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ORIGINAL ARTICLE

Levels of tumor markers CEA/CA 19–9 in serum and peritoneal lavage predict postoperative recurrence in patients with pancreatic cancer

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Abstract

Aim: This study aimed to clarify the usefulness of tumor markers from peritoneal lavage in selecting patients with a high risk of recurrence and predicting site-specific recurrence in patients with pancreatic cancer.

Methods: The levels of serum carcinoembryonic antigen (CEA) and carbohydrate antigen (CA) 19–9 (sCEA/sCA 19–9) and paired peritoneal lavage CEA and CA 19–9 (pCEA/pCA 19–9) were measured in 90 patients with pancreatic cancer who underwent surgery. Using the cutoff values determined by maximally selected rank statistics for disease-free survival (DFS), the risk of recurrence and its patterns were evaluated in combination with different markers and different test specimens.

Results: In univariate and multivariate analysis, an elevated pCA 19–9 level (>1.3 U/mL) was an independent prognostic marker for both DFS (hazard ratio [HR], 2.391; $P = .018$) and overall survival (HR, 3.194; $P = .033$). Combination analyses contributed to further stratification of a very high risk of recurrence. Of the 58 patients with resectable pancreatic cancer who underwent curative resection, elevated pCA19–9 was also associated with inferior DFS and overall survival (OS). Patients with elevated pCA 19–9 levels were more likely to have an earlier onset of peritoneal recurrence than those with normal pCA 19–9 levels ($P = .048$, Gehan–Breslow–Wilcoxon test).

Conclusion: pCA 19–9 is a reliable marker for predicting postoperative recurrence in patients with pancreatic cancer after surgery. Further risk stratification can be achieved by using combination assays. The combination of pCA 19–9 and sCA19–9 also serves as a predictor of recurrence site-specific recurrence.

KEYWORDS
pancreatic cancer, peritoneal lavage cytology, staging laparoscopy, tumor marker

1 | INTRODUCTION

Pancreatic ductal adenocarcinoma (PDAC) is still acknowledged as a highly lethal disease, with a 5-y survival rate of <10%.[1] Owing to the lack of specific symptoms and difficulty in early diagnosis, almost 80% of newly diagnosed patients exhibit locally advanced or metastatic disease at the initial presentation. The remaining ~20% of patients with localized PDAC get scheduled for surgical resection.[2,3] However, up to 80% of the patients who have undergone resection develop disease recurrence even after seemingly curative pancreatectomy and sequential adjuvant treatment.[4] Particularly, recurrent disease developing during the early postoperative period has been attributed to the presence of occult micrometastasis beyond the margins of surgical resection at the time of surgery.[5]

Peritoneal dissemination is challenging to detect among the different recurrence patterns using imaging modalities.[4] Peritoneal dissemination is characterized by the development of intestinal obstruction and massive ascites, leading to insufficient oral intake and subsequent malnutrition. These worsening symptoms prevent the patient from receiving chemotherapy.[6] Positive peritoneal lavage cytology is a strong predictor of the development of peritoneal recurrence.[7] However, peritoneal recurrence occurs even in patients undergoing curative resection for localized PDAC and negative cytology.[8,9] Taken together, identifying a more sensitive biomarker is essential for predicting or detecting peritoneal micrometastasis.

In various gastrointestinal cancers, including PDAC, serum carcinoembryonic antigen (CEA) and carbohydrate antigen (CA) 19–9 levels are commonly utilized in clinical practice to predict prognosis and evaluate therapeutic response to anticancer treatments.[10] Furthermore, pre- and postoperative assessment of serum CA 19–9 in patients with seemingly localized PDAC undergoing curative resection have been used to evaluate the risk of recurrence.[11,12] Such tumor marker dynamics can indicate the presence of minimal residual disease before the emergence of radiographically detectable recurrent lesions.

Recent studies have evaluated the clinical significance of such tumor markers in other surrogate body fluids, such as peritoneal lavage. Indeed, these prior studies suggested their ability to predict peritoneal recurrence and long-term prognosis in patients with gastric and colorectal cancer.[13–15] In patients with PDAC, recent advances in molecular biochemistry have also enabled the detection of minimal residual disease in the peritoneal lavage fluid with superior sensitivity to conventional cytology.[16,17] Even yet, tumor markers in peritoneal lavage fluid have been rarely evaluated, and their clinical utility remains controversial.[18,19]

The present study aimed to determine the best molecular diagnostic approach using tumor markers to select the high risk of recurrence in patients with PDAC. We conducted tumor marker analyses using the serum and paired peritoneal lavages samples collected from the patients with PDAC undergoing surgery and evaluated their clinical relevance.

2 | METHODS

2.1 | Patients

This study was conducted retrospectively using prospectively collected and maintained clinical subjects and databases. Between April 2018 and August 2020, 163 consecutive patients with cytohistologically proven PDAC underwent abdominal exploration at the Tohoku University Hospital. Some patients underwent serial explorations to evaluate the therapeutic effect of chemotherapy targeting radiographically invisible metastases including positive cytology. Pancreatectomy was performed only when liver metastasis, macroscopic peritoneal dissemination, and negative peritoneal lavage cytology were not detected on abdominal exploration. Based on the workflow for patient selection, 90 patients were finally included in this study (Figure S1). The study was approved by the Medical Ethics Committee of the Tohoku University Graduate School of Medicine (Institutional Review Board approval number 2019–1-119) and was conducted according to the principles of the Helsinki Declaration. All eligible patients during the course of the study provided written informed consent before participation.

2.2 | Abdominal exploration and sample collection

Abdominal exploration and collection of peritoneal lavage fluid were performed by open laparotomy or most recent staging laparotomy within 3 wk of surgery. Under general anesthesia, peritoneal lavage cytology was routinely performed at the beginning of the abdominal exploration using 100 mL of normal saline, which was introduced into the recto-uterine pouch (Douglas pouch) and aspirated soon after gentle stirring. Afterwards, routine procedures of abdominal exploration were performed as described previously.[20] Finally, metastatic findings were addressed as the presence (positive) or absence (negative) of hepatic metastasis (HEP+ or HEP−), peritoneal dissemination (PER+ or PER−), or peritoneal lavage cytology (CY+ or CY−).

