Table II. Patient characteristics of continuously high, intermittently high and continuously low alanine aminotransferase groups on diagnosis of HCV-LC.

Characteristics	Continuously high ALAT group	Intermittently high ALAT group	Continuously low ALAT group	*p*-value
No. of cases	41	43	48	
No. of cases developed HCC	32 (78.0%)	26 (60.5%)	22(45.8%)	0.008
Age (years)				
Mean (SD)	57.7 (6.1)	58.2 (7.6)	61.3 (7.1)	0.034
Gender (M/F)	22/19	18/25	19/29	0.182*
Child classification (A/B)	41/0	43/0	48/0 1.000*	
BMI				
Mean (SD)	24.7 (2.7)	24.5 (2.9)	23.1 (3.2)	0.034
Smoking habit (+)/(−)	16/25	18/24	15/31	0.605*
Liver tests				
Albumin (g/dl)				
Mean (SD)	3.9 (0.4)	4.2 (1.2)	4.1 (1.5)	0.636**
Median (IQR)	3.8(3.5–4.2)	4.1(3.7–4.5)	3.9(3.7–4.2)	
ASAT (IU)				
Mean (SD)	107.3 (56.8)	110.9 (52.0)	64.4 (27.0)	0.000**
Median (IQR)	103.0 (75.5–133.0)	100.0 (79.5–136.3)	67.5(51.5–86.0)	
ALAT (IU)				
Mean (SD)	131.9 (65.9)	125.7 (72.3)	74.6 (48.7)	0.000**
Median (IQR)	123.0 (86.0–187.5)	109.0 (72.0–140.0)	68.0 (52.5–93.5)	
Prothrombin time (%)				
Mean (SD)	80.8 (13.3)	75.1 (13.7)	79.6 (17.1)	0.086*
Median (IQR)	83.3 (74.9–90.2)	78.7 (65.0–85.7)	82.6 (64.2–93.7)	
Platelet counts (per $mm^3 \times 10^4$)				
Mean (SD)	9.9 (3.1)	10.9 (4.1)	11.2 (3.2)	0.193**
Median (IQR)	9.6 (7.4–10.8)	10.0 (8.5–13.4)	10.7(8.6–13.5)	
AFP				
Mean (SD)	54.1 (76.7)	28.8 (45.0)	27.9 (39.9)	0.037**
Median (IQR)	24.1 (10.9–80.8)	13.1 (6.0–36.5)	13.0 (5.1–31.0)	
Observation period (years)				
Mean (SD)	7.2 (2.6)	8.1 (3.0)	8.6 (3.4)	0.165**
Median (IQR)	7.0 (5.0–8.5)	7.0 (6.0–10.0)	7.0 (6.0–11.0)	

Abbreviations: BMI = body mass index; SD = standard deviation; IQR = interquartile range; M = male; F = female; ASAT = aspartate aminotransferase; ALAT = alanine aminotransferase; AFP = α-fetoprotein; HCV-LC = hepatitis C virus-liver cirrhosis.
p-value, unpaired *t*-test; *p, χ^2 test; **p, Mann-Whitney test.

132 patients included (108.9±64.1 IU) at the beginning of the study (p=0.249). The AFP level was slightly elevated in the continuously high ALAT groups. This seemed to be the result of continuous inflammation in this group of patients. The HCV-RNA level at the beginning of the study is not cited, because in many cases estimation of the HCV-RNA level was not undertaken at these days.

Figure 1 shows the cumulative incidence of HCC starting 3 years after the diagnosis of LC in patients with continuously high, continuously low, and unclassified (intermittently high) ALAT levels. The cumulative incidence of HCC in patients with continuously high serum ALAT levels for the first 3 years after the diagnosis of LC (Child Stage A) was significantly higher than that in patients with continuously low

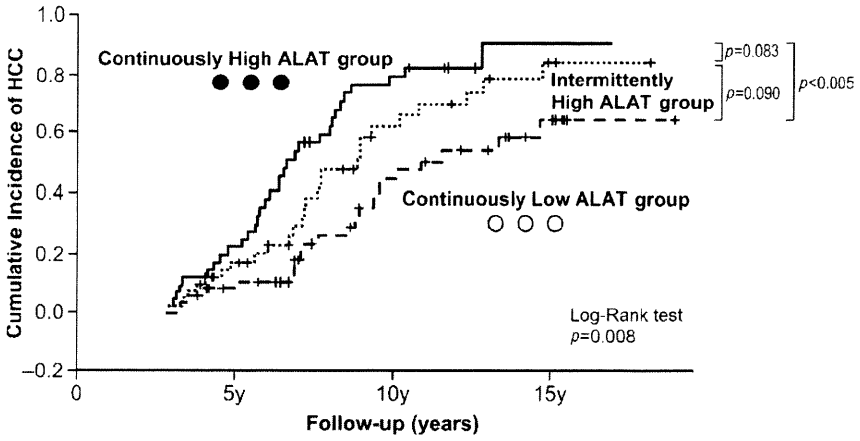

Figure 1. Cumulative incidence of hepatocellular carcinoma (HCC) starting 3 years after diagnosis of liver cirrhosis (LC) in patients with continuously high and continuously low serum alanine aminotransferase (ALAT) levels for the 3 years, and for those with intermittently high serum ALAT levels (Kaplan-Meier).

serum ALAT levels for the same period (p <0.005). The cumulative incidence of HCC in patients with intermittently high ALAT levels was higher than that in patients with continuously low serum ALAT levels, although the difference was not significant (p=0.090). There was also a tendency for the cumulative incidence of HCC in patients with continuously high ALAT levels to be higher than that in patients with intermittently high ALAT levels, but again the difference was not significant (p=0.083).

The 5-year incidence of HCC after classification of LC groups was 59% (11.8%/year) in the high ALAT group and 26% (5.2%/year) in the low ALAT group. The difference in cumulative incidence of HCC between the continuously high and continuously low groups was more marked in the period of less than 10 years after diagnosis of LC than in the period of more than 10 years after diagnosis; the ratio of incidence between these two groups at 8 years was 1.93-fold compared with 1.35-fold at 16 years after diagnosis (Figure 1).

In the univariate logistic analyses, as shown in Table III, the following four risk factors affected (p <0.20) the cumulative rate of incidence of HCC in all patients: gender, AFP, administration of SNMC, and ALAT group. As shown in Table III, the odds ratio of developing HCC in patients with continuously high serum ALAT levels was 5.1-fold that in patients with continuously low serum ALAT levels, while the odds ratio in patients with intermittently high ALAT levels was 1.5-fold that in patients with continuously low serum ALAT levels. Multivariate analysis using the logistic regression model showed that only one factor was statistically significant: the ALAT group (continuously high and continuously low ALAT group) independently contributed to HCC development (Table IV). Finally, if we assume the decrease in serum ALAT levels ≥25% to be an effective improvement, the effective improvement percentages in each drug are: SNMC (16 out of 31, 51.6%), UDCA (14 out of 36, 38.9%), Shosaiko-to (7 out of 24, 29.2%), a combination of SNMC and UDCA (18 out of 31, 58.1%).

Discussion

In this study we demonstrated that if high serum ALAT levels (≥80 IU) persisted for 3 successive years from the diagnosis of LC (Child Stage A), the 5-year incidence of HCC was markedly increased to as high as 59% (11.8%/year) in HCV-LC patients. It is clear that continuously high levels of ALAT in Child Stage A LC have a significant impact on the development of HCC. Thus, high ALAT levels (≥80 IU) for the 3 years following the diagnosis of LC can be highly predictive of the development of HCC. On this point, Mahmood et al. [28] also found that the 3-year annual average ALAT post-IFN therapy was significantly related to HCC occurrence in the HCV-associated chronic hepatitis patients with stage 3 fibrosis, although the tendency was more marked in our study with cirrhosis.

Many investigators have shown that patients with cirrhosis and high AFT levels have a high risk of developing HCC. Oka et al. [29] demonstrated that, in the cirrhotic patients without HBs-Ag, the cumulative incidence of HCC during a 5-year follow-up period was 28% in patients who had AFP levels of below 20 ng/ml at the time of entry, as compared with 44% in patients with AFP levels of 20 ng/ml or more.

In this study, we demonstrated that the continuously high serum ALAT levels for the first 3 years after diagnosis of LC was also as closely associated with the

Table III. Risk contributed to HCC development in univariate logistic model.

