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ORIGINAL ARTICLE

Collagen gel droplet-embedded culture drug sensitivity test (CD-DST) predicts the effect of adjuvant chemotherapy on pancreatic cancer

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Abstract

Purpose We evaluated the clinical effectiveness of collagen gel droplet-embedded culture drug sensitivity tests (CD-DSTs) in predicting the efficacy of adjuvant chemo-therapeutic treatments for pancreatic cancer (PC).

Methods The clinicopathological characteristics and prognoses of 22 PC patients who underwent CD-DST after pancreatectomy at Tohoku University between 2012 and 2016 were analyzed retrospectively. Eligibility criteria were resectable or borderline resectable PC, successful evaluation for 5-fluorouracil sensitivity by CD-DST, treatment with S-1 adjuvant chemotherapy, and no preoperative chemotherapy.

Results The rate of successful evaluation by CD-DST was 52.3% in PC. The optimal *T*/*C* ratio, defined as the ratio of the number of cancer cells in the treatment group (*T*) to that in the control group (*C*), for 5-fluorouracil was 85% using receiver operating characteristic curve analysis. The sensitive group (*T*/*C* ratio < 85%; $n = 11$) had a better recurrence-free survival rate than the resistant group (*T*/*C* ratio ≥ 85%; $n = 11$; $P = 0.029$). A Cox proportional hazards regression model demonstrated that sensitivity to 5-fluorouracil was an independent predictor of recurrence on multivariate analysis (hazard ratio 3.28; 95.0% CI 1.20–9.84; $P = 0.020$).

Conclusions CD-DSTs helped to predict PC recurrence after S-1 adjuvant chemotherapy.

Keywords Pancreatic cancer · Chemosensitivity test · CD-DST

Introduction

Pancreatic cancer (PC) is still associated with poor prognosis because of its early recurrence, even following curative resection [1]. To improve prognosis after resection, gemcitabine or 5-FU-based adjuvant chemotherapy is recommended by the National Comprehensive Cancer Network (NCCN) guideline [2]. Since tumor sensitivity to anticancer drugs varies widely among individuals [3, 4], the effect of chemotherapy on each patient is different. Patients with tumors less sensitive to anticancer drugs do not benefit as much from chemotherapy as those who undergo surgery alone [5]. Furthermore, adverse effects or unnecessary financial burdens can result from unsuccessful treatment. Being able to predict the effect of chemotherapy could allow for the avoidance of these undesirable outcomes. Therefore, a clinical assay as a chemosensitivity test before initial treatment is needed.

The collagen gel droplet-embedded culture drug sensitivity test (CD-DST) is a simple three-dimensional (3D) culture method [6]. This test analyzes the chemosensitivity of cancer cells after distinguishing them from fibroblast cells contaminating the sample at harvesting. It can reproduce a biological tumor microenvironment with cell–cell contact, which is an important element of tumor progression [7, 8]. This replication of in vivo environments is advantageous because it allows the analysis of anticancer drugs at physiological concentrations using extremely small clinical samples [6, 7]. The clinical efficacy of the CD-DST has been demonstrated for various cancers, including gastric cancer [4, 9, 10], lung cancer [11, 12], and colorectal cancer [5, 13]. These studies

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demonstrated that chemosensitive patients were less likely than chemoresistant patients to have recurrence after adjuvant chemotherapy. A phase II trial on patients with gastric cancer showed that S-1 adjuvant chemotherapy improved the relapse-free survival of patients highly sensitive to 5-FU [4]. However, no reports have been published on the clinical importance of CD-DST for PC and this remains unclear.

In Japan, S-1 is recommended as adjuvant chemotherapy based on the results of the JASPAC-01 study [14]. This study identified a better prognosis achieved with S-1 adjuvant chemotherapy than with gemcitabine chemotherapy [14]. S-1 is an oral anticancer drug that combines tegafur (a prodrug of 5-FU) with 5-chloro-2,4-dihydropyrimidine (CDHP) and potassium oxonate in a molar ratio of 1:0.4:1. CDHP works as a biochemical modulator (BCM) that inhibits the degradation of 5-FU by reversibly blocking the catabolic enzyme dihydropyrimidine dehydrogenase (DPD). Thus, S-1 is a drug designed to potentiate the effects of 5-FU. We conducted this study to evaluate the clinical effectiveness of CD-DST to evaluate the efficacy of 5-FU-based S-1 adjuvant chemotherapy for PC. We then investigated whether recurrence could be predicted by the results of CD-DST.

