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ORIGINAL ARTICLE – HEPATOBILIARY TUMORS

Significance of Alpha-Fetoprotein and Des-γ-Carboxy Prothrombin in Patients with Hepatocellular Carcinoma Undergoing Hepatectomy

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ABSTRACT

Background. Alpha-fetoprotein (AFP) and des-γ-carboxy prothrombin (DCP) are well-known tumor markers of hepatocellular carcinoma (HCC). The aims of this study are to calculate the sensitivity/specificity of AFP and DCP measurement for the diagnosis of HCC, measure response rates of the markers following curative-intent resections, determine the correlations between the marker levels and clinicopathological prognostic variables, and determine the correlations between the marker levels before hepatectomy and those at diagnosis of recurrence.

Methods. A retrospective cohort study of 714 consecutive patients with HCC undergoing hepatectomy was carried out.

Results. The areas under the receiver operating characteristic curves were 0.79 versus 0.91 for AFP and DCP, respectively ($P < 0.001$). Positive AFP and DCP status became negative at 6 months post surgery in 184/229 (80.3%) and 245/246 (99.6%) patients, respectively (cutoff values being 20 ng/ml for AFP and 40 mAU/ml for DCP; $P < 0.0001$). No correlation was found between marker levels ($rs = 0.23$). The level of DCP, but not that of AFP, showed a close correlation with tumor size ($rs = 0.51$ and 0.19, respectively). They were associated with indices of tumor invasiveness without showing any specific associations. AFP and DCP levels in patients showing recurrence in ≤6 months correlated with the levels measured before surgery ($rs = 0.78$ and 0.49, respectively) but not in those showing recurrence after 2 years ($rs = 0.31$ and 0.30, respectively).

Conclusions. DCP is a more accurate, albeit complementary, HCC marker than AFP. While the levels of both markers increased with advancing tumor growth, no specific associations were found. The marker values at recurrence indicated the type of recurrence.

Early diagnosis remains the key to effective therapy in cases of hepatocellular carcinoma (HCC).[1] Although serum alpha-fetoprotein (AFP), a biological tumor marker of HCC, has long been used as a tool for HCC surveillance, it is not an ideal screening test due to its low sensitivity/specificity.[2–5] Liebman et al. first reported, in 1984, an increase in the plasma levels of des-γ-carboxy prothrombin (DCP), which is an abnormal prothrombin and also otherwise known as protein induced by vitamin K deficiency or antagonist-II (PIVKA-II), in patients with HCC.[6] Since then, the significance of DCP has been examined by many investigators and it was introduced as a routine laboratory test for HCC during the early 1990s in Japan.[7–9] In addition, a two-step enzyme immunoassay method was developed and has been in use since 1997; it shows a tenfold higher sensitivity for detection as compared with the conventional enzyme immunoassay method.[10] Consensus appears to have been reached on both DCP and AFP being independent tumor markers in HCC.[8,11–17] However, it still remains controversial whether or not DCP is superior to AFP as a single marker.[12,16–22]

The second role of tumor markers is in the monitoring of response to therapy. Ideally, the levels of tumor markers should fall to within normal range after effective treatment. This aspect is especially important in the case of

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transcatheter arterial embolization, because radiological findings do not necessarily reflect the degree of biological remission achieved by necrosis or fibrosis.[23] Comparisons of AFP and DCP in this regard have not been conducted.

Thirdly, elevation of tumor marker levels reportedly represents specific clinicopathological variables identified as prognostic factors.[14,21,22,24–26] Although high plasma levels of DCP reportedly indicate the presence of portal venous thrombosis and increased serum AFP levels are associated with a poor degree of differentiation of the tumor cells, in particular, these studies failed to comprehensively investigate the relationships with various parameters.[14,21,22,24,27]

Finally, another use of tumor markers is in the prediction of tumor recurrence. In theory, patients with HCC with elevated levels of AFP and/or DCP before treatment should also show elevated levels of the respective markers at the time of recurrence if the recurrence is metastatic. On the other hand, de novo secondary tumors also contribute to postoperative intrahepatic HCC recurrence.

In the present study, taking into account these unaddressed aspects of tumor markers of HCC, we comprehensively investigated the clinical significance of measurement of two tumor markers in cases of HCC, i.e., AFP and DCP, in a large cohort.

PATIENTS AND METHODS

Patients

The base population consisted of 714 consecutive patients who underwent curative liver resections for HCC at the Division of Hepato-Biliary-Pancreatic Surgery, Tokyo University Hospital, between January 1998 and November 2006. Curative resection was defined as removal of all recognizable tumors with a clear margin. The diagnosis of HCC was finally confirmed by pathological examination of the resected specimens in all cases.

Background characteristics of the patients are presented in Table 1. After discharge, monthly follow-up by tumor markers (AFP and DCP) and ultrasound as well as dynamic computed tomography (CT) scan every 4 months were conducted for 1 year. Then, we screened patients by tumor marker measurement and ultrasound every 2 months and dynamic CT scan every 6 months thereafter. We defined recurrence as the appearance of new lesions with radiological features typical of HCC, as confirmed by at least two imaging methods.[28]

AFP and DCP Assay

Samples for AFP and DCP were taken within 7 days prior to the liver resection. Serum AFP level was measured by commercially available immunometric assay (ST AIA-PACK AFP, Tosoh, Tokyo, Japan). Plasma DCP level was measured by two-step enzyme immunoassay (Picolumi PIVKA-II, Eizai, Tokyo, Japan).[10]

TABLE 1 Background characteristics of 714 patients with HCC

Variables	$n = 714$
Sex	
Male	556 (77.9%)
Female	158 (22.1%)
Age (years)[a]	67 (19–90)
Hepatitis B virus infection[b]	
No	560 (78.4%)
Yes	154 (21.6%)
Hepatitis C virus infection[b]	
No	250 (35.0%)
Yes	464 (65.0%)
Child–Turcotte–Pugh grade[c]	
A	601 (84.2%)
B	113 (15.8%)
Background liver status[d]	
Normal liver	14 (2.0%)
Chronic hepatitis	295 (41.3%)
Cirrhosis	405 (56.7%)

[a] Median with range

[b] Five patients were positive for both hepatitis B and C virus infections and 101 patients were negative for both hepatitis B and C virus infections

[c] No patient was Child–Turcotte–Pugh grade C

[d] Pathological findings assessed in the resected specimen

Assessment

Sensitivity/Specificity of AFP and DCP for Presence of HCC At 6 months post surgery, 25 out of the 714 patients were lost to follow-up in terms of serial tumor marker measurements, 190 had developed recurrence, 9 were disease-free at <6 months of follow-up, and the remaining 490 patients were confirmed to be disease free at this time point. The AFP and DCP values in 714 patients before the liver resection were defined as those of patients with HCC, while the values of these 490 patients at 6 months post surgery were defined as those of patients without HCC. Using these values, receiver operating characteristic (ROC) curves were constructed. The diagnostic performance of AFP and DCP was evaluated and compared through their areas under the receiver operating characteristic curves (AUROC). The cutoff values for AFP and DCP used in this study are those that have been conventionally used and/or have been proposed in previous reports: 20 ng/ml for AFP and 40 mAU/ml for DCP.[29]

AFP and DCP Levels as Tools for Evaluating Therapeutic Response to HCC In these 490 patients, complete tumor remission was thought to be achieved at 6 months after the liver resection. We examined whether this treatment response was correctly reflected in the alterations in the marker values. According to the cutoff values defined above, we classified the 490 patients into marker-positive or marker-negative status both before and at 6 months after the liver resection. We then investigated the changes of AFP- and DCP-positive/negative status following the liver resection.

AFP and DCP as Complementary Tumor Markers for HCC We first evaluated the relationship between AFP and DCP values in a total of 714 patients. Second, we classified these patients into four categories according to their positive/negative status for AFP and/or DCP according to the cutoff values.

AFP and DCP as Markers of Clinicopathological Variables Representative of Tumor Invasiveness and Prognosis We assessed the association of AFP and DCP values with clinicopathological variables that have been reported as prognostic factors for HCC in the 714 patients. The variables investigated are shown in Table 2. All variables were assessed pathologically on the resected specimens. Vascular invasion was defined as presence of portal vein invasion, venous invasion or biliary invasion. Multiple primary tumor nodules and intrahepatic metastases were differentiated using the guidelines proposed by the Liver Cancer Study Group of Japan.[30]

AFP and DCP Levels as Indices for Predicting the Pattern of Recurrence At the time of data collection, recurrence was observed in 444 patients. We classified these patients with recurrence into two groups, i.e., a group in which the recurrence occurred ≤6 months post surgery ($n = 190$),

TABLE 2 Tumor-related factors

Variables	$n = 714$	AFP (ng/ml)[a]	DCP (mAU/ml)[a]
Tumor size (mm)			
≤20	223 (31.2%)	18.0 (7.0–69.0)	24.0 (16.0–61.0)
20–50	335 (46.9%)	22.0 (7.0–144.0)	57.0 (21.0–328.0)
>50	156 (21.9%)	57.0 (8.5–3007)	1251.0 (118.5–7486.0)
		$rs = 0.19$	$rs = 0.51$
Tumor number			
1	483 (67.7%)	19.0 (1.0–216.0)	55.0 (20.0–456.0)
2	138 (19.3%)	26.0 (8.0–177.5)	53.0 (19.50–254.0)
≥3	93 (13.0%)	49.0 (13.5–162.5)	59.0 (19.5–329.5)
		$P = 0.07$	$P = 0.73$
Capsular formation			
No	169 (23.7%)	25.0 (8.0–148.0)	32.0 (18.0–163.0)
Yes	545 (76.3%)	21.0 (7.0–207.5)	72.0 (21.0–489.5)
		$P = 0.83$	$P < 0.05$
Capsular infiltration[b]			
No	137 (25.1%)	14.0 (6.0–78.5)	64.0 (10.0–364.0)
Yes	408 (74.9%)	27.0 (7.0–278.0)	83.5 (21.5–579.5)
		$P < 0.01$	$P = 0.21$
Vascular invasion[c]			
No	495 (69.3%)	17.0 (7.0–76.0)	38.0 (18.0–189.0)
Yes	219 (30.7%)	88.0 (12.0–1271.0)	233.0 (31.0–2110.0)
		$P < 0.0001$	$P < 0.0001$
Intrahepatic metastases			
No	601 (84.2%)	19.0 (7.0–137.0)	44.0 (10.0–310.5)
Yes	113 (15.8%)	81.0 (9.5–1261.0)	235.0 (40.0–2544.0)
		$P < 0.001$	$P < 0.0001$
Tumor differentiation			
Well	104 (14.5%)	12.5 (6.0–31.0)	29.0 (17.0–87.5)
Moderate	511 (71.6%)	20.0 (1.0–174.0)	63.0 (10.0–441.0)
Poorly	99 (13.9%)	165.0 (25.0–2326.0)	145.0 (26.0–2455.0)
		$P < 0.0001$	$P < 0.0001$

[a] Median with interquartile range

[b] We assessed 545/714 patients who had capsular formation

[c] Macroscopic invasion was observed in 45/219 (20.5%) patients, while microscopic invasion was found in 174/219 (79.5%) patients

and another in which the recurrence occurred >6 months post surgery ($n = 254$). We first compared the preoperative levels of AFP and DCP as well as the levels at time of recurrence between the two groups of patients. Then, we further classified the two groups of patients into two subgroups according to site of recurrence, i.e., intrahepatic or extrahepatic recurrence. We investigated the correlations between the preoperative marker values and the site of recurrence.