2.3 | Sample and data collection

Harvested peritoneal lavage samples were immediately centrifuged at 2500 rpm for 10 min. The entire precipitate was used in the cytological diagnosis and the remaining supernatant was applied to evaluate the biochemistry of peritoneal lavage CEA (pCEA) and peritoneal lavage CA 19–9 (pCA 19–9). Blood samples were collected from all included patients, and their serum CEA (sCEA) and serum CA 19–9 (sCA 19–9) concentrations were also measured within 1 wk before abdominal exploration. Postoperative measurements of sCA 19–9 were taken within the 1 mo of the surgery. Levels of all tumor markers in the serum and peritoneal lavage fluid were measured using automated electrochemiluminescence immunoassays on the molecular analytical systems (Cobas8000, Roche Diagnostics, Nutley, NJ, USA). According to

the manufacture's instruction, the dynamic measurable range of CA 19–9 ranged from 0.6 to 1000 U/mL. In this study, patients with sCA 19–9 values of <2 U/mL were excluded because they were judged to be nonsecretors of CA 19–9 owing to the absence of Lewis antigen.[21,22]

Demographic, pathological, and clinical data were collected from prospectively maintained databases and medical records. Resectability classifications were based on the NCCN guidelines (version 2.2021).[23] Pathological examinations were performed in accordance with the International Union Against Cancer (UICC) TNM classification 8th edition.

2.4 | Statistics

Continuous variables were described as the medians with ranges and were compared using the nonparametric Mann–Whitney *U* test. The cutoff values of tumor markers were determined according to disease-free survival (DFS) and then dichotomized into "low" and "high" groups using the R package "Maxstat" (titled Maximally Selected Rank Statistics, https://CRAN.R-project.org/package=maxstat), which iteratively tests all possible cutoff points to find the one achieving the maximum log-rank statistic using the R software program (version 4.1.2, Vienna, Austria). Categorical variables were compared using Fisher's exact test. DFS and overall survival (OS) analyses were performed using the Kaplan–Meier method, and the differences were compared by a log-rank test. When we analyzed the peritoneal recurrence-free survival, the data for patients who died due to other recurrences were censored. The Gehan–Breslow–Wilcoxon test was performed to compare the risk of early peritoneal recurrence; this test gives more weight to events at early timepoints. The Cox proportional hazards model was used for multivariate analysis after relevant prognostic variables were identified using univariate analysis. Two-sided $P < .05$ was considered statistically significant. All statistical analyses were performed using JMP Pro 15.0.0 statistical software (SAS Institute, Cary, NC, USA) and GraphPad Prism version 9.3.1 (GraphPad Software, San Diego, CA, USA).

3 | RESULTS

3.1 | Patient demographics and tumor characteristics

Patient characteristics are summarized in Table 1. Of all 90 patients, 17 (19%) patients underwent upfront surgery without any preoperative therapy. Nine (10%) patients lost the chance of postoperative adjuvant chemotherapy due to their poor general condition. Only three patients with pathological stage IV disease were included; they all harbored the histologically proven para-aortic lymph node metastasis harvested by en-bloc dissection. The median follow-up period after surgery was 25.8 mo or until death.

TABLE 1 Patient characteristics

Characteristics	n = 90
Age (y), median (range)	70 (42–89)
Sex, n (%)	
Male	53 (58.9)
Female	37 (41.1)
Location, n (%)	
Head	53 (58.9)
Body and tail	37 (41.1)
Tumor diameter (mm), median (range)	20 (4–44)
Resectability, n (%)	
R	63 (70.0)
BR (SMV/PV invasion alone)	5 (5.6)
BR (arterial invasion)	13 (14.4)
UR (locally advanced)	9 (10.0)
Preoperative therapy, n (%)	
Absent (upfront surgery)	17 (18.9)
Chemotherapy (neoadjuvant)	48 (53.3)
Chemo(radio)therapy (conversion)	25 (27.8)
Abdominal exploration and peritoneal lavage sampling	
Staging laparoscopy	29 (32.2)
Open laparotomy	61 (67.8)
Procedures, n (%)	
PD	55 (61.1)
DP	18 (20.0)
DP-CAR	11 (12.2)
TP	6 (6.7)
PV/SMV resection, n (%)	
Present	16 (17.8)
Absent	74 (82.2)
Pathological UICC stage, n (%)[a]	
0	1 (1.1)
IA	17 (18.9)
IB	12 (13.3)
IIA	2 (2.2)
IIB	35 (38.9)
III	20 (22.2)
IV	3 (3.3)
Adjuvant chemotherapy, n (%)	
None	9 (10.0)
S-1	76 (84.4)
GEM	4 (4.4)
FFX	1 (1.1)

Abbreviations: BR, borderline resectable; DP, distal pancreatectomy; DP-CAR, distal pancreatectomy with celiac axis resection; FFX, FOLFIRINOX (5-FU/leucovorin, irinotecan, and oxaliplatin); GEM, gemcitabine; PD, pancreaticoduodenectomy; PV, portal vein; R, resectable; SMV, superior mesenteric vein; TP, total pancreatectomy; UICC, Union for International Cancer Control; UR, unresectable.

[a]According to the UICC 8th edition.

First, although the subjects with any positive findings of HEP/PER/CY were excluded from the study workflow prior to the analyses (Figure S1), the pCEA and pCA 19–9 were compared according to the presence or absence of each HEP/PER/CY finding across all tested 163 subjects, to assess the correlation between the presence of occult metastases and the elevation of tumor markers in peritoneal lavage (Figure S2). Both pCA 19–9 and pCEA were significantly elevated in patients with PER+ (Figure S2B,E) and CY+ (Figure S2C,F), respectively.

This study included six patients who underwent pancreatectomy after negative conversion from CY+ to CY− based on the longitudinal investigations. Paired comparisons were performed using each tested marker value in the six patients between before and after the negative conversion of cytology. All tested marker values appeared to decrease after negative conversion; however, significant differences were not observed, likely owing to the small number of patients (Figure S3).