Methods

Ethics statement

This nonrandomized, retrospective study was approved by the Institutional Review Board of Tohoku University (Sendai, Japan) on December 18, 2017 (approval no., 2017-1-832). The need to collect informed consent was waived due to the retrospective design of this study, and an "opt-out" method was used. The research was conducted in accordance with the principles outlined in the Declaration of Helsinki.

Eligibility criteria

The subjects of this retrospective study were 215 patients who underwent pancreatectomy for PC in the Department of Surgery at Tohoku University Graduate School of Medicine, Sendai, Japan, between January 1, 2012 and December 31, 2016 (Fig. 1). CD-DSTs were performed in 88 patients, but the number of tests was low in the first 3 years in the trial period ($n=21$), after which the test was performed for almost all patients in the later 2 years ($n=67$). CD-DSTs were not done for patients with small tumors from which it was difficult to take samples. CD-DST results were successfully obtained from 46 (52.3%) patients. The reason for assay failure was always an insufficient number of cancer cells for the assay. Among these 46 patients, 6 with diagnosed unresectable PC (UR-PC), defined by the Classification of Pancreatic Carcinoma (4th English edition) by

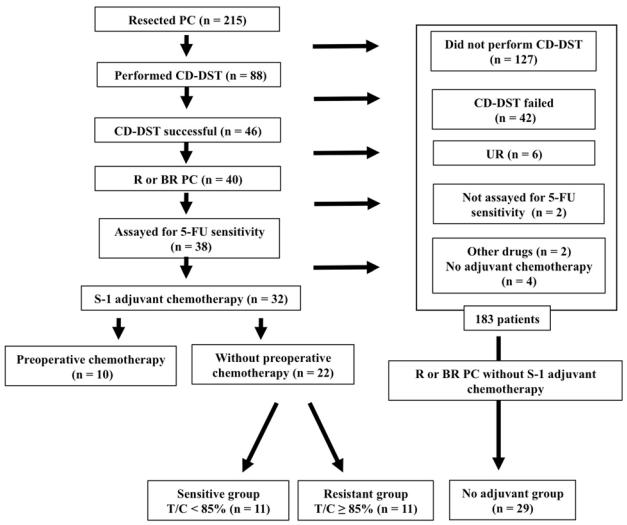


Fig. 1 Flow diagram of patient selection and enrollment in this study. *PC* pancreatic cancer, *CD-DST* collagen gel droplet-embedded culture drug sensitivity test, *UR* unresectable, *R* resectable, *BR* borderline resectable, *5-FU* 5-fluorouracil

the Japanese Pancreatic Society, were excluded [15]. Two patients who were not successfully assayed for 5-FU sensitivity and six patients who did not receive S-1 adjuvant chemotherapy were also excluded. Ten patients who had received preoperative chemotherapy were also separated for subgroup analysis. Thus, the final cohort of this study comprised 22 patients. Of the 183 patients excluded from the study design, 29 who had diagnosed resectable or borderline resectable pancreatic cancer (BR-PC) and did not undergo adjuvant chemotherapy were assigned to the "no adjuvant therapy" group.

Data collection

All data and information about the patients' clinical courses were collected and reviewed from medical records, operative reports, and pathological reports maintained by our institute. Data collection was performed on January 1, 2019.

CD-DST method

CD-DSTs were performed to assess the in vitro sensitivity of PC tumors to 5-FU and gemcitabine. LSI Medience Corporation (Tokyo, Japan) conducted the assays. The researchers of this study were not involved in the assessment of these tests.

Briefly, fresh surgical specimens from PC tumors were cut and collected aseptically. The samples were suspended in Dulbecco's modified Eagle medium/nutrient mixture F-12 (DMEM/F-12) and transported immediately to LSI Medience Corporation, after which they were digested with a cell

dispersion enzyme solution. Cells were cultured preliminarily in PCM-1 medium (Kurabo Bio-Medical Department, Tokyo, Japan) for 24 h. The condition for testing was adequate when the cell number exceeded 2×10^5 to 5×10^5 cells/mL. The growth rate at the time of testing needed to be ≥ 0.8 times the rate before preliminary culturing began. When the cells could not satisfy this condition, the test was defined as a failure. After preliminary culturing, the cells were added to collagen gel droplets, and anticancer drugs were added. The concentrations of these drugs in the culture medium was measured so as to exhibit the same area under the curve value as that observed in the serum during the first 24 h after intravenous administration of the corresponding drug at the standard clinical dosage, as reported in the literature [6, 16]: 5-FU (1.0 μg/mL, 24 h) and gemcitabine (8.0 μg/mL, 24 h). Following contact, the culture medium in each well was removed by suction. After removal of the culture medium, each well was rinsed and cultured in a serum-free medium for 7 days. In vitro sensitivity was expressed as the *T*/*C* ratio, calculated as the ratio of the number of cancer cells in the treatment group (*T*) to that in the control group (*C*). The treatment group was exposed to anticancer drugs. The control group was not exposed to anticancer drugs and was cultured for only 7 days.

