patients with adenocarcinoma ($n = 101$) after surgical resection. In contrast, the authors of a recent large study, where 32.6 % of the patients with ASC underwent surgery and 16.5 % of those with adenocarcinoma underwent surgery, insisted that the natural history of ASC of the pancreas is similar to that of adenocarcinoma [8]. Radiation and chemotherapy were administered to similar proportions of these patients. This difference in resectability could have affected the outcomes, because surgical resection itself is regarded as a favorable prognostic factor [6, 9, 10].

The evaluations of the pathological features in the present study addressed venous invasion, lymphatic invasion, intrapancreatic nerve invasion and the growth patterns of tumors infiltrating the surrounding tissue, according to the JPS classification [3], which has a more detailed description system than the UICC classification. These factors have been commonly examined and rigorously evaluated in Japan. Since these factors are known to be related to the histological grades of tumors, we re-analyzed the patients after dividing them into four types (three TAC subtypes and ASC). Among the four pathological types, only the venous invasion showed a prominent relationship with the histological subtypes of Stage IIA and IIB patients (Fig. 3a–d). These results were surprising, because it had been thought that all the features would become more severe according to the histological grade.

There was a higher percentage of v3 in the G3 patients compared to that in the G1 or G2 patients (Fig. 3a). Furthermore, more than 70 % of the patients with ASC were categorized as v3, which was the highest rate among the four categories (G1–G3 and ASC). Among the patients with ASC, none was categorized into v0 or v1.

In addition, the type of recurrence suggests that hematogenous invasion leads to the poor outcomes in ASC. As shown in Fig. 1, not only the OS but also the DFS of ASC patients were shorter than those of TAC patients. This indicates that there is an early relapse in ASC patients after surgical resection. The sites of distant metastasis, including the liver, lungs and brain, suggest that the cancer cells develop metastasis hematogenously (no patient with bone metastasis was detected in this study). Although statistical significance was not confirmed, the higher occurrence rate of distant metastasis might be related to the high venous invasion rate in ASC patients. Mrstik et al. [25] reported that microscopic venous invasion is the most important predictor of relapse in renal cell carcinoma, apart from tumor extension, and that standard histological grading and nuclear grading also had a significant impact on the DFS in a retrospective analysis. In a proteomic analysis with clinicopathological data about hepatocellular carcinoma recurrence, mortalin (encoded by *HSPA9*) was found to be closely correlated with early recurrence, and mortalin is also associated with venous invasion and tumor stages [26]. Based on these results, we assumed that marked microscopic venous invasion results in the early distant metastasis of ASC of the pancreas, which leads to the poor prognosis.

The higher incidence of ASC in larger tumors is also one of the keys to disclose the biology of ASC (Fig. 4). This result suggests that the squamous component is likely not included in the early stage of tumor occurrence, and that the squamous component likely appears as the tumor size increases. Concerning the carcinogenesis of ASC, several possible theories have been proposed, which are divided into monoclonal or polyclonal pathways. The latter is also known as the collision theory, which proposes that two histologically distinct tumors emerge independently and unify, although only a few articles have supported this theory [17, 23, 27]. Regarding the former theory, the existence of pancreatic cancer stem cells has been suggested since the 1980s [28]. Recently, some studies have claimed the existence of ASC progenitor cells in some cancers, based on the genetic and immunohistological similarities that coexisted in tubular and squamous components in ASC [15, 29–32]. If this were the case, a monoclonal pathway would seem logical for the development of ASC. Given the assumption of such a monoclonal pathway, there must be specific gene(s) that promote the squamous differentiation of the tubular adenocarcinoma from progenitor cells.

The reason why ASC showed a higher venous invasion rate was not clarified in this analysis, but some articles have been published that have partly addressed this issue. In human gastric cancer cells, alteration of the E2F-4 gene was implicated in the transformation of adenocarcinoma into squamous cell carcinoma [33]. Another study on breast cancer in mice suggested that stabilization of beta-catenin, which is involved in the Wnt signaling pathway, induces squamous metaplasia and loss of epithelial cell differentiation [34]. Furthermore, squamous metaplasia is also induced by hepatocyte growth factor, known for its association with a poor prognosis, through the stabilization of beta-catenin [35, 36]. Collectively, these studies suggest that ASC of the pancreas might be a form of TAC that gains additional gene alterations associated with marked venous invasion, leading to its poor prognosis. Although further studies are needed to elucidate the pathways underlying the carcinogenesis of ASC of the pancreas, the suppression of this biological behavior might be helpful in prolonging the patient's survival.

In conclusion, the overall survival and disease-free survival periods after surgical resection in the patients with ASC were significantly shorter than those in patients with TAC. We also revealed some of the intriguing clinicopathological features associated with ASC of the pancreas.

Conflict of interest The authors declare that they have no conflicts of interest in association with this study.

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2014 SSAT PLENARY PRESENTATION

Reappraisal of Peritoneal Washing Cytology in 984 Patients with Pancreatic Ductal Adenocarcinoma Who Underwent Margin-Negative Resection

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Abstract

Objective The objective of the present study was to reappraise the clinical value of peritoneal washing cytology (CY) in 984 pancreatic ductal adenocarcinoma patients who underwent margin-negative resection.

Methods In a 2001–2011 database from seven high-volume surgical institutions in Japan, 69 patients (7 %) had positive CY (CY+ group) indicative of M1 disease and 915 patients had negative CY (CY− group). Clinicopathological data and survival were compared between groups.

Results Significant correlations between CY+ and high CA19-9 level, pancreatic body and tail cancer, lymph node metastasis, and a lower frequency of R0 resection were observed. Overall survival (OS) of CY+ patients was significantly worse than that of CY− patients (median survival time [MST], 16 vs. 25 months; 3-year OS rate, 6 vs. 37 %; $p<0.001$). CY+ patients had a significantly higher rate of post-operative peritoneal carcinomatosis than CY− patients (48 vs. 21 %; $p<0.001$). Administration of adjuvant chemotherapy did not provide a favorable survival outcome to CY+ patients. The current study showed that patients with M1 disease had acceptable MST after margin-negative resection and a high incidence of peritoneal carcinomatosis within 3 years after surgery, resulting in decreased long-term survival. The development of a new strategy to control peritoneal carcinomatosis when surgical resection is performed in such patients is required.

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Keywords Pancreatic ductal adenocarcinoma · Peritoneal washing cytology · Peritoneal carcinomatosis · Survival analysis · Adjuvant chemotherapy

Introduction

Pancreatic ductal adenocarcinoma (PDAC) is associated with a poor prognosis, even in patients who have undergone margin-negative resection. The results of surgery alone for ductal pancreatic adenocarcinoma are disappointing, and the 5-year actual survival rate ranges from 3 to 17 %, even after surgical resection.[1–5] Positive peritoneal washing cytology (CY) status in patients with resectable PDAC is defined as M1 disease in the American Joint Committee on Cancer (AJCC) guidelines.[6] Ferrone et al. reported that positive cytology in patients who have undergone surgical resection was associated with poor survival (median survival time [MST], 8 vs. 16 months in patients with negative cytology; $p<0.001$); their survival time did not significantly differ from that of patients with metastatic (7 months) or locally advanced disease (6 months).[7] Although some studies have reported no significant difference in overall survival (OS) between patients with positive (CY+) and negative (CY−) peritoneal washing cytology who underwent surgical resection, only a small number of CY+ patients (10–20) were evaluated.[8–10] Thus, the clinical role of surgical resection in PDAC patients with CY+ is still being debated.

In Japan, many institutions routinely perform curative surgical resection in patients with CY+ when PDAC is diagnosed as resectable or borderline resectable. We have recently established a common database by collecting data from patients who underwent margin-negative resection for pancreatic ductal adenocarcinoma from seven high-volume centers in Japan (Multicenter Study Group of Pancreatobiliary Surgery [MSG-PBS]). The objective of the present study was to retrospectively reappraise the clinical role of surgical resection in patients with CY+ PDAC based on this large-scale database.

Methods

Study Design and Patient Selection

This was a case-control study that compared the surgical results of PDAC patients with CY+ and CY− who underwent surgical resection. The primary endpoint was OS rate, and secondary endpoints included disease-free survival (DFS) rate and primary site of recurrence. After several discussions, the MSG-PBS collected the data from 1,389 patients who had undergone R0 or R1 pancreatic resection from 2001 to June 2011 for pancreatic ductal adenocarcinoma according to our guidelines for correct registration of patients into the database. Among these patients, 37 patients with initially unresectable PDAC who underwent curative surgical resection after long-term favorable responses to chemotherapy or chemoradiotherapy, 13 patients with anaplastic carcinoma, and 12 patients with mucinous carcinoma were excluded. In addition, 343 patients whose CY had not been sampled for pathological examination were also excluded. A total of 984 patients were included in the present study.

Clinical data included gender, age, body mass index (BMI), neoadjuvant treatment, adjuvant chemotherapy, pre- and post-operative serum CA19-9 level, tumor location, resectability status defined by the National Cancer Comprehensive Network (NCCN),[11] status of post-operative CA19-9 normalization,[12] and operation type. For tumors, pathological data included T and N status according to the seventh AJCC/International Union Against Cancer (UICC) TNM classification, tumor size, histological type, surgical margin status, and CY.[13,14] Typically, post-operative peritoneal carcinomatosis was defined when patients had significant ascites and/or multiple peritoneal tumors found on the planned high-quality CE-CT during the post-operative follow-up period (every 3 months for 2 years after surgical resection and every 6 months over 2 years after surgical resection).

