ORIGINAL ARTICLE

Gemcitabine-based Chemoradiotherapy Followed by Surgery For Borderline Resectable and Locally Unresectable Pancreatic Ductal Adenocarcinoma: Significance of the CA19-9 Reduction Rate and Intratumoral Human Equilibrative Nucleoside Transporter-1 Expression

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Objectives: To evaluate the efficacy of gemcitabine-based chemoradiotherapy followed by surgery (gem-CRTS) for pancreatic ductal adenocarcinoma (PDAC) for borderline resectable (BR) and locally unresectable (UR).

Methods: One hundred PDAC patients who underwent the gem-CRTS protocol were classified into three groups: resectable (R:14), BR (44) and UR (42). After chemoradiotherapy, the patients were reassessed for curative-intent resection.

Results: At reassessment, distant metastases became apparent in 27% of R patients, 12% of BR patients and 18% of UR patients. Multivariate analysis of preoperative factors indicated that the CA19-9 reduction rate was an independent prognostic factor in the BR group. Among reassessed patients, the resection rate was 63.6% in R, 83.7% in BR and 50.0% in UR patients. In 63 patients that underwent curative-intent resection, the 3-year survival rate was 83.3% in R, 33.0% in BR and 7.8% in UR patients. Using multivariate analysis, the independent prognostic factor was found to be the surgical margin in BR and hENT1 expression in UR patients.

Conclusion: We consider that our gem-CRTS protocol, even for locally unresectable PDAC, allows for the identification of candidates for aggressive resection at the time of reassessment and improved prognosis in the patients with positive hENT1 expression.

Key words: pancreatic cancer; preoperative chmoradiotherapy; resectabilty; gemcitabine; human equilibrative nucleoside transporter-1 expression.

(Pancreas in press)

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Received for publication Jan 23, 2013; accepted September 23, 2013.

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Abbreviation list

AC: adjuvant chemotherapy; BR: borderline resectable; CTV: clinical target volume; DP: distal pancreatectomy; EUS-FNA: endoscopic ultrasonography guided fine-needle aspiration; gem-CRTS: gemcitabine-based chemoradiotherapy followed by surgery; GTV: gross tumor volume; hENT-1: human equilibrative nucleoside transporter-1; NCCN: National Comprehensive Cancer Network; NCRT: neoadjuvant CRT; MDCT: multi-detector computed tomography; MSTs: median survival times; PD: pancreaticoduodenectomy; PDAC: pancreatic ductal adenocarcinoma; R: resectable; RECIST: Response Evaluation Criteria in Solid Tumors; UR: unresectable

INTRODUCTION

Pancreatic ductal adenocarcinoma (PDAC) has been suggested to be a systemic disease at the time of diagnosis because of the exceedingly high rates of distant metastatic recurrence, even after successful surgical resection of early-stage tumors [1]. Therefore, multimodality treatment of PDAC is required because surgical treatment alone does not greatly improve survival. Chemoradiotherapy (CRT) prior to surgery for PDAC may provide for early treatment of micrometastatic disease, allow for the identification of patients with metastatic disease at the time of reassessment, and increase the R0 resection rate resulting in a reduced risk of local tumor recurrence [2,3].

Nevertheless, surgical resection is the only potentially curative technique for managing PDAC. R0 resection is a strong prognostic indicator for long-term patient survival [4,5]. With respect to margin status, the survival benefits of an incomplete resection (R1 or R2) may be comparable to definitive CRT without surgery [6]. The National Comprehensive Cancer Network (NCCN) have developed guidelines to define tumor resectability in PDAC [7], so as to improve patient selection for surgery and increase the likelihood of an R0 resection. Using their criteria, PDAC is classified as resectable (R), borderline resectable (BR) or unresectable (UR); for example, locally advanced or metastatic disease. The probability of achieving an R0 resection is a key criterion for determining whether or not a patient is a potential candidate for resection. In this setting, a BR tumor can be defined as one which increases the likelihood of an incomplete resection. According to a previous report [8], R and BR tumors have been categorized as potentially resectable tumors. On the contrary, UR tumors are locally advanced PDACs including: tumors with superior mesenteric artery (SMA) or celiac artery (CA) encasement greater than 180 degrees; unreconstructable portal vein (PV)/superior mesenteric vein (SMV) occlusion; or aortic invasion or encasement in addition to distant metastases or nodal metastasis beyond the field of resection.

With respect to the recommended treatment for the three groups, in group R initial surgical resection is defined as the standard therapeutic strategy, but some reports have suggested that neoadjuvant CRT (NCRT) for R tumors is feasible [9-11], because PDAC is a systematic disease. Even if R0 resection is achieved for an R tumor, 10-15% of them will exhibit early recurrence; thus, NCRT could help to select patients who might not benefit from surgical resection. In the BR group, although there is no high-level evidence supporting treatment with NCRT, some institutions prefer an initial approach involving neoadjuvant therapy [7]. In the UR group, NCRT is not usually used but it has been reported that UR tumors may be downstaged by CRT to allow for surgical resection [12-14]. Consequently, there has been no consensus or clear evidence concerning the indication of NCRT for R, BR and UR tumors.

Our institution has introduced gemcitabine-based CRT followed by surgery (gem-CRTS) for the treatment of PDAC since February 2005. Gemcitabine (20,20-difluoro-2-2-deoxycitidine analog) inhibits DNA replication and repair. Recently, intratumoral human equilibrative nucleoside transporter 1 (hENT1), which is the major transporter responsible for gemcitabine uptake into cells, has been reported as an important predictive marker of chemosensitivity for gem-based adjuvant chemotherapy (AC) for PDAC [15]. We previously reported that hENT1 expression was an independent predictor of overall survival after gem-CRTS in patients with Union Internationale Contre le Cancer (UICC) T3–T4 PDAC [16]. However, there have been no previous reports describing the significance of gem-CRTS for PDAC according to resectability based on the National Comprehensive Cancer Network (NCCN) guidelines, especially focusing on hENT1 expression.

The aim of the present study was to evaluate the efficacy of gem-CRTS for the treatment of PDAC regarding the three resectability groups (R, BR and UR) defined by the NCCN pancreatic cancer guidelines (2010), with spe-

cial attention to serum CA 19-9 alternation and hENT1 expression.