Items	*p*-value	Odds ratio	95% Confidence interval
Age	0.367	1.024	0.973–1.078
Gender (female)	0.167	0.592	0.282–1.246
BMI	0.997	1.000	0.879–1.138
Smoking habit	0.662	1.184	0.556–2.520
Albumin	0.356	0.862	0.629–1.182
Platelet counts	0.334	0.949	0.853–1.055
AFP	0.067	1.009	0.999–1.020
SNMC	0.101	1.864	0.886–3.922
UDCA	0.275	1.515	0.719–3.191
Sho-saiko-to	0.855	1.082	0.466–2.511
Juzen-taiho-to	0.913	0.921	0.210–4.047
Intermittently high ALAT	0.325	1.536	0.654–3.609
(reference: continuously low ALAT)			
Continuously high ALAT	0.002	5.120	1.816–14.433
(reference: continuously low ALAT)			

Abbreviations: HCC = hepatocellular carcinoma; BMI = body mass index; AFP = α-fetoprotein; SNMC = stronger-neo-minophagen C; UDCA = ursodeoxycholic acid; ALAT = alanine aminotransferase.

Table IV. Risk contributed to HCC development in a multivariate logistic model.

Items	*p*-value	Odds ratio	95% Confidence interval
Gender (female)	0.310	0.664	0.301–1.464
AFP	0.210	1.007	0.996–1.017
SNMC	0.501	1.327	0.581–3.029
Intermittently high ALAT	0.506	1.354	0.554–3.307
(reference: continuously low ALAT)			
Continuously high ALAT	0.013	3.931	1.336–11.565
(reference: continuously low ALAT)			

Abbreviations: HCC = hepatocellular carcinoma; AFP = α-fetoprotein; SNMC = stronger neo-minophagen C; ALAT = alanine aminotransferase.

development of HCC as the high AFP levels in the study by Oka et al. [29]. The odds ratio increased to 5.1-fold in patients with continuously high serum ALAT levels for the 3 years after diagnosis as compared with patients with continuously low ALAT levels for those years. In contrast, the odds ratio in patients with intermittently high ALAT levels was only 1.5-fold that of patients with low ALAT levels. Moreover, multivariate analysis confirmed that the ALAT group was independently associated with the development of HCC. Furthermore, the difference in cumulative incidence of HCC is more marked in the early period of follow-up than in the late period, suggesting the late occurrence of HCC in patients with continuously low ALAT levels for the 3 years following diagnosis of LC.

Recently many studies have demonstrated the close association between ALAT levels and the development of HCC. Ishiguro et al. [30] demonstrated that serum ALAT concentration was dependently associated with an increased risk of HCC in both virus-positive and virus-negative participants in a large population-based cohort study in Japan. Kurokawa et al. [31] studied the long-term effects of INF-alpha-2b plus ribavirin therapy on the incidence of HCC in patients with chronic hepatitis C and found that the cumulative incidence of HCC was significantly lower in patients who had average serum ALAT levels of <40 IU/L than in those who showed average serum ALAT levels of ≥40 IU/L after combination therapy. Moreover, Kumada et al. [32,33] surveyed the risk factors involved in the development of HCC in patients with chronic

HCV infection who had normal ALAT levels (<40 IU/L) over 10 years, and found that a slightly high ALAT level (>20 IU/L) was closely associated with the development of hepatocarcinogenesis.

The next issue is why the risk of developing HCC was increased so markedly in the continuously high ALAT group, as demonstrated in this study. It is likely that genetic alterations accumulate rapidly as inflammation persists and that the multistep process of carcinogenesis or promotion of tumor growth progresses more rapidly in patients with continuously high ALAT levels. In this respect, Ferenc et al. [34] demonstrated significant differences in the p53 expression between mildly, moderately, and severely inflamed biopsy samples in ulcerative colitis. 8-hydroxy-2′-deoxyguanosine (8-OHdG) is a pro-mutagenic DNA lesion produced by oxygen (hydroxy) radicals [35,36], and is known to be a parameter of genetic risk for hepatocarcinogenesis [37]. Furthermore, 8-OHdG was demonstrated to be involved in the initiation of rat liver hepatocarcinogenesis by low doses of N-nitrosodiethylamine (DEN) [38].

Shimoda et al. [39] examined the levels of 8-OHdG in patients with chronic hepatitis, liver cirrhosis, and HCC and found that the OHdG level in liver affected by chronic hepatitis was significantly higher than that in normal liver, and that the OHdG level in liver affected by cirrhosis also tended to be higher than that in normal liver. They also found a significant correlation between the OHdG content in non-cancerous liver tissue and individual serum ALAT levels, and concluded that chronic inflammation in the liver might produce oxidative DNA damage, which would increase the risk of genomic alterations causing hepatocarcinogenesis. If high-grade inflammation persists in the liver for many years, as in the continuously high ALAT group of patients in our study, the level of 8-OHdG might be high throughout the cirrhotic liver, resulting in the development of HCC.

Nowadays, patients with chronic hepatitis C in all countries are generally treated with IFN, and more than 50% of patients become HCV-RNA negative following PEG-IFN plus ribavirin therapy, but unfortunately, the IFN therapy is not effective in about 70% of patients with HCV-associated liver cirrhosis. Moreover, patients with HCV-associated cirrhosis carry a high risk of HCC, and in Japan, HCC actually develops in about 7% of those patients every year [40]. A strategy for preventing HCC development other than IFN therapy is therefore urgently needed for those patients.

In conclusion, we demonstrated that if the serum ALAT level was high (≥80 IU) for 3 successive years following the diagnosis of LC, then the risk of subsequently developing HCC increased markedly as compared with the continuously low ALAT group in Child A HCV-LC patients. Multivariate analysis confirmed that the ALAT group of LC was independently associated with HCC development. Thus, continuously high ALAT levels for 3 years following the diagnosis of LC (Child Stage A) can be highly predictive of the development of HCC. However, prospective trials using therapeutic approaches to decrease ALAT levels are necessary to confirm a positive impact of ALAT reduction on the incidence of HCC in patients with HCV-LC. The present study suggests that serum ALAT levels in HCV-LC patients must be lowered to below 80 IU by anti-inflammatory drugs as soon as a diagnosis of LC is confirmed.

Acknowledgements

The present work was supported by the Fund for Cancer Research from the Ministry of Welfare of Japan, the Mitsui Life Social Welfare Foundation, the Viral Hepatitis Research Foundation of Japan, and the Kanagawa Healh Foundation.

Declaration of interests: No conflict of interests existed in our present work.

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Cancer Chemother Pharmacol (2009) 65:113–120
DOI 10.1007/s00280-009-1011-z

ORIGINAL ARTICLE

A phase II study of uracil-tegafur plus doxorubicin and prognostic factors in patients with unresectable biliary tract cancer

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Received: 27 December 2008 / Accepted: 13 April 2009 / Published online: 29 April 2009

Abstract
Purpose The purpose of this study was to clarify the safety and efficacy of combination chemotherapy of uracil-tegafur (UFT) and doxorubicin (UFD regimen), and to identify the prognostic factors in patients with unresectable advanced biliary tract cancer who received systemic chemotherapy.
Methods Patients with histologically or cytologically confirmed, measurable biliary tract cancer, including intrahepatic or extrahepatic cholangiocarcinoma, gallbladder cancer, and ampullary cancer, who were not suitable candidates for surgery, were eligible for the study. Patients received oral UFT at 300 mg/m^2 per day divided into two doses on days 1–14 and intravenous doxorubicin at 30 mg/m^2 on day 1. This cycle was repeated every 21 days. The relationship between the patient characteristics and the prognosis was examined. Univariate and multivariate analyses were conducted to identify the prognostic factors associated with survival.
Results Sixty-one patients from 12 institutions were enrolled in the late phase II study between April 2005 and March 2006. Of the 61 patients, 4 patients had partial responses, for an objective response rate of 6.6% (95% CI: 1.8–15.9%); 28 patients had stable disease, 27 had progressive diseases, and 2 patients were not evaluated. The median progression-free survival was 1.6 months, and the overall median survival time was 6.5 months. In the 85 patients who received this UFD chemotherapy in previous and late phase II studies, multivariate analysis revealed the ECOG performance status 1 ($P = 0.001$), gallbladder as the

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primary cancer site ($P = 0.014$), T-factor 4 of the TNM classification ($P = 0.035$), and elevated serum lactate dehydrogenase levels ($P = 0.043$) as being associated with a significantly shorter survival.

Conclusions Combination chemotherapy of UFT and doxorubicin had minimum activity against advanced biliary tract cancer. Performance status was identified as the most important prognostic factor in patients who received systemic chemotherapy.

Keywords Biliary tract cancer · Systemic chemotherapy · Uracil-tegafur · Doxorubicin · Phase II study · Prognostic factor

Introduction

Biliary tract cancer consists of cholangiocarcinoma (CC), gallbladder cancer (GBC), and ampullary cancer (AC) [1]; intrahepatic cholangiocarcinoma is often included in clinical trials for biliary tract cancer. Each type of cancer has characteristic features, and the treatment strategy and prognosis are different. This heterogeneity has made it difficult to conduct and evaluate chemotherapy for biliary tract cancer. Biliary tract cancer is relatively uncommon in western countries, but it is a common cause of cancer-related death in Asia. In Japan, the mortality is estimated to be 16,000 deaths annually [2]. While surgery currently remains the only potentially curative treatment, most patients are found to have an unresectable advanced stage of disease. Although patients with unresectable disease receive various palliative treatments, including systemic chemotherapy, the prognosis remains extremely poor.