Surgical indications at the seven institutions were based on resectable and borderline resectable PDAC as defined by NCCN resectability status.[11] As an exception, patients who underwent distal pancreatectomy with celiac axis resection (6 %) despite having unresectable disease as defined by the NCCN were also included in this analysis. Directly after opening the abdominal cavity for a planned margin-negative resection, it was washed with physiologic saline solution (40–100 mL) into the pelvis and/or subhepatic space, and subsequently, cytologic washings (20–50 mL) were obtained for pathological examination. Smears were made from the centrifuged deposit and examined by experienced pathologists following conventional Papanicolaou and/or Giemsa staining. The CY results were given to the surgeons before resection (pancreatectomy) in some centers and post-operatively in the other centers. Surgical resection was routinely performed even in CY+ patients due to the policy at the seven institutions. Neoadjuvant or adjuvant therapy was performed based on each institution's policies. Neoadjuvant therapy regimens included gemcitabine chemotherapy, gemcitabine plus S-1 chemotherapy, gemcitabine chemotherapy with concurrent radiation, S-1 chemotherapy with concurrent radiation, and gemcitabine plus S-1 chemotherapy with concurrent radiation. Chemotherapeutic agents used in adjuvant therapy included gemcitabine alone, S-1 alone, gemcitabine plus S-1, and gemcitabine plus 5-fluorouracil. This study was approved by the institutional review board of each center.

Statistical Analysis

The database from seven institutions was carefully checked for clerical errors by YM and SS for 1 month before the

statistical analyses started in each institution. A total of 13 continuous variables and 45 categorical variables from the database were required for the statistical analyses conducted in this study. Missing values were observed in only 0.32 % of cases in the database. Data are expressed as median values and ranges. The clinicopathological parameters were compared between CY+ and CY− patients. Continuous or categorical variables were compared by Mann-Whitney *U* test, chi-squared test, or Fisher's exact test as appropriate. OS was defined as the time from the date of surgery or neoadjuvant treatment to death or the last follow-up date (June 30, 2013); OS and DFS were compared using the log-rank test. All patients were followed up for at least 1 year. In addition, factors identified by univariate analysis were further examined by multivariate Cox proportional hazards models to identify independent significant factors for survival. Hazard ratios and 95 % confidence intervals were calculated for all estimates. A two-tailed p value of <0.05 was considered to be statistically significant. Statistical analyses were performed using SPSS Version 18.0 for Windows (Chicago, IL, USA).

Results

Among 984 patients who underwent tumor resection, 69 patients (7 %) had a positive CY status (CY+ group), and 915 patients had a negative CY status (CY− group). Overall, 38 % of patients underwent portal or superior mesenteric vein resection. Morbidity was observed in 39.5 % of patients and in-hospital mortality was noted in 2.1 % of this population. With respect to therapy, 27 % of patients underwent neoadjuvant treatment, and 82 % received post-operative adjuvant chemotherapy consisting of gemcitabine- or S-1-based regimens depending on the physicians' or patients' choice.

Comparison of Clinicopathological Factors Between the CY+ and CY− Groups

As shown in Table 1, patients in the CY+ group had a significantly higher frequency of pancreatic body and tail cancer, higher pre- and post-operative CA19-9 levels, a lower incidence of post-operative CA19-9 normalization, and greater tumor diameter on CT scan relative to patients in the CY− group ($p<0.05$). Biliary drainage was more frequently performed in patients in the CY− group compared to patients in the CY+ group ($p<0.001$). No significant differences in several other parameters, including age, gender, BMI, diabetes mellitus, albumin and C-reactive protein levels, NCCN resectability status, neoadjuvant therapy, or adjuvant therapy, were observed between groups.

Comparisons of surgical parameters (Table 2) revealed that pathologically, patients in the CY+ group had a significantly higher frequency of lymph node metastasis and higher lymph node ratios and R1 rates relative to patients in the CY− group ($p<0.05$). Significant differences in tumor differentiation and pathological tumor stage were also observed between the two groups. No significant differences in type of surgery, operative time, extent of blood loss, frequency of concomitant resection of the portal/superior mesenteric vein, or overall morbidity or mortality were observed between groups.

Table 1 Comparison of pre- and post-operative parameters

Parameter	CY− n=915	CY+ n=69	p value
Age, years (range)	68 (27–91)	65 (41–85)	0.19
Gender, male: female (%)	490 (54):425 (46)	35 (51):34 (49)	0.865
Body mass index, ≥25:<25 (%)	118 (13):761 (87)	6 (9):62 (91)	0.352
Diabetes mellitus, +:− (%)	382 (42):525 (58)	28 (41):41 (59)	0.899
Biliary drainage, +:− (%)	430 (47):485 (53)	15 (22):54 (78)	<0.001
Albumin, g/dL (range)	3.8 (1.8–5.5)	3.9 (2.2–4.7)	0.739
CRP, mg/dL (range)	0.2 (0–16.7)	0.1 (0–14.3)	0.066
Pre-op CA19-9, U/L (range)	109 (0–42,060)	171 (1.0–47,470)	0.023
Post-op CA19-9, U/L (range)	17 (0–10,061)	52 (1–2,339)	0.001
Rate of post-op CA19-9 normalization	69 %	46 %	<0.001
Location of cancer, Ph:Pbt (%)	664 (73):251 (27)	27 (39):42 (61)	<0.001
Tumor diameter, mm (range)	27 (5–86)	33 (14–200)	<0.001
NCCN resectability status R:BR/UR (%)	613 (67):302 (33)	41 (59):28 (41)	0.234
Neoadjuvant therapy, +:− (%)	247 (27):668 (73)	20 (29):49 (71)	0.779
Adjuvant therapy, +:− (%)	746 (82):167 (18)	59 (86):10 (14)	0.517

CY peritoneal washing cytology, *CRP* C-reactive protein, *CA19-9* carbohydrate antigen 19-9, *Ph* pancreatic head, *Pbt* pancreatic body and tail, *NCCN* National Comprehensive Cancer Network, *R* resectable, *BR* borderline resectable, *UR* unresectable

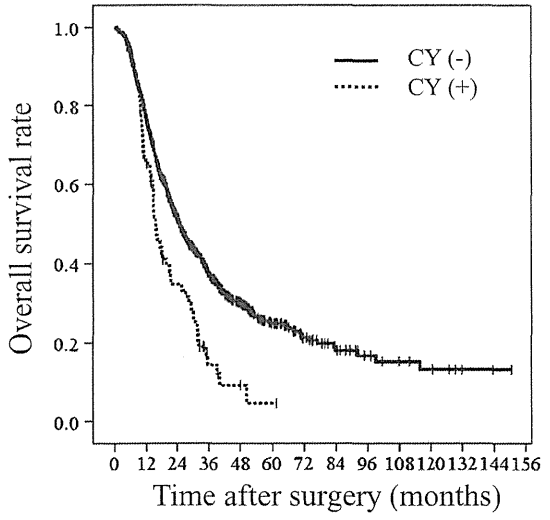
Table 2 Comparison of surgical and pathological parameters

Parameter	CY (−) n=915	CY (+) n=69	p value
Type of procedure, PpPD/PD:DP:TP (%)	300/330 (69):256 (28):29 (3)	8/15 (34):42 (60):4 (6)	<0.001
Operative time, minutes (range)	423 (101–1,160)	395 (137–727)	0.095
Extent of blood loss, mL (range)	1,000 (52–9,639)	1,160 (110–9,163)	0.146
Blood transfusion, +:− (%)	328 (36):584 (64)	25 (37):43 (63)	0.896
Concomitant resection of the artery, +:− (%)	76 (8):839 (92)	13 (19):56 (81)	0.007
Concomitant resection of the PV/SMV, +:− (%)	345 (38):570 (62)	26 (38):43 (62)	1.000
Overall complication, +:− (%)	357 (39):558 (61)	32 (46):37 (54)	0.251
In-hospital death, +:− (%)	18 (2.0):897	3 (4.3):66	0.178
Clavien score, 0-IIIA:IIIB-V (%)	868 (95):47 (5)	65 (94):4 (6)	0.777
Tumor differentiation, well:mod/por (%)	238 (26):677 (74)	12 (17):57 (83)	0.150
Lymph node metastasis, +:− (%)	593 (65):321 (35)	60 (87):9 (13)	<0.001
Lymph node ratio (range)	0.05 (0–0.88)	0.1 (0–0.67)	<0.001
R0:R1 (%)	706 (77):209 (23)	40 (58):29 (42)	0.001
T1/2:3/4 (%)	91 (10):824 (90)	3 (4):66 (96)	0.198
Stage 1:2:3:4 (%)	59 (7):786 (85):10 (1):60 (7)	0:0:0:69 (100)	<0.001

CY peritoneal washing cytology, *PpPD* pylorus preserving pancreaticoduodenectomy, *PD* pancreaticoduodenectomy, *DP* distal pancreatectomy, *TP* total pancreatectomy, *PV* portal vein, *SMV* superior mesenteric vein, *mod* moderate, *por* poor

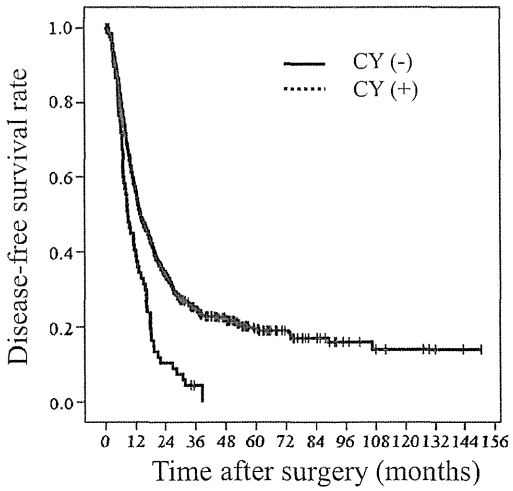
Comparisons of OS and DFS Between the CY+ and CY− Groups