MATERIALS AND METHODS

Between February 2005 and October 2010, 100 patients with PDAC who were diagnosed as having UICC-T3 and -T4 tumors using multi-detector computed tomography (MDCT) were enrolled for our gem-CRTS protocol. All patients were warned of the risks of treatment, especially concerning the possibility of developing distant metastases after gem-CRT treatment. They all gave their written informed consent for inclusion in the study. The diagnosis of pancreatic cancer was confirmed by means of cytologic or histologic analysis of biopsy specimens obtained using endoscopic ultrasonography guided fine-needle aspiration (EUS-FNA). Patients were excluded when the tumor extension determined by MDCT was categorized as UICC-T1 or UICC-T2 and/or they showed evident distant metastatic lesions. The study protocol was approved by the Medical Ethics Committee of Mie University, and the study was performed in accordance with the ethical standards established in the 1964 Declaration of Helsinki.

All patients underwent pretreatment examination using 64-slice MDCT. CT was performed according to a defined pancreas protocol as four-phasic contrast-enhanced MDCT with thin slices at intervals of 1 mm. In the present study, all of these 100 patients were reclassified into the three groups (R, BR and UR) according to NCCN guidelines (2010) [7] based on MDCT findings at the initial visit to our hospital. The CT criteria of the NCCN guidelines are as follows. R criteria: (1) no evidence of SMV and PV abutment, distortion, tumor thrombus or venous encasement; and (2) clear fat planes around the CA, hepatic artery and SMA. BR criteria: (1) venous involvement of the PV/SMV demonstrating tumor abutment without impingement and narrowing of the lumen, encasement of the PV/SMV without encasement of the nearby arteries, or short segment venous occlusion resulting from either tumor thrombus or encasement but with suitable vessel proximal and distal to the area of vessel involvement, allowing for safe resection and reconstruction; (2) gastroduodenal artery encasement up to the hepatic artery with either short segment encasement or direct abutment of the hepatic artery, without extension to the CA; and (3) tumor abutment of the SMA not exceeding >180 degrees of the circumference of the vessel wall. UR criteria: (1) >180 degrees of SMA encasement, celiac involvement (any abutment of the head with >180 degrees encasement of the body or tail); (2) unreconstructive PV/SMV occlusion and (3) aortic invasion. On the basis of the objective CT criteria, the patients enrolled in our study were classified as follows: 14 patients with R, 44 with BR and 42 with UR.

Treatment plan and assessment of gem-CRTS

Our treatment protocol for gem-CRTS has been reported previously [16]. All patients were treated with three-dimensional conformal radiotherapy using the four-field box technique from directions that avoided exposure of the kidney, which was an organ at risk. Based on the CT images the gross tumor volume (GTV), which included the main tumor and lymph nodes of ˃1 cm in diameter, was defined. The clinical target volume (CTV) was defined as the GTV plus a 5-mm margin in all directions. The planning target volume was basically defined as the CTV plus a 5-mm margin, and an additional 10-mm margin was added in the cranial-caudal direction. The total radiation dose delivered was 45 Gy in 25 fractions (5 fractions/week). Patients were administered an infusion of gemcitabine at a dose of 800 mg/m2 on days 1, 8, 22 and 29. The patients underwent reassessment at 4-6 weeks after completion of gem-CRTS; when we determined that curative-intent resection was possible, they were scheduled to undergo pancreatectomy.

Radiographic responses were determined by means of a comparison of pretreatment CT and post-CRT scans. Response was judged according to the Response Evaluation Criteria in Solid

Tumors (RECIST) criteria [17]. We used serum CA 19-9 levels as an index of response to gem-CRT. Serum CA19-9 levels were measured just before the initiation of gem-CRT (pre-CA19-9) and every 4 weeks thereafter. In the patients with obstructive jaundice, drainage was achieved using endoscopic retrograde biliary drainage, endoscopic nasobiliary drainage or percutaneous transhepatic cholangiodrainage before gem-CRT. We compared the level of pre-CA19-9 with that measured at the time of re-assessment (post-CA19-9). The reduction rate was calculated as follows: (pre-CA19-9 - post-CA19-9) / pre-CA19-9 (%). When the reduction rate was >50% regardless of the pre-CA19-9 level, gem-CRT was defined as being effective. The numbers of patients within the limit of the normal value (<37 U/ml) were four (36%) in the R group, eight (19%) in BR group and nine (23%) in UR group. CA 19-9 level may not be an accurate reflection of disease status in patients who express the Lewis a- b- genotype [18]. However, genotyping was not performed routinely, and thus we could not determine the patients with Lewis a- b-; these patients usually presented with values below the assay sensitivity threshold (1 U/ml). There was one (9.1%) patient who possessed no detectable serum CA19-9 in the R group, two (4.7%) in the BR group and one (2.5%) in the UR group, and all of them were determined as non-effective (CA19-9 reduction rate of <50%).

Indication of resection, surgical procedure and postoperative complications

At the time of reassessment, especially in the case of UR patients, we determined that curative-intent resection was possible when the following findings on MDCT were observed: no stenosis or change of shape in the celiac truncus and SMA and the absence of metastatic lesions in other distant organs. Intraoperatively, curative-intent resection was avoided when distant metastatic disease was detected on histological examination of frozen sections of suspicious lesions and of distant lymph nodes, including paraaortal lymph nodes. Curative-intent resection was also avoided when the tumor was found to be appreciably locally advanced, showing unreconstructable PV/SMV occlusion even if an external iliac vein graft had been used, and/or severe tumor invasion around SMA (which was impossible to dissect without remnant tumor) was evident.

Pancreaticoduodenectomy (PD) or distal pancreatectomy (DP) was performed as previously described [19]. Resection and reconstruction of the PV/SMV were performed when the surgeon could not separate the pancreatic head or the uncinate process from these vessels without leaving gross tumor on the vessel. When limited involvement of the common hepatic artery was identified, segmental resection of this vessel was performed with primary anastomosis. The patients who had unresectable disease at surgery, usually because of the presence of distant metastasis, underwent surgical bypass as clinically indicated.