A previous report showed improved survival in patients with biliary tract cancer treated with 5-fluorouracil (5-FU)-based chemotherapy compared to the best supportive care [3]. Efforts have been made to develop promising regimens for biliary tract cancer using clinical trials of systemic chemotherapy [4]. In various reports on chemotherapy for biliary tract cancer, fluoropyrimidines have been considered as the basis of chemotherapy [5–7]. Furthermore, cisplatin or anthracycline antitumor antibiotic agents such as doxorubicin and epirubicin have been used as combination chemotherapy with 5-FU [8–10]. Recently, clinical trials of gemcitabine show moderate activity against biliary tract cancers, and gemcitabine-based regimens have been investigated [11–22]. However, no standard chemotherapy has currently been identified that can clearly prolong survival.

In Japan, until 2006, only three anticancer agents—uracil–tegafur (UFT), doxorubicin, and cytarabine—had been approved by the Ministry of Health, Labour, and Welfare for biliary tract cancer. Uracil-tegafur is an orally administered drug that is a combination of uracil and tegafur in a 4:1 molar concentration ratio. Tegafur is a 5-FU prodrug that is hydroxylated and converted to 5-FU by hepatic microsomal enzymes. Uracil prevents degradation of 5-FU by inhibiting dihydropyrimidine dehydrogenase, which leads to an increased level of 5-FU in plasma and tumor tissues [23, 24]. Doxorubicin is an anthracycline antibiotic that induces various biologic effects and has one of the widest spectra of antitumor activity against lymphomas, leukemias, soft tissue sarcomas, and a variety of carcinomas. Because, UFT + doxorubicin is the only doublet regimen currently covered by health insurance in Japan, we investigated the combination of UFT and doxorubicin (the UFD regimen) in patients with unresectable advanced biliary tract cancer as an early phase II study in 2004. In that study, the UFD showed modest activity; the response rate was 12.5%, the median progression-free survival (PFS) was 2.5 months, and the median overall survival (OS) was 7.6 months [25]. To examine the safety and efficacy in a larger number of patients, a multicenter late phase II study was conducted in a Japanese chemotherapy study group for biliary tract and pancreatic cancers. The objectives of the study were to evaluate response rate, toxicity, PFS, and OS. As an additional exploratory analysis, we examined the prognostic factors in patients with unresectable biliary tract cancer who had received the UFD regimen in the early and current phase II studies.

Patients and methods

Patient eligibility

The eligibility criteria for enrollment in this late phase II study were: (1) histologically or cytologically confirmed biliary tract cancer consisting of intrahepatic CC (ICC), extrahepatic CC (ECC), GBC, or AC; (2) measurable disease on computed tomography (CT) or magnetic resonance imaging (MRI); (3) unresectable disease; (4) no prior chemotherapy; (5) age $\geq$ 20 years, with a set upper limit of 74 years according to another Japanese trials of gemcitabine and S-1 [13, 26]; (6) Eastern Cooperative Oncology Group (ECOG) performance status (PS) of 0–2; (7) adequate bone marrow function (leukocyte count $\geq$ 4,000 cells/mm^3, platelet count $\geq$ 100,000 cells/mm^3, and hemoglobin $\geq$ 9.0 g/dL), renal function (serum creatinine concentration $\leq$ upper limit of normal range), and hepatic function [serum bilirubin level $\leq$ 2.0 mg/dL, serum albumin level $\geq$ 3.0 g/dL, and serum aspartate transaminase (AST) and alanine transaminase (ALT) levels $\leq$ 2.5 times the upper limit of normal range]; (8) life expectancy $\geq$ 8 weeks; and (9) written informed consent from the patient. Percutaneous biliary drainage was performed in patients with obstructive jaundice and these patients were

required to have serum bilirubin levels of ≤ 3.0 mg/dL, and serum AST and ALT levels ≤ 5 times the upper limit of normal before enrollment. Exclusion criteria were: serious complications such as active infection, active gastrointestinal ulcer, cardiac disease, or renal disease; central nervous system metastasis; marked pleural effusion or ascites; symptomatic interstitial pneumonitis; and pregnancy or lactation for women. This study was approved by the local institutional review boards at all participating centers.

In addition, prognostic factors were analyzed in patients treated with the UFD regimen in the earlier and current phase II studies. The eligibility criteria for enrollment in the previous study were the same as those mentioned above for the current study, except that the upper age limit of 74 years for enrollment was not set.

Treatment methods

Uracil-tegafur was administered orally at a dose of 300 mg/m^2 per day (400 mg/day in patients with body surface < 1.50 m^2 and 500 mg/body per day in patients with body surface ≥ 1.50 m^2) divided into two dosages, for 14 consecutive days followed by 1 week of rest. Doxorubicin was given as a 10-min intravenous infusion on day 1 of each cycle at a dose of 30 mg/m^2. This cycle was repeated every 21 days provided that patients had recovered sufficiently from the drug-related side effects.

Patients continued to receive additional courses of this regimen until a maximum of 15 courses, evidence of disease progression, or the appearance of unacceptable toxicity. When hematological toxicity greater than grade 3 or nonhematological toxicity greater than grade 2 was observed, treatment was delayed until the toxicity subsided to grade 1 or less. If the daily dose of UFT was considered to be intolerable, the dose was reduced by 100 mg/day (one capsule/day). In general, patients were treated as outpatients and admitted to the hospital only for management of toxicities and disease-related complications.

Assessment of response and toxicity

Physical examination, complete blood cell counts, serum chemistries, and urinalysis were performed at baseline and at least twice in 3 weeks after initiating treatment. Patients underwent dynamic CT or MRI to evaluate response at 4–6-week intervals after the start of treatment. Computed tomography or MRI was performed by obtaining contiguous transverse sections using the helical scanning method at a section thickness of 5 mm. Tumor response was assessed using the Response Evaluation Criteria in Solid Tumors [27]. Objective responses were confirmed by a second evaluation performed at least 4 weeks later. Toxicity was graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events, version 3.0.

Study designs

The primary end point of this study was the overall response rate and the secondary endpoints were adverse events, OS, and PFS. In this study, the threshold response rate was defined as 5%, the expected response rate was set as 15%, and a sample size of 40 would ensure that there was a 74% power at a one-sided significance level of 5% in the late phase II study. The accrual period was set at 1 year and follow-up period was set at 1 year. When 40 patients were enrolled, the enrollment was extended until the end of the accrual time to improve the statistical power.

Factors analyzed

Twenty-three clinical variables were chosen at the time of study enrollment for the univariate and multivariate analyses. Each variable was divided into two categories as follows: age (<64 or ≥64 years), sex (male or female), PS (0 or 1), pretreatment (surgery or no treatment), biliary drainage (yes or no), diagnosis (GBC or non-GBC including ICC, ECC, and AC), white blood cell count (<8,000 or ≥8,000/mL), hemoglobin level (<11.0 or ≥11.0 g/dL), platelet count (<150,000 or ≥150,000/mL), serum total bilirubin level (<2.0 or ≥2.0 mg/dL), serum albumin level (<3.5 or ≥3.5 g/dL), serum lactate dehydrogenase (LDH) level (<300 or ≥300 IU/L), serum AST and ALT levels (<40 or ≥40 IU/L), serum alkaline phosphatase (<400 or ≥400 IU/L), size of maximum targeted tumor (<60 mm or ≥60 mm), T-factor of TNM classification (Tx-3 or T4) [1], extent of disease (locally advanced and local recurrence after surgery, or metastatic), liver metastasis (presence or absence), ascites or peritoneal dissemination (presence or absence), lymph node metastasis (presence or absence), serum carcinoembryonic antigen (CEA) level (<10 or ≥10 ng/mL), and serum carbohydrate antigen 19-9 (CA 19-9) level (<1,000 or ≥1,000 U/mL). The size of the primary tumor was measured by enhanced CT. Peritoneal dissemination was defined as recognition of peritoneal nodules in CT scans or accumulation of ascites.

Statistical analysis

Progression-free survival was calculated from the first day of treatment until evidence of tumor progression, clinical progression, or death due to any cause. Overall survival was calculated from the first day of treatment until death due to any cause. Survival data were analyzed using the Kaplan–Meier method. The tumor response, toxicity, and survival were evaluated on an intention-to-treat basis.