The median duration of follow-up was 19.7 months. All patients were followed up for at least 1 year. OS in the CY+ group was significantly worse than that of the CY− group (MST, 16 vs. 25 months; p<0.001) (Fig. 1). OS rates at 1, 3, and 5 years were 77, 37, and 25 %, respectively, in the CY− group, and 45, 6, and 1 %, respectively, in the CY+ group. Overall, 59 of 69 patients in the CY+ group and 572 of 915 patients in the CY− group died. Similar results were observed upon comparison of DFS in the CY− and CY+ groups (8.8 vs. 13.8 months; p<0.001) (Fig. 2). DFS rates at 1, 3, and 5 years were 57, 25, and 19 %, respectively, in the CY− group, and



Patients at risk	1y	2y	3y	4y	5y	7.5y	10y	12.5y
CY (-) 915	697	355	204	120	64	16	6	1
CY (+) 69	65	35	14	9	5	0		

Fig. 1 Comparison of OS between patients with positive (n=69) and negative (n=915) cytology. OS in patients with CY+ (*dotted black line*) was significantly worse than that in patients with CY− (*solid black line*) (MST, 16 vs. 25 months; p<0.001). OS rates at 1, 3, and 5 years were 77, 37, and 25 %, respectively, in the CY− group, and 45, 6, and 1 %, respectively, in the CY+ group



Patients at risk	1y	2y	3y	4y	5y	7.5y	10y	12.5y
CY (-) 915	489	228	137	91	49	14	4	1
CY (+) 69	27	7	1	0				

Fig. 2 Comparison of DFS between patients with positive (n=69) and negative (n=915) cytology. DFS in patients with CY+ (*dotted black line*) was significantly worse than that in patients with CY− (*solid black line*) (median DFS, 8.8 vs. 13.8 months; p<0.001). DFS rates at 1, 3, and 5 years were 57, 25, and 19 %, respectively, in the CY− group, and 27, 1, and 0 %, respectively, in the CY+ group

27, 1, and 0 %, respectively, in the CY+ group. Overall, 65 of 69 patients in the CY+ group and 648 of 915 patients in the CY− group experienced disease recurrence.

Identification of Prognostic Factors in All Patients

As shown in Table 3, significant prognostic factors identified using univariate analysis included cytology status, pre-operative CA19-9 levels, tumor location, NCCN resectability status, arterial resection, portal/superior mesenteric vein resection, residual tumor grading, tumor differentiation, lymph node metastasis, and adjuvant therapy. In addition, multivariate analysis revealed CY− status, CA19-9 <115 U/L, resectable disease, R0 resection, negative lymph node metastasis, well-differentiated pathology, and use of post-operative adjuvant therapy to be significantly independent prognostic factors for better survival.

Primary Site of Recurrence

Patients in the CY+ group had a significantly higher frequency of post-operative peritoneal carcinomatosis as the primary site of recurrence compared to patients in the CY− group (49 vs. 21 %, $p<0.001$; Table 4). No differences in the rates of local recurrence or recurrence in the liver, lung, or other sites were observed between the two groups.

Identification of Prognostic Factors in the CY+ Group

Cox proportional hazards analysis in the CY+ subgroup revealed that the only independent significant factor for survival

Table 3 Univariate and multivariate cox proportional hazards analysis for overall survival: association with overall survival and patient, tumor, and treatment characteristics

Variable	No. (%)	MST (months)	Univariate analysis		Multivariate analysis			
	CY− vs. +	CY− vs. +	*p* value	Hazard ratio (95 % CI)	Estimate	SE	*p* value	Hazard ratio (95 % CI)
Group, CY− vs. +	915 vs. 69	24.9 vs. 16.0	<0.001	0.57 (0.44–0.75)	−0.31	0.15	0.035	0.74 (0.55–0.98)
Pre-op CA19-9, IU/L			<0.001	1.83 (1.56–2.15)	0.52	0.08	<0.001	1.68 (1.43–1.98)
≥115	447 vs. 41	18 vs. 16						
<115	464 vs. 28	34 vs. 15						
Tumor location			0.013	0.80 (0.67–0.96	−0.15	0.11	0.16	0.86 (0.70–1.06)
Pancreas body and tail	251 vs. 42	34 vs. 19						
Pancreas head	664 vs. 27	24 vs. 13						
NCCN resectability status			<0.001	0.56 (0.48–0.66)	−0.37	0.11	0.001	0.69 (0.56–0.86)
Resectable	613 vs. 41	32 vs. 19						
BR/UR	302 vs. 28	18 vs. 15						
Arterial resection			0.025	0.74 (0.58–0.96	−0.05	0.16	0.74	0.95 (0.70–1.30)
No	839 vs. 56	25 vs. 16						
Yes	76 vs. 13	21 vs. 14						
Portal vein resection			<0.001	0.64 (0.55–0.75)	−0.02	0.11	0.82	0.98 (0.79–1.21)
No	570 vs. 43	31 vs. 16						
Yes	345 vs. 26	18 vs. 16						
Residual tumor grading			<0.001	0.77 (0.71–0.84)	−0.25	0.09	0.006	0.78 (0.65–0.93)
R0	706 vs. 40	28 vs. 17						
R1	209 vs. 29	18 vs. 15						
Tumor differentiation			<0.001	0.65 (0.54–0.79)	−0.38	0.10	<0.001	0.69 (0.57–0.83)
Well	238 vs. 12	34 vs. 22						
Mod/por	677 vs. 57	22 vs. 16						
Lymph node metastasis			<0.001	0.72 (0.66–0.79)	−0.50	0.10	<0.001	0.61 (0.50–0.73)
No	321 vs. 9	40 vs. 29						
Yes	593 vs. 60	21 vs. 15						
Adjuvant therapy			<0.001	1.25 (1.14–1.38)	0.57	0.10	<0.001	1.77 (1.46–2.16)
No	167 vs. 10	14 vs. 10						
Yes	746 vs. 59	27 vs. 16						

MST median survival time, *CY* peritoneal washing cytology, *CA19-9* carbohydrate antigen 19-9, *NCCN* National Comprehensive Cancer Network, *BR* borderline resectable, *UR* unresectable, *mod* moderate, *por* poor, *CI* confidential interval, *SE* standard error

Table 4 Primary site of recurrence and median survival time according to metastatic site between the two groups

	Number of events	Primary site of recurrence				
		Peritoneum	Liver	Local	Lung	Others
CY+, n=69 (%)	65	31 (49 %)*	13 (20 %)	13 (20 %)	7 (11 %)	0
MST, month		11	17	26	30	-
CY−, n=915 (%)	648	132 (21 %)	210 (33 %)	180 (28 %)	86 (14 %)	26 (4 %)
MST, month		13	17	25	37	22

Data of the primary site of recurrence were missing in 1 patient in the CY+ group and 14 patients in the CY− group

CY peritoneal washing cytology, *MST* median survival time

*CY+ vs. CY−, p<0.001

was the presence of post-operative peritoneal carcinomatosis (hazard ratio, 2.65; 95 % confidential interval, 1.52–4.60; p=0.001). OS in patients with post-operative peritoneal carcinomatosis (n=31; MST, 11 months) was significantly worse than that in patients with other sites of recurrence (n=33; MST, 22 months; p<0.001, Fig. 3a). Data for the primary site of recurrence were missing for one patient. Adjuvant chemotherapy was administered to 86 % of patients in the CY+ group and 82 % of patients in the CY− group. No significant differences in OS were observed between patients who did (n=59; MST, 16 months) and did not (n=10; MST, 10 months) receive adjuvant therapy in the CY+ group (p=0.21) (Fig. 3b, Table 3). However, OS was significantly longer in patients who received adjuvant chemotherapy in the CY− group compared to patients who did not (MST, 27 vs. 14 months; p<0.001) (Table 3).

Discussion

PDAC is still associated with a dismal prognosis, and potential cure can only be achieved when the primary tumor is completely resected. However, the 5-year survival rate in patients who undergo resection is <20 %. Surgical resection is not usually indicated for PDAC with M1 disease, because the MST in patients with metastatic disease is only 6–8 months.[15,16] The AJCC/TNM staging system classifies positive cytology in the washing peritoneal fluid (CY+) as M1 disease,[13,14] and the NCCN pancreatic adenocarcinoma guidelines[11] state that positive cytology from washings (CY+) obtained at laparoscopy or laparotomy is equivalent to M1 disease. Even if resection has been performed in such patients, they should be treated as having M1 disease.

To date, only a limited number of studies on the post-operative prognosis of patients with CY+ have been

