staining. A wide range of immunoreactivity was observed in the surgical cases, varying from the cytoplasm only (focal or diffuse) to strong accumulation in the nuclei. The peripheral nerves constantly showed intense SNCG staining and therefore served as an internal positive control.

Of the total of 62 resected pancreatic cancer samples, 38 (61%) were positive and 22 (39%) were negative for SNCG staining. χ^2 analysis revealed that perineural invasion (grades 2 and 3) and other histologic markers of aggressive disease, including tumor size > 20 mm, positive lymph node metastasis, UICC stage > IIB, and vascular invasion (grades 2 and 3), were significantly associated with SNCG overexpression (Table 1).

During a median follow-up of 25 months (range, 4-171 months), the overall 5-year survival rate was 32%, with a median survival of 29 months [95% confidence interval (95% CI), 11-47 months]. Log-rank analysis showed that pancreatic cancer with SNCG overexpression had a significantly decreased overall survival, with a median of 15 months (95% CI, 9-22 months) compared with SNCG-negative tumors (median survival not reached; $P = 0.002$; Fig. 2). Positive lymph node metastasis, UICC stage ≥ IIB, moderately or poorly differentiated histology, and lymphatic invasion (grades 2 and 3) were also associated with a poor prognosis by univariate analysis (Table 2). Multivariate analysis based on the Cox proportional hazard model including these five factors revealed SNCG overexpression to be the only independent negative prognostic variable of overall survival (hazard ratio, 3.4; 95% CI, 1.51-7.51; $P = 0.003$). Meanwhile, patients having tumors with positive lymph node metastasis, UICC stage ≥ IIB, lymphatic/vascular invasion (grades 2 and 3), perineural invasion (grades 2 and 3), and SNCG overexpression were found to have significantly shorter disease-free survival (Table 2). By multivariate analysis, SNCG overexpression was the strongest negative predictor of disease-free survival (hazard ratio, 2.8; 95% CI, 1.26-6.02; $P = 0.011$), followed by positive lymph node metastasis (hazard ratio, 2.4; 95% CI, 1.03-5.57; $P = 0.044$).

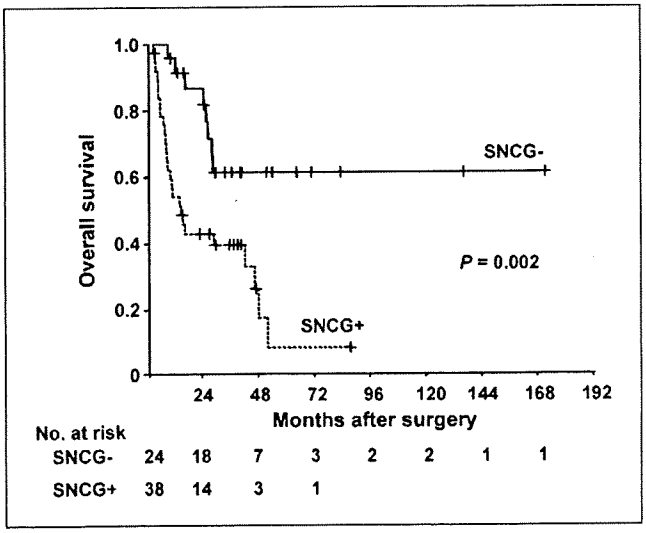

Fig. 2. Comparison of Kaplan-Meier survival curves. Patients with SNCG xoverexpression (*dotted line*) had a significantly poor prognosis compared with patients with negative SNCG expression (*solid line*).

Table 2. Log-rank univariate analysis of overall and disease-free survival

Variables	Overall survival		Disease-free survival	
	Median, mo	*P*	Median, mo	*P*
Tumor size, mm		0.059		0.060
≤20	NR		NR	
>20	25		13	
Serosal invasion		0.65		0.70
Absent	29		35	
Present	15		25	
Retroperitoneal extension		0.68		0.38
Absent	42		40	
Present	29		18	
Portal vein involvement		0.24		0.20
Absent	42		35	
Present	15		10	
Lymph node metastasis		0.016		0.006
Negative	NR		NR	
Positive	17		10	
Resection status		0.052		0.12
R0	42		35	
R1	12		8	
UICC stage		0.043		0.021
IA/IB/IIA	NR		NR	
IIB/III/IV	17		13	
Histologic differentiation		0.022		0.14
Well	NR		49	
Moderate, poor	25		18	
Lymphatic invasion		0.016		0.021
0-1	NR		40	
2-3	15		10	
Vascular invasion		0.23		0.030
0-1	42		NR	
2-3	18		13	
Perineural invasion		0.087		0.031
0-1	NR		NR	
2-3	26		12	
Intraoperative radiation		0.54		0.78
Yes	46		25	
No	28		28	
Adjuvant chemotherapy		0.14		0.98
Yes	42		18	
No	25		25	
SNCG expression		0.002		0.001
Negative	NR		NR	
Positive	15		10	