As an additional and unplanned analysis, the Cox proportional hazards model was used to evaluate prognostic variables associated to survival in patients with unresectable biliary tract cancer who received the UFD regimen in two phase II studies. Forward and backward stepwise regression procedures based on the partial likelihood ratio were used to determine the major independent predictors of survival. Statistical analyses were performed using the SPSS II 11.0 J software package for Windows (SPSS Japan, Tokyo, Japan). The statistical significance of differences between the survival curves was determined using the log-rank test. Two-sided *P*-values of less than 0.05 were considered significant.

Results

Patient characteristics

A total of 61 patients were enrolled between April 2005 and March 2006 in the late phase II study. Patient characteristics are shown in Table 1. The 61 patients received 244 cycles of the UFD regimen. The median number of cycles administered per patient was two (range 1–16 cycles). All patients discontinued this treatment: 50 experienced disease progression, six patients refused further treatment, two patients experienced serious adverse events of disseminated intravascular coagulation (DIC), or thrombocytopenia, and in three patients doxorubicin reached the upper limit dose. After abandoning the UFD treatment, 28 (45.9%) patients received second-line treatment; 30 patients had systemic chemotherapy with gemcitabine in 18 patients, UFT in 7, doxorubicin in 1; 1 patient had chemoradiotherapy and the other had immunotherapy. Three patients were unknown because of moving to another hospital. The remaining 30 (49.2%) patients received only best supportive care after the UFD treatment.

Table 1 Patient characteristics

	Current phase II study	Previous phase II study
N	61	24
Sex		
Male	27 (44%)	13 (54%)
Female	34 (56%)	11 (46%)
Median age (range)	65 (46–74) years	63 (46–75) years
ECOG performance status		
0	45 (74%)	16 (67%)
1	16 (26%)	8 (33%)
Location of primary tumor		
Gallbladder cancer	29 (48%)	13 (54%)
Intrahepatic cholangiocarcinoma	18 (30%)	10 (42%)
Extrahepatic cholangiocarcinoma	11 (18%)	1 (4%)
Ampullary cancer	3 (5%)	0 (0%)
Extent of disease		
Locally advanced or local recurrence after surgery	10 (16%)	5 (21%)
Metastatic	51 (84%)	19 (79%)
Metastatic sites		
Lymph node	43 (70%)	15 (63%)
Liver	35 (57%)	16 (67%)
Lung	6 (10%)	4 (17%)
Peritoneum	7 (11%)	1 (4%)
Bone	2 (3%)	1 (4%)
Adrenal grand	1 (2%)	0 (0)
Pleura	1 (2%)	0 (0)
Pretreatment		
No	44 (72%)	18 (75%)
Surgery	17 (28%)	6 (25%)

Tumor response

Partial response was achieved in 4 of the 61 patients (2 with GBC and 2 with ECC), but no complete response was observed. Overall response rate was thus 6.6% [95% confidence interval (CI), 1.8–15.9%], and 8.7% (95% CI, 2.6–14.7%) in 85 patients including 24 patients in the early phase II study. Stable disease (SD) was noted in 28 (45.9%) of the 61 patients and progressive disease (PD) was noted in 27 patients (44.3%). The remaining two patients who refused the treatment before the evaluation were not evaluated for response.

Toxicity

Toxicities of the 61 patients are shown in Table 2. During treatment, the most common toxicities were gastrointestinal effects such as anorexia in 38 patients (62.3%) and nausea in 35 patients (57.4%). Other major symptoms were fatigue in 35 patients (57.4%), hematological toxicities of anemia in 23 patients (32.8%), and leukopenia in 17 patients (27.9%). Grade 3 or 4 toxicity was observed in 4 of the 61 patients (6.6%), with anorexia, nausea, fatigue, DIC, and/or hematological toxicities. There were no treatment-related deaths during the study.

Survival

Disease progression was finally observed in 57 of the 61 patients. The progression pattern was progression of target lesions in 24 patients (42.1%), developments of new lesions in 10 (17.5%), both of these in 11 (19.3%), symptomatic deterioration without objective evidence of disease progression in 9 (15.8%), progression of non-target lesion and new

Table 2 Toxicity ($n = 61$)

Toxicity	Grade 1–4	Grade 3	Grade 4
Hematological			
Leukopenia	17 (28%)	2 (3%)	0 (0)
Neutropenia	14 (23%)	0 (0%)	0 (0)
Anemia	23 (38%)	1 (2%)	2 (3%)
Thrombocytopenia	9 (15%)	2 (3%)	0 (0)
Non-hematological			
Anorexia	38 (62%)	5 (8%)	1 (2%)
Nausea	35 (57%)	2 (3%)	0 (0)
Fatigue	35 (57%)	3 (5%)	1 (2%)
Alopecia	19 (31%)	0 (0)	0 (0)
Vomiting	13 (21%)	0 (0)	0 (0)
Abdominal pain	12 (20%)	0 (0)	0 (0)
Mucositis	10 (16%)	0 (0)	0 (0)
Fever	7 (11%)	0 (0)	0 (0)
Diarrhea	5 (8%)	0 (0)	0 (0)
Transaminase elevation	4 (7%)	0 (0)	0 (0)
Rash	4 (7%)	0 (0)	0 (0)
Pigmentation	3 (5%)	0 (0)	0 (0)
Arrhythmia	2 (3%)	0 (0)	0 (0)
Taste disturbance	1 (2%)	0 (0)	0 (0)
Edema	1 (2%)	0 (0)	0 (0)
Constipation	1 (2%)	0 (0)	0 (0)
Total bilirubin	1 (2%)	0 (0)	0 (0)
Sore throat	1 (2%)	0 (0)	0 (0)
Hand–foot skin reaction	1 (2%)	0 (0)	0 (0)
BW loss	1 (2%)	0 (0)	0 (0)
DIC	1 (2%)	1 (2%)	0 (0)

BW body weight, *DIC* disseminated intravascular coagulation

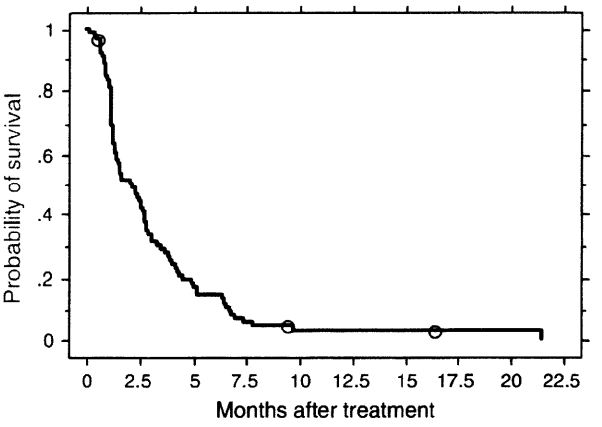

Fig. 1 Progression-free survival of all 85 patients. The median progression-free survival was 2.2 months and the 6-month survival rate was 14.3%

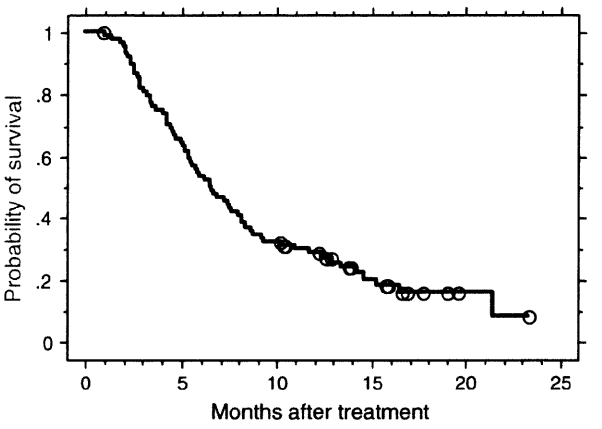

Fig. 2 Overall survival of all 85 patients. The median overall survival was 6.6 months and the 1-year survival rate was 28.2%

lesions in 3 (5.3%). Fifty of the 61 patients died: 49 patients died of cancer progression, and in the case of the other patient, the death was reported and the cause was unknown. The median PFS was 1.6 months in the 61 patients. The median OS time was 6.5 months and the 1-year survival rate was 30.0%.

Univariate and multivariate analyses

Among the 23 variables in 85 patients who received the UFD chemotherapy in the early and late phase II studies, six variables were identified as being significantly associated with shorter survival time: PS of 1, diagnosis of GBC, serum CA 19–9 level of >1,000 U/mL, T-factor of 4, serum LDH level of ≥300 IU/L, and serum total bilirubin level of ≥2.0 mg/dL by univariate analysis. The median PFS was 2.2 months in the 85 patients (Fig. 1). The median OS time was 6.6 months and the 1-year survival rate was 28.2% (Fig. 2). The median OS of patients with PS 0 was 8.2 months and that of patients with PS 1 was 4.3 months. There was a statistically significant difference in the survival curves between the two groups ($P < 0.0001$). Figure 3 shows survival curves for patients with non-GBC of ICC, ECC, or AC and for patients with GBC. The median OS of the patients with GBC was 5.4 months and that of the patients without GBC was 8.4 months. There was a statistically significant difference in the survival curves between the two groups ($P = 0.0019$). On the other hand, there was no statistically significant difference in the survival among patients with ICC, ECC, or AC.