Postoperative complications including morbidity and mortality were graded according to the Clavien-Dindo classification [20]. Postoperative pancreatic fistula, which is a complication specific to pancreatectomy, was graded according to the International study group of postoperative pancreatic fistula classification [21]. Postoperative mortality was defined as all causes of death in hospital.

Postoperative chemotherapy and follow-up

From 6 weeks after resection, we planned to start the postoperative chemotherapy regimen, consisting of gemcitabine at a dose of 800 mg/m2 biweekly for at least 6 months. After pancreatectomy, all patients were evaluated as follows: physical examination every month; laboratory tests including CEA serum levels (normal <5 ng/mL) and CA19-9 levels (normal <37 U/mL) every 2 or 3 months; and MDCT every 3 months within 2 years, and thereafter every 6 months. However, when the serum levels of the tumor markers increased they were immediately evaluated using MDCT.

Evaluation of histological response

The resected specimens were fixed in a formalin solution, sliced into 5-mm sections and embedded in paraffin blocks. A 3-μm section was obtained from each block and stained with hematoxylin and eosin. Sections were routinely examined for lymph node status, degree of lymphatic invasion (ly: 0, 1, 2 and 3), degree of venous invasion (v: 0, 1, 2 and 3), degree of intrapancreatic nerve invasion (ne: 0, 1, 2 and 3) and status of the surgical margin (R0, R1 and R2). Histological response to gem-CRT was evaluated according to Evans' histological criteria [22]. We defined patients with grade IIb or higher (51–100% tumor destruction) as high responders and patients with grade I or IIa (0–50% tumor destruction) as low responders [23].

Immunohistochemical analysis and evaluation of hENT1 expression

The paraffin-embedded blocks were also used for the assessment of intratumoral hENT1 expressions using immunohistochemistry. The hENT1 staining was carried out as detailed in a previous report [16]. The scoring for hENT1 was carried out on the basis of the relative intensities of staining of the pancreatic tumor, with reference to the normally strong hENT1 staining of cell membranes within the islets of Langerhans cells as internal controls. The degree of hENT1 expression was defined by the intensity as well as extent of positive staining as high, intermediate and low. All of the patients were classified into two groups: positive hENT1 expression (high and intermediate) and negative hENT1 expression (low) groups.

Statistical analyses

In all patients who came for reassessment, the date of the initial treatment was chosen as the starting point for the measurement of survival time. Disease-free survival time was calculated in the patients that underwent resection, and defined as the time from the date of initial treatment to the date of first relapse or death. Survival was calculated using the Kaplan-Meier method and was compared between the groups using the log rank test. The day of final follow-up was July 31, 2012, and there was no loss of follow-up. All variables were dichotomized for analyses. A multivariate analysis was performed

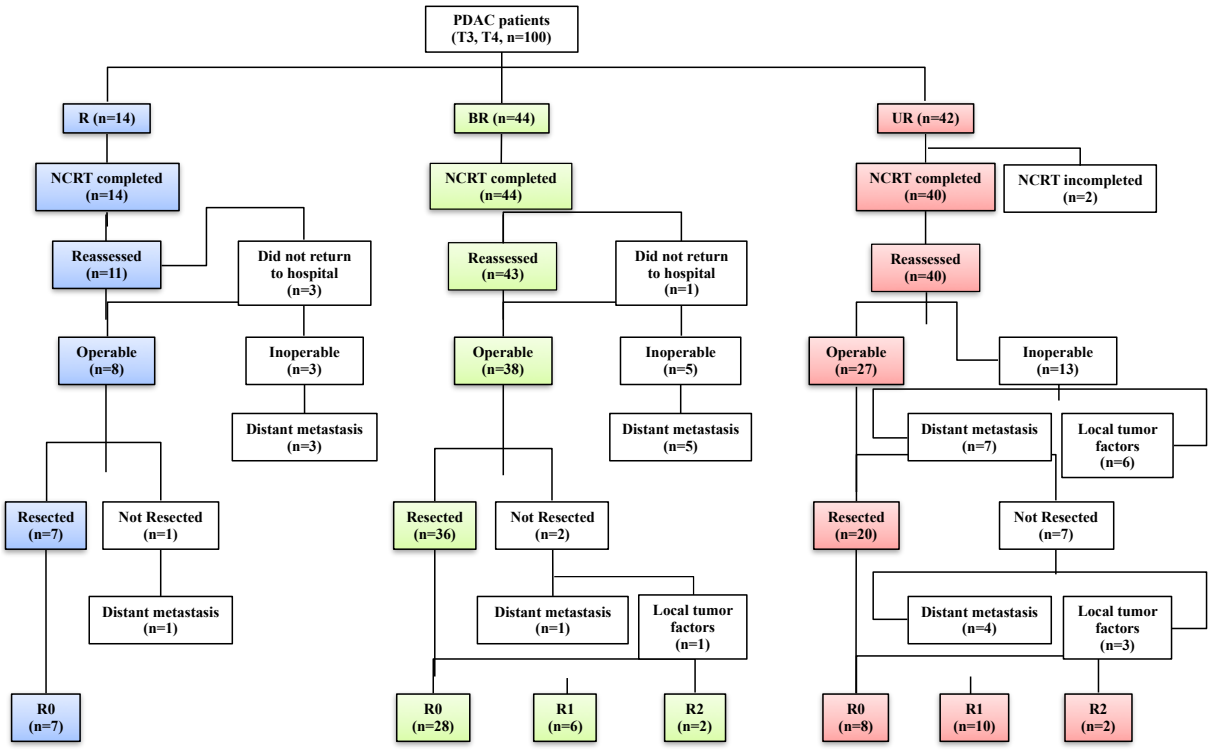

Figure 1. Algorithm illustrating patient flow through gem-CRTS. One hundred patients with stage T3–4 PDAC were classified into resectable (R), borderline resectable (BR) and locally unresectable (UR) groups according to the National Comprehensive Cancer Network (NCCN) guidelines.

using Cox's proportional hazard model. Variables with a significance of P<0.1 in the univariate analysis were entered into the multivariate analysis. Comparisons were performed using the chi-square test with Yates' correction in the univariate analysis. All statistical analyses were performed using SPSS version 18 (SPSS Inc., Chicago, IL) software. A P value <0.05 was considered as being statistically significant.

