinoembryonic antigen; ly, lymphatic; ne, intrapancreatic nerve; v, venous.

count, which was generally used to evaluate immunohistochemical staining.

Tumor-associated macrophages identified as CD68- or CD204-positive cells were defined as cells with oval to round nuclei that showed strong membranous/cytoplasmic staining but no nuclear staining. After scanning the immunostained slide at a magnification of ×100, the three areas with the greatest number of macrophages in both the center of the lesion and the peripheral site were selected as hot spots. The Automeasure function in Axio Vision 4.7.1 software (Carl Zeiss, Oberkochen, Germany) was used to distinguish the immunopositive area and to objectively calculate the summed areas of the immunopositive cells in each hot spot at a magnification of ×400. The IHC % (summed area of CD68- or CD204-positive cells/measured area ×100) was then calculated for each site (Fig. 1).

The number of macrophages was counted in three hot spots at ×400 magnification using a micrometer. The mean number of infiltrating macrophages was then calculated.

Statistical analysis. Correlations between IHC% and macrophage count for CD68 and CD204 in the center of the lesion and the peripheral sites were evaluated using Spearman's rank correlation coefficients. Differences in macrophage infiltration between the two groups were evaluated using the Mann–Whitney *U*-test. Overall survival time was calculated from the date of pancreaticoduodenectomy to August 24, 2010. Parameters that were significantly associated with disease-free survival (DFS) or overall survival rates evaluated in univariate analyses using log–rank tests were further analyzed with multivariate analysis using the Cox proportional hazard regression model. Crude overall survival curves were plotted using the Kaplan–Meier method. All *P*-values were two-sided, and the significance level was set at $P < 0.05$. All statistical analyses were carried out using the Statistical Package for the Social Sciences 11.5 J for Windows software (SPSS Inc., Chicago, IL, USA).

Results

Comparison of the area ratio of IHC-positive cells and IHC-positive macrophage count. To validate auto-measurement of IHC-positive cell areas, the correlation between IHC-positive cell numbers and IHC% was examined. The median CD68 count was 21.0 (range, 1.7–64.0) at the center and 42.0 (range, 13.3–94.3) at the periphery of the lesion. The median CD204 count was 14.0 (range, 0.3–48.3) at the center and 24.7 (range, 4.0–75.3) at the periphery. The CD68% and CD204% strongly correlated with the number of CD68- and CD204-positive cells at the center and the periphery of the tumor in pancreatic IDCs ($P < 0.001$, R [correlation coefficient] >0.4). To ensure objectivity, auto-measurement of the IHC% was used to quantify immunoreactivity in this study (Fig. S1).

Distribution of CD68- and CD204-positive cells in pancreatic IDC and CP. A series of 107 IDC specimens of the pancreas and 11 specimens of CP were examined for CD68 and CD204 expression in the center and periphery of the lesion. In the IDC series, the median CD68% was 3.65% (range, 0.05–18.6%) at the center of the lesion and 9.92% (range, 0.37–25.1%) at the periphery, whereas the median CD68% of the CP series was 1.62% (range, 0.55–6.20%) at the center of the lesion and 2.29% (range, 1.13–19.5%) at the periphery ($P = 0.031$ at the center, $P = 0.002$ at the periphery). The median CD204% was 1.64% (range, 0.06–18.1%) at the center of the lesion and

Table 3. Distribution of central and peripheral CD204-positive cell area ratios in pancreatic tumors according to clinicopathological features