Multivariate regression analysis was conducted for the six variables found to have prognostic significance in the univariate analysis. The four factors of PS, disease site, T-factor, and serum LDH were identified as independent prognostic factors (Table 3).

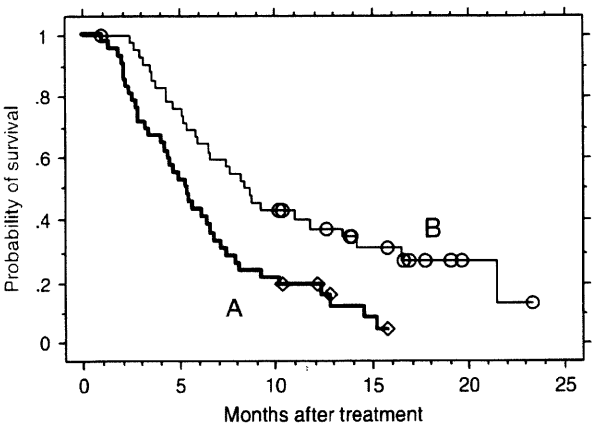

Fig. 3 Survival curves of patients with gallbladder cancer (**a**, $n = 42$) and with non-gallbladder cancer (intrahepatic cholangiocarcinoma, extrahepatic cholangiocarcinoma, or ampullary cancer (**b**, $n = 43$) ($P = 0.0019$)

Table 3 Multivariate analysis of prognostic factors in patients with unresectable biliary tract cancer

Variables	*N*	Median OS (mo)	Hazard ratio	95%CI	*P*-value
ECOG PS					
0	61	8.2	1		0.001
1	1	4.3	2.52	1.44–4.42	
Disease site					
ICC/ECC/AV	43	8.4	1		0.014
GB	42	5.4	1.88	1.14–3.12	
T-factor					
T1–3	62	8.1	1		0.035
T4	23	5.0	1.93	1.05–3.56	
LDH					
<300	67	8.1	1		0.043
≥300	18	4.8	1.85	1.02–3.35	
CA19-9					
<1,000	59	8.1	1		0.067
≥1,000	26	5.2	1.73	0.96–3.11	
T-Bil					
<2.0	77	6.6	1		0.27
>2.0	8	5.2	1.85	0.70–3.49	

OS overall survival, *CI* confidence interval, *PS* performance status, *ICC* intrahepatic cholangiocarcinoma, *ECC* extrahepatic cholangiocarcinoma, *GB* gallbladder cancer, *AV* ampullary cancer, *LDH* lactate dehydrogenase, *CA19-9* carbohydrate antigen 19-9, *T-Bil* serum total bilirubin

Discussion

Chemotherapy is generally indicated in patients with unresectable advanced cancer and patients with recurrence after resection. However, no standard chemotherapy for biliary tract cancer has yet been established, because only few randomized controlled trials with large numbers of patients have been conducted till date. Since only UFT and doxorubicin had been approved for biliary tract cancer for more than 20 years in Japan, the efficacy and safety of combinations of UFT and doxorubicin were examined in two phase II studies. The expected response rate was set as 15%, because biliary tract cancer was considered to be chemoresistant. The overall response rate in the two phase II studies was 8.7% (95% CI, 2.6–14.7%). The upper limit of the 95% confidence interval did not reach 15%, and the combination of UFT and doxorubicin was decided to have minimum activity against biliary tract cancer.

Response rate is sometimes not correlated with OS. Eckel et al. reported a pooled analysis of clinical trials in biliary tract cancer [28]. Based on the analysis of 104 phase II studies comprising of 112 trial arms, there was a highly significant correlation between time to progression (TTP) and OS ($r = 0.73$, $P = 0.000$), but there was a significant weak correlation between response rate and OS ($r = 0.2$, $P = 0.043$). Furthermore, it was reported that the pooled tumor control rate was 57.3% (95% CI: 55.3–59.3%), the median TTP was 4.1 months, and the median OS was 8.2 months. In the current studies, the tumor control rate (CR + PR + SD) was 56.4% (95% CI: 44.1–66.1%), which was almost equal to the pooled TCR, but the median PFS and OS were inferior to those of the pooled analysis, only 2.2 months and 6.6 months, respectively. The TTP or PFS seems appropriate as a surrogate marker of OS compared to the TCR.

It is difficult to conduct clinical trials consisting of a large number of patients with biliary tract cancer, because complications such as obstructive jaundice or cholangitis make it difficult to recruit eligible patients. Therefore, most of the clinical trials of chemotherapy for biliary tract cancer consist of less than 50 patients. Owing to the lack of clinical trials with large patient numbers, few analyses of prognostic factors in patients with advanced biliary tract cancer who received chemotherapy have been conducted till date. In the current phase II studies, 85 patients who received the same regimen of chemotherapy were enrolled and the patient characteristics in the two studies were almost the same. Therefore, we tried to determine the prognostic factors with univariate and multivariate analyses. Although some limitations of these methods should be recognized, such as insufficient patient number to allow adequate statistical power to be obtained, four factors, namely, the PS, disease site, T-factor, and serum LDH were identified as independent prognostic factors; PS was the most important prognostic factor with a hazard ratio of 2.52 ($P = 0.001$).

It has been reported for the advanced stage of various cancers, including pancreatic cancer, that the survival differs significantly depending on the extent of disease, that

is, depending on whether the disease is locally advanced or metastatic. In the current study, the median OS of the patients with locally advanced cancer was longer than that of patients with metastatic disease (8.2 months vs. 5.8 months), although there was no statistically significant difference in survival between the two patient groups ($P = 0.18$). We believe that this could possibly be explained by the smaller number of patients with locally advanced disease ($n = 15$) compared to that with metastatic disease ($n = 70$).

Performance status is often mentioned as an important independent prognostic factor in various cancers such as pancreatic cancer and hepatocellular carcinoma. The clinical practice guideline for the management of biliary tract cancer in Japan recommends that patients with a PS of two or more should not receive chemotherapy at the present time [29]. Since most clinical trials of chemotherapy for biliary tract cancer conducted till date have included patients with a PS of 2, the protocol of the current study also allowed the entry of patients with a PS of 2. However, only patients with a PS of 0 or 1 were actually enrolled. We investigated the prognostic factors to distinguish between PS 0 and 1, and found a statistically significant difference in survival between PS 0 and 1. The median OS in patients with a PS of 0 was 8.2 months and in patients with a PS of 1 was 4.3 months. Patients with a PS of 1 may be candidates for chemotherapy, but the survival is shorter than that in patients with a PS of 0.

The heterogeneity of biliary tract cancer is recognized to be one of the most important issues in considering prognosis of patients with biliary tract cancer. Regarding the primary site, the median OS in patients with gallbladder cancer was statistically significantly shorter than that in patients with intrahepatic cholangiocarcinoma, extrahepatic cholangiocarcinoma or ampullary cancer in the current study ($P = 0.014$). Some other trials showed this tendency [16, 20] but some did not [11, 12, 26]. The reason for this discrepancy is not clear but the small number of patients in each trial may be one of the reasons. In a retrospective analysis of a large number of patients ($n = 179$) [30], the median OS was 8.44 months for intrahepatic cholangiocarcinoma, 10.15 months for extrahepatic cholangiocarcinoma, and 6.50 months for gallbladder cancer. There was a statistically significant difference between extrahepatic cholangiocarcinoma and gallbladder cancer ($P = 0.029$). In the current study, a multivariate analysis in patients with unresectable biliary tract cancer who received the same regimen revealed that the site of disease was one of the significant prognostic factors. Therefore, PS and tumor site of gallbladder cancer or non-gallbladder cancer should be considered in randomized clinical trials for unresectable biliary tract cancer.

No standard chemotherapy for biliary tract cancer has yet been established till date. In Japan, recently, two registration phase II studies of a single agent, gemcitabine and S-1, have been reported [13, 26]. Gemcitabine achieved a better response rate, PFS, and OS compared with the UFT or UFD regimens. Furthermore, S-1 also seems active. Both gemcitabine and S-1 were well tolerated. Based on these results, gemcitabine and S-1 were approved for the treatment of biliary tract cancer in June 2006 and August 2007, respectively.

In conclusion, combination chemotherapy with UFT and doxorubicin (the UFD regimen) was well tolerated but showed minimum activity against advanced biliary tract cancer. Further studies of gemcitabine, S-1, and other cytotoxic or molecular targeted agents are expected to lead to the establishment of a standard chemotherapy for biliary tract cancer.