RESULTS

The flow of all 100 patients through the treatment protocol is illustrated in Figure 1. Gem-CRT was completed in all 14 (100%) of the R patients, in all 44 (100%) of the BR patients and in 40 (95.2%) of the 42 UR patients. Two (2.0%) out of the 100 patients could not complete gem-CRT because of a decline in performance status related to disease progression. During gem-CRT grade 3 gastrointestinal toxicities occurred in one of the R patients and in three of the UR patients. Thirty-eight patients (R: 6; BR: 12; UR: 20) had grade 3 hematologic toxicities, and five patients (R: 1; BR: 3; UR: 1) had grade 4 hematologic toxicities. Three R patients and one BR patient who completed gem-CRT did not return to hospital for surgery. Finally, 94 patients (R: 11; BR: 43; UR: 40) could be reassessed; 73 were operable (R: 8; BR: 38; UR: 27) and 21 were inoperable (R: 3; BR: 5; UR: 13). The reasons that patients were inoperable in the R, BR and UR groups were distant metastases in three (3/11; 27%), five (5/43; 12%) and seven (7/40: 18%), respectively, and local tumor factors in zero, zero and six (6/40: 15%), respectively. Among the 73 patients who presented for surgery, 10 patients (R: 1; BR: 2; UR: 7) were found to have unresectable disease owing to the presence of radiographically occult distant metastases (R: 1; BR: 1; UR: 4) or local tumor factors (BR: 1; UR: 3), and thus the curative-intent resection rate was 87.5% (7/8) in R patients, 94.7% (36/38) in BR patients and 74.0% (20/27) in UR patients, respectively.

The types of pancreatectomy in the R, BR and UR groups were PD in four, 32 and 16 patients, respectively, and DP in three, four and four patients, respectively. The combined resection rate of PV/SMV was significantly higher in the BR (32/36; 88.9%) and UR (19/20; 95.0%) groups, as compared with the R group (2/7; 28.6%) (P < 0.001). In five patients (3 in the BR and 2 in the UR groups), an external iliac vein graft was used as an interpositional venous graft to reconstruct the PV/SMV. Combined resection of the celiac trunk was performed in three UR patients, and resection of the hepatic artery in two BR and two UR patients. The R0 resection rate was significantly higher in R (7/7; 100%)

TABLE 1. Characteristics of Patients Enrolled for the Gem-CRT Protocol and the Outcome of Gem-CRT

Variable	R (n=14)	BR (n=44)	UR (n=42)	P
Age (years)	66.4±9.9	68.8± 9.1	66.1± 8.8	0.8142
Size of tumor before gem-CRT (cm)	3.1±1.2	3.0±0.9	3.6±1.1	0.1562
Cancer involvement of large vessels	0 (0%)	44 (100%)	42 (100%)	
PV/SMV	0	37	32	
SMA	0	8	27	
Ceriac artery	0	2	29	
Hepatic artery	0	11	20	
IVC, Aorta	0	0	2	
Gem-CRT completion rate	100% (14/14)	100% (44/44)	95% (40/42)	0.3064
Did not return to hospital	3	1	0	
Reassessed cases	11 (78.6%)	43 (97.7%)	40 (95.2%)	
Response of gem-CRT*				
CR	0	0	0	0.5558
PR	1 (1)	5 (5)	2 (2)	
SD	7 (6)	31 (29)	30 (18)	
PD	3 (0)	7 (2)	8 (0)	
Distant metastasis after gem-CRT	27% (3/11)	12% (5/43)	18% (7/40)	0.4167
CA 19-9 levels, median				
Pre-CA19-9 (U/ml)	95.1	275.2	160.6	0.2467
Post-CA19-9 (U/ml)	32.0	77.7	129.2	0.1858
Degree of reduction rate in CA19-9 level*				
50% or more	3 (2)	23 (23)	14 (9)	0.1295
Less than 50%	8 (5)	20 (13)	26 (11)	

Data are expressed mean ± SD. PV: portal vein; SMV: superior mesenteric vein; SMA: superior mesenteric artery; IVC: inferior vena; CR: complete response; PR: partial response; SD: stable disease; PD: progressive disease. *: the numbers of resected cases are shown in parentheses.

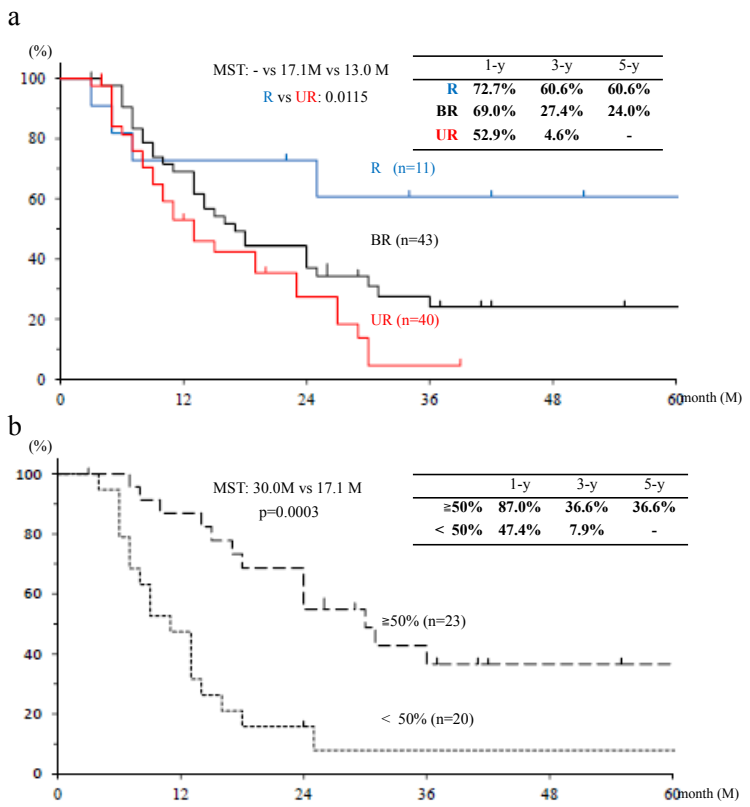

Figure 2. Survival curves after initial treatment in reassessed patients.
a: Cumulative survival curves according to the three groups in 94 patients who were reassessed. b: Cumulative survival curves according to the CA19-9 reduction rate in 43 BR patients who were reassessed.

and BR patients (32/36: 77.8%), as compared with UR patients (8/20: 40.0%) (P=0.0023).