Etiological Association Between the Primary and Recurrent Tumors Investigated Through AFP and DCP Marker Values We investigated the correlations of the tumor marker values at the time of recurrence with those measured before the liver resection. We classified 444 patients who developed recurrences into four groups according to time to recurrence, as follows: recurrence at ≤6 months ($n = 190$), recurrence between 7 and 12 months ($n = 70$), recurrence between 13 and 24 months ($n = 70$), and recurrence after 2 years ($n = 114$). Then, we examined the chronological alterations in the correlation of values of the respective tumor markers measured before the liver resection with those measured at the time of recurrence.

Statistical Analysis

Marker values are expressed as median with interquartile range. The AUROC for markers was compared by Wilcoxon's rank-sum test.[31] Correlations between marker values were analyzed by Spearman's rank correlation. Categorical binary variables were compared by Fisher's exact test. Associations between marker values and clinicopathological variables were analyzed by Wilcoxon's rank-sum test or by the Kruskal–Wallis test, as appropriate. P values of < 0.05 were accepted as statistically significant. All statistical analyses were performed using the GraphPad Prism® computer software, version 5 (GraphPad Software Inc., San Diego, CA).

RESULTS

Sensitivity/Specificity of AFP and DCP for Presence of HCC

The median (interquartile range) AFP and DCP levels in 714 patients before liver resection were as follows: 22.0 (7.0–195.0) ng/ml and 55.0 (20.0–443.0) mAU/ml. The AFP and DCP levels in 490 patients who had no evidence of tumor recurrence at 6 months post surgery were 5.0 (3.0–9.0) ng/ml and 11.0 (10.0–15.0) mAU/ml, respectively. The sensitivity and specificity of AFP and DCP were assessed by ROC curves (Fig. 1). The AUROC (95% confidence interval, CI) for AFP and DCP were 0.79 (0.76–0.81) and 0.91 (0.89–0.92), respectively ($P < 0.001$). The sensitivities and specificities at various cutoff values including those adopted in the present study (AFP, 20 ng/ml; DCP, 40 mAU/ml) and proposed in previous reports are presented in Table 3.

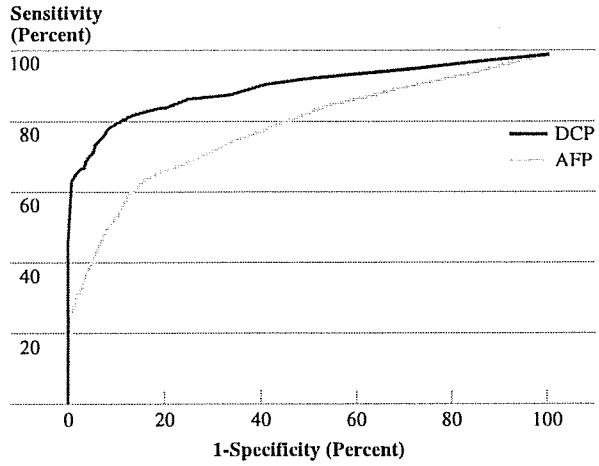

FIG. 1 ROC curves for AFP and DCP. The yellow line represents AFP and the blue line represents DCP. The AUROC (95% CI) for AFP and DCP were 0.79 (0.76–0.81) and 0.91 (0.89–0.92), respectively ($P < 0.001$)

TABLE 3 Sensitivities and specificities of AFP and DCP values according to various cutoff values

AFP (ng/ml)	11	13	20	100	200
Sensitivity (%)	64.9	60.8	51.3	30.4	24.7
Specificity (%)	82.9	86.1	90.8	98.6	99
DCP (mAU/ml)	20	30	40	100	125
Sensitivity (%)	73.4	62.8	55.9	41.9	39.1
Specificity (%)	94.7	99.4	99.8	100	100

In the present study, the cutoff values adopted were 20 ng/ml for AFP and 40 mAU/ml for DCP

AFP alpha-fetoprotein, *DCP* des-γ-carboxy prothrombin

AFP and DCP as Tools for Evaluating Response to Therapy of HCC

Among the 490 patients who were confirmed to be disease free at 6 months postoperatively, 229 (46.7%) and 246 (50.2%) were classified as AFP positive and DCP positive, respectively, before the liver resection under the present cutoff values. At 6 months post surgery, when complete tumor remission was thought to have been achieved, marker-negative status was achieved in 184/229 (80.3%) and 245/246 (99.6%) patients for AFP and DCP, respectively ($P < 0.0001$) (Table 4). Out of 45 patients

TABLE 4 Pre- and postoperative marker status in 490 disease-free patients at 6 months

Preoperative status		Postoperative status	
AFP			
(+)	229/490 (46.7%)	(−)	184/229 (80.3%)
		(+)	45/229 (19.7%)
(−)	261/490 (53.3%)	(−)	261/261 (100%)
		(+)	0/261 (0%)
DCP			
(+)	246/490 (50.2%)	(−)	245/246 (99.6%)
		(+)	1/246 (0.4%)
(−)	244/490 (49.8%)	(−)	244/244 (100%)
		(+)	0/244 (0%)

Cutoff values were set at 20 ng/ml for AFP and 40 mAU/mL for DCP, respectively

AFP alpha-fetoprotein, *DCP* des-γ-carboxy prothrombin

who showed AFP-positive status without recurrence at 6 months post surgery, 33 remained disease free at 12 months post surgery, whereas 12 had developed recurrence by this time point. In retrospect, the AFP values at 6 months post surgery were not thought to be indicative of recurrence at least in 6/12 patients. A single patient positive for DCP at 6 months post surgery was also disease free 5 years later. In all the 261 (53.3%) and 244 (49.8%) patients who were negative for AFP and DCP, respectively, before the surgery, the marker status for both of these markers remained negative at 6 months post surgery (Table 4).

AFP and DCP as Complementary Tumor Markers for HCC

The correlation between the levels of these markers in the 714 patients is shown in Fig. 2; no association was seen ($rs = 0.23$). These patients were classified into four categories by the cutoff values used in the present study, as follows: AFP(+)/DCP(+): 229 (32.1%), AFP(+)/DCP(−): 137 (19.2%), AFP(−)/DCP(+): 170 (23.8%), and AFP(−)/DCP(−): 178 (24.9%) (Fig. 2).

AFP and DCP as Markers of Clinicopathological Variables Representative of Tumor Invasiveness and Prognosis

The correlations of the AFP and DCP levels with clinicopathological findings are shown in Table 2. Although the DCP levels increased with increasing tumor size ($rs = 0.51$), this relationship was not found for AFP ($rs = 0.19$). While no statistical correlation was found between DCP levels and tumor number ($P = 0.73$), AFP levels tended to increase with increasing tumor number

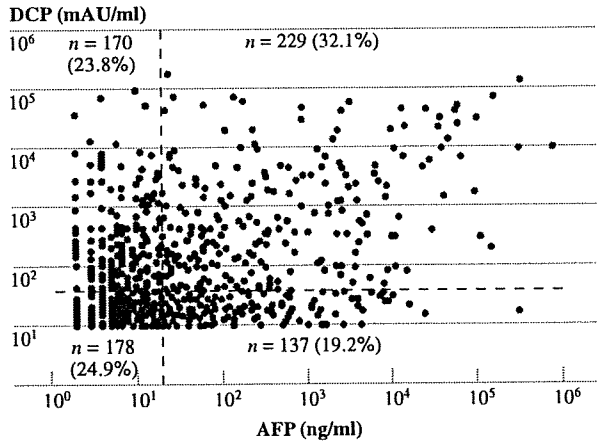

FIG. 2 Correlation between AFP and DCP values in 714 patients. No correlation was found between the two markers ($rs = 0.23$, $P < 0.0001$). Dotted line represents cutoff values, i.e., 20 ng/ml for AFP and 40 mAU/ml for DCP. Patients were placed into four categories: either positive or negative for AFP and/or DCP according to these cut-off values. Number of patients in the each category was shown

($P = 0.07$). AFP and DCP levels increased to similar extent in the presence of indices of tumor invasiveness, such as vascular invasion and intrahepatic metastases. Likewise, both marker levels increased with increasing tumor cell differentiation.

AFP and DCP Levels as Indices for Predicting the Pattern of Recurrence

The preoperative AFP and DCP values in HCC patients who developed recurrence ≤6 months ($n = 190$) versus patients who developed recurrence >6 months post surgery ($n = 254$) are shown in Table 5. Patients who developed recurrence ≤6 months post surgery showed higher preoperative AFP and DCP values than those who developed recurrence >6 months post surgery. Similarly, the AFP and DCP values measured at the time of recurrence in the two groups are shown separately in Table 5. Again, patients who developed HCC recurrence ≤6 months post surgery showed higher AFP and DCP values at the time of recurrence.