Acknowledgments This study was supported in part by Grants-in-Aid for Cancer Research from the Ministry of Health, Labour, and Welfare of Japan.

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The Usefulness of Perfusion-Weighted Magnetic Resonance Imaging in Advanced Pancreatic Cancer

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Objectives: Perfusion-weighted magnetic resonance imaging (MRI) can detect the changes of signal intensity in tumors. We evaluated the prognostic value of perfusion-weighted MRI in patients with advanced pancreatic cancer (PC).

Methods: Perfusion-weighted MRI was performed before treatment on 27 consecutive patients with advanced PC. The American Joint Committee on Cancer (AJCC) stages of patients were as follows (8, stage III; 19, stage IV). Imaging acquisition was continually repeated with echo planar sequence every 2 seconds for 2 minutes after a bolus injection of gadolinium. We made a time intensity curve of PC and calculated the signal ratio (SR) on perfusion-weighted imaging. We assessed the relation between SR and clinical factors including tumor stage, lymph node metastasis, liver metastasis, and so on. Patients were divided into low and high SR group and compared SR with the overall survival.

Results: All cases showed transient decreases signal intensity (SR, 6.9–55.7%). These patients were classified into 2 groups at cutoff median SR of 22.0% The high SR group significantly correlated with the higher stage ($P = 0.03$) and the presence of lymph node metastasis ($P = 0.04$). The high SR group had significantly shorter overall survival ($P = 0.04$).

Conclusions: Perfusion-weighted MRI may predict the survival in advanced PC patients.

Key Words: MRI, pancreatic cancer, prognosis

Abbreviations: PC - pancreatic cancer, MRI - magnetic resonance imaging, SR - signal ratio, BSA - body surface area, VEGF - vascular endothelial growth factor

(*Pancreas* 2009;38: 644–648)

Pancreatic cancer (PC) has a poor prognosis, and the 5-year survival rate is less than 5%[1] because of the difficulty of early detection and the relative chemoresistance of this tumor. Currently, gemcitabine is the most standard cytotoxic agent.[1,2] However, the objective response rate of this drug is less than 10%.[2] Although some clinical trials of systemic chemotherapy that include combinations of gemcitabine have been recently performed, most trials could not show the superiority to gemcitabine.[3–5] Considering the poor prognosis of advanced PC, it is important to predict the survival of the patients with this cancer.

Recently, perfusion-weighted magnetic resonance imaging (MRI) has been used in the diagnosis of brain and liver tumors.[6–10] When a bolus of MR contrast agent passes through the intravascular space, it creates local magnetic susceptibility (T2*) effects which cause a transient signal drop during the first pass of the contrast agent. This negative enhancement is directly proportional to the hemodynamic blood volume map.[6] To our knowledge, the usefulness of perfusion-weighted MRI in PC has not been evaluated yet. We examined the usefulness of perfusion-weighted MRI in inoperable advanced PC.

MATERIALS AND METHODS

Patients' Characteristics and Follow-Up

Twenty-seven consecutive patients with advanced PC were examined by perfusion-weighted MRI at the Kanagawa Cancer Hospital, Kanagawa, Japan between May 2003 and June 2004 (Table 1). Especially lymph node metastasis was defined as lymph node size over 10 mm on the MR and computed tomography (CT) images. Fully informed consents were obtained from all patients before the examinations. This study was permitted by the independent ethical committee of Kanagawa Cancer Center. The diagnosis was histologically confirmed by examining specimens obtained from 24 cases. If no histological confirmation could be done, the diagnosis was made on the basis of clinical and imaging findings. Metastasis was diagnosed with CT and MRI comprehensively.

MRI Examinations

Magnetic resonance imaging examinations were performed with a 1.5-T superconducting MR system (Excelart XGS; Toshiba Medical System, Tokyo, Japan) in the supine position after an overnight fasting. Before perfusion-weighted MRI, transverse T1-weighted fast gradient echo image (repetition time [TR], 187 ms; echo time [TE], 4 ms) and transverse T2-weighted fast spin echo image (TR, 3150 ms; TE, 10 ms; echo train length, 19) were acquired. The perfusion-weighted MRI was performed by the single-shot gradient echo, echo planar pulse sequence with the following scan parameters: TR, 2000 ms; TE, 45 ms; matrix, 144 × 144; field of view, 30 × 30 cm; slice thickness, 8 mm; inter-slice gap, 1 mm; number of excitations, 1, 13 slices. The acquisition time was 2 seconds. The contrast agent of gadopentetate dimeglumine (Magnevist; Nihon Schering, Osaka, Japan) was automatically injected as a bolus infusion (at a dose of 0.2 mL/kg, 4 mL/s) through a 20-gauge cannula placed in the cubital vein, followed by physiological saline (0.4 mL/kg) to flush the cannula with automated power injector. The patients were asked for hold their breath for 30 minutes. At 30-second intervals, the patient could take a new breath for 5 seconds. The total acquisition time for perfusion-weighted imaging was 2 minutes. Finally, post-contrast transverse T1-weighted fast gradient echo images (TR, 187 ms; TE, 4 ms) were acquired.

Data Analysis

Three regions of interests (ROIs) were placed in APC, and the averaged signal was measured in all dynamic images. All

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Received for publication August 28, 2008; accepted March 17, 2009.
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This work was supported by the Fund for Cancer Research from Kanagawa Prefecture.
The results of this work were presented in part at ASCO, Orlando (abstract #4109) in 2005.

TABLE 1. Categorial Distributions of the Baseline Characteristics in All Patients

Age, y		63 ± 8.9
Males/females		16/11
CA19-9 change >20% decrease, ≤20% decrease		20/7
Tumor stage III/IV		8/19
Tumor location (head/body-tail)		7/20
Median tumor diameter (mm)		40
Primary tumor	T3/T4	11/16
Lymph nodes	N0/N1	23/4
Distant metastasis liver: lymph nodes: peritoneum: others		11: 4: 3: 3
Chemotherapy		
Gemcitabine		20
S-1		3
Gemcitabine + S-1		4

the ROI measurements were done on the workstation. Dynamic perfusion images as well as CT scans, pre- and post-contrast T1-weighted images, and T2-weighted images were referred for tumor location and extent. The vessels and cystic areas were avoided with reference to the T1-weighted, T2-weighted, and post-contrast T1-weighted images for signal intensity measurement.

The ROI placement and size of the ROI were chosen precisely for each lesion so as to use the maximum ROI without volume averaging. To ensure that the same areas were measured, ROI was copied and pasted onto each dynamic image. When ROI was not the same portion of the tumor in the dynamic images with the patients' breathing, ROI was carefully moved manually so as to measure the signal at the same portion of the tumor in the dynamic images. All the ROI measurements were performed with 1 experienced radiologist who had no background about the clinical data.

We calculated the signal ratio (SR) using a time-intensity curve. The formula of SR is as follows: SR = (unenhanced signal intensity − maximal enhanced signal intensity) / unenhanced signal intensity. We classified the patients into 2 groups according to the median SR as follows: high SR, low SR (Figs. 1, 2).

Chemotherapeutic Regimens

Most patients were treated with gemcitabine and the remains with S-1, or a combination of both as clinical trials. Twenty patients received chemotherapy with gemcitabine (1000 mg/m^2), which was administered once weekly for 3 weeks followed by 1 week of rest. Three patients were treated with S-1 (80–120 mg/body), which was given twice daily for 28 days followed by 2 weeks of rest. Based on previous studies,[11] the body surface area (BSA) was used to determine the dose of S-1