3.2 | Measurement of tumor marker levels in serum and peritoneal lavage

The peritoneal lavage samples of 68 (76%) patients had the lowest limit of the normal range of CEA level (0.2 ng/mL). The median pCA 19–9 level was 1.6 U/mL. The actual values of pCEA and pCA 19–9 were much lower than those of the paired sCEA and sCA 19–9 (Figure 1). Only three patients exhibited higher values of pCA 19–9 than the paired sCA 19–9 (Table S1). A unique cutoff value was defined based on the maximum difference in DFS using the maximally selected rank test (Figure S4). The cutoff values of pCEA and pCA 19–9 were calculated as 0.6 ng/mL and 1.3 U/mL, respectively. Besides the well-recognized universal cutoff value of 5.0 ng/mL for sCEA and 37.0 U/mL for sCA 19–9, the unique cutoff values of sCEA and sCA 19–9 were set at 4.1 ng/mL and 55.0 U/mL, respectively (Figure S4).

The clinicopathological features associated with elevated pCA 19–9 levels were compared. A positive correlation was observed between preoperative sCA 19–9 and pCA 19–9 levels (Figure S5). Additionally, a higher postoperative sCA 19–9 level was associated with the elevated pCA 19–9 level ($P = .003$, Table 2). Moreover, patients with PDAC harboring a larger tumor diameter ($P = .017$) and histological nerve plexus invasion ($P = .040$) exhibited elevated pCA 19–9 levels (Table 2). When comparing patients with a low pCEA level (≤0.6 ng/mL, n = 77) and high pCEA level (>0.6 ng/mL, n = 13), elevated pCEA levels was found to be associated with younger patients ($P = .017$), larger tumor diameter ($P = .012$), and more aggressive PDAC in terms of the resectability classification and histological invasiveness (Table S2). The factors associated with the postoperative sCA 19–9 were also evaluated. Nonnormalized sCA 19–9 (>37 U/mL) in the postoperative period was more frequently observed in patients with margin-positive resection than in those with margin-negative resection (Table S3).

3.3 | DFS analysis

Using the predefined cutoff values, the DFS was compared between dichotomized patients with high and low tumor marker levels in the serum (sCEA and sCA 19–9) and peritoneal lavage (pCEA and pCA 19–9). Although patients with higher sCEA levels were more likely to have worse DFS than those with lower sCEA levels, no significant difference was observed between the two groups

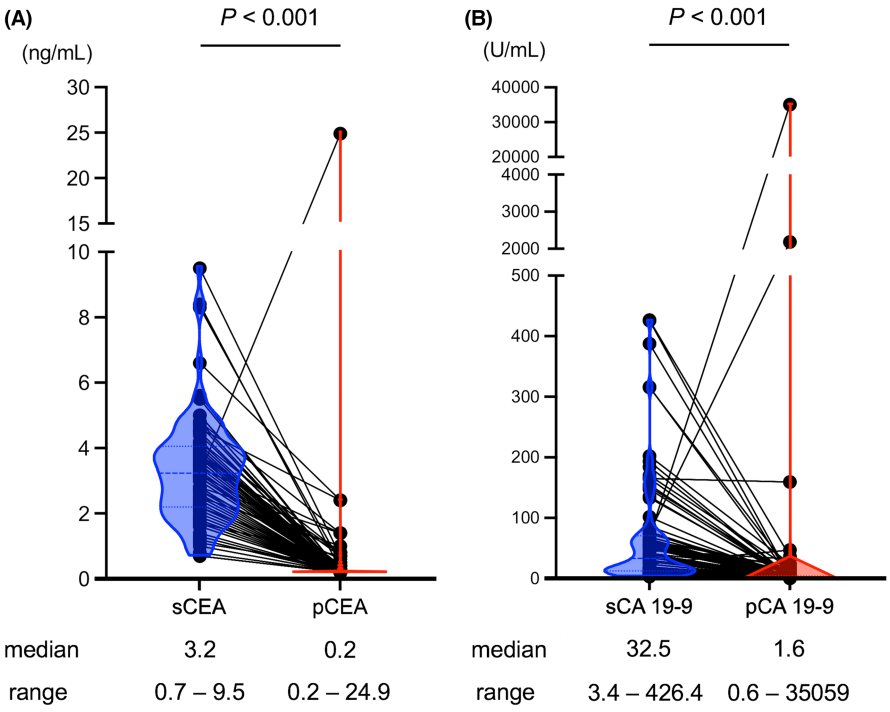

FIGURE 1 The levels of CEA and CA 19–9 in serum and paired peritoneal lavage fluid in 90 patients with pancreatic cancer and their paired comparison by a Wilcoxon matched-pairs signed-rank test. Merged volcano plots in each subgroup are highlighted by blue (serum) and red (peritoneal lavage) colors. Thick and thin dotted lines indicate median and 25%–75% percentile, respectively. sCEA, serum CEA; sCA 19–9, serum CA 19–9; pCEA, peritoneal lavage CEA; pCA 19–9, peritoneal lavage CA 19–9

TABLE 2 Clinicopathological features according to the dichotomized peritoneal lavage CA 19–9 levels

	pCA 19–9 (low) [n = 35]	pCA 19–9 (high) [n = 55]	*P*
Age (y), median (range)	71 (55–80)	69 (42–89)	.734
Sex, n (%)			
Male	22 (62.9)	31 (56.4)	.661
Female	13 (37.1)	24 (43.6)	
Location, n (%)			
Head	19 (54.3)	34 (61.8)	.516
Body and tail	16 (45.7)	21 (38.2)	
Tumor diameter (mm), median (range)	18 (4–40)	22 (5–44)	**.017**
Resectability, n (%)			
R	25 (71.4)	38 (69.1)	.620
BR	8 (22.9)	10 (18.2)	
UR (locally advanced)	2 (5.7)	7 (12.7)	
Preoperative sCEA (ng/mL), median (range)	2.8 (0.7–5.6)	3.4 (0.8–9.5)	.097
Preoperative sCA 19–9 (U/mL), median (range)	15.2 (3.4–387.7)	51.1 (4.1–426.4)	**.003**
Preoperative therapy, n (%)			
Absent (upfront surgery)	5 (14.3)	12 (21.8)	.568
Chemotherapy (neoadjuvant)	21 (60.0)	27 (49.1)	
Chemo(radio) therapy (conversion)	9 (25.7)	16 (29.1)	
Procedures, n (%)			
PD	20 (57.1)	35 (63.6)	.343
DP/DP-CAR	14 (40.0)	15 (27.3)	
TP	1 (2.9)	5 (9.1)	
Histological PV/SMV invasion, n (%)			
Absent	23 (65.7)	31 (56.4)	.508
Present	12 (34.3)	24 (43.6)	
Histological arterial invasion, n (%)			
Absent	30 (85.7)	46 (83.6)	.999
Present	5 (14.3)	9 (16.4)	
Histological nerve plexus invasion, n (%)			
Absent	28 (80.0)	32 (58.2)	**.040**
Present	7 (20.0)	23 (41.8)	
Histological lymph node metastasis, n (%)			
Absent	16 (45.7)	16 (29.1)	.120
Present	19 (54.3)	39 (70.9)	
Histological residual tumor, n (%)			
Absent	31 (88.6)	46 (83.6)	.760
Present	4 (11.4)	9 (16.4)	
Postoperative sCA 19–9 (U/mL), median (range)	9.7 (4.1–96.7)	16.3 (3.5–1387.0)	**.003**
Adjuvant chemotherapy, n (%)			
Absent	2 (5.7)	7 (12.7)	.473
Present	33 (94.3)	48 (87.3)	