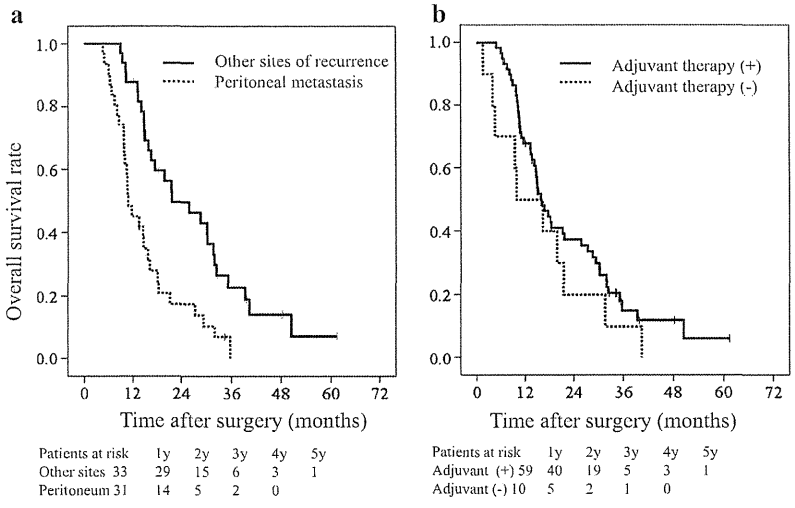


Fig. 3 Comparisons of OS by primary site of recurrence and adjuvant therapy in patients with positive cytology (n=69). **a** OS in patients with post-operative peritoneal carcinomatosis (n=31, *dotted black line*) was significantly worse than that in patients with recurrence in other sites (n=33, *solid black line*) (p<0.001). Data regarding the primary site of recurrence were missing for one patient in the CY+ group; the remaining four patients were free of disease. **b** No significant difference in survival was observed between patients who did (n=59, *solid black line*) and did not (n=10, *dotted black line*) receive adjuvant chemotherapy

conducted, as shown in Table 5. CY+ in patients with resectable[7] and unresectable[17] PDAC has been reported to negatively influence their prognosis. Some studies from Japan reported no significant difference in survival between CY+ and CY− patients.[8–10] The investigators in these studies concluded that CY status in the absence of other distant metastasis was not a contraindication for radical surgery, as surgical resection remained the only modality that offers a chance for long-term survival. However, as only approximately 10–20 patients with CY+ were statistically evaluated in these previous studies,[8–10] their statistical power was not sufficiently strong. Very recently, Yamada et al. re-evaluated the clinical impact of CY+ in patients who underwent surgical resection from 1991 to 2012.[18] The OS of patients with resected CY+ tumors (n=51) was worse than that of patients with resected CY− tumors (n=339). The current study also showed that from 2001 to 2011, survival in the 69 patients with CY+ was significantly worse than that of the 915 patients with CY−. Moreover, the present multivariate analysis revealed that cytology status was an independent prognostic factor. Thus, this large-scale study clearly demonstrated the negative impact of surgical resection in patients with CY+ on their prognosis. However, Yamada et al.[18] stated that the survival of 51 resected patients with CY+ (MST, 14 months) was significantly better than that of 133 unresected patients regardless of CY status (MST, 7 months). The MST of the CY+ group in the present study was 16 months, which appears to be better than the 6–12 months reported for modern chemotherapy regimens in patients with unresectable PDAC.[15,16,19–21] However, the MST in locally advanced PDAC patients who underwent chemoradiation was reported to be approximately 16 months in recent phase II studies.[22,23] Thus, the current study showed that the MST in patients with M1 disease with CY+ who underwent margin-negative resection was acceptable compared to MSTs achieved with other treatment modalities.

Although margin-negative resection provides the only chance for long-term survival in patients with pancreatic ductal adenocarcinoma, the 5-year survival rates range from 3 to 17 %.[1–5] In the present study, the 3- and 5-year OS rates were 37 and 25 %, respectively, in the CY− group, and 6 and 1 %, respectively, in the CY+ group. Patients in the CY+ group were less likely to be alive >3 years after surgical resection, as evidenced by 3- and 5-year DFS rates of only 1 and 0 %, respectively. With a median follow-up duration of 19.7 months, only 4 of 69 patients (5.8 %) in the CY+ group were free of disease, compared to 267 of 915 patients (29.2 %) in the CY− group. Similar 3-year OS and DFS rates of <10 % in CY+ patients have also been observed in other studies.[7,8,18] Thus, CY+ patients appear to have a high incidence of recurrent disease at 3 years after surgical resection, resulting in fewer long-term survivors. Moreover, patients in the CY+ group were significantly more likely to develop peritoneal carcinomatosis as the primary site of recurrence compared to patients in the CY− group (49 vs. 21 %). Among patients in the CY+ group, no pre-operative, surgical, or pathological factors were identified as prognostic factors; development of peritoneal carcinomatosis as the primary site of recurrence was the only prognostic factor identified. Moreover, survival in patients with peritoneal carcinomatosis was significantly worse than that in patients with other sites of recurrence. Motoi et al.[12] reported that even in patients who underwent R0 resection, sustained elevation of post-operative CA19-9 levels was accompanied by poor prognosis which was associated with distant metastases. In the present study, the significantly lower incidence of post-operative CA19-9 normalization in the CY+ group may reflect the potential micrometastasis that occurs during margin-negative resection and may therefore be associated with the higher incidence of disease recurrence relative to that of the CY− group. Thus, CY+ status should be considered as a specific subgroup in future survival analyses of PDAC patients.

The introduction of advanced adjuvant chemotherapy regimens may prevent potential microscopic residual disease and micrometastasis during or immediately after surgical resection. A potentially beneficial effect of adjuvant therapy in CY+ patients has been observed in previous studies.[8,18] The authors of these studies concluded that as surgical resection remains the only treatment modality that offers a chance for long-term survival, and considering the efficacy of modern chemotherapy regimens in eliminating micrometastases, margin-negative resection should be offered to patients with

Table 5 Recent reports on pancreatic cancer patients with positive peritoneal washing cytology who underwent surgical resection

First author	Publication year	Study period	CY(+)/surgical resection	MST (CY+ vs. CY−, months)	p value
Yachida[10]	2002	1990–1999	16/130 (12.3 %)	18 vs. 15	0.347
Ferrone[7]	2006	1995–2005	10/217 (4.6 %)	8 vs. 16	<0.0001
Yamada[9]	2007	1991–2006	21/157 (13.4 %)	14 vs. 14	0.269
Yoshioka[8]	2012	2003–2010	20/254 (7.9 %)	24 vs. 27	0.302
Yamada[18]	2013	1991–2012	51/390 (13.1 %)	14 vs. 18	0.009
Current study	2014	2001–2012	69/984 (7.0 %)	16 vs. 25	<0.001

CY peritoneal washing cytology, *MST* median survival time

PDAC, regardless of CY status. However, in the present study, administration of gemcitabine- or S-1-based adjuvant chemotherapy did not provide a favorable survival outcome in CY+ patients. This result may be indicative of the limitations of current adjuvant chemotherapy regimens. Recent progress in chemotherapy regimens has been closely associated with the improved prognosis of patients with unresectable PDAC.[15,16,19–21] However, no studies regarding the clinical efficacy of chemotherapy in patients with peritoneal carcinomatosis of pancreatic origin have been published. Thomassen et al.[24] stated that the biological response to systemic chemotherapy may differ between patients with peritoneal carcinomatosis and patients with liver metastasis due to the different blood supplies to the metastatic sites and/or different biological responses to cytotoxic agents. A CY+ finding indicates a risk of development of peritoneal carcinomatosis; thus, controlling the development of peritoneal carcinomatosis in patients with CY+ is an important issue. There are two approaches for treating such patients in the clinical setting. In some centers, the use of diagnostic laparoscopy is limited and intraoperative cytology is not available. In such a situation, we advocate surgical resection followed by effective adjuvant chemotherapy using extensive intraoperative peritoneal lavage,[25] heated intraperitoneal chemotherapy (HIPEC),[26,27] or systemic and intraperitoneal chemotherapy[28] in patients with CY+ in a clinical trial setting. We also advocate the selective use of diagnostic laparoscopy in patients at risk for occult distant organ metastasis. Chemotherapy for controlling peritoneal carcinomatosis should be first administered in such patients, and surgical resection should be selectively performed in patients with long-term favorable responses to chemotherapy. These approaches should be appropriately used according to the availability of staging laparoscopy and intraoperative cytology in each institution. This issue must be addressed in future randomized control trials.

The current study has some limitations. The first limitation is that it was conducted in a retrospective fashion with a hidden bias; however, it should be noted that prospective data collection is difficult in patients with CY+ who undergo surgical resection. The second limitation is that this study included only a small number of patients with CY+ (n=69); however, this is the largest database of such patients to date. It is particularly notable that our database had a relatively small amount of missing data. Other limitations include a lack of central review of cytologic results, a lack of quantification of the degree of cytologic abnormalities, and the varieties of neoadjuvant and adjuvant chemotherapy regimens that were used, which can influence survival in patients.

In conclusion, the results of the current study showed that PDAC patients with CY+ who underwent margin-negative resection had an acceptable MST. However, a high rate of peritoneal carcinomatosis was observed within 3 years after margin-negative resection, resulting in decreased long-term survival. Therefore, the development of a new strategy to control post-operative peritoneal carcinomatosis is required to obtain long-term survival when surgical resection is performed.

Conflict of Interest The authors have no conflicts of interest to disclose.

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Discussant

Dr. Steven J Hughes (Gainesville, FL):

The peritoneum is a rich soil for cancerous seeds, and carcinomatosis is a devastating development for pancreatic cancer patients. The authors' findings that CY+ is a profound indicator of poor prognosis are intuitive, and I rise to applaud your thoughtful study design and careful analysis of data from a truly impressive series of pancreas cancer patients. I would also like to thank the authors for providing me with a copy of the manuscript well in advance of the meeting. I have two questions.

Question #1: I am inclined to think that positive cytology should be indicative of subsequent carcinomatosis. I can understand why carcinomatosis was not subsequently identified in approximately half of the CY+ patients; cross-sectional imaging struggles to accurately diagnose this condition. Nonetheless, your data would suggest that current methods of cytology are not particularly specific for the development of clinically relevant carcinomatosis. More importantly in my mind, 21 % of CY− patients did subsequently develop carcinomatosis. Thus, this data suggests this particular assay may lack sensitivity. What are the authors' opinions regarding the sensitivity and specificity of peritoneal washings and how should that impact our interpretation of the data.

Question #2: For pancreatic adenocarcinoma, the NCCN guidelines characterize positive cytology as M1 disease, yet some authors have advocated proceeding with curative resection in this setting based upon data that your study would suggest suffered from Type II error. In your series, 7 % of patients proved to have positive cytology. How would you suggest we apply your findings to current clinical practice? Specifically, do you advocate diagnostic laparoscopy with peritoneal washings as a separate procedure? Are there barriers to intraoperative cytology?