Patient follow-up

Postoperative follow-up was conducted at least once every 2–3 months until the date of death or date of censoring (final day of data collection: January 1, 2019). Follow-up included laboratory testing for carcinoembryonic antigen, cancer antigen 19-9 [CA19-9], Duke pancreatic monoclonal antigen type 2 and s-pancreas-1 antigen levels, and computed tomography (CT) scans. Radiographic findings were interpreted by surgeons and radiologists. If recurrence was suspected, magnetic resonance imaging, 18-fluorodeoxyglucose positron emission tomography/CT scan, or cytology from ascites was performed. Recurrence was confirmed by surgeons, based on these results.

Adjuvant chemotherapy

Patients with adequate postoperative recovery were given S-1 twice a day for 4 weeks, followed by 2 weeks of rest. The dosages of S-1 were assigned according to body surface area as follows: < 1.25 m^2, 80 mg daily; 1.25 m^2–1.5 m^2, 100 mg daily; and > 1.5 m^2, 120 mg daily. This 6-week cycle was repeated for 6 months after adjuvant chemotherapy was started [14]. Treatment was discontinued if any of the following occurred: recurrence, serious adverse events, adverse events requiring more than two levels of dose reduction, patient's request, or other medical conditions as judged by the patient's physicians; for example, loss of daily activity great enough to require a hospital visit.

Statistical analyses

All data were entered in a Microsoft Excel spreadsheet (Redmond, WA, USA), and statistical analyses were performed using JMP Pro software, version 13.0 (SAS Institute Inc., Cary, NC, USA). The differences between groups were evaluated using Pearson's Chi-squared tests for categorical data and the Mann–Whitney *U* test or the Kruskal–Wallis test for continuous data. Receiver operating characteristic (ROC) curve analysis was used to determine the optimal *T*/*C* cutoff ratio for predicting recurrence after curative resection. The accuracy of the predictive score was calculated in terms of the sensitivity and specificity of the cutoff ratio. Survival curves were estimated using the Kaplan–Meier method, and comparison was performed by the log-rank test. Disease-specific survival (DSS) was measured from the date of surgical resection until the date of death or censoring. Recurrence-free survival (RFS) was defined as the duration from the date of surgical resection to the date of initial recurrence. A Cox proportional hazards regression model was used for multivariate analysis to evaluate the factors associated with recurrence. $P < 0.05$ was considered significant.

Results

Optimal *T*/*C* cutoff ratio for predicting recurrence

To evaluate the effect of S-1 (TS-1, Taiho Pharmaceutical, Tokyo, Japan) adjuvant chemotherapy, the appropriate *T*/*C* cutoff ratio for predicting efficacy was set and ROC curve analyses were performed to establish the optimal cutoff values for predicting recurrence. Recurrence developed in 18 patients, and 15 patients died of PC recurrence during the median follow-up time of 37.0 months. The median RFS of the patients without recurrence ($n = 18$) was 38.2 months, which was significantly longer than that of the patients with recurrence (11.0 months, $n = 15$). The areas under the curve (AUCs) and cutoff values for 3 RFS outcomes were 0.547 and 88.6, 0.600 and 87.6, and 0.778 and 85.3 for 1-year, 2-year, and overall recurrence, respectively. These findings demonstrate that the most significant cutoff value was 85 for overall recurrence, with sensitivity and specificity of 61.1% and 100%, respectively (Supplemental Fig. 1). Based on these results, the 22 patients were divided into two groups: the sensitive group (S-group; *T*/*C* ratio < 85%, $n = 11$) and the resistant group (R-group; *T*/*C* ratio ≥ 85%, $n = 11$).

Clinicopathological characteristics

Table 1 shows the clinical and pathological characteristics of the 22 patients. The distribution between the S- and R-groups was balanced. Seven patients (31.8%) did not complete adjuvant chemotherapy, the primary reason for which was the patient's request in the S-group (2 of 3 cases) and tumor recurrence during treatment in the R-group (3 of 4 cases).