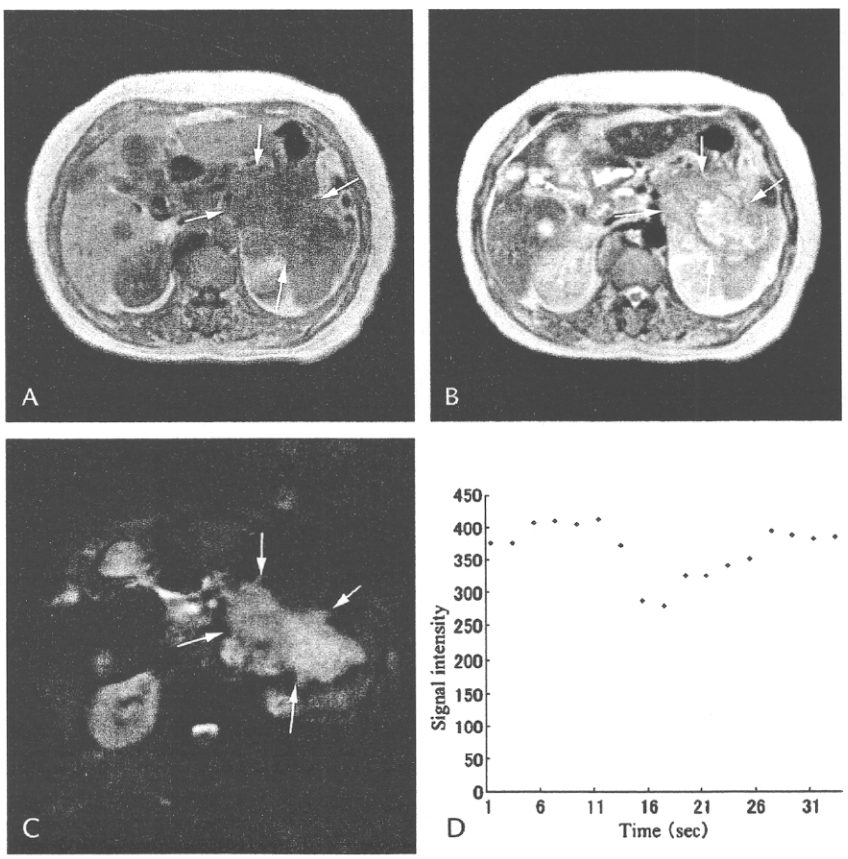

FIGURE 1. Perfusion-weighted MRI (with negative enhancement) showing high SR in a 63-year-old women with advanced pancreatic body cancer. A, T1-weighted fast gradient echo image (187/4). B, T2-weighted fast spin echo image (3150/10). C, perfusion-weighted MR pre-contrast image. D, the time intensity curve of SR. These MR images show an irregular tumor in the pancreatic body (A, B, C, arrows). The time intensity curve (D) shows a large signal drop. The SR was 33%.

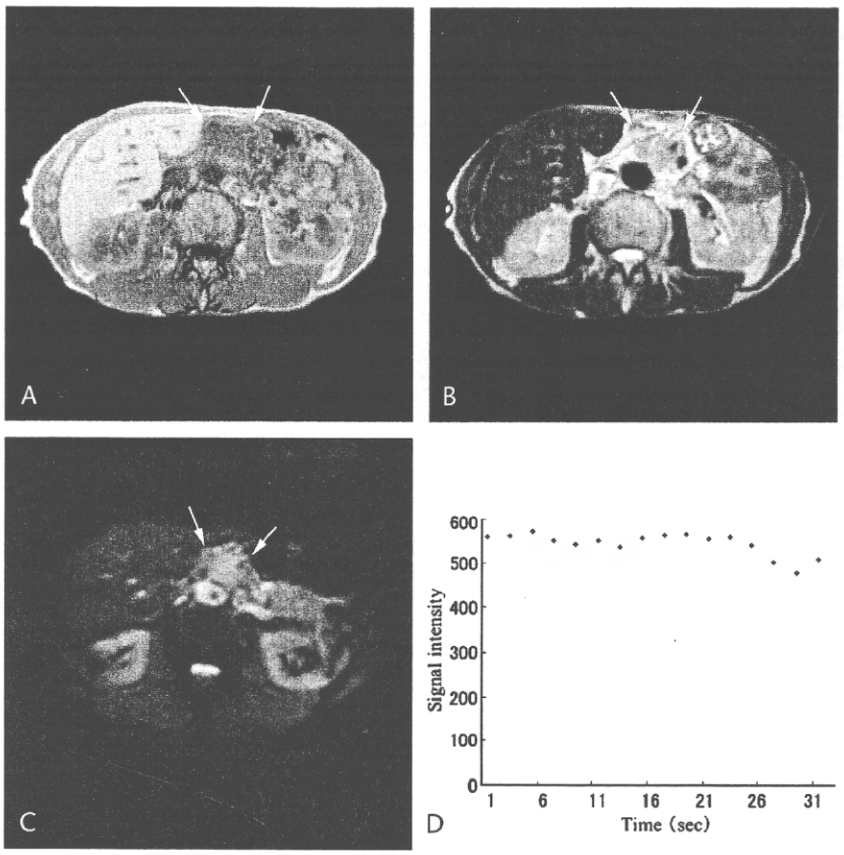

FIGURE 2. Perfusion-weighted MRI (with negative enhancement) showing low SR in a 68-year-old man with advanced pancreatic body cancer. A, T1-weighted fast gradient echo image (187/4). B, T2-weighted fast spin echo image (3150/10). C, perfusion-weighted MR pre-contrast image. D, the time intensity curve of SR. These MR images show an irregular tumor in the pancreatic body (A, B, C, arrows). The time intensity curve (D) shows a small signal drop. The SR was 12%.

as follows; BSA less than 1.25 m^2, 80 mg; BSA 1.25 to 1.5 m^2, 100 mg; BSA greater than 1.5 m^2, 120 mg. The remaining 4 patients received combination chemotherapy with gemcitabine (400–1000 mg/m2) and S-1 (40–100 mg/body) conducted as a phase I trial at our hospital. Dose escalation was performed in a stepwise manner in which the dose of 1 drug was escalated, whereas the dose of the other drug was kept constant. Chemotherapy was continued until disease progression, death, or unacceptable toxicity.

Statistical Analysis

The relations between SR and clinical factors were assessed using Fisher exact test. The examined factors were as follows: sex, age (median of 63 years), AJCC stage (III and IV), primary tumor (T3, T4), tumor location (head/body-tail), tumor diameter, lymph node metastasis, liver metastasis, CA19-9 response (divided at cutoff value of 20% decrease after the first cycle of chemotherapy), and chemotherapy (gemcitabine, others).

Survival curves were plotted according to Kaplan-Meier. The continuous SR values were divided according to cutoff value (median). The age and tumor diameter were divided at cutoff value (mean). Differences in the overall survival were calculated with the log-rank test. The overall survival was measured from the first day of chemotherapy to the date of death. Two-sided $P < 0.05$ were considered as statistically significant. All computations were performed using the Statistical Package for Social Sciences (version 11.0; SPSS Inc, Chicago, Ill).

RESULTS

Patients' Characteristics

The patients' characteristics were as follows: sex, 16 men and 11 women; age, ranged from 43 to 79 years with a median of 63 years; tumor stage (III/IV), 8/19; primary tumor (T3/ T4), 11/16; tumor location (head/ body-tail), 7/20. The median tumor diameter was 40 mm. Lymph node metastases were recognized in 4 patients (in the regional lymph nodes and around the abdominal aorta). Liver metastases were recognized in 11 patients. Other distant metastases were detected in the peritoneum, 3; ovary, 1; and bone, 2 (Table 1).

Quantitative Analysis of Signal Intensity by Perfusion-Weighted MRI

We noticed MRI signal change of tumor intensity in all patients. The mean size of ROIs was 199 ± 73 mm^2. None of the patients experienced complications such as allergic reactions or renal dysfunctions to the contrast agent. Signal ratios ranged from 6.9% to 55.7% (median = 22.0%). The patients were classified to high/low SR groups according to their SRs greater/ less than median value. The high SR group was consisted of 13 patients, whereas the low SR group was consisted of 14 patients (Figs. 1, 2).

TABLE 2. Relation Between Perfusion MRI SR and Various Factors

Variable	Class	SR < 22.0	SR ≥ 22.0	*P*
Sex	Male	9	6	0.45
	Female	5	7	
Age, y	≤63*	7	7	1.00
	>63*	7	6	
Tumor stage	III	7	1	0.03†
	IV	7	12	
Primary tumor	T3	4	7	0.07
	T4	10	6	
Tumor location (head/body-tail)	Head	6	1	0.07
	Body-tail	8	12	
Tumor diameter (mm)	≤40	7	6	1.00
	>40	7	7	
Lymph node metastasis	Positive	0	4	0.04†
	Negative	14	9	
Liver metastasis	Positive	3	8	0.054
	Negative	11	5	
CA19-9 change	>20% decrease	6	2	0.21
	≤20% decrease	8	11	
Chemotherapy	Gemcitabine	11	8	0.42
	Others	3	5	

*Median value.
†Statistically significant.

The Relation Between SR and Clinical Factors

The high SR group significantly correlated with the higher stage ($P = 0.03$) and the presence of lymph node metastasis ($P = 0.04$) (Table 2).

Patients' Survival

The overall survival ranged from 38 to 612 days with a median of 217 days. The median overall survival was 130 days in the high SR group and 262 days in the low SR group. The high SR group had a shorter overall survival than the low SR group as calculated with the log-rank test ($P = 0.04$; Fig. 3).

DISCUSSION

In perfusion-weighted MRI, gadolinium, an extracellular compartment contrast agent, initially enters the intravascular space and then rapidly distributes into the extracellular space. This paramagnetic contrast agent enters the intravascular space by bolus injection and generates a drop in the signal intensity with perfusion-weighted imaging. After the first pass, it distributes rapidly into the extracellular component and the signal intensity rises.[7]

Perfusion-weighted MRI has been widely used in neuroimaging diagnosis.[12] In case of abdominal imaging, this technique has been applied for liver tumors where the degree of tumor perfusion correlated with the vascularity in angiography, and accordingly, it is useful to evaluate the effectiveness of transarterial chemoembolization.[7] To our knowledge, this is the first report about the perfusion-weighted MRI of the PC.