Abbreviation: NR, not reached.

Effect of SNCG suppression **in vitro** *and* **in vivo.** To determine whether SNCG is an instigator of invasion and metastasis or merely a correlative product during pancreatic cancer progression, the effect of SNCG suppression on high-perineural invasion group of human pancreatic cancer cell lines was further evaluated. Capan-1 was selected for the study because the infection rate of recombinant retroviruses with shRNA for SNCG was considerably lower in Capan-2 compared with Capan-1 (data not shown). Of the two stable SNCG-suppressed Capan-1 cells, a significant decrease of SNCG mRNA and protein levels in sh-A was shown by quantitative reverse transcriptase-PCR and Western blot analysis compared with sh-Control *in vitro* ($P < 0.0001$; Fig. 3A,). In sh-B, the decline was subtle and the difference was not statistically significant ($P = 0.096$; Fig. 3A).

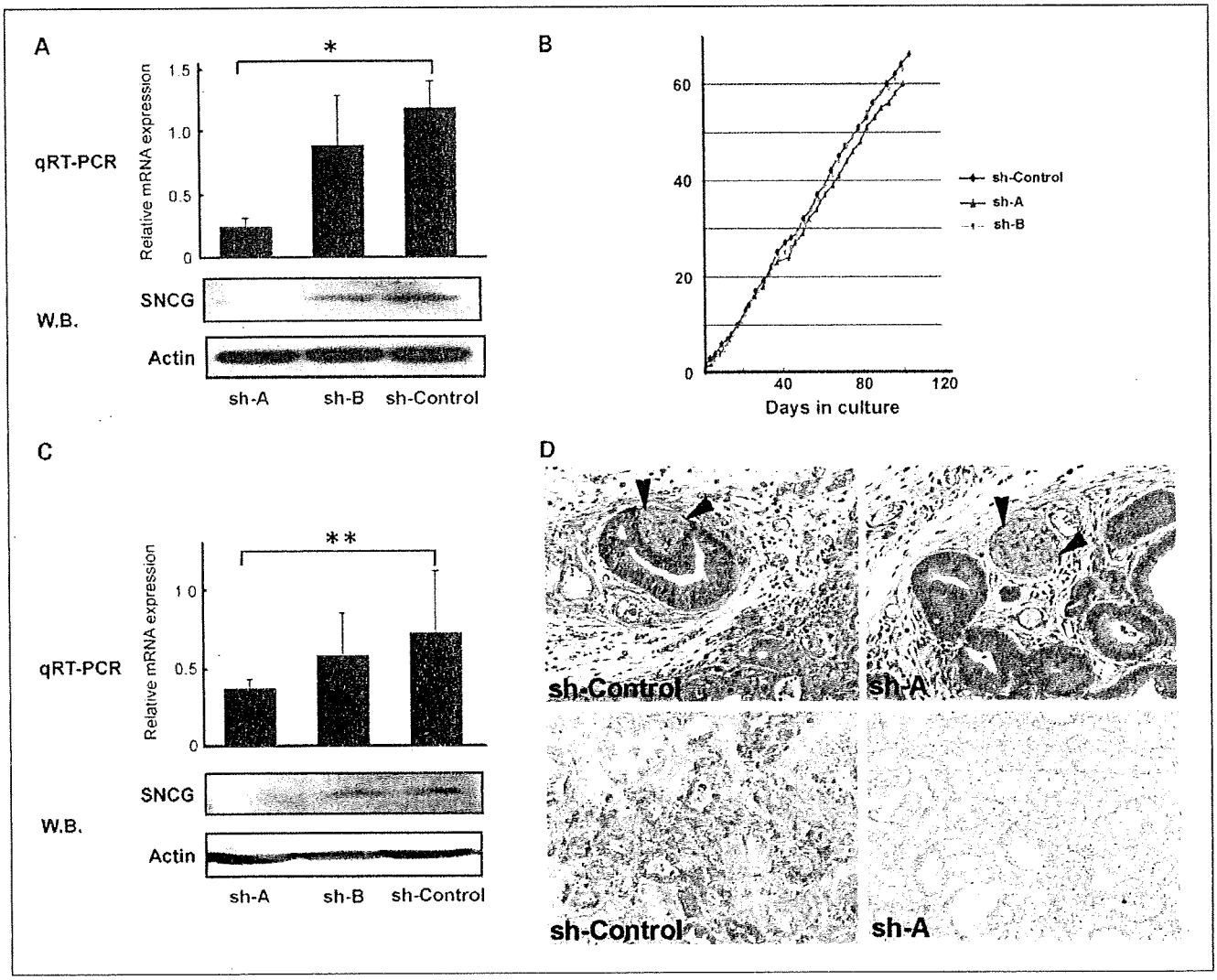

Fig. 3. *A*, quantitative reverse transcriptase-PCR analysis and Western blotting of SNCG in gene suppression study *in vitro* confirmed a substantial decrease in SNCG expression in sh-A compared with sh-Control. *, $P < 0.0001$. The difference was not statistically significant in sh-B ($P = 0.096$). *B, in vitro* growth curves of SNCG knockdown cells and control cells. The population doubling time of sh-A (*triangles*) was slightly extended compared with sh-Control (*diamonds*) and sh-B (*circles*). PD, population doubling time. *C*, quantitative reverse transcriptase-PCR and Western blotting of the s.c. tumors in mouse perineural invasion models revealed a stable *SNCG* gene knockdown effect in sh-A compared with sh-Control. **, $P = 0.018$. *D*, microscopic findings in mouse perineural invasion models. The tumor cells of sh-Control easily invaded the s.c. nerve (*arrowheads, top left plate*) and showed diffusely positive staining to SNCG (*bottom left plate*). In contrast, sh-A – derived tumors exhibited no perineural invasion, although the nerve (*arrowheads*) was involved within the tumor (i.e., nerve involvement, cancer nest includes nerves without direct contact between the tumor cells and the perineurium; *top right plate*). They were generally negative for SNCG (*bottom right plate*).