Prognostic value of CD-DST

Figure 2a, b shows the DSS and RFS curves for the S- and R-groups. Although the DSS rates did not differ significantly between the groups ($P=0.521$; Fig. 2a), the RFS rates were better in the S-group ($P=0.029$; Fig. 2b). The 1-year, 2-year, and overall recurrence rates were 36.4%, 54.5%, and 63.6%, respectively, in the sensitive group and 45.5%, 81.2%, and 100%, respectively, in the resistant group.

Table 1 Clinicopathological characteristics of the upfront surgery patients in the sensitive and resistant groups

	Total ($n=22$)	Sensitive group (*T/C* ratio < 85%) ($n=11$)	Resistant group (*T/C* ratio ≥ 85%) ($n=11$)	*P* value
Age (year) (median)	71.5	68	74	0.158
Sex (male:female)	10:12	5:6	5:6	1.000
Resectability status (R:BR)	15:7	7:4	8:3	0.647
CA19-9 before initial treatment (median)	72.0	63.4	149.8	0.108
Tumor position (ph:pbt)	13:9	8:3	5:6	0.193
Time to adjuvant chemotherapy (days)	42.5	53	39	0.123
Complete adjuvant chemotherapy	15 (68.2%)	8 (72.7%)	7 (63.6%)	0.647
Histology (well:mod:poor:other)	3:15:0:4	1:8:0:2	2:7:0:2	0.819
Median tumor size (mm)	30	26	31	0.309
Anterior serosal invasion	15 (68.2%)	8 (72.7%)	7 (63.6%)	0.647
Retroperitoneal invasion	19 (86.4%)	10 (90.9%)	9 (81.8%)	0.534
Portal vein invasion	6 (27.3%)	2 (18.2%)	4 (36.4%)	0.338
Artery invasion	1 (4.5%)	0 (0%)	1 (9.1%)	0.306
Lymph node metastasis	14 (63.6%)	7 (63.6%)	7 (63.6%)	1.000
Residual cancer (R1)	6 (27.3%)	3 (27.3%)	3 (27.3%)	1.000

T/C tumor growth inhibition ratio, *R* resectable, *BR* borderline resectable, *CA19-9* cancer antigen 19-9, *n.s.* not significant, *ph* pancreatic head, *pbt* pancreatic body and tail, *int* intermediate, *sci* scirrhous

*$P<0.05$

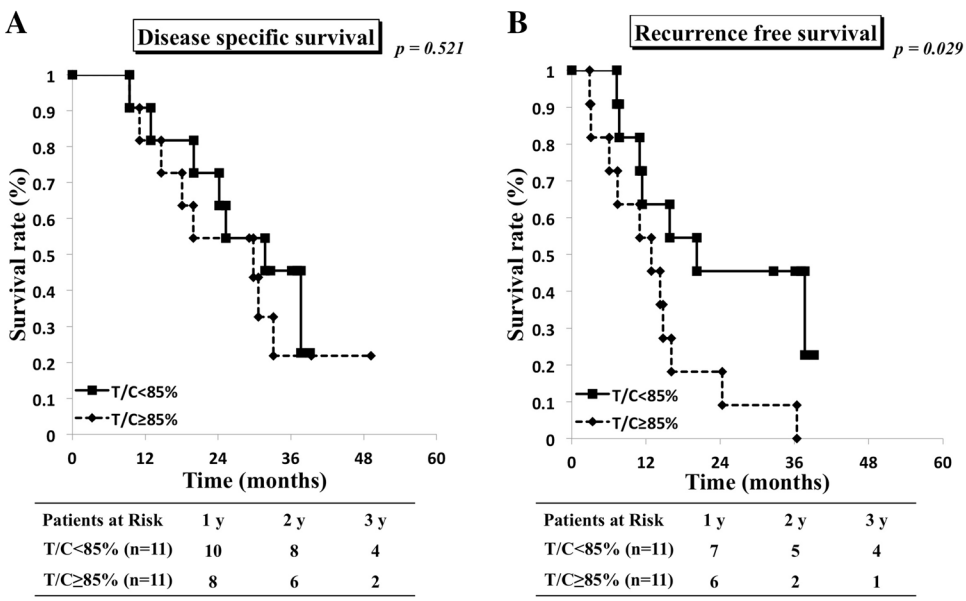


Fig. 2 **a** Disease-specific survival. **b** Recurrence-free survival of the patients ($n=22$). Patients classified as sensitive (*T/C* ratio < 85; $n=11$) and resistant (*T/C* ratio ≥ 85%; $n=11$) to S-1 are represented by rectangles and diamonds, respectively