Out of 190 recurrences observed ≤6 months post surgery, 32 (16.8%) were extrahepatic: 18/32 (59 %) in the lung, 6/32 (19%) in the lymph node, 4/32 (13%) in the bone, 2/32 (6%) in the peritoneal membrane, and 1/32 (3%) in the adrenal gland.

On the other hand, the overall rate of extrahepatic recurrence in the patients who developed recurrence >6 months post surgery was 3/254 (1.2 %). Since extrahepatic recurrence was a rare event >6 months post surgery, we analyzed the correlations between the

TABLE 5 AFP and DCP values in patients who developed HCC recurrence ≤6 months ($n = 190$) and >6 months ($n = 254$) post surgery

	Preoperative values		Values at recurrence	
	Recurrence ≤6 months	Recurrence >6 months	Recurrence ≤6 months	Recurrence >6 months
AFP (ng/ml)	54.0 (9.0–624.5)[a]	18.5 (7.0–76.0)	17.5 (6.0–163.5)[a]	13.0 (6.0–43.0)
DCP (mAU/ml)	237.5 (22.8–2553.0)[b]	37.5 (19.0–142.0)	25.0 (14.0–131.0)[c]	18.0 (13.0–34.3)

Values are expressed as median (interquartile range)

[a] $P < 0.0001$ compared with recurrence >6 months

[b] $P < 0.005$ compared with recurrence >6 months

[c] $P < 0.0005$ compared with recurrence >6 months

TABLE 6 Preoperative AFP and DCP values in patients who developed intrahepatic ($n = 158$) and extrahepatic ($n = 32$) recurrence ≤6 months post surgery

	Intrahepatic recurrence	Extrahepatic recurrence
AFP (ng/ml)	50.0 (9.0–337.8)[a]	255.0 (10.8–9636.0)
DCP (mAU/ml)	188 (22.8–184.0)[b]	543.0 (34.3–10179.0)

Values are expressed as median (interquartile range)

One patient who developed intra- and extrahepatic recurrences simultaneously was classified into those with extrahepatic recurrence

[a] $P < 0.05$ compared with extrahepatic recurrence

[b] $P = 0.08$ compared with extrahepatic recurrence

preoperative marker values and the site of recurrences exclusively in the 190 patients who developed recurrence ≤6 months post surgery (Table 6). Patients who developed intrahepatic recurrence ($n = 158$) showed higher preoperative marker values than those who developed extrahepatic recurrence ($n = 32$).

AFP and DCP as Markers Reflecting the Association Between the Primary and Recurrent Tumors

The values of AFP and DCP measured before the liver resection are plotted against the values measured at the time of recurrence separately according to their time to recurrences in Fig. 3A–D and Fig. 4A–D, respectively. The AFP values in patients with recurrence at ≤6 months showed a close relationship with those measured before the liver resection ($rs = 0.78$, Fig. 3A). The strength of this relation became weaker in the groups with longer time to recurrence (Fig. 3B–D).

A similar trend was found in regard to the relationship of DCP values, although the correlations were weaker than those observed for AFP (Fig. 4A–D).

DISCUSSION

The diagnostic accuracy of tumor markers should be evaluated on the basis of a trade-off between sensitivity and specificity, ideally by drawing ROC curves.[31] To date, three cross-sectional studies have compared the accuracy of AFP and DCP levels for the diagnosis of HCC through ROC curves, each using the present sensitive assay method for measuring DCP.[17,19,20] Two studies reported superiority of DCP.[17,20] However, a third reported better overall diagnostic accuracy of AFP.[19] The distribution of the etiology of the underlying liver disease in the present study population was similar to that in the populations studied by Marrero et al. and Nakamura et al., except that the former included a quantifiable proportion of alcoholic patients.[19,20] In regard to the distribution of the Child–Turcotte–Pugh (CPT) grade, our cohort is thought to lie in between the study cohorts of Marrero et al. and Nakamura et al., since 84.2% of our patients were classified into CPT grade A.[19,20]

In this study, we defined patients without recurrence at 6 months post surgery as a cohort without HCC. Although this approach may be different from that of former studies, this is advantageous in that the background characteristics are uniform in the patients with and without HCC.[17,19,20] This situation, which is an essential requirement in prospective screening studies of tumor markers, is not necessarily guaranteed in a cross-sectional study.[32] This study showed similar ROC results to those reported by Marrero et al. and Wang et al., which demonstrated superiority of DCP by approximately 10% (0.73–0.83 versus 0.85–0.93 for AFP versus DCP) (Fig. 1).[17,20]

In the present study, we used the cutoff values for AFP (20 ng/ml) and DCP (40 mAU/ml) proposed by previous studies and used most commonly in clinical settings.[29] Considering that much higher AFP values, e.g., 100 ng/ml or 200 ng/ml, have often been proposed as cutoff points, it is noteworthy that the present cutoff value showed better performance than these cutoff values, and even lower cutoff values can be adopted in terms of ROC performance (Table 3, Fig. 1). The cutoff value for DCP in the present study (40 mAU/ml), showing similar sensitivity to that of AFP, was thought to be the lowest among the values proposed until now (40–125 mAU/ml). Again, analysis of the ROC curve revealed that this value can be reduced even further in terms of a trade-off between sensitivity and specificity.

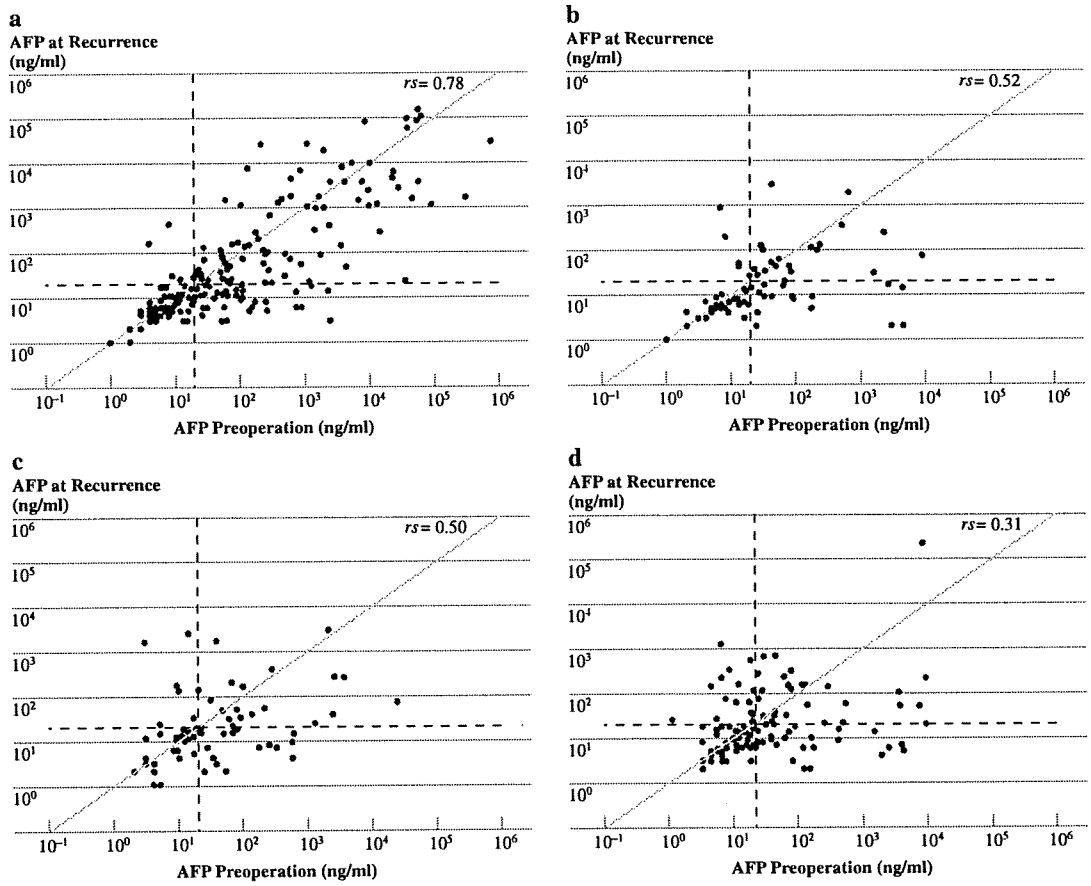

FIG. 3 Correlations between preoperative AFP values and AFP values at recurrence stratified according to period of recurrence: (**a**) recurrence ≤6 months (n = 190), (**b**) recurrence from 7 to 12 months (n = 70), (**c**) recurrence from 13 to 24 months (n = 70), and (**d**) recurrence >2 years (n = 114). The dotted lines represent 20 ng/ml

In addition, DCP is a superior marker for monitoring response to therapy, that is, it was confirmed that positive DCP status converted to negative status in 99.6% (245/246) of patients at 6 months post surgery in the absence of tumor recurrence; in contrast, conversion from AFP-positive to AFP-negative status was achieved in only 80.3% of the patients (184/229). This high false-positive rate of AFP is thought to reflect the observed elevation in the levels of this marker also in conditions such as acute and/or chronic hepatitis and cirrhosis, which is an inherent drawback of AFP as a HCC-specific tumor marker.[3] Whereas high DCP values have been reported in patients with vitamin K deficiency, such as in cases of obstructive jaundice or cases receiving vitamin K antagonists, e.g., warfarin, these uncommon clinical situations can be easily discriminated in HCC patients.[12,33] Rather, it must be noted that patients with chronic alcoholism, another high-risk cohort for HCC, often show nonspecific DCP elevation, reportedly in 5–8% of patients.[34,35] The higher DCP cutoff value adopted by Marrero et al. in their study (125 mAU/mL) may be partially ascribed to the fact that their cohort included a considerable proportion of alcoholic patients (5%).[20]

In the present study, no correlation was found between the levels of AFP and DCP. This observation is consistent with previous reports.[11–17,21] These results strongly suggest that these markers are complementary to each other and that, although DCP might be superior to AFP as a single marker, the two should be evaluated in combination in clinical practice.