Patient characteristics and the outcomes of gem-CRT treatment are summarized in Table 1. There were no statistically significant differences in age and size of tumor before gem-CRT therapy among the three groups. According to the evaluation using MDCT before gem-CRT, all of the BR and UR patients had cancer involvement in large vessels, but no R patient did. The response status after gem-CRT did not differ among the three groups. Although CA19-9 levels (median) decreased after gem-CRT in all three groups, pre- and post-CA19-9 levels did not differ among the three groups. The incidence of patients with a >50% reduction in CA19-9 level was 27.3% (3/11) in the R, 53.5% (23/43) in the BR and 35.0% (14/40) in the UR groups (these values did not differ significantly).

Cumulative survival curves for 94 patients in the three groups who were reassessed are shown in Figure 2a. The 3-year survival rate of R, BR and UR patients was 60.6%, 27.4% and 4.6%, respectively (R vs. UR: P=0.0115).

Univariable and multivariable analyses of the effect of preoperative factors on survival time are summarized in Table 2. Statistically significant variables in the univariable analyses were the CA19-9 reduction rate (P=0.0003) and cancer involvement of a major artery (P=0.0421) in the BR group, and gender (P=0.0306) in the UR group. Multivariable analysis indicated the CA19-9 reduction rate in

TABLE 2. Univariable and Multivariable Analyses of the Effect of Preoperative Factors on Survival Time in Reassessed Cases

Variable	R (n=11) *	BR (n=43)		UR (n=40) *
	Univariable P value	Univariable P value	Multivariable HR (95%CI)	Univariable P value
Gender				
male vs. female	0.3278	0.4760	-	**0.0306**
Age				
<65 vs. ≥65	0.9919	0.2778	-	0.9636
Tumor location				
head vs. body/tail	0.2853	0.1628	-	0.4820
Tumor size				
<3.0 vs. ≥3.0	0.5181	0.8812	-	0.8062
Cancer involvement of PV/SMV				
positive vs. negative	-	0.4258	-	0.1668
Cancer involvement of major artery				
positive vs. negative	-	**0.0421**	1.430 (0.599-3.415)	-
RECIST				
PR vs. SD,PD	-	0.1241	-	0.6141
Reduction rate in serum CA19-9 level				
≥50% vs. <50%	0.8658	**0.0003**	**3.445 (1.559-7.613)**	0.4003

Major artery including the SMA and/or celiac artery.*: multivariable analysis were not assessed.
HR: hazard ratio; CI: confidence interval.

TABLE 3. Postoperative Mortality and Morbidity

	R (n=7)		BR (n=36)		UR (n=20)	
	PD (n=4)	DP (n=3)	PD (n=32)	DP (n=4)	PD (n=16)	DP (n=4)
Clavien-Dindo classification						
Grade IIIa	1	-	4	-	-	1
Grade IIIb	-	-	1	-	1	-
Grade V	-	-	1	-	-	1
ISGPF classification						
Grade A	-	-	-	-	-	1
Grade B	-	-	-	-	-	-
Grade C	-	-	1	-	-	1
Complications						
Abdominal abscess	1	-	-	-	-	-
Intractable ascites	-	-	3	-	-	-
Pneumonia	-	-	2	-	-	-
Gastric hemorrhagic ulcer	-	-	1	-	-	
Anastomotic leakage	-		2	-	1	-
Thrombosis in the IVC	-	--	-	-	-	1
Sepsis	-	-	-	-	-	1

PD: pancreaticoduodenectomy; DP: distal pancreatectomy; IVC: inferior vena cava.

the BR group as the single significant independent factor.

Figure 2b shows the survival curves for the BR group according to the CA19-9 reduction rate. The 3-year survival rate was significantly higher in patients with a CA19-9 reduction rate of >50% than in patients with a CA19-9 reduction rate of <50% (36.6% vs. 7.9%).

Postoperative complications (Clavien grades IIIa-V) occurred in one of seven (14.3%) R patients, six of 36 (16.7%) BR patients and three of 20 (15.0%) UR patients (Table 3). There were no significant differences in postoperative complications (Clavien grades IIIa-V) between the three groups. Postoperative 30-day mortality occurred in two patients owing to pneumonia (a BR patient who underwent pancreatoduodenectomy) and sepsis (a UR patient who underwent distal pancreatectomy) caused by grade C pancreatic fistula according