Predictors of recurrence

To elucidate the clinical potential of CD-DST for recurrence, predictors of recurrence were examined in the 22 patients (Table 2). Univariate analysis showed significant differences in three factors: age (≤75 years) ($P=0.008$), completion of adjuvant chemotherapy ($P<0.001$), and 5-FU sensitivity ($P=0.032$). Multivariate analysis using these three factors demonstrated that complete adjuvant chemotherapy [hazard ratio (HR) 17.3, 95.0% confidence interval (CI) 4.00–120.0; $P<0.001$], and sensitivity to 5-FU (HR 2.93, 95.0% CI 3.28–9.84; $P=0.020$) were predictors of recurrence.

Effect of preoperative chemotherapy on the CD-DST result

The effect of preoperative chemotherapy on the CD-DST was evaluated, targeting ten selected patients. The chemotherapy regimens were gemcitabine with S-1 therapy in nine patients and S-1 monotherapy in one patient. The median *T/C* ratio for 5-FU was not significantly different between the preoperative group (59.0%) and the upfront surgery group (84.1%, $P=0.350$) (Table 3). The ratio for gemcitabine was also not significant, at 53.9% in the preoperative group and 54.4% in the upfront surgery group ($P=0.824$).

Prognosis was analyzed in subgroup analyses of the preoperative chemotherapy group. The clinicopathological characteristics were distributed equally (Supplemental Table 1). Although this cohort was too small for statistical analysis to be performed, the RFS of the S-group was longer than that of the R-group (Fig. 3a). On the other hand, analysis of the combined upfront surgery and preoperative chemotherapy group ($n=32$) revealed significantly better RFS of the S-group than the R-group ($P=0.012$). The 1-year, 2-year, and overall recurrence rates were 33.3%, 44.4%, and 55.6%, respectively, in the S-group and 50%, 78.6%, and 92.9%, respectively, in the R-group. Furthermore, multivariate analysis of the combined group also demonstrated that sensitivity to 5-FU (HR 4.03, 95.0% CI 1.57–10.8; $P=0.004$) was a predictor of recurrence (Supplemental Table 2).

Table 3 The treatment-to-control ratio (*T/C* ratio) for several anticancer drugs in the preoperative chemotherapy and upfront surgery groups

	Preoperative chemotherapy group ($n=10$)	Upfront surgery group ($n=22$)	*P* value
5-Fluorouracil (median; range)	59.0 (7.6–100)	84.1 (26.3–100)	0.350
Gemcitabine (median; range)	53.9 (21.5–87.1)	54.6 (3.3–84.6)	0.824

Prognosis compared with the no adjuvant chemotherapy group

The no adjuvant group ($n=29$) including patients who received preoperative chemotherapy, was compared with

Table 2 Predictive risk factors for recurrence in the patients treated with S-1 adjuvant chemotherapy

	Univariate analysis		Multivariate analysis	
	HR (95% CI)	*P* value	HR (95% CI)	*P* value
Age (≤75 years)	4.42 (1.48–13.9)	0.008	1.18 (0.27–4.57)	0.818
Sex (male)	1.23 (0.47–3.15)	0.667		
Resectability status (BR)	0.50 (0.16–1.34)	0.173		
CA19-9 before initial treatment (≤100 U/mL)	2.34 (0.88–6.15)	0.088		
Tumor position (ph)	0.76 (0.29–2.09)	0.577		
Time to adjuvant chemotherapy (≤57 days [8 weeks])	1.90 (0.68–5.16)	0.214		
Completion of adjuvant chemotherapy (no)	17.3 (4.00–120.0)	<0.001	18.4 (3.36–160.5)	<0.001
Tumor size (≤20 mm)	2.91 (0.80–18.7)	0.112		
Anterior serosal invasion (positive)	1.36 (0.52–3.95)	0.534		
Retroperitoneal invasion (positive)	0.43 (0.13–1.95)	0.243		
Portal vein invasion (positive)	2.63 (0.88–7.02)	0.080		
Artery invasion (positive)	20.5 (0.81–517.8)	0.063		
Residual cancer (R1)	1.21 (0.38–3.25)	0.727		
Lymph node metastasis (positive)	1.30 (0.50–3.75)	0.597		
5-FU sensitivity (≤85%)	2.93 (1.10–8.60)	0.032	3.28 (1.20–9.84)	0.020