Closing Discussant

Dr. Satoi:

Answer #1. I greatly appreciate your very important suggestions. The current study was retrospectively performed in a multi-center setting, and cytology analysis was conducted using Papanicolou and May-Giemsa staining, not with molecular techniques. As Dr. Hughes indicated, I agree that current cytology methods may not be particularly specific. The current method of cytology for predicting subsequent peritoneal carcinomatosis as the primary site of recurrence showed sensitivity of 15 %, specificity of 95 %, a positive predictive value of 49 %, and a negative predictive value of 21 %. However, it is difficult to accurately evaluate the sensitivity and specificity of peritoneal washing because the second site of recurrence was not recorded in this database. Therefore, we could not evaluate the overall rate of peritoneal recurrence. We should conduct a prospective study to evaluate this issue.

We would like to share the data from a Korean multi-center randomized control trial that showed that extended lymph node dissection was significantly associated with a high rate (25 %) of peritoneal carcinomatosis relative to the 9 % rate in patients who underwent standard lymphadenectomy. In the current study, extended lymph node dissection had been routinely performed at the seven institutions. I therefore think that patients with CY− may have had a high frequency of peritoneal carcinomatosis due to extended lymph node dissection.

Answer #2. First, we strongly suggest that peritoneal washing cytology should be done in pancreatic cancer patients who undergo planned surgical resection in all centers to select patients with CY+. Although we recognize that CY+ can be a prognostic factor and a risk factor for the development of peritoneal carcinomatosis after surgical resection, margin-negative resection might not be a good surgical indication in patients with CY+ but should not be a contraindication. The important issue for such patients is to control the development of peritoneal carcinomatosis and malignant ascites. There are two potential approaches for treating such patients. The availability of diagnostic laparoscopy is limited and intraoperative cytology is not available in some centers. In such a situation, we advocate surgical resection followed by effective adjuvant chemotherapy such as hyperthermic intraperitoneal chemotherapy or intravenous and intraperitoneal chemotherapy in patients with CY+ in a clinical trial setting. We also advocate the selective use of diagnostic laparoscopy in patients at risk for occult distant organ metastasis. Chemotherapy for controlling the development of peritoneal carcinomatosis should be first administered in such patients, and surgical resection should be selectively performed in patients with long-term favorable responses to chemotherapy. These approaches should be appropriately used according to the availability of staging laparoscopy and intraoperative cytology in each institution.

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TECHNICAL VIEW

Tips and tricks of the surgical technique for borderline resectable pancreatic cancer: mesenteric approach and modified distal pancreatectomy with en-bloc celiac axis resection

Seiko Hirono · Hiroki Yamaue

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Abstract Borderline resectable (BR) pancreatic cancer involves the portal vein and/or superior mesenteric vein (PV/SMV), major arteries including the superior mesenteric artery (SMA) or common hepatic artery (CHA), and sometimes includes the involvement of the celiac axis. We herein describe tips and tricks for a surgical technique with video assistance, which may increase the R0 rates and decrease the mortality and morbidity for BR pancreatic cancer patients. First, we describe the techniques used for the "artery-first" approach for BR pancreatic cancer with involvement of the PV/SMV and/or SMA. Next, we describe the techniques used for distal pancreatectomy with en-bloc celiac axis resection (DP-CAR) and tips for decreasing the delayed gastric emptying (DGE) rates for advanced pancreatic body cancer. The mesenteric approach, followed by the dissection of posterior tissues of the SMV and SMA, is a feasible procedure to obtain R0 rates and decrease the mortality and morbidity, and the combination of this aggressive procedure and adjuvant chemo(radiation) therapy may improve the survival of BR pancreatic cancer patients. The DP-CAR procedure may increase the R0 rates for pancreatic cancer patients with involvement within 10 mm from the root of the splenic artery, as well as the CHA or celiac axis, and preserving the left gastric artery may lead to a decrease in the DGE rates in cases where there is more than 10 mm between the tumor edge and the root of the left gastric artery. The development of safer surgical procedures is necessary to improve the survival of BR pancreatic cancer patients.

Keywords Borderline resectable pancreatic cancer · Mesenteric approach · Modified distal pancreatectomy with en-bloc celiac axis resection

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Instructions

Complete surgical resection is the sole curative treatment for pancreatic cancer patients; however, only 15–20% of the pancreatic patients are eligible for surgery [1, 2]. Therefore, aggressive surgical procedures, including concomitant portal vein and/or superior mesenteric vein (PV/SMV) resection and/or resection of major arteries during pancreatectomy, have been performed for advanced pancreatic cancer patients [3, 4] since Fortner et al. reported these aggressive surgical procedures in 1973 [5]. Nevertheless, these aggressive procedures have been considered to be contraindicated because of the high associated morbidity and mortality rates [4]. Recently, the surgical techniques, perioperative management and adjuvant chemotherapy or chemoradiation therapy have improved, and the national consensus of "borderline resectable (BR) pancreatic cancer" has widely been used worldwide [6–8].

BR pancreatic cancer involves the PV/SMV and/or major arteries (determined based on computed tomography [CT]) and is associated with a high risk of harboring radiographically occult metastases. Therefore, it is difficult for BR pancreatic cancer patients to obtain pathologically negative surgical margins to obtain survival benefits even if they undergo extended surgical resection. The definition of the National Comprehensive Cancer Network (NCCN) guideline 2013 described that: (1) no distant metastasis; (2) venous involvement of the PV/SMV with distortion or narrowing of the vein or occlusion of the vein with suitable vessels proximal and distal, allowing for safe resection and replacement; (3) gastroduodenal artery encasement up to the hepatic artery with either short segment encasement or direct abutment of the hepatic artery without extension to the celiac axis; and (4) tumor abutment of the superior mesenteric artery not to exceed more than 180 degrees of the circumference of the vessel wall [9]. However, the definitions of BR pancreatic cancer have subtle differences, and vary by institution [9–11]. For example, distal pancreatec-

tomy with en-bloc celiac axis resection (DP-CAR) has been widely performed for BR pancreatic cancer patients with radiological abutment of the celiac axis in Japan [12–15].

Although some studies have reported the advantages and disadvantages of aggressive surgical procedures for a small number of BR pancreatic cancer patients, it has not been known whether these aggressive surgical procedures, including concomitant dissection of the nerve plexus along major arteries and the resection of major vessels, lead to improvements in the survival of patients with BR pancreatic cancer. Recently, neoadjuvant therapy has been recommended for BR pancreatic cancer; however, it is unknown what regimen is the most effective and safest, and whether the neoadjuvant therapy could impact the survival [16–19].

The aim of this article is to suggest surgical techniques that can be used to increase the R0 rates and survival benefits, as well as to decrease the morbidity and mortality rates for BR pancreatic cancer using surgical videos.

Methods

We selected two operative procedures for BR pancreatic cancer patients in this article: one was mesenteric approach during pancreaticoduodenectomy (PD) for pancreatic head cancer, and another was DP-CAR with preservation of the left gastric artery (modified DP-CAR) for pancreatic body cancer, and trimmed each operation videos. The tips and tricks of the two operative procedures will be introduced with videos assistance and some published research in this article.

Results

Mesenteric approach for BR pancreatic cancer located in the pancreatic head (Video S1)

In Video S1, the patient was a 50-year-old male, and had the cancer located in the head of the pancreas with abutment of the SMA with 90 degrees of the circumference. Therefore, he was diagnosed as BR pancreatic cancer, and underwent neoadjuvant chemotherapy using gemcitabine and S-1.

First, the mesentery of the jejunum is incised at the line between the Treiz ligament and the third portion of the duodenum in order to identify the SMV and SMA at the line. Next, the J1 and J2 arteries are approached at the root of the SMA (Fig. 1), and the inferior pancreaticoduodenal artery (IPDA) is also identified. After the J1 artery and IPDA are ligated and divided, the posterior tissues of the SMA and SMV are dissected completely (Fig. 2). In cases with the involvement of the SMA, right side semicirumferential dissection of the nerve plexus of the SMA may be required in addition to this procedure in order to obtain negative surgical margins. The dissection of the tissues along the SMV and SMA is promoted cephalad toward the inferior border of the pancreatic body.

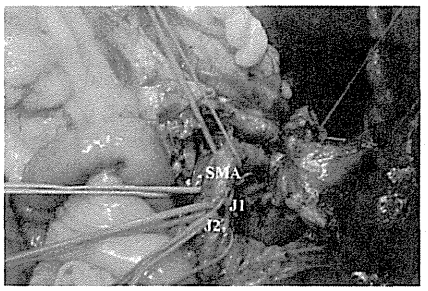

Fig. 1 After identification of the superior mesenteric artery (SMA) and the superior mesenteric vein (SMV) at the line between the Treiz ligament and the third portion of the duodenum, we approached the J1 and J2 arteries at the roots of the SMA to proceed with the posterior approach for the SMA

Fig. 2 The dissection of the posterior fat tissues of the superior mesenteric artery (SMA) and superior mesenteric vein (SMV) is completed via the mesenteric approach

After transection of the stomach or duodenum, the lymph node dissection around the common hepatic artery and hepatoduodenal ligament is performed. The dissection of the nerve plexus around the common hepatic artery may sometimes be required for pancreatic cancer with involvement of the common hepatic artery and/or the root of the gastroduodenal artery, especially for pancreatic neck cancer. After the dissection around the common hepatic artery and the hepatoduodenal ligament, the bile duct and the pancreas are transected. In cases of pancreatic neck cancer, the invasion of the confluence of the PV and SMV and even splenic vein invasion are sometimes found behind the tumor, and a procedure is needed that allows the range of the tumor to be cleared by intraoperative ultrasound and dissection of the posterior tissues between the pancreas and splenic artery from the PV toward the left side of the tumor [20].

Next, the dissection of the nerve plexus from the celiac trunk and the root of the SMA at the aorta to the pancreatic head is performed, and the pancreatic head is dissected

from the retroperitonum by the Kocher maneuver. After transection of the jejunum, the pancreatic head is connected with only the PV/SMV, if the tumor involves the PV/SMV. After the specimen is removed with the resection of the PV/SMV, PV/SMV reconstruction is performed. If the length of the resected PV/SMV is long, the splenic vein should be divided and/or an autologous graft should be interposed for a tension-free anastomosis in order to prevent the development of vessel thrombosis after reconstruction [21].