Although the association of tumor markers with various clinicopathological variables has been evaluated in many studies, the majority of these works assessed the associations solely with variables of interest and/or exclusively for AFP or DCP. Bearing this in mind, we investigated these associations in a comprehensive manner. While serum DCP values increased with increasing tumor size, no similar association was found for AFP (Table 2). This result is consistent with the results of previous

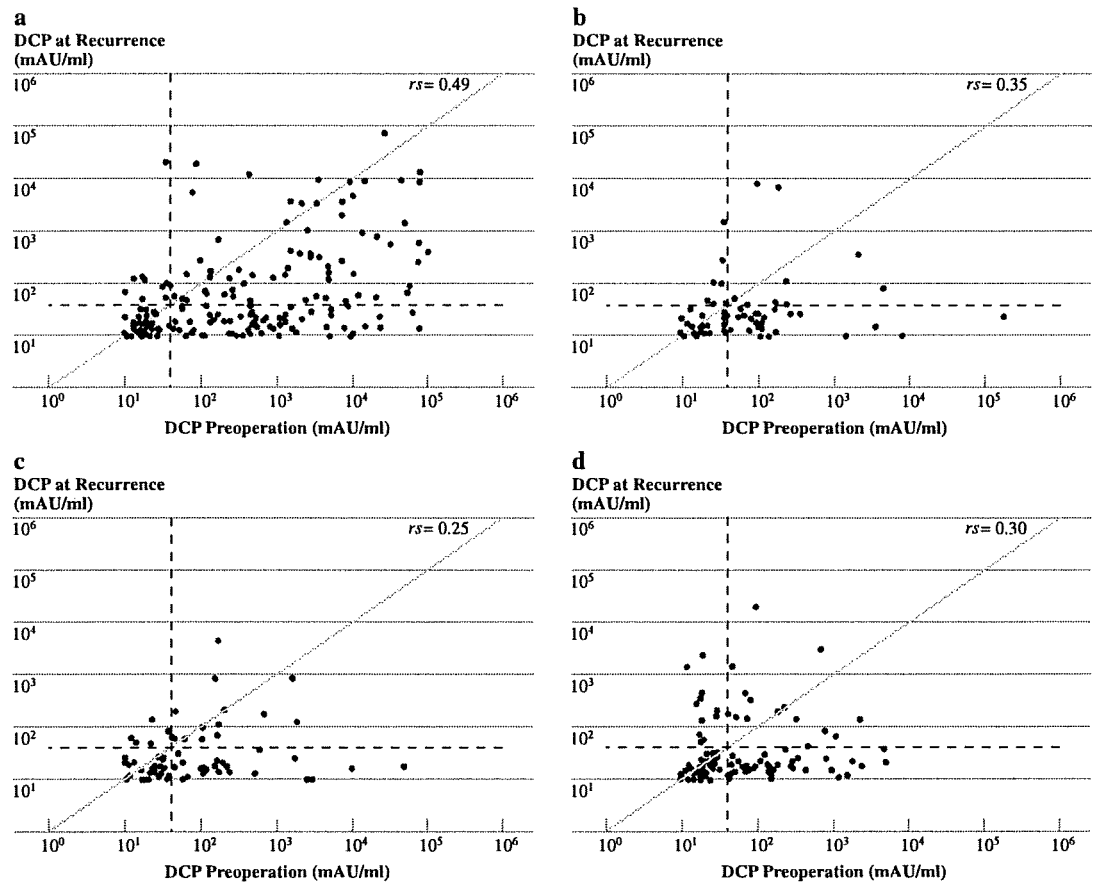

FIG. 4 Correlations between preoperative DCP values and DCP values at recurrence stratified according to period of recurrence: (**a**) recurrence ≤6 months (n = 190), (**b**) recurrence from 7 to 12 months (n = 70), (**c**) recurrence from 13 to 24 months (n = 70), and (**d**) recurrence >2 years (n = 114). The dotted lines represent 40 mAU/ml

studies.[8,13,14,16,17,26,36] These findings suggest that the interindividual variations in the capacity of the tumor cells to synthesize AFP far exceed the elevation in the marker values with increasing tumor cell number.

While serum AFP levels tended to increase with increasing tumor number, this association was not observed for plasma DCP (Table 2). This finding is consistent with those of Kasahara et al. and Carr et al., who found a significant relationship between AFP and tumor number.[13,22] Considering that tumor number is thought to be a variable representing the degree of carcinogenicity in the background liver, the finding of the association for AFP but not for DCP is most probably explained by the elevation of AFP with advancing severity of background liver disease.[3,36,37]

In the present cohort (n = 714), both increased AFP and DCP values were related to presence of indices of tumor invasiveness, such as vascular invasion, and intrahepatic metastases. To date, several studies with 72–161 patients have investigated the association of AFP and/or DCP with these indices, three of which assessed these pathological variables on surgically resected specimens.[14,21,24] A closer and/or specific relationship between these indices and DCP has been reported. Thus, the results of the present and former studies were partially contradictory. In our study, the AFP and DCP values were associated to a similar extent with the tumor cell differentiation grade (Table 2). Again, this observation is partially contradictory to the results of previous studies with 56–354 patients that claimed a specific close association with AFP or DCP.[24,26,27] The results of the present large cohort strongly suggests that both increased levels of AFP and DCP indicate the overall presence of pathological indices representing tumor invasiveness and/or increased malignant potential; however, they do not necessarily signify the presence of any specific entity.

Elevated preoperative AFP and/or DCP levels were correlated with early postoperative recurrence (≤6 months), and recurrence in the early phase was characterized by high serum levels of tumor markers. These results can most reasonably be interpreted as follows: high tumor marker levels signify an increased malignant

potential of the tumor, and the majority of recurrences in the early phase represent recurrence by metastasis, while the later phase of recurrences most often represent secondary de novo tumors whose malignant potential has not yet increased during the process of multistep carcinogenesis. This contention is further supported by the observed association of elevated tumor marker levels with a higher frequency of extrahepatic recurrence.

Two different underlying mechanisms are thought to contribute to postoperative HCC recurrence. In theory, recurrence by metastasis takes place in the early period after surgery, whereas recurrence in the late phase largely represents a new primary lesion.[37,38] Likewise, it can be hypothesized that (1) metastatic recurrence exhibits similar tumor characteristics to the primary lesion, while de novo lesions are independent of the primary tumors in terms of the marker expression profile, and (2) tumor marker levels in recurrent tumors in the early phase show a close relationship with those before hepatectomy, while this relationship becomes obscure in recurrent tumors in the late phase. Chronological alterations in the correlation coefficients (Figs. 3 and 4) support this hypothesis. Moreover, this correlation was stronger for AFP than for DCP across all the study groups. This observation suggests that the increased AFP values both before hepatectomy and at the time of recurrence are at least partially accounted for by the background liver diseases.

A limitation of this investigation is that all of the study patients underwent curative liver resections. They would therefore be supposed to exhibit relatively well-preserved liver function, despite the presence of cirrhosis, from the viewpoint of screening. Likewise, they would be expected to have relatively early stage of HCC as compared with patients undergoing transcatheter arterial embolization, from the standpoint of prediction of response to therapies.

In conclusion, although DCP might be more accurate than AFP for the differentiation of HCC from nonmalignant chronic liver disease, the two markers are complementary to each other. The levels of both markers increased with tumor growth, but no specific association of either with any specific pathological entities was noted. The observed relationship between the preoperative marker values and the values measured at the time of recurrence may serve as a basis for predicting the pattern of recurrence of HCC, i.e., recurrence by metastasis or de novo secondary lesions.

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A randomised phase III trial comparing gemcitabine with surgery-only in patients with resected pancreatic cancer: Japanese Study Group of Adjuvant Therapy for Pancreatic Cancer

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BACKGROUND: This multicentre randomised phase III trial was designed to determine whether adjuvant chemotherapy with gemcitabine improves the outcomes of patients with resected pancreatic cancer.

METHODS: Eligibility criteria included macroscopically curative resection of invasive ductal carcinoma of the pancreas and no earlier radiation or chemotherapy. Patients were randomly assigned at a 1 : 1 ratio to either the gemcitabine group or the surgery-only group. Patients assigned to the gemcitabine group received gemcitabine at a dose of 1000 mg m^{-2} over 30 min on days 1, 8 and 15, every 4 weeks for 3 cycles.

RESULTS: Between April 2002 and March 2005, 119 patients were enrolled in this study. Among them, 118 were eligible and analysable (58 in the gemcitabine group and 60 in the surgery-only group). Both groups were well balanced in terms of baseline characteristics. Although heamatological toxicity was frequently observed in the gemcitabine group, most toxicities were transient, and grade 3 or 4 non-heamatological toxicity was rare. Patients in the gemcitabine group showed significantly longer disease-free survival (DFS) than those in the surgery-only group (median DFS, 11.4*versus* 5.0 months; hazard ratio = 0.60 (95% confidence interval (CI): 0.40–0.89); $P = 0.01$), although overall survival did not differ significantly between the gemcitabine and surgery-only groups (median overall survival, 22.3 *versus* 18.4 months; hazard ratio = 0.77 (95% CI: 0.51–1.14); $P = 0.19$).

CONCLUSION: The current results suggest that adjuvant gemcitabine contributes to prolonged DFS in patients undergoing macroscopically curative resection of pancreatic cancer.

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Pancreatic cancer remains to be one of the most challenging malignancies to treat. Surgical resection offers the only opportunity for cure. However, as no valid method for early detection of this disease has been established, 80% or more of patients present with unresectable disease at the time of diagnosis. Furthermore, even when resection is performed, the recurrence rate is extremely high, resulting in the 5-year survival rate of patients with resected pancreatic cancer being no more than 20% (Evans *et al*, 1997). As surgical resection alone has limitations, development of non-surgical treatments, including adjuvant therapy, is needed to improve the prognosis of patients with pancreatic cancer.