In this study, higher SRs on perfusion-weighted MRI correlated with the tumor stage and lymph node metastasis. High SR indicated poor prognosis. Recently, it has been reported that perfusion-weighted MRI correlated with the expression of the vascular endothelial growth factor (VEGF) in various cancers.[13] And in the PC, the expression of VEGF is reportedly associated with poor prognosis.[14,15] In this regard, the signal changes of perfusion-weighted MRI may also correlate with the VEGF expression in the PC.

It has been reported that the survival was longer for the patients having avascular tumors as compared with those having vascular tumors on the contrast-enhanced ultrasonography.[16] Ohshima et al[17] investigated the relation between contrast-enhanced Doppler signals and the VEGF expression in PCs and reported that the VEGF expression was significantly higher in the vascular tumors than in the avascular tumors. Our results support these studies. However, we could not compare the histology with perfusion-weighted MRI because the biopsy specimens were small, and this study was performed on inoperable patients. Further investigation on operable patients will be needed for validation in the future.

Several prognostic factors have been reported. Ikeda et al[18] reported that the performance status of 0–1 ($P < 0.01$), absence of regional lymph node metastasis ($P < 0.01$), and serum CA 19-9 level of less than 1000 ($P = 0.02$) were independent favorable prognostic factors. Furthermore, the histologic grade and tumor stage are reportedly prognostic factors of PC.[19,20] Considering these reports, the correlation of SRs on perfusion-weighted MRI with lymph node metastasis and tumor stage may support the possibility of using perfusion-weighted MRI as a prognostic factor. However, our study has several limitations. Although high SR patients had poor prognosis, our study was preliminary, and the subjects were few. Therefore, prognostic factors including SR and clinical factors should be examined on more patients.

In the imaging study, we diagnosed PC from the surrounding tissues and normal parenchyma and detected lymph node metastasis, liver metastasis, and other metastasis using mainly MRI and CT comprehensively. As a limitation of MRI, tumors less than 1 cm in size cannot be reliably detected because of poor spatial resolution, and huge tumors (>15 cm) cannot be imaged simultaneously. However, most tumors were between these sizes. Furthermore, the signal-to-noise ratio of echo planar imaging was relatively low in the current MR scanners. Yet, we

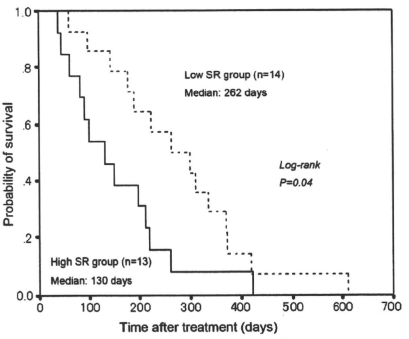

FIGURE 3. Survival curves comparing the high and low SR groups in advanced PC. Based on the Kaplan-Meier method and the log-rank test, the high SR group (solid line) had a shorter overall survival than the low SR group (dotted line) ($P = 0.04$).

could detect the changes of signal intensity in all cases in this study and make a time intensity curve.

Regarding ROIs, 3 ROIs were placed in APC, and the vessels and cystic areas were avoided with reference to the T1-weighted, T2-weighted, and post-contrast T1-weighted images for signal intensity measurement. Nonetheless, it was impossible to avoid the desmoplastic change and edema perfectly. Although it may be more reproducible to include the entire tumor within the ROI, we considered that this method was not accurate because of the unclear tumor margins and the possibility of including the tumor margin vessels. Moreover, the PCs on MRI move with the patients' breathing. We located ROIs carefully and manually so as to measure the same position on all dynamic images in this study.

Regarding the parameters, we investigated only SR as a preliminary study because the target lesions move with breathing. In the future, motion-correction should be performed to allow calculation of parameters such as blood flow, blood volume, mean transit time using K-trans model. The pre- and post-chemotherapy perfusion changes should also be assessed.

In conclusion, we suggest that perfusion-weighted MRI may predict the survival in the APC patients, although this study is preliminary and further investigations on large groups are needed in the future.

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Ruptured pseudoaneurysm of the splenic artery complicating endoscopic ultrasound-guided fine-needle aspiration biopsy for pancreatic cancer

Endoscopic ultrasound-guided fine-needle aspiration (EUS-FNA) is a safe procedure, although major complications do rarely occur. Pseudoaneurysm rupture is an emergency that can cause life-threatening hemorrhage. An exceedingly rare case of ruptured pseudoaneurysm of the splenic artery following EUS-FNA is described.

A 62-year-old man was referred for investigation of a pancreatic tumor. On abdominal computed tomography (CT), a low-density area was seen running from the pancreatic body, involving the celiac and splenic arteries (© **Fig. 1**). Angiography showed encasement of the splenic artery (© **Fig. 2**). Therefore, EUS-FNA was performed with two separate passes into the lesion using a 22-gauge needle. Cytological analysis showed a pancreatic adenocarcinoma. Nineteen days later abdominal CT showed a pseudocyst in the pancreatic body and tail (© **Fig. 3**), and 30 days later (i.e., 30 days after EUS-FNA) the patient developed hematemesis and hemorrhagic shock. Upper gastrointestinal endoscopy showed a hemorrhagic gastric ulcer in the posterior wall of the middle body (© **Fig. 4**). Since endoscopic treatment was unsuccessful, angiography was performed and showed a pseudoaneurysm of the splenic artery. Coil embolization was performed in and around the pseudoaneurysm. After 7 days the pancreatic pseudocyst had narrowed (© **Fig. 5**); there was no further recurrence.

The overall complication rate of EUS-FNA is 1%–2% [1]. The major complications are postaspiration infection in cystic lesions, bleeding, pancreatitis, and cervical and duodenal perforation [2]. Three different mechanisms are recognized as causing pseudoaneurysm formation [3], but all are called pseudoaneurysm because the end result is a cystic vascular structure surrounded by a fibrous wall. Pseudoaneurysms can rupture into the peritoneal cavity, retroperitoneum, gastrointestinal tract, biliary tract, and pancreatic duct. The most common vessel involved is the splenic artery as it runs along the pancreatic bed before reaching the spleen [4]. To our knowledge, this is the

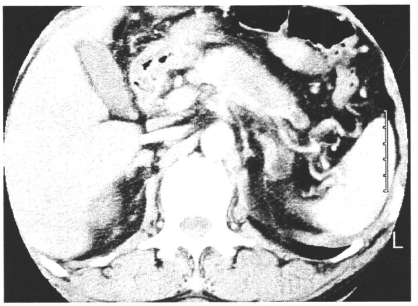

Fig. 1 On abdominal CT, a low-density area was seen running from the pancreatic body, involving the celiac and splenic arteries.

Fig. 2 Angiography showed encasement of the splenic artery.

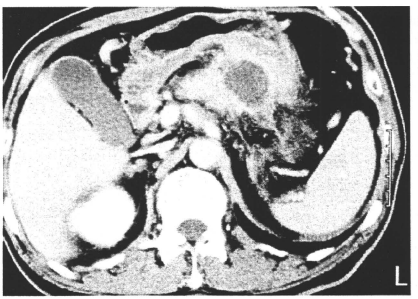

Fig. 3 Abdominal CT 19 days later showed a pseudocyst in the pancreatic body and tail.

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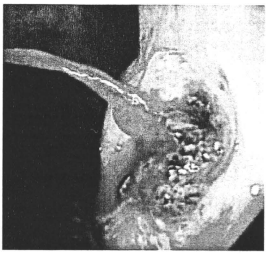

Fig. 4 Upper gastrointestinal endoscopy showed a hemorrhagic gastric ulcer in the posterior wall of the middle body.

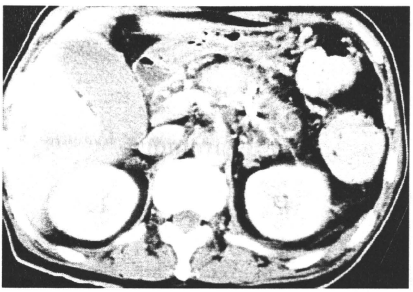

Fig. 5 Coil embolization in and around the pseudoaneurysm was performed, and 7 days later the pancreatic pseudocyst had narrowed.

first case report of a ruptured pseudoaneurysm of the splenic artery complicating EUS-FNA.

Endoscopy_UCTN_Code_CPL_1AL_2AD

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DOI 10.1055/s-0029-1215323

Endoscopy 2010; 42: E27–E28

ISSN 0013-726X

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