Modified DP-CAR procedure for BR pancreatic cancer located in the pancreatic body and/or tail (Video S2)

The patient was a 72-year-old male, and had the cancer located in the body of the pancreas with radiographic invasion of nerve plexus around the confluence of splenic artery and common hepatic artery and celiac axis. Therefore, he was diagnosed as having BR pancreatic cancer, and underwent neoadjuvant chemotherapy using gemcitabine and S-1. After neoadjuvant treatment, the tumor was stable disease, and his common hepatic artery was preoperatively embolized by angiographic coiling to increase arterial blood flow to the liver via the pancreatoduodenal arcades from the SMA.

The DP-CAR procedure includes en-bloc resection of the celiac axis, common hepatic artery and left gastric artery, in addition to the distal pancreatectomy. The nerve plexus and ganglions around the celiac axis and the SMA, and the retroperitoneal fat tissues, are also dissected. No reconstruction of the arterial system is required because of the development of the collateral arterial pathways via the pancreatoduodenal arcades from the SMA. Preoperative coil embolization of the common hepatic artery is often performed in order to enlarge the collateral pathways and prevent ischemia-related complications. Moreover, the right gastric vein, which usually joins to the portal vein, should be preserved for the prevention of the congestive gastropathy. If PV/SMV invasion is found and resection is required, the use of an autologous graft should be considered for a tension-free anastomosis after PV/SMV reconstruction to prevent thrombosis in the reconstructed PV/SMV [21].

Furthermore, we reported DP-CAR with preservation of the left gastric artery, named "modified DP-CAR", and found that the incidence of postoperative delayed gastric emptying was lower in the modified DP-CAR than standard DP-CAR. Therefore, we recommend the modified DP-CAR procedure over the standard DP-CAR, if the length between the edge of the tumor and the root of the left gastric artery is longer than 10 mm [15].

Discussion

Some artery-first approaches have been reported for PD [22], including the right posterior approach [23, 24], left posterior approach [25] and mesenteric approach [26, 27]. BR pancreatic cancer located in the pancreatic head has often required PV/SMV resection and lymph node dissection along the SMV and the SMA, and/or the dissection of the nerve plexus along the SMA, in order to obtain negative surgical margins. Therefore, the combination of the mesenteric approach and the left posterior approach to the SMA may be the most appropriate procedure for BR pancreatic cancer patients, because this approach makes it easy to dissect the lymph nodes and nerve plexus along the SMA, even for BR pancreatic cancer with PV/SMV involvement. This approach also makes it easy to determine the resectability at the beginning of the operation. However, there is currently no evidence whether the mesenteric approach and/or left posterior approach have clinical and survival benefits for BR pancreatic cancer patients. Therefore, further large studies, including randomized clinical trials, are needed to confirm the optimal approach.

Distal pancreatectomy with en-bloc celiac axis resection is sometimes performed to obtain R0 resection for pancreatic cancer with involvement of the celiac axis and/or common hepatic artery [12–15], although the NCCN guidelines classify pancreatic cancers with involvement of the celiac axis as unresectable [9]. Some studies have reported that DP-CAR is a safe and feasible procedure, and this procedure may have survival benefits for patients with pancreatic body and/or tail cancer [13–15]. Furthermore, our data showed that the DP-CAR procedure might lead to increased R0 rates and improve survival for patients with pancreatic body/tail cancer within 10 mm from the root of the splenic artery [14]. However, further large studies are needed to determine whether this aggressive surgery has survival benefits.

We also reported that the incidence of postoperative delayed gastric emptying was lower in the modified DP-CAR, which means DP-CAR with preservation of the left gastric artery, than standard DP-CAR [15]. Therefore, the pancreatic body/tail cancer, where the length between the edge of the tumor and the root of the left gastric artery is longer than 10 mm may be indicated for the modified DP-CAR, because it is important to decrease morbidity rate and postoperative adjuvant therapy starts as soon as possible for advanced pancreatic cancer.

To obtain negative surgical margins for BR pancreatic cancer, these aggressive surgical procedures are often required. However, it remains unknown whether these aggressive procedures improve the clinical and survival benefits. Recent studies have reported the effectiveness of neoadjuvant therapy to decrease the rates of the lymph node metastasis, and the activity of the tumor cells [16–19, 28]; however, it is also controversial what regimen is the most appropriate as neoadjuvant therapy, and whether chemotherapy or chemoradiation therapy is better for the BR pancreatic cancer patients.

In conclusion, the combination of safe R0 surgical resection and adjuvant therapies, including preoperative and postoperative chemo(radiation) therapy, is essential to improve the survival of the BR pancreatic cancer patients. The development of safer and more effective multimodality treatments is necessary for the BR pancreatic cancer patients.

Conflict of interest None declared.

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Supporting information

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Video S1 Mesenteric approach for BR pancreatic cancer located in the pancreatic head.
Video S2 Modified DP-CAR procedure for BR pancreatic cancer located in the pancreatic body and/or tail.

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Association of Pancreatic Fatty Infiltration With Pancreatic Ductal Adenocarcinoma

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OBJECTIVES: Fatty infiltration (FI) in the pancreas is positively correlated with high body mass index (BMI) or obesity, and the prevalence of diabetes mellitus (DM), which are well-known risk factors of pancreatic cancer. However, the association of FI in the pancreas with pancreatic cancer is unclear. Recently, we have shown that Syrian golden hamsters feature FI of the pancreas, the severity of which increases along with the progression of carcinogenesis induced by a chemical carcinogen. To translate the results to a clinical setting, we investigated whether FI in the pancreas is associated with pancreatic cancer in a series of patients who had undergone pancreatoduodenectomy.

METHODS: In the series, we identified 102 cases with pancreatic ductal adenocarcinoma (PDAC) and 85 controls with cancers except for PDAC. The degree of FI was evaluated histopathologically from the area occupied by adipocytes in pancreas sections, and was compared between the cases and controls.

RESULTS: The degree of FI in the pancreas was significantly higher in cases than in controls (median 26 vs. 15%, $P<0.001$) and positively associated with PDAC, even after adjustment for BMI, prevalence of DM and other confounding factors (odds ratio (OR), 6.1; $P<0.001$). BMI was identified as the most significantly associated factor with FI in the pancreas.

CONCLUSIONS: There is a positive correlation between FI in the pancreas and pancreatic cancer.

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INTRODUCTION

Pancreatic cancer is one of the most lethal human cancers with a 5-year survival rate of <5% in both Japan and the United States.[1] Thus, the development of useful predictive markers for individuals with a high risk of pancreatic cancer would be of great help in detecting pancreatic cancer at its early stages, and might contribute to a significant reduction of mortality. Epidemiological studies have shown that a family history of pancreatic cancer, cigarette smoking, age, obesity, and diseases such as chronic pancreatitis and diabetes mellitus (DM) increase the risk of pancreatic cancer.[2–4] A few pathologic studies of patients with pancreatic cancer have demonstrated fatty infiltraton (FI) in the pancreas parenchyma.[5,6] FI in the pancreas is positively correlated with age, body mass index (BMI), and a history of DM.[7–9]

Recently, we have shown that in Syrian golden hamsters, which exhibit a substantial age-related increase of hypertriglyceridemia and FI in the pancreas, there is further progression of pancreatic FI and carcinogenesis upon treatment with a carcinogen, *N*-nitrosobis (2-oxopropyl) amine (BOP), while the animals are fed a high-fat diet (HFD).[10] Therefore, we hypothesized that FI in the pancreas accompanied by hypertriglyceridemia might be associated with pancreatic cancer in both humans and experimental animals.

In the present case–control study, we examined whether FI in the pancreas is associated with pancreatic ductal adenocarcinoma (PDAC) in humans, independently of several other suggested risk factors for pancreatic cancer, such as obesity and DM.

METHODS

Patients and samples. Between January 2004 and December 2010, 367 patients underwent pancreatoduodenectomy for PDAC at the National Cancer Center Hospital, Japan. Among them, 102 were considered to be appropriate for the present study on the basis of the criteria detailed later. As controls, we used non-cancerous pancreas tissues from 85 patients who had undergone pancreatoduodenectomy for cancer, except for PDAC; these included 46 patients with distal bile duct cancer, 33 with cancer of the ampulla of Vater, 4 with gallbladder cancer, and 2 with duodenal cancer. DM was clinically diagnosed at the referring hospitals, using criteria of fasting blood glucose level ≥126 mg/dl and HbA1c ≥6.1%, before the patients visited our hospital to resect pancreatic cancer. BMI was calculated when the patients were admitted to our hospital. The use of each individual's material for analysis in the

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present study was approved by the Ethics Review Committee of the National Cancer Center (2010-088). The materials are from patients who had given general consent for the research use of their leftover samples, and all clinical investigations were conducted in accordance with the principles of the Declaration of Helsinki.

Pathological examination. PDACs were examined pathologically and classified according to the World Health Organization classification and TNM classification.[11,12] Surgically resected specimens were fixed in 10% formalin, and the pancreas heads were cut horizontally into serial slices 5 mm thick. In order to evaluate FI appropriately, we conducted a preliminary study to select a target area of pancreas parenchyma in 16 cases of PDAC. As FI is easily affected by any type of pancreatitis associated with cancer infiltration, including obstructive pancreatitis, we selected the FI area for measurement, avoiding any primary and/or secondary effect caused by cancer infiltration (Supplementary Figure S1 online). Thus, pancreatitis patients were ruled out from the FI evaluation. First, we selected anterior and cranial areas of the pancreas near the duodenum that correspond to the dorsal pancreas during organogenesis. Second, we chose areas of the pancreas near the ampulla of Vater if the former area was affected by cancer infiltration. If both of these areas were affected by cancer infiltration, such cases were excluded from the study. Thus, we selected one section containing non-tumorous pancreatic tissue and confirmed whether it fulfilled the above conditions. Then, FI areas were measured quantitatively as the percentage of area infiltrated by adipocytes relative to the total area on the section was calculated using the WinROOF image analysis software package (Mitani Corp, Tokyo, Japan). The reproducibility of this quantitation method was checked preliminarily by comparing the FI area of one section with another section derived from tissue immediately adjacent to the former. The difference between the two FI area values measured in 16 pairs of sections was 5.6% on average.