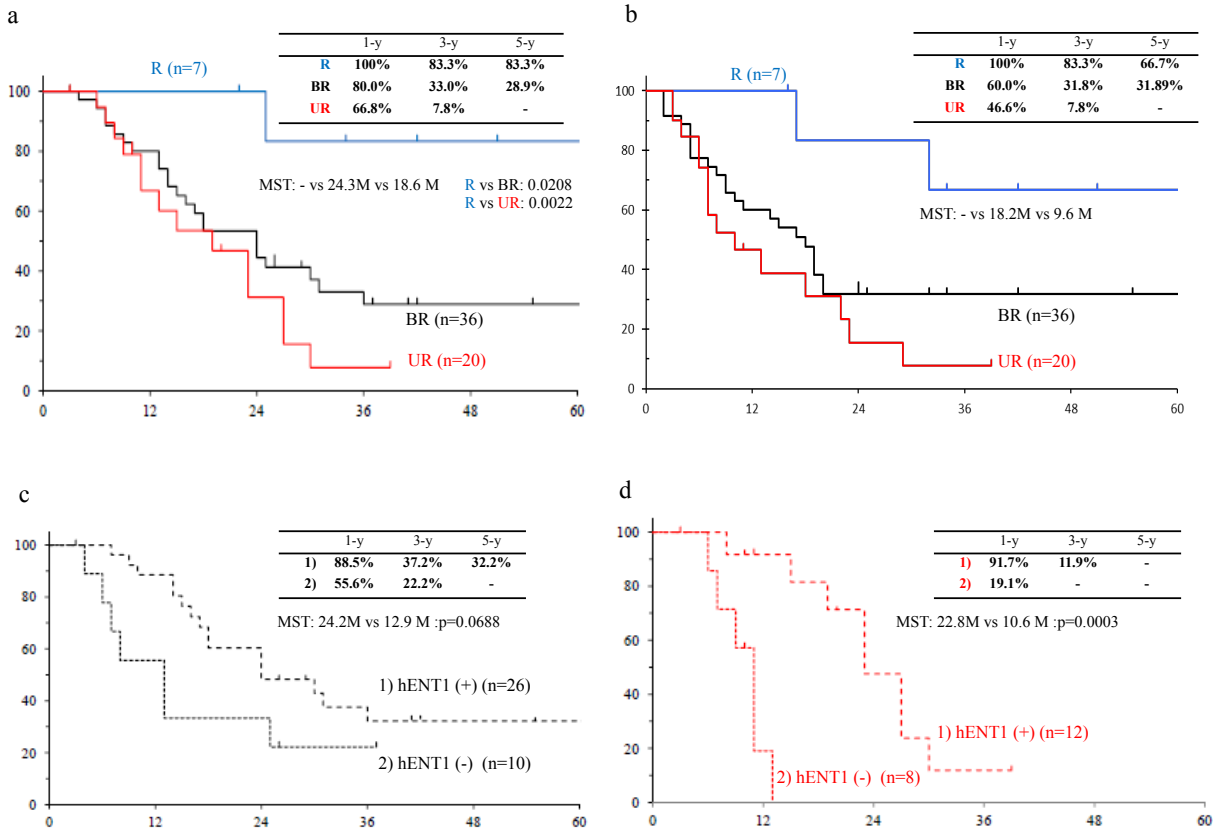

Figure 3. Survival curves after initial treatment in patients who completed gem-CRTS. a: Cumulative survival curves for the resectable (R), borderline resectable (BR) and locally unresectable (UR) groups (63 patients in total) who completed gem-CRTS (resected cases). b: Disease-free survival curves for the three groups involving the 63 patients who completed gem-CRTS (resected cases). c: Cumulative survival curves according to hENT1 expression in the 36 BR patients who completed gem-CRTS. d: Cumulative survival curves according to hENT1 expression in the 20 UR patients who completed gem-CRTS.

TABLE 4. Pathological Findings Regarding the Resectability Groups

Variable		R (n=7)	BR (n=36)	UR (n=20)	P
Histological effect of CRT					
	G I	0	15	4	0.0877
	G IIa	3	12	13	
	G IIb	2	8	3	
	G III, IV	2	1	0	
hENT1					
positive		4 (66.7%)	26 (72.2%)	12 (60.0%)	0.6065
negative		2	10	8	
Ly					
ly0		2	5	2	0.479
ly1-3		5 (71.4%)	31 (86.1%)	18 (90.0%)	
V					
v 0		5	15	9	0.350
v 1-3		2 (28.6%)	21 (58.3%)	11 (55.0%)	
Ne					
ne0		2	11	0	**0.022**
ne1-3		5 (71.4%)	25 (69.4%)	20 (100%)	

ly: degree of lymphatic invasion; v: degree of venous invasion; ne: degree of intrapancreatic nerve invasion.

to the International Study Group on Pancreatic Fistula criteria.

When we compared pathological factors according to resectability groups (Table 4), high response was observed in four (50%) R patients, nine (25%) BR patients and three (15%) UR patients. The rate of positive hENT1 expression was almost the same in each of the three groups: 67% in the R, 72% in the BR and 60% in the UR groups. There were no significant differences in the degree of ly and v, while as for ne the incidence of ne1-3 was significantly higher in the UR relative to R and BR groups (P = 0.022).

Cumulative survival curves for 63 patients in the three groups who completed gem-CRTS treatment are shown in Figure 3a. The 3-year survival rate of the R, BR and UR patients was 83.3%, 33.0% and 7.8%, respectively (R vs. BR: P = 0.0208; R vs. UR: P = 0.0022). Disease-free survival curves for the three groups after initial treatment are presented in Figure 3b. The 3-year disease-free survival rate for R, BR and UR patients was 83.3%, 31.8% and 7.8%, respectively (without any statistical difference).

Table 5 shows univariable and multivariable analyses of pre- and post-operative factors regarding survival time after gem-CRTS (excluding R patients because 6 out of 7 the patients remain alive). Statistically significant factors in the univariable analyses in BR patients were the CA19-9 reduction rate, status of the surgical

TABLE 5. Univariable and Multivariable Analyses of Pre- and Post-Operative Factors on Survival Time after Gem-CRTS

Variable	BR (n=36)		UR (n=20)	
	Univariable P value	Multivariable HR (95%CI)	Univariable P value	Multivariable HR (95%CI)
Gender				
male vs. female	0.3010	-	0.2446	-
Age				
<65 vs. ≥65	0.6543	-	0.4069	-
Tumor location				
head vs. body/tail	0.3544	-	0.5325	-
Cancer involvement of PV/SMV				
positive vs. negative	0.3945	-	0.3893	-
Cancer involvement of major artery				
positive vs. negative	0.3436	-	-	-
Reduction rate in serum CA19-9 level				
≥50% vs. <50%	**0.0145**	1.360 (0.534-3.464)	**0.0310**	3.778 (0.640-22.285)
Status of surgical margin				
R0 vs. R1,2	**<0.001**	**5.204 (1.547-17.506)**	0.3448	-
Histological effect of gem-CRT				
G I, IIa vs. G IIb, III	**0.0393**	1.965 (0.620-6.230)	0.6530	-
hENT1				
positive vs. negative	0.0688	2.585 (0.743-8.989)	**<0.001**	**18.515 (2.148-159.595)**
Ne				
ne 0 vs. ne 1~3	**0.0378**	2.847 (0.745-10.878)	-	-

ne: grade of intrapancreatic nerve invasion.

margin, histological effect of gem-CRT and ne; those in UR patients were the CA19-9 reduction rate and hENT1 expression. Multivariable analyses indicated the status of the surgical margin in the BR group and positive hENT1 expression in the UR group as the single independent significant factors.

We compared survival curves according to hENT1 expression in BR (Fig. 3c) and UR patients (Fig. 3d). The 3-year survival rate was not significantly different between positive and negative hENT1 expressions (37.2% vs. 22.2%) in the BR group (median survival time [MST]: 24.2 months vs. 12.9 months; P=0.0688), while in the UR group there was a significant difference (11.9% vs. 0%; MST: 22.8 months vs. 10.6 months; P=0.003).