Note: Bold values indicate statistical significance ($P < .05$).

Abbreviations: R, resectable; BR, borderline resectable; UR, unresectable; CEA, carcinoembryonic antigen; sCEA, serum CEA; PD, pancreaticoduodenectomy; DP, distal pancreatectomy; DP-CAR, distal pancreatectomy with celiac axis resection; TP, total pancreatectomy; PV, portal vein; SMV, superior mesenteric vein; CA 19–9, carbohydrate antigen 19–9; pCA 19–9, peritoneal lavage CA 19–9; sCA 19–9, serum CA 19–9.

($P = .080$, Figure 2A). In contrast, the patients with higher pCEA levels demonstrated a significantly shorter DFS period than those with lower pCEA levels ($P = .006$, Figure 2B). The DFS in patients with elevated sCA 19–9 and pCA 19–9 levels was significantly shorter than those with normal levels of sCA 19–9 ($P = .010$, Figure 2C) and pCA 19–9 ($P = .002$, Figure 2D), respectively. For both CEA and CA 19–9, peritoneal lavage markers showed a higher hazard ratio (HR) than the paired serum markers. Furthermore, in both test specimens of the serum and peritoneal lavage, CA 19–9 was a more reliable marker than CEA owing to the higher HR (Figure 2A–D, Table 3). The DFS times were also compared using the universal cutoff values of CEA (5.0 ng/mL) and CA 19–9 (37.0 U/mL) (Figure S6).

To examine further improvement of the predictive performance, combination analyses were conducted across the four tested markersie, sCEA, sCA 19–9, pCEA, and pCA 19–9. In combination with the double peritoneal lavage markers pCEA and pCA 19–9, the survival curves across the three subgroups appeared to have good stratification (Figure 3A). Notably, the pCEA (high)/pCA 19–9 (high) subgroup showed the worst DFS, with a median survival time (MST) till recurrence of 14.5 mo after surgery (Figure 3A). Likewise, another set of subgroups stratified by sCA 19–9 and pCA 19–9 levels showed differential survival curves (Figure 3B). The subgroup of patients with sCA 19–9 (high)/pCA 19–9 (high) showed a similar poor outcome (MST: 15.2 mo) compared with those with pCEA (high)/pCA 19–9 (high).

The univariate analysis demonstrated the association between the risk of recurrence and histological findings of vessel invasion, nerve plexus invasion, and lymph node metastasis (Table 3). In multivariate analysis, only elevated pCA 19–9 level (HR, 2.391; $P = .018$) and histologically positive lymph node metastasis (HR, 3.167; $P = .009$) were independent risk factors significantly associated with postoperative recurrence (Table 3).