Serum sample collection and assays. Peripheral blood was collected from each patient at the time of the hospital visit prior to treatment, and blood sugar, HbA1c, and serum levels of total cholesterol (TC), high-density lipoprotein (HDL), amylase, CEA, and CA19-9 were measured by participants at the National Cancer Center Hospital. For further examination, serum provided by the National Cancer Center Biobank, Japan, was stored at $-20^{\circ}C$. Serum adiponectin, leptin and insulin growth factor-I (IGF-I) (R&D Systems, Inc., Minneapolis, MN, USA), apolipoprotein A-II (apoA-II) (Assay pro, St Charles, MO, USA), insulin (Millipore, Billerica, MA, USA), and serum amyloid A (SAA; Invitrogen, Camarillo, CA, USA) were measured using enzyme-linked immunosorbent assay kits in accordance with the manufacturers' instructions. The levels of serum triglycerides (TGs), HDL, and gamma-glutamyltransferase (GGT) were analyzed using the FUJI Dri-Chem system (Fuji Film, Tokyo, Japan).

Statistical analysis. The cases and controls were classified into three subgroups, $<10\%$, 10–20%, and $\geq 20\%$, according to the area of FI. The cutoff points of 10 and 20 were nearly equal to the tertile cutoff points in the controls, namely 9.5 and 20.4. An unconditional logistic regression model was used to estimate odds ratios (ORs) and their 95% confidence intervals (CIs) of PDAC according to the three categories of FI in the pancreas, the lowest value being used as a reference. Two-sided P values <0.05 were considered to indicate statistical significance. All statistical analyses were carried out using the Statistical Analysis System (SAS), version 9.1 software package (SAS Institute, Cary, NC, USA) by a statistician (T.Y.).

RESULTS

Patient characteristics. Among 102 cases, one was classified as stage IB, 27 as stage IIA, 65 as stage IIB, and 9 as stage IV. The characteristics of the case and control patients are summarized in Table 1. Controls were older than cases ($P=0.001$), and there was a male predominance in both groups. The known risk factors for PDAC were compared between cases and controls. The prevalence of DM ($P=0.03$) and family history of pancreatic cancer ($P=0.007$) in cases was higher than in controls. The values of blood sugar ($P=0.002$) and HbA1c ($P<0.001$) in cases were also significantly higher than in the controls. The serum apoA-II level was shown to be lower in cases than in controls ($P=0.02$), as reported previously, in comparison with healthy subjects. CEA ($P=0.04$) and CA19-9 ($P<0.001$), serum tumor markers for PDAC, were also significantly higher in cases than in controls. Meanwhile, serum levels of GGT ($P<0.001$), which are associated with liver and biliary disorders, were higher in controls than in cases.

Association of FI in the pancreas with PDAC. In the human pancreas, adipocytes were observed to accumulate in the area between pancreatic lobules (interlobular fat), especially around great vessels, or to be scattered in the lobules (intralobular fat), as shown in Figure 1. The distribution pattern of FI in some patients was similar to that observed in hamster pancreas.[10] In this study, FI in the pancreas was defined as the sum of the areas showing any types of FI in the pancreas parenchyma. Table 1 shows that the area of FI in the pancreas was significantly greater in cases than in controls (median 26 vs. 15%, $P<0.001$). Types of differentiation and stages of PDACs were not associated with the degree of FI (data not shown).

Table 2 shows the association between the area of FI in the pancreas and PDAC. A significantly higher OR for PDAC was observed according to the area of FI in the pancreas ($P<0.001$). Adjusted for sex, age, BMI, history of DM, and family history of pancreatic cancer, confounding factors for pancreatic cancer, ORs for PDAC showed an increasing trend according to the area of FI ($P<0.001$). Even when patients with a BMI $>25\ kg/m^2$, a history of DM, and a family history of pancreatic cancer were excluded, positive associations between the degree of FI in the pancreas and PDAC were observed ($P<0.001$ overall).

The factors associated with FI. The characteristics of the study participants were examined in relation to the degree of FI of the pancreas in controls and cases and are shown in Supplementary Tables 1 and 2, respectively. BMI and age were positively correlated with the area of FI of the pancreas

Table 1 Selected characteristics of study subjects

Characteristic	Cases ($n=102$)	Controls ($n=85$)	P^a
Categorical variables, n *(%)*			
FI in the pancreas ≥ 20%	64 (62.7)	30 (35.2)	<0.001
Male	60 (58.8)	60 (70.5)	0.12
Ever smoking	53 (51.9)	42 (49.4)	0.77
Frequent drinking (5–7 times/week)	34 (33.3)	31 (36.9)	0.85
DM	30 (29.4)	14 (16.4)	0.03
Hypertension	36 (35.2)	27 (31.7)	0.64
Hyperlipidemia	7 (6.8)	10 (11.7)	0.30
Family history of PC	11 (10.7)	1 (1.1)	0.007
Continuous variables, median (IQR)			
FI in the pancreas, %	25.8 (14.2–40.9)	15.0 (7.7–24.8)	<0.001
Age, years	63.5 (56–69)	68.0 (63–73)	0.001
BMI, kg/m^2	22.4 (20.3–24.3)	22.7 (20.7–24.2)	0.95
Blood sugar, mg/dl	114.0 (100–141)	106.0 (93–119)	0.002
HbA1c, %	5.5 (5.1–6.4)	5.1 (4.7–5.5)	<0.001
TC, mg/dl	189.0 (162–221)	195.0 (169–227)	0.31
HDL, mg/dl	52.0 (43–62)	52.0 (42–67)	0.47
TG, mg/dl	149.0 (109–209)	155.0 (117–210)	0.38
Apo A-II, μg/ml	219.3 (136.7–397.5)	327.3 (174.0–444.4)	0.02
Adiponectin, μg/ml	5.4 (3.0–9.6)	6.3 (3.2–12.3)	0.37
Leptin, ng/ml	3.2 (2.2–4.6)	3.1 (2.4–3.8)	0.57
Insulin, mU/l	3.5 (2.5–5.8)	3.7 (2.8–6.4)	0.41
IGF-I, ng/ml	69.7 (53.2–93.5)	74.1 (54.4–96.4)	0.69
Amylase, IU/l	107.0 (75–182)	105.0 (83–141)	0.55
CEA, ng/ml	2.6 (1.6–4.1)	2.0 (1.3–3.4)	0.04
CA19-9, U/ml	96.0 (46–400)	30.0 (16–121)	<0.001
SAA, μg/ml	22.1 (8.93–54.3)	35.8 (12.7–89.8)	0.06
GGT, ng/ml	105.0 (33–311)	339.0 (101–673)	<0.001

Apo A-II, apolipoprotein A-II; BMI, body mass index; DM, diabetes mellitus; FI, fatty infiltration; GGT, gamma-glutamyltransferase; HDL, high density lipoprotein; IGF-I, insulin growth factor-I; IQR, interquartile range; PC, pancreatic cancer; SAA, serum amyloid A; TC, total cholesterol; TG, triglyceride.
[a]Based on the Fisher's exact test for percentage difference and the Wilcoxon rank-sum test for median difference.

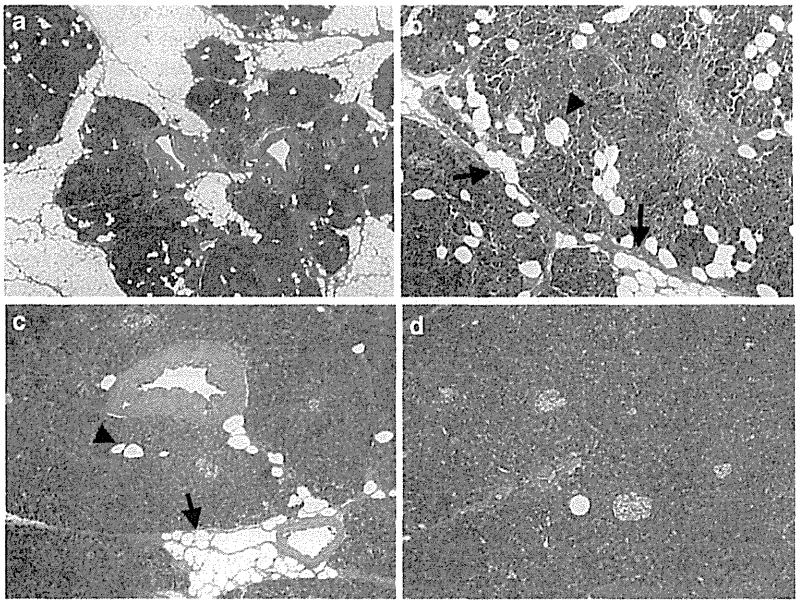

Figure 1 Histology of the human pancreas with fatty infiltration. (**a**, **b**) Pancreas tissue with moderate to severe FI. Most of the pancreas parenchyma has been replaced by adipocytes, and the remaining pancreas lobules resemble islets surrounded by a fatty lake. Most adipocytes have accumulated interlobularly (arrow in **b**), but some are scattered within the lobules (arrowhead in **b**). (**c**) Pancreas tissue with mild FI. Adipocytes have accumulated around arterioles (arrow), and several adipocytes are scattered within the lobules (arrowhead). (**d**) Pancreas tissue with minimal FI. Super-low magnification in **a**, and low magnification in (**b** to **d**).