HR hazard ratio, *CI* confidence interval, *BR* borderline resectable, *CA19-9* cancer antigen 19-9, *ph* pancreatic, *5-FU* 5-fluorouracil

*$P<0.05$

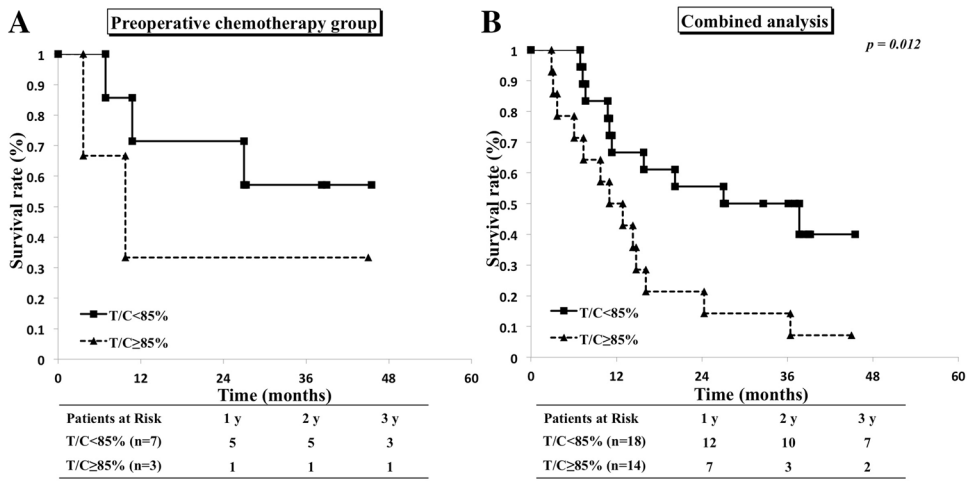
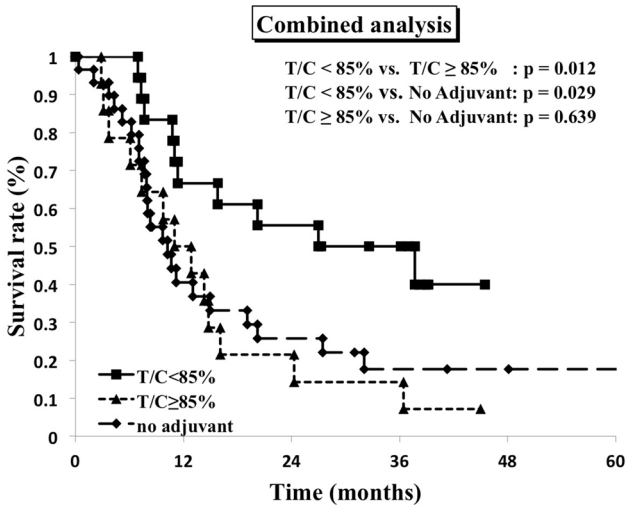


Fig. 3 **a** Recurrence-free survival in the preoperative chemotherapy group: the sensitive group (*T*/*C* ratio < 85%: $n=7$), and the resistant group (*T*/*C* ratio ≥ 85%; $n=3$) to S-1 are represented by rectangles and diamonds, respectively. **b** Recurrence-free survival for the combined group with upfront surgery and preoperative chemotherapy patients: the sensitive group (*T*/*C* ratio < 85%: $n=18$) and the resistant group (*T*/*C* ratio ≥ 85%; $n=14$) to S-1 are represented by rectangles and diamonds, respectively



Patients at Risk	1 y	2 y	3 y
T/C<85% (n=18)	12	10	7
T/C≥85% (n=14)	7	3	2
no adjuvant (n=29)	11	7	4

Fig. 4 Recurrence-free survival of three combined patients groups: the sensitive group (*T*/*C* ratio < 85%: $n=18$), resistant group (*T*/*C* ratio ≥ 85%; $n=14$), and no adjuvant group ($n=29$) are represented by rectangles, triangles, and diamonds, respectively

both the combined S- and R-groups. The S-group had a better RFS rate than the no adjuvant group ($P=0.029$; Fig. 4). On the other hand, the RFS rate of the R-group was similar to that of the no adjuvant group ($P=0.639$). The patients' characteristics were equally distributed among the groups (Supplemental Table 3). These results suggest that the R-group did not respond to S-1 adjuvant chemotherapy.