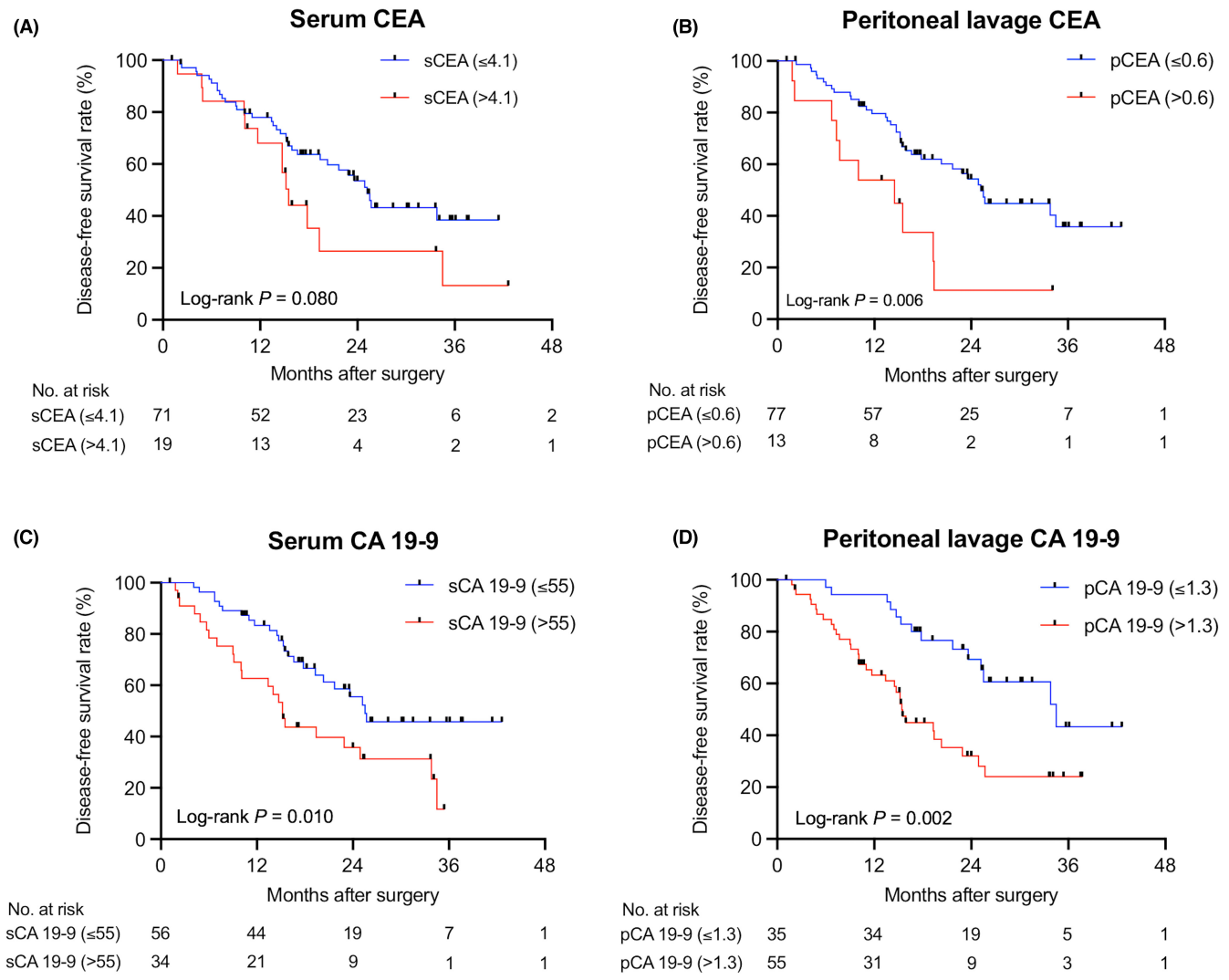

FIGURE 2 Kaplan–Meier survival curves for disease-free survival according to the dichotomized high and low levels of all four tested markers of serum CEA (A), peritoneal lavage CEA (B), serum CA 19–9 (C), and peritoneal lavage CA 19–9 (D). sCEA, serum CEA; sCA 19–9, serum CA 19–9; pCEA, peritoneal lavage CEA; pCA 19–9, peritoneal lavage CA 19–9

3.4 | OS analysis

The impact of tumor markers in the serum and peritoneal lavage of patients with PDAC on postoperative OS was evaluated using the optimized cutoff values for DFS. Univariate analyses showed that all tested markers, namely sCEA, pre/postoperative sCA 19–9, pCEA, and pCA 19–9, were associated with worse OS (Figure 4, Table S3). Consistent with the results of DFS, similar significant OS differences were observed in the histopathological findings of nerve plexus invasion and nodal metastasis (Table S4). In multivariate analysis, both sCA 19–9 (HR, 2.669; $P = .022$) and pCA 19–9 (HR, 3.194; $P = .033$) were selected as independent predictors of OS (Table S4). Furthermore, selected patients who lost the chance to receive adjuvant chemotherapy showed a significantly shorter OS, possibly owing to impaired physical condition and nonspecific cause of death.

Subgroup analyses were also stratified by the marker combinations of pCEA/pCA 19–9 and sCA 19–9/pCA 19–9. Trends similar to those in the DFS analysis were observed, wherein OS worsened in accordance with the number of markers with elevated levels (Figure S7).

3.5 | Subgroup analysis in patients with resectable PDAC who underwent pathologically curative resection

We performed a subgroup analysis of 58 patients with resectable PDAC who underwent histologically curative (R0) resection. The univariate analysis demonstrated that patients with resectable PDAC with elevated levels of pCA 19–9 showed significantly inferior DFS than those with nonelevated levels. However, in the multivariate analysis histological findings of nodal metastasis had a significantly worse impact on DFS than pCA 19–9 (Table S5). We then performed an OS subgroup analysis. A similar prognostic impact was also observed in OS and DFS analyses (Table S6).

TABLE 3 Univariate and multivariate analysis of disease-free survival

		Univariate analysis			Multivariate analysis		
Variables	n	HR	95% CI	P	HR	95% CI	P
Age, ≥70 y	46	1.046	(0.587–1.865)	.878			
Sex, male	53	1.170	(0.649–2.111)	.601			
Tumor location, head	53	0.765	(0.431–1.357)	.359			
Tumor size, >20 mm	44	0.734	(0.410–1.315)	.299			
Resectability, BR/UR	27	1.166	(0.622–2.184)	.633			
Preoperative therapy, yes	73	1.394	(0.664–2.924)	.380			
Preoperative sCEA, >4.1 ng/mL	19	1.781	(0.934–3.395)	.080			
Preoperative sCA 19–9, >55 U/mL	34	2.123	(1.197–3.767)	**.010**	1.359	(0.723–2.545)	.338
PV/SMV resection, yes	16	1.271	(0.592–2.729)	.538			
Operative time, ≥500 min	51	1.108	(0.622–1.975)	.727			
Blood loss, ≥1000 mL	44	1.605	(0.901–2.859)	.109			
pCEA, >0.6 ng/mL	13	2.731	(1.341–5.564)	**.006**	0.967	(0.430–2.175)	.936
pCA 19–9, >1.3 U/mL	55	2.790	(1.479–5.264)	**.002**	2.391	(1.165–4.909)	**.018**
Histological PV/SMV invasion, yes	36	1.964	(1.102–3.500)	**.022**	1.477	(0.795–2.745)	.217
Histological arterial invasion, yes	14	1.764	(0.897–3.468)	.100			
Histological nerve plexus invasion, yes	30	1.923	(1.074–3.441)	**.028**	0.950	(0.485–1.861)	.882
Histological lymph node metastasis, positive	58	4.204	(1.943–9.097)	**<.001**	3.167	(1.329–7.549)	**.009**
Histological residual tumor, yes	77	1.682	(0.783–3.615)	.183			
Postoperative sCA 19–9, >37 U/mL	12	2.098	(1.013–4.347)	**.046**	2.098	(0.982–4.482)	.056
Adjuvant chemotherapy, none	9	1.317	(0.468–3.713)	.602			

Note: Bold values indicate statistical significance ($P < .05$).

Abbreviations: BR, borderline resectable; CA 19–9; carbohydrate antigen 19–9; CEA, carcinoembryonic antigen; CI, confidence interval; HR, hazard ratio; pCA 19–9, peritoneal lavage CA 19–9; pCEA, peritoneal lavage CEA; PV, portal vein; sCA 19–9, serum CA 19–9; sCEA, serum CEA; SMV, superior mesenteric vein; UR, unresectable.