Parameter	Category	*n*	Central CD204%, median (range)	*P*	Peripheral CD204%, median (range)	*P*
Age (years)	≤64	58	1.54 (0.06–18.10)	0.970	3.43 (0.34–12.80)	0.846
	>64	49	1.65 (0.22–9.010)		3.27 (0.27–14.00)	
Gender	Male	63	1.51 (0.06–9.310)	0.364	3.27 (0.27–14.00)	0.552
	Female	44	1.77 (0.19–18.10)		3.59 (0.43–14.00)	
CEA (ng/mL)	≤3.5	57	1.45 (0.10–11.90)	0.064	3.31 (0.27–14.00)	0.469
	>3.5	50	2.02 (0.06–18.10)		3.80 (0.34–14.00)	
CA19-9 (U/mL)	≤109	53	1.56 (0.10–9.31)	0.983	3.37 (0.27–14.00)	0.400
	>109	54	1.66 (0.06–18.10)		3.41 (0.34–14.00)	
Differentiation	Well	31	1.38 (0.06–18.10)	0.477	3.43 (0.27–14.00)	0.995
	Moderate/Poor	76	1.69 (0.10–9.31)		3.33 (0.44–14.00)	
Tumor size (cm)	≤3.0	66	1.45 (0.13–9.31)	0.110	3.10 (0.27–14.00)	0.031*
	>3.0	41	2.10 (0.06–18.10)		3.38 (0.34–12.70)	
Serosal invasion	Absent	84	1.66 (0.06–11.90)	0.797	3.34 (0.27–14.00)	0.575
	Present	23	1.34 (0.10–18.10)		4.23 (0.55–12.20)	
Retroperitoneal invasion	Absent	9	1.68 (0.22–8.84)	0.439	6.10 (1.21–11.80)	0.346
	Present	98	1.60 (0.06–18.10)		3.37 (0.27–14.00)	
Lymphatic invasion	ly0/1	60	1.45 (0.22–11.90)	0.201	3.10 (0.27–14.10)	0.151
	ly2/3	47	1.96 (0.06–18.10)		4.26 (0.34–12.80)	
Vessel invasion	v0/1	10	1.17 (0.19–7.98)	0.309	3.33 (0.43–7.18)	0.460
	v2/3	97	1.66 (0.06–18.10)		3.43 (0.27–14.00)	
Intrapancreatic nerve invasion	ne0/1	27	1.64 (0.43–9.31)	0.659	3.38 (0.43–11.80)	0.954
	ne2/3	80	1.64 (0.06–18.10)		3.40 (0.27–14.00)	
Extrapancreatic nerve plexus invasion	Absent	48	1.73 (0.06–11.90)	0.641	3.34 (0.34–14.00)	0.925
	Present	59	1.56 (0.10–18.10)		3.43 (0.27–14.00)	
Portal vein invasion	Absent	81	1.44 (0.06–18.10)	0.012*	3.31 (0.27–14.0)	0.263
	Present	26	2.56 (0.44–9.01)		4.13 (0.55–14.0)	
Lymph node involvement	Absent	22	1.12 (0.13–4.20)	0.018*	0.94 (0.44–8.83)	0.003*
	Present	85	1.86 (0.06–18.10)		4.06 (0.27–14.0)	

*$P < 0.05$. Differences between the two groups were evaluated using the Mann–Whitney *U*-test. CA19-9, carbohydrate antigen 19-9; CEA, carcinoembryonic antigen; ly, lymphatic; ne, intrapancreatic nerve; v, venous.

3.38% (range, 0.27–14.0%) at the periphery in the IDC series, whereas the median CD204% in the CP series was 0.60% (range, 0.26–3.78%) at the center of the lesion and 1.59% (range, 0.32–3.54%) at the periphery ($P = 0.018$ at the center, $P = 0.008$ at the periphery). In each series, CD68- and CD204-positive cells were more frequently observed in the periphery than at the center of the lesions (Fig. 2). The CD204/CD68 ratios at the center and periphery were compared between IDC and CP cases to evaluate the population of cells with the M2 phenotype. In IDC cases, the median CD204/CD68 ratio was 67.6% (range, 3.6–185.4%) at the center of the lesion and 59.9 (range, 2.1–158.5%) at the peripheral sites, whereas the median CD204/CD68 ratio was 47.3% (range, 12.2–96.9%) at the center and 57.6% (range, 18.1–81.7%) at the periphery in CP cases. These differences were not significant ($P = 0.238$ at the center, $P = 0.753$ at the periphery; Fig. 2).

Distribution of CD68% and CD204% according to clinicopathological features. The relationship between clinicopathological features and macrophage infiltration was evaluated using Mann–Whitney *U*-tests (Tables 2, 3). The IDCs with lymph node involvement showed elevated expression of peripheral CD68 ($P = 0.045$), central CD204 ($P = 0.018$), and peripheral CD204 ($P = 0.003$). Cases with tumors >3.0 cm were significantly correlated with high peripheral CD204 expression ($P = 0.031$), and those with portal vein invasion were significantly correlated with high central CD204 expression ($P = 0.012$).