Factors affecting the success rate of CD-DST analysis

In this study, the failure rate of CD-DST was 47.7% (42 of 88 patients). The reason for failure to assay was consistently an insufficient number of viable cells. Table 4 summarizes the clinicopathological characteristics of the patients in whom CD-DSTs were successfully performed vs. those in whom it failed. Compared with patients in whom CD-DST yielded results, the group in which CD-DST did not yield results had a significantly higher incidence of men ($P=0.013$), unresectable PC ($P=0.008$), and preoperative chemotherapy ($P=0.003$). Tumor size was also significantly smaller ($P=0.045$) and the CA19-9 value was lower ($P=0.033$) in the patients with failed CD-DST.

Discussion

This study showed that 5-FU-sensitive patients had better RFS than FU-resistant patients and that 5-FU sensitivity could be a predictor of recurrence after S-1 adjuvant chemotherapy. From this perspective, CD-DST is useful for predicting the effect of S-1 adjuvant chemotherapy.

CD-DST is used to evaluate the effect of anticancer drugs after exposing the drugs to collagen gel droplet-coated cancer cells [6]. S-1 combines three drugs to potentiate the effects of 5-FU. Therefore, a method to predict the effect of S-1 for PC is needed. In relation to gastric cancer, Tanigawa et al. suggested that if the appropriate dose of 5-FU were identified, only the results of 5-FU could be used to predict the effect of S-1 chemotherapy [4]. They predicted prognosis by evaluating progression-free survival after establishing the appropriate 5-FU dose. In the present study, the dose of 5-FU could not be changed because this test was not performed in our institute, but by a separate company (LSI Medience). Thus, the effect of S-1 adjuvant chemotherapy was predicted by calculating the appropriate *T*/*C* ratio using

Table 4 Clinicopathological characteristics of patients who had collagen gel droplet-embedded culture drug sensitivity tests (CD-DST) performed

	Total ($n=88$)	CD-DST		*P* value
		Success ($n=42$)	Failure ($n=46$)	
Age (year) (median)	69	70	68	0.229
Sex (male:female)	53:35	22:24	31:11	0.013
Resectability status				0.218
Resectable	46	30	16	0.011
Borderline resectable	30	14	16	0.449
Unresectable	12	2	10	0.008
Preoperative chemotherapy	38 (43.2%)	13 (28.3%)	25 (59.6%)	0.003
CA19-9 before initial treatment (median)	84	86.8	77.4	0.033
Tumor position (ph:pbt)	52:36	28:18	24:18	0.722
Adjuvant chemotherapy	69 (82.1%)	39 (86.7%)	30 (76.9%)	0.245
Median tumor size (mm)	25	30	23	0.045
Histology (well:mod:poor:other)	10:59:3:16	6:31:2:7	4:28:1:9	0.480
Interstitial type (int:sci)	38:47	23:22	15:25	0.208
Anterior serosal invasion	62 (70.5%)	34 (73.9%)	28 (66.7%)	0.457
Retroperitoneal invasion	66 (75.0%)	34 (73.9%)	32 (76.2%)	0.805
Portal vein invasion	28 (31.8%)	13 (28.3%)	15 (35.7%)	0.453
Artery invasion	5 (5.7%)	3 (6.5%)	2 (4.8%)	0.722
Lymph node metastasis	51 (58.0%)	31 (67.4%)	20 (47.6%)	0.061
Peritoneal cytology (positive)	10 (11.4%)	6 (13.0%)	4 (9.5%)	0.603
Residual cancer (R1)	20 (22.7%)	12 (26.1%)	8 (19.0%)	0.431

CD-DST collagen gel droplet-embedded culture drug sensitivity test, *CA19-9* cancer antigen 19-9, *ph* pancreatic head, *pbt* pancreatic body and tail, *int* intermediate, *sci* scirrhous

*$P<0.05$

the results of the CD-DST for 5-FU. The *T*/*C* ratio calculated in the present study was valuable because it enabled us to identify which patients could or could not be expected to have longer survival after S-1 adjuvant chemotherapy.

The present study showed that S-1 adjuvant chemotherapy could not improve the RFS of 5-FU-resistant patients. These patients suffer the adverse effects of toxic chemotherapy if treated with S-1. Conversely, the present data also demonstrated that 5-FU sensitivity was not correlated with DSS, possibly because several anticancer drugs were given following recurrence, which might prolong survival. These results suggest that certain drugs or drug combinations might be effective if the tumor is defined as resistant to 5-FU. From this perspective, CD-DST might be of clinical benefit for selecting patients appropriate for S-1 adjuvant chemotherapy or other drug regimens.