To determine whether SNCG suppression affects the *in vitro* growth of Capan-1 cells, cultures of sh-Control, sh-A, and sh-B were initially seeded at 1×10^5 cells/10-cm plate in 10 mL of RPMI 1640 serum. The population doubling time was roughly equivalent in the first 40 days in culture, although slight extension was observed in sh-A thereafter (Fig. 3B). Immunofluorescence analysis of sh-Control and sh-A under a confocal laser scanning microscope (LSM 510, Carl Zeiss) are shown in Supplementary Fig. S4A and B. In sh-Control, SNCG (*green*) diffusely localized to the cytoplasm, whereas in sh-A, the cytoplasmic SNCG signal was much lower, leaving only punctuate staining (Fig. 4A and B, respectively; Supplemental Data). No apparent difference was noted in cell shape and intercellular relations.

The results of mRNA assay and Western blotting of the s.c. tumors in the mouse perineural invasion model confirmed stable SNCG suppression in sh-A (Fig. 3C). In sh-B, the degree of down-regulation was mild, correlating with the *in vitro* results (Fig. 3A and C). In the mouse perineural invasion model, sh-A and -B showed significantly lower perineural invasion rates at 25% ($P = 0.009$) and 33% ($P = 0.026$), respectively, compared with the high perineural invasion incidence (82%) in sh-Control (Table 3). Tumor growth was also inhibited to some extent in the knockdown group, exhibiting a >25% size difference in sh-A ($P = 0.016$); however, the difference was not statistically significant in sh-B ($P = 0.27$; Table 3). A remarkable difference about the affinity of tumor cells to the mouse s.c. nerves was observed between sh-Control

and sh-A (Fig. 3D). The number of SNCG-positive cells was significantly smaller in sh-A (11% ± 11%; $P < 0.0001$) and sh-B (46% ± 12%; $P = 0.0005$) compared with sh-Control (65% ± 11%; Fig. 3D). In the invasive front of sh-Control–derived tumors, the cancer cells easily infiltrated into the muscle layers, whereas sh-A–derived tumors predominantly presented expansive growth (Supplementary Fig. S4C and D, respectively).