Table 2 Association of the degree of FI in the pancreas with pancreatic ductal adenocarcinoma

	FI in the pancreas						
	<10%		≥10%, <20%		≥20%		
Population	**OR**	**95% CI**	**OR**	**95% CI**	**OR**	**95% CI**	***P*[a]**
All subjects							
Cases/controls		17/30		21/25		64/30	
Crude estimate	1	Reference	1.4	(0.6–3.4)	3.7	(1.8–7.8)	<0.001
Adjusted estimate[b]	1	Reference	2.3	(0.8–6.2)	6.1	(2.4–15.2)	<0.001
Excluding those with BMI of ≥25 kg/m²							
Cases/controls		17/28		18/22		49/24	
Adjusted estimate[c]	1	Reference	2.1	(0.7–5.9)	6.3	(2.4–16.5)	<0.001
Excluding those with past history of DM							
Cases/controls		14/28		18/21		40/22	
Adjusted estimate[d]	1	Reference	3.1	(1.0–9.3)	7.5	(2.6–21.3)	<0.001
Excluding those with family history of PC							
Cases/controls		15/29		17/25		59/30	
Adjusted estimate[e]	1	Reference	2.0	(0.7–5.5)	5.4	(2.2–13.6)	<0.001

BMI, body mass index; CI, confidence interval; DM, diabetes mellitus; FI, fatty infiltration; OR, odds ratio; PC, pancreatic cancer.
[a]Statistical tests for trend (two-sided) were assessed by assigning ordinal values to the degree of FI in the pancreas.
[b]Adjusted for sex, age (≤60, 61–70 and >70), BMI (<25, ≥25), past history of DM (yes or no) and family history of PC (yes or no).
[c]Adjusted for sex, age (≤60, 61–70 and >70), past history of DM (yes or no), and family history of PC (yes or no).
[d]Adjusted for sex, age (≤60, 61–70 and >70), BMI (<25, ≥25), and family history of PC (yes or no).
[e]Adjusted for sex, age (≤60, 61–70 and >70), BMI (<25, ≥25), and past history of DM (yes or no).

in both cases and controls. In control patients, the serum TG and amylase values were also positively correlated with the area of FI in the pancreas. Meanwhile, the levels of the serum insulin, HbA1c and blood sugar in case patients were positively correlated with the area of FI in the pancreas. To further investigate an association with FI in the pancreas, we conducted a multivariable linear regression analysis in each group, in which the above variables (BMI, serum TG, and amylase for controls; BMI, serum insulin, HbA1c, and blood sugar for cases), as well as age and sex, were included in one model. After mutual adjustment, a statistically significant association was noted only for BMI (controls, $P=0.001$; cases, $P=0.01$).

DISCUSSION

Based on epidemiological observation of human pancreatic cancers, FI in the pancreas was suggested to associate with PDAC, independently of known risk factors such as obesity and DM (Supplementary Figure S2). Although we identified BMI, a measurement of obesity, as the most significantly associated factor among several factors related to FI in the pancreas, FI in the pancreas was likely to increase the risk of pancreatic cancer beyond the effect of obesity alone. Some previous studies have evaluated pancreatic FI in humans using diagnostic modalities such as ultrasound, magnetic resonance imaging, or magnetic resonance spectroscopy.[9,13–16] FI in the pancreas has been suggested to promote dissemination and lethality of PDAC and to increase the risk of postoperative pancreatic fistula.[17,18] Here we demonstrated that the area of FI in histopathological sections of PDAC resected can be used as a quantitative indicator of the degree of FI. This is the first report to indicate an association between the area of FI and the development of PDAC.

Although mechanistic insights into how PDAC could develop from such an adipocyte-rich microenvironment are not clear, recent evidence suggests that ectopic fat accumulation produces certain adipocytokines that induce cell proliferation.[19,20] Serum adipocytokine levels were not clearly correlated with the area of FI in the present study, but the level of leptin expression was high in the pancreas of BOP-treated hamsters fed a HFD.[10] Thus, local release of adipocytokines from adipocytes in an adipocyte-rich microenvironment appeared to be correlated with PDAC development.

In the present study, serum insulin levels in cases were positively correlated with FI in the pancreas. It has also been reported that HOMA-IR is strongly correlated with FI of the pancreas except in subjects with a history of DM, pancreatic diseases and liver diseases.[9] In an *in vitro* setting, it has been shown that glucose-dependent insulinotropic polypeptide activates lipoprotein lipase, leading to TG accumulation in differentiated 3T3-L1 adipocytes in the presence of insulin.[21] Therefore, it is conceivable that induction of high glucose and insulin levels by hyperphagia could be associated with FI through activation of lipoprotein lipase in the pancreas. Conversely, it has also been suggested that increased pancreatic FI is related to β-cell dysfunction in the absence of type 2 DM,[22] and that this can lead to subsequent development of type 2 DM.[23,24] The hyperinsulinemia seen in human obesity, including the early phase of type 2 DM, may be closely related to FI in the pancreas.

Several possible mechanisms underlying the development of FI in the pancreas can be speculated. It has been shown in experimental animal models that FI can be induced in the pancreas by obstruction of the pancreatic duct or vasculature.[25,26] Smits and van Geenen[27] have showed that FI or non-alcoholic fatty pancreas disease represents fat accumulation induced by obesity and metabolic syndrome, while fatty replacement represents replacement of adipocytes induced by

death of acinar cells. We agree with their statements that pancreatic fat accumulation is mainly induced by these two factors. In this study, pancreatic FI in cases represents any type of fat accumulation caused by any type of etiology. It has been reported that lipotoxicity caused by a high TG content induces inflammatory responses and necrosis in pancreatic acinar cells *in vitro*.[28,29] It has also been shown that c-Myc activity is required for growth and maturation of the exocrine pancreas and for the transdifferentiation of acinar cells into adipocytes in mice.[30] Thus, pancreas containing scattered adipocytes might be more sensitive to acinar cell damage due to lipotoxity and other genetic factors, and scattered FI may reflect the acinar cell death or transdifferentiation after the damage.

Some limitations could be pointed out in this study. The major limitation is that it lacked normal healthy controls because pancreatic sections could be obtained only from patients who had undergone pancreatoduodenectomy. A second limitation is that we could not measure FI in more than one pancreatic section, as areas for measuring FI were limited and small because the areas of tumor and secondary inflammation were avoided. Therefore, a future study using a non-invasive method will be required to evaluate FI in a large area/volume of pancreas from healthy and case subjects. Previously, we have reported a case of PDAC that was associated with marked FI in the pancreas, as seen on computed tomography images.[31] Computed tomography imaging of the pancreas would be a useful approach for accurate evaluation and follow-up of pancreatic FI in normal subjects, as well as in cohort studies. The third limitation is that we did not exclude the areas of pancreas with PanINs from the sections for measuring FI because it is known that PanINs are sometimes found in pancreatic tissue of the elderly, and also that a large number of PanINs with various grades are found in the pancreas of the patients with PDAC. Therefore, it is extremely difficult to measure FI in the pancreas tissue without PanINs, especially in the limited area for measuring FI. The fourth limitation is that BMI could be underestimated in the cases, because weight loss is a very common symptom of patients suffering from pancreatic cancer even though most cases were classified as stage IIA or IIB. The fifth limitation is that there is no validation study. To confirm the observation in the present study, the same study should be repeated with the same methods in another center (hospital/institution). The final limitation is that we cannot distinguish whether FI was a risk factor or a consequence of the cancer. The only way to demonstrate that FI is a risk factor for PDAC is to perform a prospective cohort study to observe whether individuals with fatty pancreas could develop PDAC. For this purpose, we are now trying to establish the methods to evaluate FI in a large area/volume of pancreas by non-invasive method, using computed tomography and magnetic resonance imaging. In addition, studies on pancreatic carcinogenesis using animal models of fatty pancreas would be helpful to elucidate underlying mechanisms.

In conclusion, there is a positive correlation between FI in the pancreas and pancreatic cancer. The development of effective detection methods and/or markers of FI, especially "fatty pancreas" with severe FI, is warranted for mass screening of individuals at high risk of pancreatic cancer at health examinations.

CONFLICT OF INTEREST

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Specific author contributions: Mika Hori contributed to the design of the study, acquisition, analysis and interpretation of data, writing and drafting of the manuscript; Mami Takahashi contributed to the conception of the study, development of methodology and data analysis and revision of the manuscript; Nobuyoshi Hiraoka contributed to the histopathological analysis and revision of the manuscript; Taiki Yamaji contributed to the statistical analysis and revision of the manuscript; Michihiro Mutoh contributed to data analysis and revision of the manuscript; Rikako Ishigamori contributed to the histopathological analysis; Koh Furuta contributed to material supports in human serum analysis; Takuji Okusaka contributed to the clinical revision of the manuscript; Kazuaki Shimada contributed to the clinical revision of the manuscript; Tomoo Kosuge contributed to the clinical revision of the manuscript; Yae Kanai contributed to the histopathological analysis; Hitoshi Nakagama contributed to study supervision and revision of the manuscript.

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Potential competing interests: None.

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Study Highlights

WHAT IS CURRENT KNOWLEDGE

- Fatty infiltration (FI) in the pancreas is positively correlated with obesity and prevalence of DM.
- The association of FI in the pancreas with pancreatic ductal adenocarcinoma (PDAC) is unclear in humans.

WHAT IS NEW HERE

- FI in the pancreas is associated with PDAC development in humans.
- Body masss index (BMI) was identified as the most significantly associated factor with FI in the pancreas.
- FI in the pancreas may increase the risk of PDAC beyond the effect of obesity alone.

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