3.6 | Tumor marker elevation and site of recurrence

In this study, 47 of 90 patients exhibited recurrence after surgery. For both pCA 19–9 and sCA 19–9, higher marker levels were associated with an increased likelihood of hepatic recurrence (Figure S8). We then compared the cumulative incidence of site-specific recurrence according to dichotomized high and low levels of pCA 19–9 (Figure 5A,C) and sCA 19–9 (Figure 5B,D). Survival curve analyses also revealed that patients with elevated levels of both markers had a significantly higher cumulative incidence of hepatic recurrence.

Patients with elevated pCA 19–9 levels were more likely to have an earlier onset of peritoneal recurrence than those without elevated pCA 19–9 levels, with a significant difference in the timing of early events tested by the Gehan–Breslow–Wilcoxon test ($P = .048$, Figure 5C). In contrast, similar cumulative incidences of peritoneal recurrence were observed between patients with elevated sCA 19–9 and those of the nonelevated controls (Figure 5D).

4 | DISCUSSION

Early postoperative recurrence even after the curative resection for localized PDAC has been attributed to occult micrometastatic disease at the time of resection.[24] Highly reliable biomarkers for predicting the minimal residual disease enable selecting the patients who will benefit from surgery. Even in cases with pancreatic resection, such markers help identify subgroups with more intensive postoperative treatment and surveillance. To the best of our knowledge, the present study is the first to demonstrate that elevated pCEA/pCA 19–9 levels in patients with localized PDAC harboring HEP – PER–CY– are significantly associated with worse DFS, suggesting that pCEA/pCA 19–9 can be a surrogate for minimal residual disease.

This study examined for each of 2 × 2 combinations of the target (CEA/CA 19–9) and test specimens (serum/peritoneal lavage). CA 19–9 was identified as a more reliable marker than CEA in predicting shorter DFS with earlier recurrence. This is supported by the strong evidence of CA 19–9 being used as the most common and useful biomarker in managing patients with potentially resectable PDAC.[25] Otherwise, the actual values of pCEA in most of the included patients were below the measurable range, potentially due to the dilution effect during the collection of peritoneal lavage fluid samples. The difficulty in absolute quantification of pCEA was attributable to its lower reliability than pCA 19–9. For such cases, highly sensitive quantification using reverse transcription-polymerase chain reaction targeting the CEA mRNA might improve the performance, as reported previously.[26–29] However, in this study the elevated pCEA

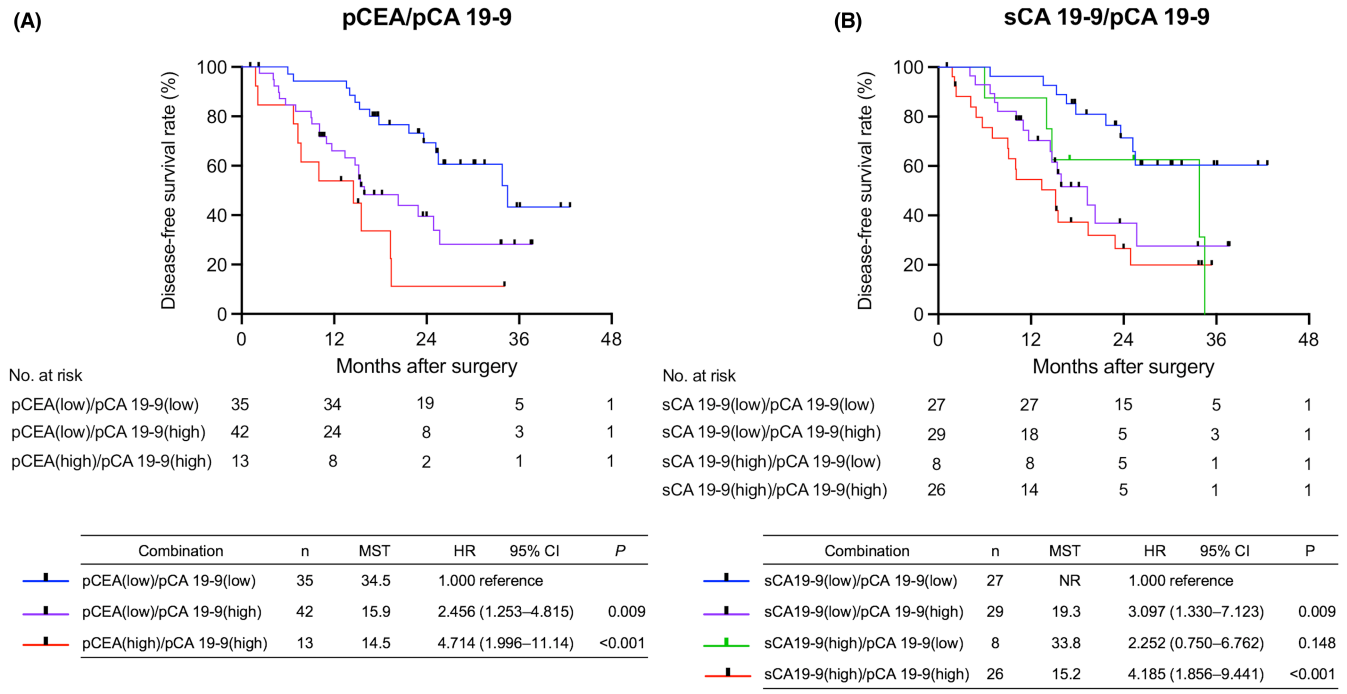

FIGURE 3 Kaplan–Meier survival curves for disease-free survival according to high and low combination patterns between pCEA/pCA 19–9 (A) and sCA 19–9/pCA 19–9 (B). sCEA, serum CEA; sCA 19–9, serum CA 19–9; pCEA, peritoneal lavage CEA; pCA 19–9, peritoneal lavage CA 19–9; MST, median survival time; HR, hazard ratio; CI, confidence interval