Several previous studies have suggested the efficacy of adjuvant chemoradiotherapy and/or chemotherapy for the treatment of resected pancreatic cancer (Kalser and Ellenberg, 1985; Neoptolemos *et al*, 2004; Stocken *et al*, 2005; Hazard *et al*, 2007). In the United States, adjuvant chemoradiation with fluorouracil has become the standard of care after the Gastrointestinal Tumour Study Group study showed a statistically significant improvement in survival as compared with surgery-only (median overall survival, 20 *versus* 11 months; 2-year survival rate, 42 *versus* 15%) (Kalser and Ellenberg, 1985). Recently, an evaluation of Medicare patients derived from the SEER database showed a survival advantage for patients who received adjuvant chemo-radiotherapy as compared with patients who did not (3-year survival rate, 45 *versus* 30%) (Hazard *et al*, 2007). On the other

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hand, no survival benefit of adjuvant chemoradiation was shown by the European Study Group for Pancreatic Cancer (ESPAC)-1 trial, a large-scale phase III study conducted in Europe (Neoptolemos *et al*, 2004). In the ESPAC-1, chemotherapy using fluorouracil plus leucovorin, but not chemoradiation, showed efficacy in the adjuvant setting: for patients who received postoperative chemotherapy compared with those who did not, the 2-year survival rates (40 *versus* 30%) and the 5-year survival rates (21 *versus* 8%) were significantly greater. Therefore, although the benefit of adjuvant therapy has become more apparent in recent years, the optimal treatment modality remains controversial (Ueno and Kosuge, 2008; Zuckerman and Ryan, 2008).

As for unresectable advanced pancreatic cancer, gemcitabine has been widely employed, since Burris *et al* (1997) reported results of a phase III study. The results of this study suggested that patients receiving gemcitabine experienced improved survival as compared with those receiving fluorouracil (median overall survival, 5.65*versus* 4.42 months; $P=0.0025$). The efficacy and tolerability of gemcitabine for advanced pancreatic cancer have been confirmed by several subsequent studies (Berlin *et al*, 2002; Moore *et al*, 2003; Rocha Lima *et al*, 2004), and gemcitabine has become the standard therapy for unresectable pancreatic cancer. These facts led investigators to evaluate gemcitabine in the adjuvant setting for patients with resected pancreatic cancer.

In 2005, a large phase III study, CONKO-001 (Charité Onkologie), was presented at the American Society of Clinical Oncology (ASCO) Annual Meeting by a German group (Oettle *et al*, 2007). CONKO-001 compared a gemcitabine therapy group with a surgery-only group after macroscopically curative resection of pancreatic cancer. In CONKO-001, disease-free survival (DFS) was significantly longer in the gemcitabine than in the observation group (median DFS, 13.4 *versus* 6.9 months; $P<0.001$). However, overall survival did not differ significantly between the gemcitabine and surgery-only groups, although the survival period tended to be longer in the gemcitabine than in the observation group (median, 22.1 *versus* 20.2 months; $P=0.06$).

Coincidentally, at approximately the same time as the CONKO-001, our multicentre randomised phase III trial, JSAP-02 (Japanese Study Group of Adjuvant Therapy for Pancreatic Cancer), was being conducted to test whether the addition of adjuvant gemcitabine to surgery would improve the outcomes of patients with resected pancreatic cancer. The JSAP-02 study design basically resembled that of CONKO-001, except for the planned number of gemcitabine cycles: six cycles of gemcitabine were used in CONKO-001 and three cycles in our study. To our knowledge, this is the first randomised phase III trial of adjuvant gemcitabine in an Asian population.

PATIENTS AND METHODS

Trial design

JSAP-02 was conducted at 10 centres in Japan. The trial was supported by funding from the Health and Labour Sciences Research Grant for Clinical Cancer Research from the Ministry of Health, Labour and Welfare, Japan.

The primary end point was overall survival. Secondary end points were DFS and gemcitabine safety. The ethics boards of all institutions approved the protocol and all patients provided a written, informed consent. The trial was conducted in accordance with the World Medical Association Declaration of Helsinki and Japanese Good Clinical Practice guidelines. The trial was monitored for excessive toxicity by the Data Monitoring Committee, which functions independently of the JSAP. Data were collected using the web-based clinical trial management system at the data centre (EPS Co., Ltd., Osaka, Japan), and additional changes were locked out of the database on 31 March 2009.

Patient eligibility

Patients who underwent macroscopically curative resection of pancreatic cancer were enrolled in the study 3 to 10 weeks after surgery. The other eligibility criteria were histologically proven invasive ductal carcinoma of the pancreas; no history of earlier chemotherapy or radiotherapy for pancreatic cancer except intraoperative radiotherapy; age 20–74 years; Karnofsky performance status of 50 or more; and adequate organ function (WBC count $\geqslant 4000$ and $\leqslant 12\,000\,mm^{-3}$; neutrophil count $\geqslant 2000\,mm^{-3}$; platelet count $\geqslant 100\,000\,mm^{-3}$; haemoglobin level $\geqslant 9.0\,g$ per 100 ml; serum total bilirubin level $\leqslant 3.0\,mg$ per 100 ml; serum aspartate aminotransferase and serum alanine aminotransferase level $\leqslant 5$ times the upper limit of the normal range; and serum creatinine level lesser than or equal to the upper limit of the normal range). The exclusion criteria were pulmonary fibrosis or interstitial pneumonia; clinically significant pleural effusions; presence of distant metastasis (except distant lymph node metastasis confirmed by resected specimen); other concomitant malignant disease; active infection; history of serious complications related to surgery; active gastrointestinal ulcers; history of myocardial infarction within 3 months; severe mental disorder; pregnant or lactating women; and other serious concomitant systemic disorders incompatible with the trial in the investigator's judgment.

Treatment plan

Patients were enrolled, within 10 weeks after surgery, through fax by the staff at the data centre. Patients were randomly assigned at a 1:1 ratio to either the gemcitabine group or the surgery-only group using the minimisation method stratified by resection status (R0 *versus* R1), pathological stage (I–II *versus* III–IV) and enrollment centre. Stage classification and the evaluation of resected specimens were performed in accordance with the fifth edition of the tumour–node–metastasis classification system of the International Union Against Cancer. Patients assigned to the gemcitabine group received gemcitabine at a dose of $1000\,mg\,m^{-2}$ over 30 min on days 1, 8 and 15 every 4 weeks. This 4-week cycle was repeated for 3 cycles. If patients developed leukocyte counts of $<2000\,mm^{-3}$ or $>12\,000\,mm^{-3}$, or platelet counts of $<75\,000\,mm^{-3}$ during chemotherapy, gemcitabine administration was stopped until recovery. When patients had grade 4 leukopenia or neutropenia, febrile neutropenia or infection with grade 3 leukopenia or neutropenia, a platelet count of $<25\,000\,mm^{-3}$, or non-heamatological toxic effects of grade 3 or greater, a dose reduction of gemcitabine from $1000\,mg\,m^{-2}$ to $800\,mg\,m^{-2}$ was allowed. The surgery-only group received no anticancer treatment after surgery, unless there was a confirmed relapse.

Assessments

Baseline assessments included medical history, physical examination, vital signs, chest radiography, ECG, routine laboratory tests, and the tumour markers CEA and CA19-9. Patients in the gemcitabine group underwent laboratory tests and assessment of clinical symptoms every week during the treatment period and every 3 months after completing adjuvant chemotherapy. Patients in the surgery-only group underwent similar examinations every 3 months. Adverse events were assessed according to the Common Toxicity Criteria of the National Cancer Institute (version 2.0). Patients in both groups underwent computed tomography and/or ultrasonography at 3-month intervals after surgery, unless there was a confirmed relapse. Tumour markers, CEA and CA19-9, were also measured every 3 months until relapse.

Statistical analysis

A total of 116 patients were required to detect a hazard ratio of 0.55 with 80% power at a two-sided 0.05 significance level, which

corresponds to a 20% increase in the 2-year overall survival rate in the surgery-only group *versus* the gemcitabine group (15 *versus* 35%, respectively).

All randomised and eligible patients were included in the intent-to-treat (ITT) population for efficacy analyses. Efficacy analyses were also performed in subpopulations stratified by resection status (R0 *versus* R1) and pathological stage (I–II *versus* III–IV). For safety analyses of gemcitabine, only patients who received adjuvant gemcitabine were included. Overall survival was defined as the period between randomisation and death. All deaths, including those from other diseases, were considered to be events. Disease-free survival was defined as the period between randomisation and the occurrence of an event—relapse or death—whichever came first. Data for patients who had not had an event were censored, as of the date of the final observation. The Kaplan–Meier method was used to estimate the overall survival or DFS and the log-rank test was used for comparisons between the two groups. The Wilcoxon test, Fisher's exact test and the Mantel trend test were used to compare differences among pretreatment characteristics between the two groups. *P*-values of less than 0.05 were considered to indicate statistical significance. All statistical analyses were performed using SAS version 9.1 statistical software (SAS Institute Inc, Cary, NC, USA).

RESULTS

Characteristics of patients

Between April 2002 and March 2005, 119 patients in total were enrolled at 10 centres. After randomisation, one patient in the gemcitabine group was found to be ineligible because of a low WBC count at baseline. Therefore, 118 eligible patients (58 in the gemcitabine group and 60 in the surgery-only group) were included in the ITT population for efficacy analyses (Figure 1). No patients assigned to the surgery-only group received post-operative anticancer treatment until a confirmed relapse. The two groups were well balanced with regard to baseline characteristics (Table 1). In total, 16% of the patients had a microscopically positive margin (R1) and 69% had nodal metastases (N1). The median follow-up period for surviving patients was 60.4 months (range, 40.6–77.1 months) on the analysis cut-off date of 31 March 2009.

Treatment administration

Among the 58 patients in the gemcitabine group, one withdrew from the study before treatment because of a postoperative complication. Six patients (10%) discontinued treatment within 1 cycle, 7 (12%) after 2 cycles and 44 (76%) completed the scheduled 3 cycles of treatment. The reasons for withdrawal from treatment included adverse events or complications (10 patients), the detection of recurrent disease (2 patients) and patient preference (2 patients). The dose of gemcitabine was decreased in one patient because of neutropenia. The median number of cycles and the median number of gemcitabine doses administered were 3 and 8, respectively. The median dose intensity of gemcitabine was 667 mg m^{-2} per week, and the median relative dose intensity was 89%.