Preoperative chemotherapy was found recently to have a survival benefit for resectable and borderline resectable PC [16]. Furthermore, the opportunity of conversion surgery for UR-PC was shown to be increased after chemotherapy [17, 18]. Hence, the opportunity for surgical treatment after chemotherapy should be increased. To our knowledge, CD-DST has not been performed after preoperative chemotherapy, and the impact of the *T*/*C* ratio on prognosis was unclear. Thus, our study focused on 10 patients given preoperative chemotherapy. The results showed that the *T*/*C* ratio was lower in the preoperative group than in the upfront surgery group; however, this result was the opposite to what was expected. After chemotherapy, it is possible that only resistant cancer cells would have survived and that the *T*/*C* ratio might have been increased. The small study cohort may have resulted in some selection bias. To elucidate the effect of chemotherapy, further study is needed to evaluate the change in the *T*/*C* ratio before and after chemotherapy.

The reliability of CD-DST after preoperative chemotherapy was also evaluated in subgroup analysis. Though RFS in the sensitive group was longer than that in the resistant group, the number of cases in the subgroups was too small to achieve statistical significance. When combined with the upfront surgery group, the sensitive group had longer RFS than the resistant group or the no adjuvant group, and 5-FU sensitivity was also a predictor for recurrence. Taken together, CD-DST could predict the effect of S-1 adjuvant chemotherapy, even when preoperative chemotherapy patients were included, although it is difficult to confirm that CD-DST predicts the effect of S-1 adjuvant therapy targeting only patients after preoperative chemotherapy. Further study is needed to determine the effect of CD-DST following preoperative chemotherapy after increasing the size of

the cohort, because the chance of surgical resection after preoperative chemotherapy will increase.

The small number of patients in this study is attributed to the low rate of successful CD-DST evaluation in PC. Kobayashi et al. demonstrated that the overall successful CD-DST evaluation rate was around 80% in 554 patients [6]. Thus, the successful rate for PC (52.3%) in the present study was very poor. PC cells are usually surrounded by fibroblasts and connective tissues, making it difficult to obtain a sufficient number of cancer cells for assay, even if the same amount of sample blocks was obtained as with other tumors [19]. The present study also showed that tumor size, resectability, and preoperative treatment were correlated with failure to assay. Tumor size may have made it technically difficult to obtain samples, while preoperative treatments may have affected tumor viability. Hence, to perform CD-DST successfully, a process that collects enough viable cancer cells needs to be developed. We are now trying to collect cancer cells using core needle biopsy, which could punch out the central area of the tumor without disturbing the pathological structure if it were performed from various areas around the tumor. Even after preoperative chemotherapy, enough cancer cells could be obtained from the resected specimens after surgical treatment if the number of punctures was 20–30. Since the utility of this method is now being examined in a trial, the data were not available for this study. However, a technical trial would improve the chance to collect enough samples for this test and will raise the CD-DST success rate.

Because this was a retrospective, single-center study, it had several limitations. First, the number of subjects was very small, partially because of the low success rate of the test. Increasing the success rate of this test is indispensable to provide predictive information in the clinical setting. A clinical trial to improve the success rate is now underway. Second, the cutoff value of the *T*/*C* ratio in the present study was set by ROC analysis; however, a validation analysis of this cutoff ratio could not be performed due to the small study population. This could be a limitation of this study. Finally, the retrospective nature of this study caused selection bias.

Considering these limitations, this study was only a pilot study and cannot provide a definite conclusion. Despite these limitations, it is the first study to prove the clinical importance of CD-DST for predicting PC prognosis. Our findings offer the possibility of predicting the effect of chemotherapy by using the cancer cells from resected specimens for an in vitro chemosensitivity test. These results are useful for patient selection and could minimize unfavorable outcomes, such as to avoid adverse effects caused by toxic chemotherapy for non-sensitive patients. Future large-scale prospective studies could reduce the potential for bias and provide more conclusive data regarding the utility of the CD-DST to predict the prognosis of PC patients after adjuvant chemotherapy.

In conclusion, CD-DST might predict the response to 5-FU-based S-1 adjuvant chemotherapy, with possible implications in precision medicine. To further define the clinical value of CD-DST for PC, methods for collecting adequate samples need to be established and large-scale trials should be conducted.

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Compliance with ethical standards

Conflict of interest We have no conflicts of interest to disclose and we did not receive any financial support in relation to this study.

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