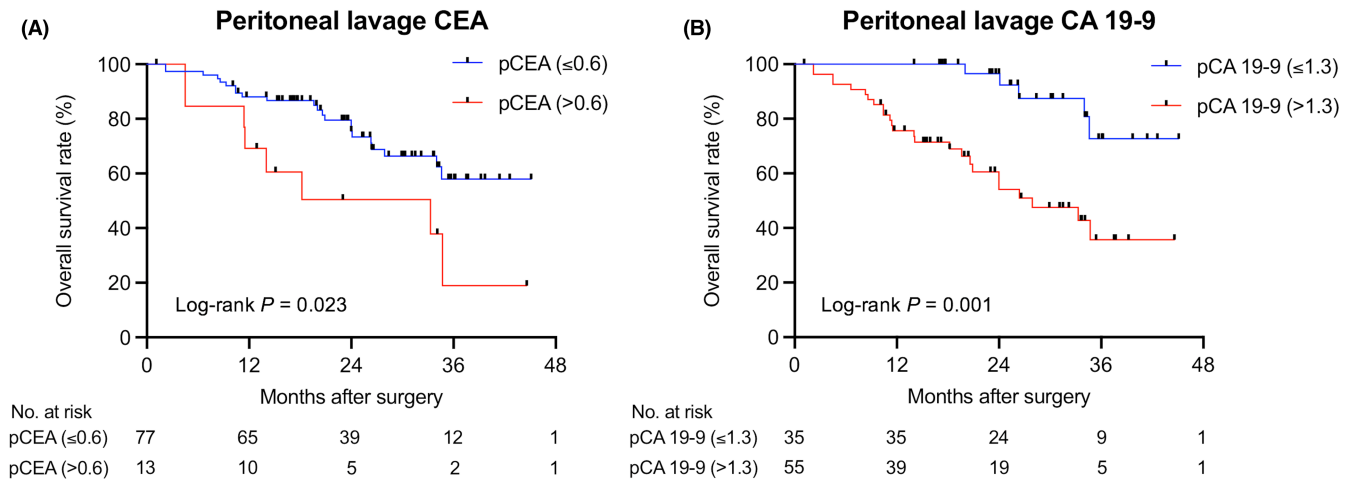

FIGURE 4 Kaplan–Meier survival curves for overall survival according to dichotomized high and low levels of peritoneal lavage CEA (A), peritoneal lavage CA 19–9 (B). sCEA, serum CEA; sCA 19–9, serum CA 19–9; pCEA, peritoneal lavage CEA; pCA 19–9, peritoneal lavage CA 19–9

level provided additional information in combination with pCA 19–9 to further subdivide classification, facilitating the identification of patients at an extremely high risk of recurrence.

This study showed considerably higher values of sCA 19–9 than paired pCA 19–9 and a positive correlation between them. This is explained by the mechanism that the tumor markers present at high concentrations in the systemic circulation may exude into the peritoneal cavity. Moreover, the current study demonstrated a worse prognostic impact of tumor markers in the peritoneal lavage than in paired serum samples. One possible underlying explanation is that the tumor marker in peritoneal lavage might be produced and secreted by the floating cancer cells in the peritoneal cavity.[30] Peritoneal lavage cytology has the potential limitation of relatively low sensitivity and the subsequent risk of false-negative results. Actually, some patients showing negative results in peritoneal lavage cytology suffered from recurrent disease.[8,31] Actually, all three patients with higher levels of pCA 19–9 than sCA 19–9 in this study had a cancer-specific death following locoregional and/

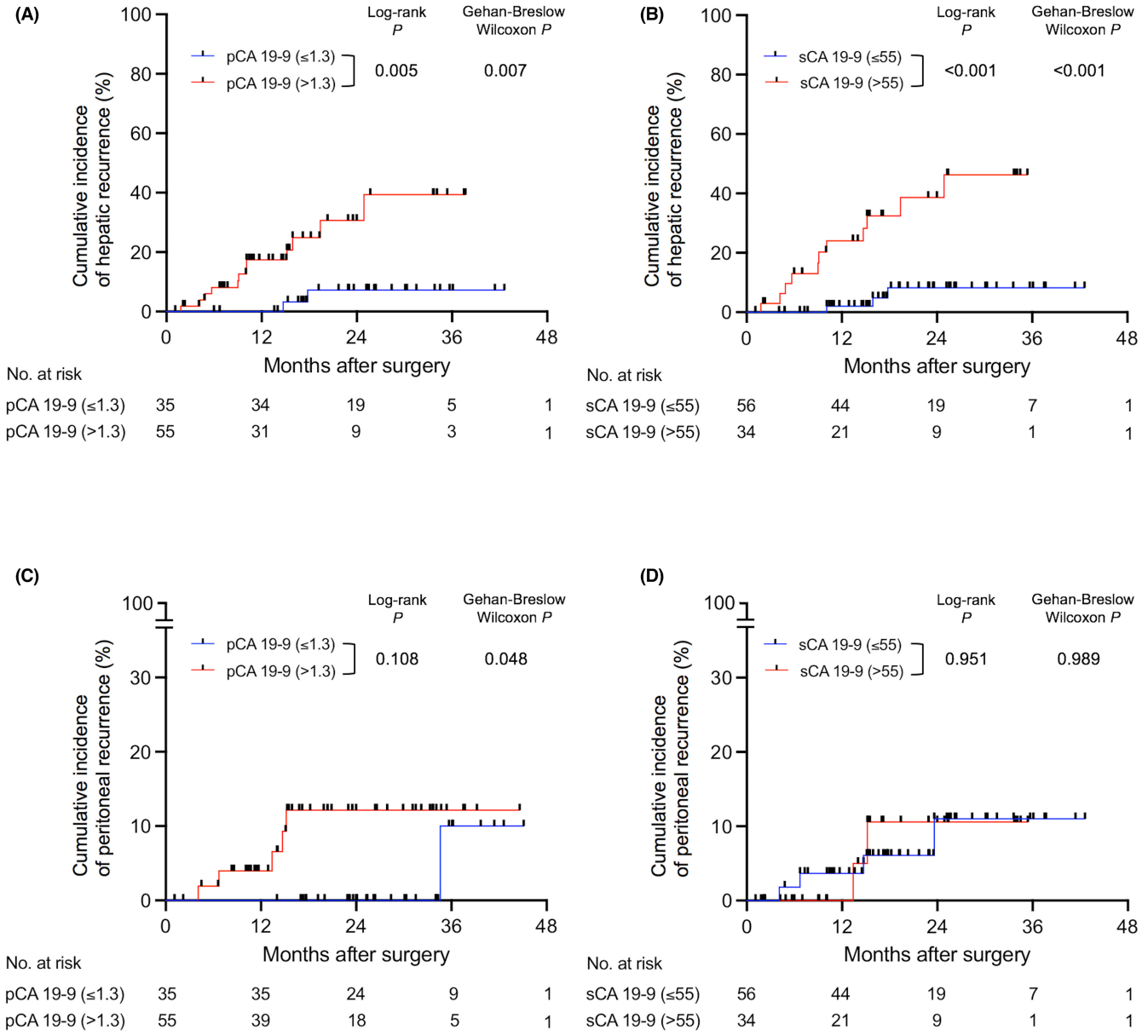

FIGURE 5 Cumulative incidence of hepatic recurrence (A,B) and peritoneal recurrence (C,D) in patients with PDAC according to high and low levels of pCA 19–9 (A,C) and sCA 19–9 (B,D). sCA 19–9, serum CA 19–9; pCA 19–9, peritoneal lavage CA 19–9