Safety

Of the 58 eligible patients assigned to the gemcitabine group, adverse events were analysed in 57 patients who received at least one dose of gemcitabine. Major grade 3 or 4 adverse events observed during the treatment are listed in Table 2. Adjuvant gemcitabine was generally well tolerated. Although high frequencies of grade 3 or 4 leukopenia and neutropenia were experienced (25 and 70%, respectively), most myelosuppression resolved promptly without complications.

Three fatal events occurred during the study period: two in the gemcitabine group and one in the surgery-only group. Of the two, an association with gemcitabine could not be ruled out in one patient who developed an abdominal abscess without neutropenia after two treatment cycles and died from gastrointestinal bleed 183 days after the final gemcitabine administration.

DFS and overall survival

At the time of analysis, 44 patients in the gemcitabine group and 53 in the surgery-only group had recurrent disease. The common sites of first recurrence were the liver, peritoneum and local recurrence (Table 3). The recurrence pattern was similar in the two groups. DFS was significantly longer in the gemcitabine group than in the surgery-only group, with an estimated hazard ratio of 0.60 (95% confidence interval (CI), 0.40–0.89; $P=0.01$;

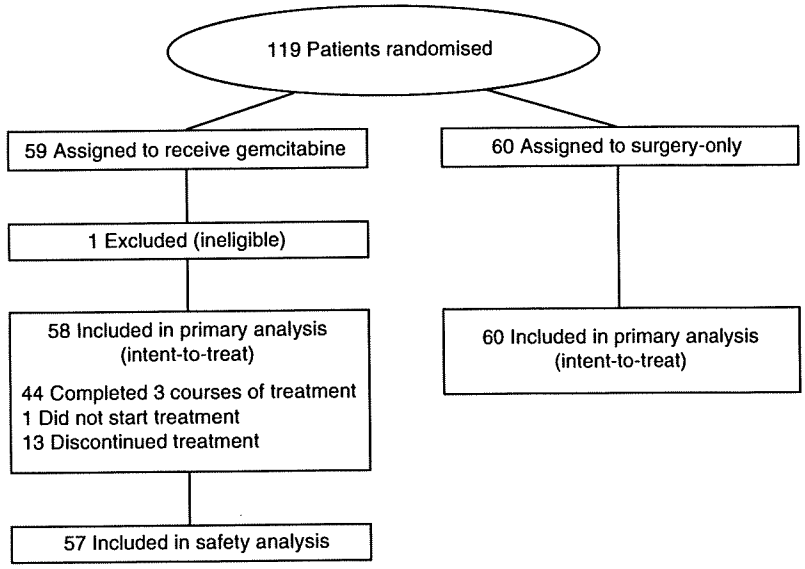


Figure 1 Flow chart of study subjects.

Table 1 Baseline characteristics

Characteristic	Gemcitabine ($n=58$) No.	%	Surgery-only ($n=60$) No.	%	*P*-value
Age (years)					
Median	65		64		0.62
Range	41–74		36–74		
Sex					
Women	18	31	26	43	0.19
Men	40	69	34	57	
Days from surgery to randomisation (days)					
Median	44		47		0.45
Range	22–71		22–70		
Karnofsky performance status					
Median	90		90		0.83
Range	70–100		70–100		
Intra-operative radiotherapy					
Yes	27	47	34	57	0.36
No	31	53	26	43	
Primary site					
Head	42	72	42	70	0.84
Body-tail	16	28	18	30	
Maximal tumour size (cm)					
Median	3.5		3.5		0.27
Range	1.0–10.0		1.2–7.0		
Resection status					
R0	47	81	52	87	0.46
R1	11	19	8	13	
Primary tumour size					
T1	6	10	6	10	0.90
T2	1	2	4	6	
T3	31	53	28	47	
T4	20	35	22	37	
Nodal status					
N0	19	33	18	30	0.84
N1	39	67	42	70	
Pathological stage[a]					
I	3	5	4	7	0.82
II	10	17	10	17	
III	21	36	22	37	
IV	24	41	24	40	
Grading					
1	18	31	16	27	0.80
2	33	57	36	60	
3	5	9	4	7	
Unknown	2	3	4	7	
Histology					
Adenocarcinoma	56	97	56	93	0.68
Other	2	3	4	7	
CEA ($ng\,ml^{-1}$)					
Median	3.7		4.6		0.34
Range	0.9–252		0.5–74		
CA19-9 ($U\,ml^{-1}$)					
Median	33.2		37.5		0.40
Range	0–10435		0–46100		

Abbreviations: CEA = carcinoembryonic antigen; CA 19–9 = carbohydrate antigen 19–9. [a]UICC fifth edition.

Table 2 Grade 3 and 4 adverse events in the gemcitabine group ($n=57$)

Adverse event	Gemcitabine Grade 3[a] No.	%	Grade 4[a] No.	%
Heamatological				
Leukopenia	13	23	1	2
Neutropenia	32	56	8	14
Anaemia	2	4	0	0
Thrombocytopenia	1	2	0	0
Non-heamatological				
Diarrhoea	1	2	0	0
Fever	1	2	0	0
Nausea	0	0	1	2
Anorexia	1	2	1	2
Fatigue	1	2	0	0
AST	3	5	0	0
ALT	4	7	0	0
Abscess	0	0	1	2

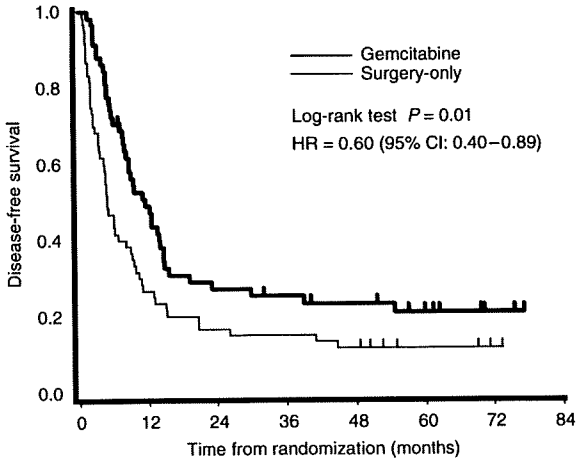
Abbreviations: ALT = alanine aminotransferase; AST = aspartate aminotransferase. [a]NCI Common Toxicity Criteria, version 2.0.

Table 3 Patterns of initial recurrence

	Gemcitabine No.	%	Surgery-only No.	%
Local	10	23	17	32
Liver	13	30	16	30
Peritoneum	8	18	7	13
Other	12	27	12	23
Unknown	1	2	1	2

Figure 2). Median DFS was 11.4 months (95% CI, 8.0–14.5) in the gemcitabine group *versus* 5.0 months (95% CI, 3.7–8.9) in the surgery-only group. The estimated DFS rates at 6, 12 and 24 months were 70.7, 49.0 and 27.2% in the gemcitabine group, and 43.3, 26.7 and 16.7% in the surgery-only group, respectively. Subgroup analyses showed that the beneficial effect of adjuvant gemcitabine on DFS was evident for R0, N0 and stage I–II patients (Table 4, Figure 3).

At the time of analysis, 98 patients (83%) had died (45 patients in the gemcitabine group and 53 in the surgery-only group). The causes of death in the gemcitabine group and surgery-only groups were as follows: relapse (41 and 52 patients, respectively), adverse events (2 and 1 patients, respectively) and unknown causes (2 and 0 patients, respectively). Log-rank analysis revealed no statistically significant difference in survival estimates between the treatment groups (hazard ratio, 0.77 (95% CI, 0.51–1.14); $P=0.19$; Figure 4). Median overall survival was 22.3 months in the gemcitabine group (95% CI, 16.1–30.7) *versus* 18.4 months in the surgery-only group (95% CI, 15.1–25.3). The estimated overall survival rates at 6, 12, 18, 24 and 60 months were 94.8, 77.6, 58.6, 48.3 and 23.9% in the gemcitabine group, and 85.0, 75.0, 53.3, 40.0 and 10.6% in the surgery-only group, respectively. Subgroup analyses failed to show the beneficial effect of adjuvant gemcitabine on overall survival, although the survival period tended to be longer in the gemcitabine than in the observation group for R0, N0 and stage I–II patients (Table 4).



No. at risk

Gemcitabine	58	27	15	13	11	6	2
Surgery-only	60	16	10	9	7	3	1

Figure 2 Kaplan–Meier estimates of disease-free survival. Intent-to-treat analysis.

DISCUSSION

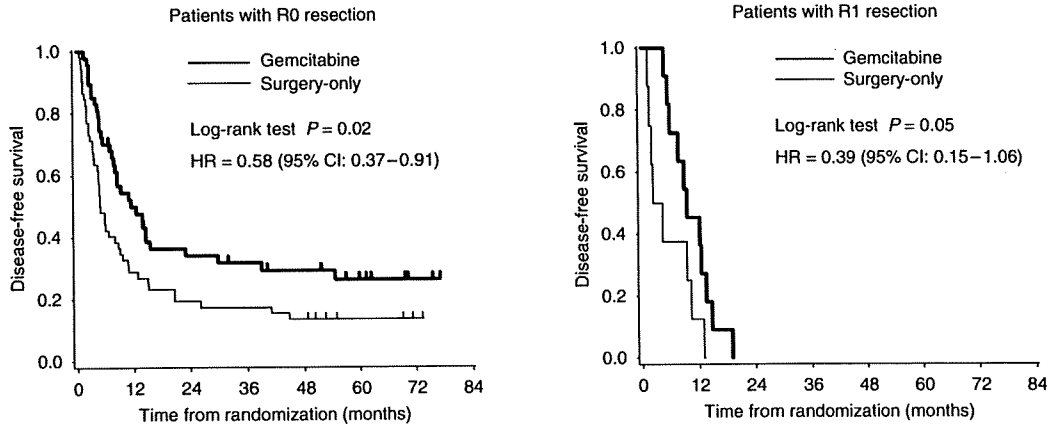
We found that DFS in patients with resected pancreatic cancer was significantly improved with three cycles of adjuvant gemcitabine as compared with surgery-only, with an estimated hazard ratio of 0.60 ($P = 0.01$). However, a statistically significant improvement in overall survival was not shown in this study, although median overall survival, and 2-year and 5-year survival rates were favourable in the gemcitabine group as compared with the surgery-only group. These results were similar to those of the previously reported phase III trial of adjuvant gemcitabine, CONKO-001 (Oettle *et al*, 2007).