Table 6 shows the CA19-9 reduction rate, histological response, status of adjuvant chemotherapy and sites of tumor recurrence according to hENT1 expression among the three groups. In the R group, we excluded one patient from the examination of hENT-1 expression because they had no residual tumor in the resected specimen after gem-CRTs. In the BR patients the CA19-9 reduction rate and histological response, which were considered as indicators of the gem-CRT effect, showed significantly higher incidences of CA19-9 reduction of >50%. In addition, there was a higher rate of positive hENT1 expression than negative hENT1 expression: 77% vs. 30%, respectively (P=0.025), and 35% vs. 0%, respectively (P=0.011). In R and UR patients, however, there was no significant difference between positive and negative hENT1 expression. It was possible to commence the planned gem-AC in 44 patients, but the remaining 18 patients could not be treated owing to a prolonged recovery time. Although the hENT1 expression did not influence the commencement of gem-AC among the three groups, because there was no significant difference in the incidence of patients who commenced gem-AC, it significantly influenced the gem-AC completion rate in UR patients (66.7% with positive expression vs. 0% with negative expression; P= 0.005). The incidences of local recurrence and distant metastasis were not significantly different between the patients with positive and negative hENT1 expression in each group. When we compared the incidences of the CA19-9 reduction rate of >50.0% in all 62 patients and in patients selected and treated with gem-AC according to hENT1 expression, there was significantly higher positive expression than negative

Table 6. CA19-9 Reduction Rates, Histological Response, Status of Adjuvant Chemotherapy and Sites of Tumor Recurrence According to hENT1 Expression Among the Three Groups

	R (n=6)[#]			BR (n=36)			UR (n=20)		
	hENT1 + (n=4)	hENT1 – (n=2)	p	hENT1 + (n=26)	hENT1 – (n=10)	P	hENT1 + (n=12)	hENT1 – (n=8)	P
CA19-9≥ 50%*	1 (25.0%)	1 (50.0%)	0.541	20 (77.0%)	3 (30.0%)	**0.025**	6 (50.0%)	3 (37.5%)	0.582
High responder	2 (50.0%)	1(50.0%)	0.541	9 (34.6%)	0 (0%)	**0.011**	2 (16.6%)	1 (12.5%)	0.812
AC commenced	2 (50.0%)	1 (50.0%)	1	19 (73.1%)	7 (70.0%)	0.820	11 (91.7%)	4 (50.0%)	0.110
AC completed	2 (50.0%)	1 (50.0%)	1	13 (50.0%)	2 (20.0%)	0.210	8 (66.7%)	0 (0%)	**0.005**
Recurrence	2 (50.0%)	0 (0%)	0.76	16 (61.5%)	7 (70.0%)	0.93	9 (75.0%)	6 (75.0%)	0.91
Local	1	0		[b]	0		2	1	
Metastasis	1	0		4	7		7	5	
Lung	1	0		14	2		1	2	
Liver	0	0		6	2		4	3	
Peritoneal	0	0		10	2		5	0	
Other	0	0		3	1		0	0	
				2					

[#]Excluding 1 patient because they had no residual tumor in the resected specimen after gem-CRTs.
*: Reduction rate in serum CA19-9 level ≥50% after gem-CRT; AC: gem-based adjuvant chemotherapy; a: remnant pancreas; b: including three cases with remnant pancreas.

expression in the former group: 64.3% (27/42) vs. 35.0% (7/20) (P=0.030) and 54.8% (23/42) vs. 15.0% (3/20) (P=0.003). Furthermore, the 15 BR patients who completed gem-AC survived longer than the 21 who did not complete gem-AC; MST was 24.4 18.8 months, respectively (P =0.014). Eight UR patients who completed gem-AC survived longer than the 12 who did not complete gem-AC; MST was 26.8 and 10.8 months, respectively (P = 0.0002).

DISCUSSION

Unexpectedly, at the time of reassessment in our study, distant metastases had occurred at a similar frequency among the three groups: 27% in the R, 12% in the BR and 18% in UR groups. To our knowledge, there have been no reports regarding the frequency of occurrence of distant metastasis after completion of gem-CRT according to resectability based on the NCCN guidelines. Previous studies have reported that the occurrence rate of distant metastasis at the time of reassessment after NCRT was 12.8% in BR patients [24] and 12.5% in potentially resectable tumors (including R and BR) [25]. However, there have been no reports concerning UR. Our results suggest that PDAC is a systematic disease regardless to its resectability status. A subgroup analysis carried out in the CONKO-001 randomized controlled trial regarding adjuvant chemotherapy after curative-intent resection [1] also suggested that PDAC is a systemic disease, even in early-stage tumors at the time of diagnosis. This was because the median disease-free survival among T1-2 patients in the observation group, which was almost the same as that among T3-4 patients in the gemcitabine group (12.9 M), was significantly shorter than in the gemcitabine group (10.0 months vs. 48.2 months). One of the advantages of NCRT is the identification of a subset of patients for whom resection will not offer a survival benefit. In fact, all of the R patients that underwent resection in our study did not recur within 2 years following gem-CRTS treatment. Although NCRT is recommended for the treatment of BR tumors as an option [7], it may also be recommended for R tumors.

Although CA 19-9 has been accepted as a measure of pancreatic cancer burden, the role of CA 19-9 in the evaluation of patients with NCRT prior to planned surgical resection has not been well evaluated. Recently, there have been two studies that have underscored CA 19-9 as a marker of resectability and survival in patients with potentially resectable PDAC treated with NCRT. One of these studies [26] indicated that a pre-CA 19-9 level of <37 U/ml had a positive predictive value (PPV) for completing PD of 86% but a negative predictive value (NPV) of 33%; in addition, a post-CA 19-9 level of <61 U/ml had a PPV of 93% and a NPV of 28%. The other study [25] used more complicated criteria by dividing the patients into three categories (I: increased; MD: modestly decreased; SD: substantially decreased) and utilizing pre-CA19-9 level and post-CA19–9 reduction rate as endpoints; the authors suggested that alteration in CA19-9 status was a single independent factor associated with prognosis. In the present study, multivariable analysis of the effect of preoperative factors on survival time in reassessed cases indicated that a CA19-9 reduction rate of more than 50% was the single significant prognostic factor in BR patients, but not in R and UR patients. In our study, all of the 15 patients (3 in the R, 5 in the BR and 7 in the UR groups) who developed distant metastases at reassessment did not show a CA19-9 reduction rate of >50%, suggesting that the CA19-9 reduction rate is associated with the systematic progression of PDAC.