or peritoneal recurrence. Taken together, pCA 19–9 and its combination with sCA19–9 or pCEA can be a powerful diagnostic tool to identify a subgroup of patients at high risk of recurrence, especially in CY– cases.

Although several previous studies have suggested the use of peritoneal lavage CEA as a potential predictor of peritoneal recurrence and worse OS in patients with PDAC,[26–29] only a limited number of studies have focused on peritoneal lavage CA 19–9. Recently, Yonkus et al demonstrated a significant association between pCEA/pCA 19–9 and positive abdominal metastatic findings, such as radiographically invisible hepatic or peritoneal metastasis in patients with PDAC.[18] However, the authors also concluded that the prognostic impact of pCEA/pCA 19–9 remains controversial. One strength of the present study is the association of pCA 19–9 with peritoneal recurrence, even in patients with HEP−PER−CY−, whereas patients with elevated levels of both pCA 19–9 and sCA 19–9 were found to be more likely to develop hepatic recurrence. These results suggest that the combination of sCA 19–9 and pCA 19–9 may serve as a promising prognosticator for further personalized management, such as through the prediction of site-specific recurrence. Specifically, pCA 19–9 elevation can be a risk factor for hepatic and peritoneal recurrence, while sCA 19–9 is a risk factor for hematogenous hepatic recurrence.[12]

This study included the resected PDAC with different resectability classifications at the initial diagnosis. Considering the prognostic impact of resectability status, our subgroup analysis of only resectable PDAC with R0 resection showed persistently better predictive performance of pCA 19–9 than sCA 19–9 in terms of the DFS and OS.

Furthermore, one of the current topics of multidisciplinary therapy for PDAC is the clinical utility of conversion surgery for borderline resectable (BR)/unresectable (UR) PDAC after successful disease control, owing to a good response to chemotherapy/chemoradiotherapy. This study suggests that pCA 19–9 can help decision-making in selecting patients with BR/UR PDAC who really benefit from conversion surgery after excluding those with a high risk of early recurrence.

Even though the pCA 19–9 cutoff value of 1.3 U/mL was within the dynamic range of the electrochemiluminescence immunoassay, the measured levels of peritoneal lavage tumor markers were greatly lower than those of the paired serum markers. The quantification of tumor markers in peritoneal lavage fluid depends considerably on the physiological amount of the ascites. Furthermore, the dilution effect of the washing with saline during sample collection should also be considered. The different physiological amounts of the ascitic fluid affect the different dilution effects, even with the standardized amount of washing saline (100 mL). Usually, the amount of malignant ascites appears to be excessive, indicating that it is radiographically detectable in the peritoneal cavity. In such cases, pCA 19–9 is more prone to be diluted by the physiological ascites. Otherwise, pCA 19–9 is more concentrated because of the secretion from rich viable tumor cells. It is unclear which mechanisms are more predominant in the peritoneal cavity. However, precise quantification of the total amount of physiological ascitic fluid is not technically difficult, and the assessment of the dilution effect on the pCA 19–9 value was theoretically unevaluable. In this study we performed peritoneal lavage sampling using standardized procedures with a unified amount of the washing saline. Nonetheless, the dilution effect on the measured pCA 19–9 value could not be entirely eliminated. Future validations should be required to assess the reliability of the cutoff value defined in this study.

This study has several limitations. First, the study was limited by its retrospective design, relatively small sample size, and heterogeneity of the postoperative treatment. Furthermore, the postoperative follow-up period was limited in terms of duration. Second, difficulty in determining peritoneal recurrence may affect its actual incidence. Indeed, not all patients in the study had histological confirmation of peritoneal recurrence. Finally, as discussed above, the difficulty in determining the optimal cutoff values for molecular markers detected in the peritoneal lavage fluid can be attributed to the dilution of the target molecules by physiological ascites and washing with saline solution.[32] Furthermore, a prior study showed that peritoneal lavage tumor markers were measurable in cases with chronic pancreatitis as well as PDAC.[33] This study included only the patients with PDAC; therefore, much better cutoff values should be examined in future studies taking into account normal and disease control.

In conclusion, pCA 19–9 is a reliable marker for predicting early recurrence in patients with PDAC after surgery. When combined with pCEA, pCA 19–9 enables the identification of a subgroup at a very high risk of early recurrence. Furthermore, combination analysis of pCA 19–9 and sCA 19–9 can serve in risk stratification and predict site-specific recurrence. Using standardized predefined cutoff values and sampling methods, future prospective validations with larger cohorts are needed to determine whether the dynamics of tumor markers in peritoneal lavage can be translated to clinical settings during the entire period of clinical care for patients with PDAC.

AUTHOR CONTRIBUTIONS

Tatsuo Hata designed the study. Tatsuo Hata and Kazuharu Chiba collected the cilinical samples. Tatsuo Hata, Kazuharu Chiba, Masamichi Mizuma, Kunihiro Masuda, Hideo Ohtsuka, Kei Nakagawa, Takanori Morikawa, and Hiroki Hayashi collected and assessed the clinical data. Tatsuo Hata wrote the first draft. Masamichi Mizuma, Fuyuhiko Motoi, and Michiaki Unno revised the manuscript. Michiaki Unno supervised the study. All authors have approved the final version of the article.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest for this article.

ETHICAL APPROVAL

The study protocol was approved by a suitably constituted Ethics Committee of the institution and it conforms to the provisions of the Declaration of Helsinki. It was approved by the Ethics Committee of the Tohoku University Graduate School of Medicine (Sendai, Japan), Approval No. 2019–1-119.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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