CONKO-001 compared six cycles of gemcitabine with surgery-only after macroscopically curative resection of pancreatic cancer. Table 5 shows a comparison of our study (JSAP-02) and CONKO-001. The study design of JSAP-02 basically resembled that of CONKO-001 except for the planned sample size, number of gemcitabine cycles, weeks from surgery to randomisation and eligibility criteria determined by postoperative tumour markers. Baseline patient characteristics, including resection status and nodal status, were similar between the two studies. As for efficacies, although both studies failed to show a statistically significant improvement in overall survival, a significantly better DFS was shown with the adjuvant gemcitabine. The data on DFS and overall survival reported in JSAP-02 were comparable with those in CONKO-001.

Table 4 Disease-free and overall survivals in the total entire population and subgroups

	No.		Disease-free survival				Overall survival			
			Median (months)				Median (months)			
	GEM	Surgery-only	GEM	Surgery-only	HR (95% CI)	*P*-value	GEM	Surgery-only	HR (95% CI)	*P*-value
All patients	58	60	11.4	5.0	0.60 (0.40–0.89)	0.01	22.3	18.4	0.77 (0.51–1.14)	0.19
R0	47	52	11.4	5.1	0.58 (0.37–0.91)	0.02	26.8	19.1	0.70 (0.45–1.09)	0.11
R1	11	8	9.5	3.4	0.39 (0.15–1.06)	0.05	18.3	17.6	1.05 (0.41–2.72)	0.92
N0	19	18	—	9.0	0.38 (0.16–0.86)	0.02	32.0	28.4	0.63 (0.29–1.37)	0.24
N1	39	42	8.6	4.5	0.73 (0.46–1.16)	0.19	17.1	17.3	0.84 (0.53–1.34)	0.84
Stage I–II	13	14	—	10.0	0.27 (0.08–0.85)	0.02	67.8	—	0.42 (0.15–1.22)	0.10
Stage III–IV	45	46	8.9	4.6	0.68 (0.44–1.05)	0.08	18.3	16.3	0.82 (0.53–1.26)	0.36

Abbreviations: CI = confidence interval; GEM = gemcitabine; HR = hazard ratio.



No. at risk

Gemcitabine	47	22	15	13	11	6	2
Surgery-only	52	15	10	10	7	3	1

No. at risk

Gemcitabine	11	5
Surgery-only	8	1

Figure 3 Kaplan–Meier estimates of disease-free survival in patients with R0 or R1 resection. Intent-to-treat analysis.

The CONKO-001 data were re-analysed in March 2008, and presented at the ASCO Annual Meeting of that year as the final results (Neuhaus *et al*, 2008). Although the improvement in overall survival did not reach statistical significance ($P=0.06$) in the previous report (Oettle *et al*, 2007), the new CONKO-001 report showed a significant difference in overall survival between the gemcitabine and surgery-only groups after long-term observation (median overall survival, 22.3 months *versus* 20.2 months; 5-year survival rate, 21.0% *versus* 9.0%; $P=0.005$). In contrast to these final results of CONKO-001, our study, though the data were analysed after an adequate observation period, failed to show a survival benefit of adjuvant gemcitabine. As the JSAP-02 survival curve itself resembled that of CONKO-001, the main reason for this discrepancy may be the underpowered nature of our study. The planned sample size for the JSAP-02, which was less than one-third that of CONKO-001, might have been too small to detect a significant difference in overall survival. Other factors that differed between the two studies, including race, number of gemcitabine cycles and patient selection based on postoperative tumour markers, may also have influenced the outcome of our study. Further study is needed to clarify the impacts of these factors on adjuvant gemcitabine. Although intra-operative radiotherapy was allowed only in our study and 52% of patients actually received this treatment, its influence may be very small because a recent phase III trial failed to show any benefits of intra-operative radiotherapy in patients with resected pancreatic cancer (Kinoshita *et al*, 2009).

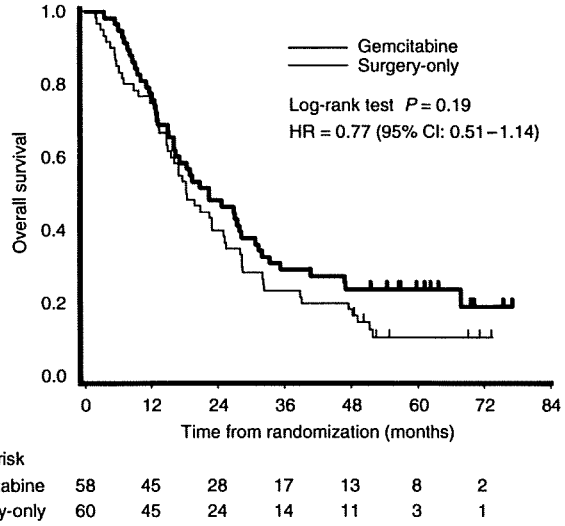


Figure 4 Kaplan–Meier estimates of overall survival. Intent-to-treat analysis.

Adjuvant gemcitabine was well tolerated in our study. A total of 44 patients (76%) completed the three scheduled treatment cycles, and the median relative dose intensity of gemcitabine was as good as 89%. Although 70% of patients experienced grade 3 or 4 neutropenia during adjuvant gemcitabine therapy, most of these toxicities were transient, and serious adverse events were rare. The frequencies of grade 3 or 4 neutropenia induced by gemcitabine monotherapy are reportedly 20–30% (Aapro *et al*, 1998). The reasons for marked heamatological toxicities occurring in our study are unclear, although surgical stress might have exacerbated myelosuppression. Onoue *et al* (2004) reported that administering gemcitabine to patients after surgical resection resulted in more severe leukopenia, as compared with patients not undergoing resection (grade 3 or 4 leukopenia, 57 *versus* 25%; $P=0.048$). Although our study, similar to CONKO-001, showed the safety of adjuvant gemcitabine, cautious selection of patients and careful observation of treatment will be necessary when giving this agent to patients with resected pancreatic cancer.

Other than gemcitabine, fluorouracil-based chemotherapy is now considered to be an option for adjuvant therapy for resected pancreatic cancer based on the results of ESPAC-1. The ESPAC-1

Table 5 Comparison between the current Japanese study and CONKO-001

	Current study (JSAP-02)		CONKO-001[a]	
	Gemcitabine	**Surgery-only**	**Gemcitabine**	**Surgery-only**
Study design				
Planned sample size	116		368	
Planned number of gemcitabine cycles	3	—	6	—
Planned weeks from surgery to randomisation	⩽10		⩽6	
Selection based on postoperative tumour markers	No requirement		⩽2.5 times the upper limit of normal	
Baseline patient characteristics				
No. of patients	58	60	179	175
Median age (years)	65	64	62	61
Sex: men	69%	57%	59%	56%
Median Karnofsky PS	90	90	80	80
Resection status: R0	81%	87%	81%	85%
Nodal status: N0	33%	30%	29%	27%
Median days from surgery to randomisation	44	47	22	24
Results				
Median DFS (months)	11.4	5.0	13.4	6.9
1-year DFS rate	49%	27%	58%	31%
2-year DFS rate	27%	17%	31%	15%
P-value	0.01		<0.01	
Median OS (months)	22.3	18.4	22.1	20.2
1-year OS rate	78%	75%	73%	73%
2-year OS rate	48%	40%	48%	42%
5-year OS rate	24%	11%	23%	12%
P-value	0.19		0.06	

Abbreviations: DRF = disease-free survival; OS = overall survival; PS = performance status. [a]Previously reported in Oettle *et al* (2007).

study showed a survival benefit of adjuvant fluorouracil plus leucovorin in 289 patients with resected pancreatic cancer (Neoptolemos *et al*, 2004). The ESPAC group also performed a pooled analysis using data from 458 patients who were enrolled in ESPAC-1, ESPAC-1 plus or early ESPAC-3(v1) (Neoptolemos *et al*, 2009a). The overall survival was superior in patients randomised to fluorouracil plus leucovorin, as compared with those randomised to observation (pooled hazard ratio, 0.70; $P=0.003$, median overall survival, 23.2 *versus* 16.8 months), indicating the validity of using fluorouracil plus leucovorin as adjuvant therapy.

With regard to the comparison between gemcitabine and fluorouracil-based chemotherapy in the adjuvant setting, two large phase III trials, RTOG 97-04 and ESPAC-3(v2), were recently reported (Regine *et al*, 2008; Neoptolemos *et al*, 2009b). RTOG 97-04 examined whether survival could be extended by substituting gemcitabine for fluorouracil before and after fluorouracil-based radiation. When the data from the entire population were analysed, no significant difference in the survival period was noted between the fluorouracil and gemcitabine groups, but the gemcitabine group had better outcomes when the analysis was confined to patients with pancreatic head cancer (median overall survival, 20.5 *versus* 16.9 months, $P=0.033$). ESPAC-3(v2) was designed to compare fluorouracil plus leucovorin and gemcitabine in patients with resected pancreatic cancer. In total, 1088 patients were randomised in ESPAC-3(v2), and Neoptolemos *et al* reported no significant difference in survival between adjuvant fluorouracil plus leucovorin and adjuvant gemcitabine at the 2009 ASCO Annual Meeting (hazard ratio, 0.94; $P=0.39$, median overall survival 23.0 *versus* 23.6 months). Although no significant difference in survival was shown, gemcitabine may be suitable for clinical use as adjuvant therapy because the rate of serious adverse events in patients treated with gemcitabine was significantly lower than that in patients treated with fluorouracil plus leucovorin (7.5 *versus* 14%, $P<0.001$).