Univariate and multivariate analyses of parameters significantly associated with overall survival and DFS. The median IHC% of infiltrating macrophages was used to divide the cases into two groups, high (above the median value) and low (equal to or below the median value). Univariate analyses using log–rank tests were carried out to compare survival according to IHC% (Table 4), and overall survival curves were obtained with the Kaplan–Meier method (Fig. 3). Univariate analysis (Table 4) produced the following candidates for predicting prognosis: tumor size > 3.0 cm ($P = 0.0001$); lymph node involvement ($P = 0.0106$); lymphatic invasion ($P = 0.0171$); extrapancreatic nerve plexus invasion ($P = 0.0025$); and high central and peripheral CD204 expression ($CD204^{high}$) ($P = 0.0248$ at the center, $P < 0.0001$ at the periphery). Multivariate analysis (Table 5) revealed the following independent prognostic factors: tumor size > 3.0 cm (hazard ratio [HR], 2.017; $P = 0.002$); extrapancreatic nerve plexus invasion (HR, 1.992; $P = 0.002$); and peripheral $CD204^{high}$ (HR, 2.781; $P < 0.001$).

Univariate analysis (Table 4) showed that tumor size > 3.0 cm ($P = 0.0058$), serosal invasion ($P = 0.0427$), extrapancreatic nerve plexus invasion ($P = 0.0057$), and peripheral $CD204^{high}$ ($P = 0.0010$) were correlated with shorter DFS. Multivariate analysis (Table 5) revealed that extrapancreatic nerve plexus invasion (HR, 1.882; $P = 0.008$) and peripheral $CD204^{high}$ (HR, 1.864; $P = 0.010$) were independent risk factors for DFS. Initial recurrent sites of IDC were considered to be liver metastasis ($n = 38$), local recurrence ($n = 38$), or peritoneal dissemination ($n = 20$). The DFS curves for these groups were plotted using the Kaplan–Meier method to determine any significant impact of high CD204 expression at the peripheral site. The peripheral $CD204^{high}$ group had a significantly shorter DFS period than the peripheral $CD204^{low}$ group when stratified by initial liver metastasis and local recurrence (Fig. 4).

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Table 4. Univariate analyses of overall survival (OS) and disease-free survival (DFS) in patients with invasive ductal carcinoma of the pancreas

Factor	Category	*n*	OS, median (range)	*P* (uni)	DFS, median (range)	*P* (uni)
Age (years)	≤64	58	15.0 (3–145)	0.1561	7.5 (2–145)	0.1678
	>64	49	16.0 (1–90)		8.0 (1–90)	
Gender	Female	44	14.0 (1–77)	0.6205	8.0 (1–77)	0.4528
	Male	63	16.0 (2–145)		8.0 (1–145)	
CEA (ng/mL)	≤3.5	57	16.0 (2–145)	0.1757	11.0 (1–145)	0.1374
	>3.5	50	11.5 (1–90)		5.5 (1–90)	
CA19-9 (U/mL)	≤109	53	19.0 (1–90)	0.9288	8.0 (1–90)	0.3710
	>109	54	13.0 (3–145)		7.5 (2–145)	
Differentiation	Well	31	20.0 (1–77)	0.2594	15.0 (1–77)	0.1694
	Moderate/Poor	76	12.5 (2–145)		6.5 (1–145)	
Tumor size (cm)	≤3.0	66	19.0 (2–145)	0.0001*	11.0 (2–145)	0.0058*
	>3.0	41	10.0 (1–52)		6.0 (2–34)	
Serosal invasion	Absent	84	16.0 (1–145)	0.1058	10.0 (1–145)	0.0427*
	Present	23	12.0 (2–39)		6.0 (2–34)	
Retroperitoneal invasion	Absent	9	8.0 (4–53)	0.6294	6.0 (2–53)	0.5389
	Present	98	15.5 (1–145)		8.0 (1–145)	
Portal vein invasion	Absent	81	16.0 (1–90)	0.0745	8.0 (1–90)	0.4140
	Present	26	12.0 (3–145)		6.5 (2–145)	
Lymphatic invasion	0/1	60	20.0 (1–145)	0.0171*	9.5 (1–145)	0.1598
	2/3	47	11.0 (2–63)		6.0 (1–64)	
Vessel invasion	0/1	10	26.0 (6–77)	0.1072	17.0 (3–77)	0.2669
	2/3	97	13.0 (1–145)		8.0 (1–145)	
Intrapancreatic nerve invasion	0/1	27	15.0 (4–145)	0.1198	10.0 (2–145)	0.1001
	2/3	80	15.5 (1–77)		8.0 (1–77)	
Lymph node involvement	Absent	22	26.0 (4–90)	0.0106*	12.0 (1–90)	0.0645
	Present	85	13.0 (1–145)		8.0 (1–145)	
Extrapancreatic nerve plexus invasion	Absent	48	19.0 (3–145)	0.0025*	11.5 (2–145)	0.0057*
	Present	59	12.0 (1–53)		7.0 (1–53)	
CD68% at center	≤3.65%	53	19.0 (2–90)	0.5247	8.0 (1–90)	0.6641
	>3.65%	54	13.0 (1–145)		7.5 (1–145)	
CD68% at periphery	≤6.92%	54	19.0 (3–145)	0.3471	8.0 (2–145)	0.4213
	>6.92%	53	12.0 (1–77)		8.0 (1–77)	
CD204% at center	≤1.64%	54	19.0 (1–90)	0.0248*	8.0 (1–90)	0.6195
	>1.64%	53	11.0 (3–145)		6.0 (1–145)	
CD204% at periphery	≤3.39%	54	21.0 (3–90)	<0.0001*	13.5 (1–90)	0.0010*
	>3.39%	53	10.0 (1–145)		6.0 (1–145)	