Several previous studies have indicated that margin resection status is a very important prognostic factor, and that the survival benefits of R1 resection may be comparable to palliative CRT without surgery [27]. There have been few studies that have evaluated the R0 resection rates in BR according to the NCCN guidelines, and we could find only two studies that had evaluated the R0 resection rates in BR patients. One of these studies [28] emphasized the effect of NCRT on the R0 resection rate by retrospec-

tively comparing patients that had and had not undergone NCRT for BR tumors; the R0 resection rate was significantly higher in patients who had received NCRT than in those who had not (59% vs. 11%). The other study which was Japanese [29] compared the R0 resection rate between R (n = 109) and BR (n = 24) patients who were retrospectively classified according to NCCN guidelines, and reported rates of 81% and 71%, respectively. In our study, the R0 resection rate was 100% in R, 78% in BR and 40% in UR patients. Our results suggested that gem-CRT increased the R0 resection rate in R and BR patients, and moreover that the R0 resection rate was an independent prognostic risk factor in BR patients. As for the R0 resection rate in UR patients who underwent curative-intent resection after CRT, there have been no reports because UR patients are usually not candidates for resection. Only a few studies [12-14] have reported that 8.6–32% of locally advanced (including BR and UR) patients who received CRT had undergone resection. To the best of our knowledge, the present study is the first to have evaluated the R0 resection rate by performing curative-intent resection after gem-CRT in UR patients. Among 42 UR patients enrolled for gem-CRTS, curative-intent resection could be performed in 20 (48%), of whom only eight (40%) had undergone R0 resection.

The rate of high responders to gem-CRT has been getting worse in line with resectability: 57% in R, 25% in BR and 15% in UR patients. Although the mechanism for resistance to CRT has not been fully explored, it has been reported that pancreatic cancer stem cells are a fundamental reason for this resistance [30-32]. A recent study [33] has revealed that chemoradioresistant pancreatic cancer cells are rich in stem-cell-like tumor cells and undergo epithelial-mesenchymal transition (EMT). It is known that N-cadherin is associated with a high invasiveness potential in cancer. A previous study [34] has also demonstrated that overexpression of N-cadherin in PDAC, which was significantly correlated with the degree of ne, was is involved in EMT. In the present study, the degree of ne was significantly higher in UR than in R and BR patients. Based on these findings, we hypothesize that pancreatic cancer cells in UR had become chemoradioresistant by undergoing EMT.

Several previous reports have demonstrated that hENT1 expression in the resected specimen is a significant predictive marker of chemosensitivity for gem-based AC in PDAC [15,35,36]. As for neoadjuvant therapy, we could find only two previous reports, one of which was ours, that have described the relationship between hENT1 expression and prognosis for PDAC. Our previous study [16] demonstrated that hENT1 expression was the independent predictor of overall survival after gem-CRTS in 55 patients with UICC T3–T4 PDAC. On the contrary, the other study [37] found that hENT1 expression was not associated with prognosis in 63 patients who underwent gem-CRTS. The significant difference between the two studies involving gem-CRTS was the type of AC: gem-AC in the former and postoperative liver perfusion chemotherapy using continuous infusion of 5-fluorouracil in the latter. In our previous study [16], the percentage of patients who completed gem-AC had significantly lower negative hENT1 expression than positive expression, and in addition the patients who completed gem-AC had a longer MST than those who did not complete gem-AC (24.9 months vs. 12.8 months). In the present study, the survival rate of BR patients did not differ significantly between those with positive and negative hENT1 expression, while UR patients with positive hENT1 expression had a significantly higher survival rate. Furthermore, the rate of completion of gem-AC treatment in UR patients was significantly higher in those with positive hENT1 expression than in those with negative expression (66.7% vs. 0%), while in BR patients there was no significant difference between the two groups. These results suggested that the status of hENT1 expression highly influenced the gem-AC completion rate, which in turn affected patient survival.

Pretreatment evaluation of hENT1 expression in pancreatic cancer tissue can be benefi-

cial in predicting the efficacy of gemcitabine therapy before initial treatment. The EUS-FNA specimens that we used for cytological/histological diagnosis might be suitable for evaluating hENT1 expression; however, the analysis of hENT1 expression in the pancreatic tumor tissue taken by EUS-FNA has not been established. Recently, we evaluated the availability of EUS-FNA samples for hENT1 expression and compared the status of hENT1 expression in the resected specimen in the 55 PDAC patients treated with gem-CRTS [38]. Among the 55 patients, only 23 (41.8%) who were histologically diagnosed as PDAC could be evaluated for hENT1 expression in the EUS-FNA samples: positive for hENT1 expression in 16 (69.6%) and negative for hENT1 expression in seven (30.4%) samples. hENT1 expression in 87% of EUS-FNA samples was identical with that in resected specimens after gem-CRT. The 16 patients with positive hENT1 expression in the EUS-FNA samples had significantly longer overall and recurrence-free survival rates than the seven with negative hENT1 expression (2-year survival and recurrence-free survival rates: 67.5% and 29.2%, respectively, vs. 35.7% and 0%, respectively). Our data provide the evidence that intratumoral hENT1 expression in EUS-FNA samples can be used to predict treatment outcome before gem-CRT. However, improvement in the rate of acquisition of specimens by EUS-FNB and further modification of the protocol for assay of hENT1 are needed.

In conclusion, a CA19-9 reduction rate of more than 50% after gem-CRT and R0 status were the significant prognostic factors in BR PDAC. Positive expression of hENT1 in the resected specimen was the significant prognostic factor in UR PDAC. We consider that our gem-CRTS protocol, even for locally unresectable PDAC, allows for the identification of candidates for aggressive resection at the time of reassessment; thus, facilitating an increase in the R0 resection rate, and improving prognosis in patients with positive hENT1 expression.

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