In recent years, new approaches, including novel cytotoxic or molecular-targeting agents, have been actively applied in the adjuvant setting for pancreatic cancer. In Japan, S-1, an oral fluoropyrimidine derivative, has attracted the attention of investigators on the basis the promising results of clinical trials for advanced pancreatic cancer (Ueno *et al*, 2007; Okusaka *et al*, 2008). We are now conducting a phase I/II trial of gemcitabine plus S-1 for resected pancreatic cancer (JSAP-03 trial). As well as developing new effective treatments, individualised approaches based on individual differences in drug metabolism are also important in selecting patients who are more likely to benefit from adjuvant gemcitabine. Several recent studies have suggested that tumour-specific expression of human equilibrative nucleoside transporter 1 may be a promising predictive biomarker of outcome in pancreatic cancer patients receiving gemcitabine chemotherapy (Spratlin *et al*, 2004; Giovannetti *et al*, 2006; Farrell *et al*, 2009; Maréchal *et al*, 2009). Further investigation of and progress in these new strategies are expected in the future.

In conclusion, adjuvant chemotherapy with gemcitabine significantly improved DFS, as compared with surgery-only in patients with resected pancreatic cancer. Our study supports the conclusions of the CONKO-001 as well as the validity of using gemcitabine as adjuvant therapy for resected pancreatic cancer.

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Conflict of interest

The authors declare no conflict of interest.

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Full Paper

Homozygous *CDA**3 is a major cause of life-threatening toxicities in gemcitabine-treated Japanese cancer patients

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Among 242 Japanese pancreatic cancer patients, three patients (1.2%) encountered life-threatening toxicities, including myelosuppression, after gemcitabine-based chemotherapies. Two of them carried homozygous *CDA**3 (*CDA*208G>A [Ala70Thr]), and showed extremely low plasma cytidine deaminase activity and gemcitabine clearance. Our results suggest that homozygous *3 is a major factor causing gemcitabine-mediated severe adverse reactions among the Japanese population.
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Keywords: gemcitabine; toxicity; *CDA*208G>A; pancreatic cancer; pharmacogenomics; polymorphism

Gemcitabine (2′,2′-difluorodeoxycytidine) is a nucleoside anticancer drug for various solid tumours (Noble and Goa, 1997). Gemcitabine exerts its cytotoxic effect through phosphorylation by nucleotide kinases, including the deoxycytidine kinase (DCK), whereas most of the administered gemcitabine is rapidly degraded by cytidine deaminase (CDA) into its inactive metabolite, 2′,2′-difluorodeoxyuridine (Plunkett *et al*, 1995). Various genetic variations have recently been reported in human *DCK* and *CDA* genes (Ueno *et al*, 2007).

Our earlier prospective pharmacogenetic study using 256 Japanese cancer patients treated with gemcitabine-based chemotherapies revealed that one of the *CDA* single-nucleotide polymorphisms (SNPs), *CDA**3 (*CDA*208G>A [Ala70Thr], rs60369023), showed significant associations with reduced CDA activity, reduced gemcitabine clearance, increased gemcitabine area under the concentration–time curve (AUC), and an increased incidence of severe neutropaenia (Sugiyama *et al*, 2007). Most notably, one patient who had developed life-threatening toxicities, including severe myelosuppression, was found to be homozygous for *CDA**3 (*CDA**3/*3), and excessive exposure to gemcitabine was considered responsible for the severe toxicities (Yonemori *et al*, 2005; Sugiyama *et al*, 2007).

Owing to a low allele frequency of *CDA**3 (3.7% in the Japanese population), only one homozygous patient was found in the earlier study, necessitating further examination. For this purpose, we have carefully monitored toxicities in gemcitabine-treated patients in the National Cancer Center Hospital for 4.5 years. Three patients with life-threatening adverse reactions, including serious myelosuppression, were identified, and their *CDA* genotypes, plasma CDA activities, and pharmacokinetic parameters (when available) were determined and compared with those of the earlier cases.

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PATIENTS AND METHODS

The ethics committees of the National Cancer Center and the National Institute of Health Sciences approved this study. Written informed consent was obtained from each participant. Of 176 and 66 pancreatic cancer patients who received gemcitabine monotherapy and gemcitabine-based combination chemotherapies, respectively, at the National Cancer Center Hospital between 1 September 2003 and 31 March 2008, three showed severe and prolonged myelosuppression with other complications. Characteristics of the three patients designated A, B, and C are summarised in Table 1. All these patients received a 30 min intravenous gemcitabine infusion at an initiation dose of 1000 mg m^{-2}. Patient A initially received gemcitabine and S-1 combination therapy, whereas patients B and C were given gemcitabine alone.

Measurement of plasma CDA activity towards gemcitabine and genotyping of *CDA* and *DCK* were carried out in the three patients as reported earlier (Sugiyama *et al*, 2007; Kim *et al*, 2008). After recovery from severe adverse reactions, chemotherapy was resumed in two patients: patient A received gemcitabine monotherapy instead of gemcitabine plus S-1, whereas the gemcitabine dose was reduced in patient C. Gemcitabine was not resumed in patient B because of disease progression. Pharmacokinetics were carried out when 1000 and 450 mg m^{-2} of gemcitabine were administered to patients A and C, respectively. Blood sampling schedule and the measurement method of gemcitabine in plasma were reported earlier (Sugiyama *et al*, 2007). Pharmacokinetic parameters were estimated using WinNonlin ver 4.01 (Pharsight Corporation, Mountain View, CA, USA).

RESULTS

Although 30 patients (about 12.4%) among the 242 developed grade 4 neutropaenia, the toxicity was transient and required no supportive treatment in most patients except in patients A, B, and C. Pretreatment organ functions, including bone marrow, renal, and hepatic functions, were preserved in the three patients (Table 1). Observed toxicities in the patients are summarised in Table 2. The serious haematotoxicities requiring intensive supportive treatments during hospitalisation were recognised in these patients: patient A was treated with antibiotics because of febrile neutropaenia, patient B received a platelet transfusion, and patient C received a red blood cell transfusion, a platelet transfusion, and a granulocyte colony-stimulating factor. Both the neutrophil and platelet nadir appeared at approximately day 15 of the first course of treatment in patients A and B, whereas in patient C, the nadir occurred on day 15 of the second course of treatment that was resumed after reducing the dose of gemcitabine. The symptomatic non-haematologic toxicities shown in Table 2 appeared before severe myelosuppression.

Patients B and C were found to be *CDA*3/*3*, whereas patient A did not have *CDA*3* (Table 2). No SNPs of *DCK*, including *DCK*364C>T (Pro122Ser), which were reported to have reduced enzymatic activity (Lamba *et al*, 2007), were identified in our three patients. Plasma CDA activities of patients A, B, and C were compared with those of 121 patients in our earlier study (Sugiyama *et al*, 2007) (Figure 1A). Patient A without *CDA*3* showed relatively high plasma CDA activity, whereas plasma CDA activities in the *CDA*3/*3* patients (patients B and C) were comparably low to those in the earlier reported *CDA*3/*3* patient.

Pharmacokinetic parameters of patients A and C were also shown in Table 2, and their gemcitabine clearances were compared with those of the earlier reported 250 patients (Sugiyama *et al*, 2007) (Figure 1B). Although the gemcitabine dose for patient C was low (450 mg m^{-2}), her gemcitabine AUC was higher than the average value in the *CDA*3*-negative patients who were administered 1000 mg m^{-2} gemcitabine. When it was assumed that patient

Table 1 Patient characteristics at baseline

	Patient A	Patient B	Patient C
Sex	Female	Male	Female
Age (years)	57	70	70
Performance status	1	2	1
Stage[a]	IV	IV	IV
Previous treatment	Surgery	None	None
Body surface area (m^2)	1.3	1.9	1.1
Laboratory data			
Leukocyte (mm^{-3})	6300	9700	4300
Neutrophil (mm^{-3})	4200	6400	2700
Haemoglobin (g dl^{-1})	12.4	16.0	10.8
Platelet (mm^{-3})	116 000	163 000	185 000
Total bilirubin (mg dl^{-1})	0.6	1.4	0.8
ALT (IU l^{-1})	26	35	24
Creatinine (mg dl^{-1})	0.6	1.2	0.4
Initial regimen	Gemcitabine+S-1[b]	Gemcitabine alone	Gemcitabine alone

ALT = alanine aminotransferase. [a]UICC sixth edition. [b]An oral product containing tegafur, gimeracil, and oteracil potassium.

Table 2 Toxicities, treatment, genotype, and PK analysis

	Patient A		Patient B		Patient C	
	Grade	Value	Grade	Value	Grade	Value
Haematologic toxicities[a]						
Leukocyte (mm^{-3})	4	800	3	1100	3	1000
Neutrophil (mm^{-3})	4	200	4	300	4	100
Haemoglobin (g dl^{-1})	2	8.1	1	13.2	4	6.3
Platelet (mm^{-3})	3	26 000	4	10 000	3	28 000
Non-haematologic toxicity[a]						
Fatigue	2		3		2	
Anorexia	3		3		2	
Diarrhoea	3		0		1	
Stomatitis	3		0		0	
Rash	2		0		2	
Febrile neutropaenia	3		0		0	
Treatment						
Resumption of chemotherapy	Yes		No		Yes	
Total number of gemcitabine doses	10		2		10	
Final dose (mg m^{-2})	600		1000		270	
Genotype						
CDA haplotype[b]	*1a/*1j		*3a/*3a		*3a/*3a	
DCK haplotype[c]	*1a/*1a		*1a/*1a		*1a/*1a	
PK analysis						
Regimen at PK study	Gemcitabine alone				Gemcitabine alone	
Dose of gemcitabine (mg m^{-2})	1000				450	
C_{max} (mg l^{-1})	29.0		Not available		22.5 (49.7[d])	
AUC (mg h l^{-1})	16.2				26.8 (59.0[d])	
Clearance (l h^{-1} m^{-2})	61.8				16.6	

AUC = area under the concentration–time curve; C_{max} = maximum plasma concentration; CDA = cytidine deaminase; DCK = deoxycytidine kinase; PK = pharmacokinetics. [a]Toxicities were assessed according to the National Cancer Institute Common Terminology Criteria for Adverse Events version 3.0. [b]*CDA* haplotype was reported earlier in Sugiyama *et al* (2007). [c]*DCA* haplotype was reported earlier in Kim *et al* (2008). [d]On the basis of the assumption that patient C received 1000 mg m^{-2} of gemcitabine.