*$P < 0.05$. Univariate analysis (uni) was carried out using the log–rank test. CA19-9, carbohydrate antigen 19-9; CEA, carcinoembryonic antigen; CD68%, summed area of CD68-positive cells/measured area ×100; CD204%, summed area of CD204-positive cells/measured area ×100.

Discussion

This was the first study to evaluate the distributions of M2 macrophages (CD204-positive cells) in pancreatic IDC and CP. M2 macrophages preferentially accumulated in peripheral rather than central sites in pancreatic IDC and CP. This finding may indicate that non-cancerous cells play an important role in the recruitment of macrophages and the polarization toward M2 macrophages in pancreatic IDC and CP. In CP, macrophages are recruited using chemoattractants produced by myofibroblasts.[31] Myofibroblasts are considered to be the activated state of pancreatic stellate cells (PSCs), and PSCs are activated by pancreatitis[31] and pancreatic cancer cells.[32] Macrophages in pancreatic IDC may have infiltrated because of chemoattractants produced by myofibroblasts derived from PSCs. The polarization toward M2 macrophages may be responsible for the cells producing IL-4 and IL-10 in both IDC and CP tumors. We considered mast cells and PSCs as candidates. Mast cells accumulate in peripheral areas of IDC[33] and intestinal areas of CP[34] and can produce IL-10.[35] Activated PSCs are abundant in IDC and CP tumors and lead to IL-4 production by T cells.[36] Mast cells and PSCs may play important roles in M2 accumulation in IDC and CP. In this study, most peripheral M2 macrophages in pancreatic IDC were dense along the stroma but not along tumor cells, a finding that may reinforce the above speculation.

Accumulated M2 macrophages in pancreatic IDC were more numerous than in CP. In pancreatic IDC, a large tumor was significantly correlated with dense peripheral M2 macrophages. These results indicate that the tumor volume affects accumulation of M2 macrophages. Recent studies have shown that monocyte recruitment is driven by several chemoattractants such as MIP-2, CCL3, and hypoxia-inducible factor-2α, which are secreted by malignant cells and stromal cells and induced by tumor hypoxia.[26,27,37] Tumor-associated macrophages are recruited to tumors by multiple growth factors and chemokines that are often produced by tumor cells themselves.[38,39] Tumor necrosis is increased in large tumors,[7] and TAMs are attracted to and retained in avascular and necrotic areas where they are exposed to tumor hypoxia.[26,27] Large tumors may increase expression of inflammatory mediators from tumor cells, stroma cells, and tumor hypoxia. Thus, increased tumor volume may promote accumulation of M2 macrophages.

The independent impact of M2 macrophages on survival and time to relapse was first revealed with multivariate analysis in pancreatic IDC. Dense accumulation of peripheral M2 macrophages was established as a good predictive mar-

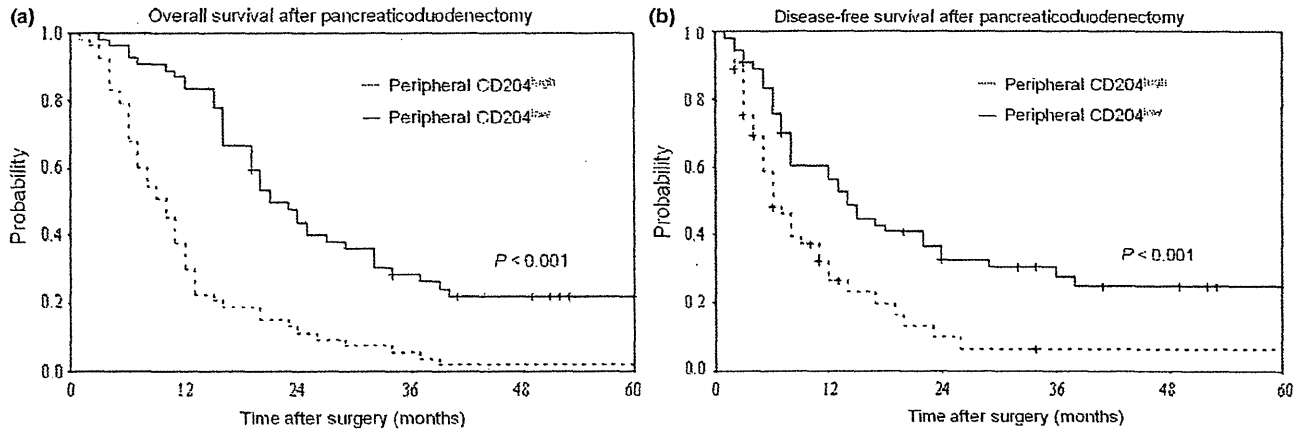

Fig. 3. Overall survival (a) and disease-free survival (b) curves for invasive ductal carcinoma of the pancreas according to the area ratio of peripheral CD204-positive cells. Disease-free survival periods were significantly shorter in patients with peripheral CD204^{high} than in patients with CD204^{low}. Prognosis was significantly worse in patients with peripheral CD204^{high} than for those with CD204^{low}.

ker of survival and recurrence. According to the type of initial recurrence, dense peripheral M2 macrophages were associated with early relapse in liver and the manipulated area of the pancreaticoduodenectomy. This suggests that M2 macrophages may accelerate liver metastasis and local recurrence. Tumor-associated macrophages are important producers of proteases, including MMPs, and of a wide variety of growth factors, such as fibroblast growth factor and epidermal growth factor (EGF) receptor family ligands that can stimulate the growth and motility of tumor cells.[38] Tumor-associated macrophages have been reported to be the most significant source of EGF in tumors,[40] and they are associated with EGF receptor expression and poor outcome in breast cancer.[41] Pollard *et al.* showed that tumor cells respond to macrophage-produced EGF ligands *in vivo* by chemotaxis and invasion, and that macrophages are often associated with vessels.[38,42] Thus, M2 macrophages may provide chemotactic signals that recruit tumor cells to blood vessels and enhance their egress into vasculature, leading to tumor hematogenous metastasis and further local invasion. These effects of M2 macrophages may shorten DFS and overall survival.

Lymph node involvement was significantly correlated with high CD204 expression in peripheral sites of the lesion. Tumor-associated macrophages within the invasive tumor front have a profound influence on the regulation of tumor angiogenesis and lymphangiogenesis by production of vascular endothelial growth factor-C and -D.[9,37,41,43] Elevated lymphangiogenesis by TAMs may promote lymph node metastasis.

The independent prognostic values of large tumor size and extrapancreatic nerve plexus invasion were reported in our previous study[7] and reconfirmed by this study. Time to recurrence was associated with the presence of extrapancreatic nerve plexus invasion. Large tumor size did not show an impact on DFS, because high accumulation of peripheral M2 macrophages correlated with large tumor size.

In conclusion, dense M2 macrophages in peripheral sites were significantly correlated with large tumor size, lymph node involvement, and poor prognosis due to accelerated liver metastasis and local recurrence. The number of accumulated M2 macrophages was associated with tumor volume, but the distribution of M2 macrophages in CP was similar to that in IDC.

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Disclosure Statement

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Table 5. Multivariate analyses of independent significant factors associated with overall survival and disease-free survival in patients with invasive ductal carcinoma of the pancreas

	Overall survival			Disease-free survival		
	HR	95% CI	*P* (multi)	HR	95% CI	*P* (multi)
Tumor size (>3.0 cm)	2.017	1.301–3.127	0.002*	1.492	0.920–2.419	0.105
Serosal invasion present				1.667	0.960–2.896	0.070
Lymph node involvement present	1.112	0.612–2.020	0.727			
Extrapancreatic nerve plexus invasion present	1.992	1.283–3.095	0.002*	1.882	1.176–3.013	0.008*
Central CD204^{high}	1.035	0.673–1.592	0.874			
Peripheral CD204^{high}	2.781	1.740–4.445	<0.001*	1.864	1.164–2.986	0.010*

*P < 0.05. Multivariate analyses (multi) were carried out using the Cox regression hazard model. Central CD204^{high}, percentage of CD204-positive cells area over 1.64%; CI, confidence interval; HR, hazard ratio; Peripheral CD204^{high}, percentage of CD204-positive cells area over 3.39%.

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