Because the clinicopathologic analysis of surgical cases suggest a strong correlation between SNCG overexpression and lymph node metastasis (Table 1), we constructed an orthotopic (pancreas) transplantation model to examine the SNCG knockdown effect on the metastatic potential of Capan-1 cells. In sh-A, the incidence of liver and lymph node metastasis remarkably decreased compared with sh-Control, developing in 0% (0 of 9; $P = 0.019$) and 22% (2 of 9; $P = 0.020$) of transplanted mice, respectively (Table 3). Although sh-B–derived tumors showed a mild reduction in the metastatic rate against sh-Control, the differences were not statistically significant (liver, $P = 0.28$; lymph node, $P = 0.51$). Representative microscopic findings of the liver and lymph node metastasis are shown in Supplementary Fig. S4E and F, respectively.

Discussion

This is the first study to provide *in vivo* evidence that SNCG is significantly correlated with perineural invasion as well as other major invasive parameters, including tumor size, vascular invasion, lymph node metastasis, and UICC stage, in patients with pancreatic cancer. The prognostic impact of SNCG overexpression was impressive; it was the only independent predictor of diminished overall survival and the strongest negative indicator of disease-free survival by multivariate analysis. Furthermore, SNCG gene silencing in mouse models of perineural invasion and orthotopic transplantation using human pancreatic cancer cell lines was associated with a dramatic reduction of perineural invasion as well as liver and lymph node metastasis, the main homing organs of pancreatic cancer cells. Our series shed light on the critical role of SNCG overexpression in acquiring invasive and metastatic properties.

SNCG has been shown to be involved in tumorigenesis and metastasis of a wide range of malignancies; nevertheless, only one report has documented SNCG overexpression in pancreatic cancer to date (16, 19–22). In breast cancer cell lines and mammary glands, chaperone-like activity of SNCG has been described (23, 24). SNCG may potentially exert various oncogenic roles in pancreatic cancer as a chaperone protein through stimulation of signal transduction pathways that regulate cell proliferation, invasion, and metastasis.

Zhu et al. (25) suggested that pancreatic cancer cells and nerves may interact in an autocrine/paracrine manner to provide microenvironment conducive for perineural invasion. Pancreatic cancers with overexpression of nerve growth factor in the cytoplasm of tumor cells and its high-affinity receptor, tyrosine kinase receptor A, in the perineurium of pancreatic nerves exhibited significantly higher perineural invasion rates and degree of pain. Meanwhile, in human pancreatic cancer cell lines, nerve growth factor–induced pancreatic cancer cell growth seems to be mediated by phosphorylation of tyrosine kinase receptor A and mitogen-activated protein kinase (26). Because SNCG overexpression was described to modulate mitogen-activated protein kinase pathways, leading to cell survival by inhibition of apoptotic activities (27), it may also promote nerve growth factor–tyrosine kinase receptor A signaling through its chaperone-like activity, contributing to perineural invasion in pancreatic cancer.

Previous studies have shown stage-specific SNCG up-regulation in advanced breast carcinomas and other malignancies, and our results are consistent with their observations (19–22). SNCG may be involved in pancreatic cancer progression by the induction of matrix metalloproteinases and its association in tumor cell-to-stroma interaction (28, 29). Meanwhile, SNCG was suggested to stimulate disassembly of neurofilament network and to interact with microtubule-associated proteins, thus influencing cytoskeletal integrity (30, 31). In our study, the mouse models apparently exhibited infiltrative and exaggerated growth of tumors with SNCG overexpression, although no obvious differences in cell proliferation or morphology were detected *in vitro* between cells overexpressing SNCG and knockdown cells. These findings indicate that SNCG overexpression may lead to a more malignant phenotype by altering the cell architecture, growth, and motility of pancreatic cancer cells as a chaperone protein in association with the tumor microenvironment.

Currently, proteomic analysis is emerging as a novel and powerful method of detecting proteins associated with pancreatic cancer progression (32, 33). We focused on the regulatory element of perineural invasion by comparing the proteomic profiles between high- and low-perineural invasion groups in human pancreatic cancer cell lines. Previously, proteomic

Table 3. Incidence of perineural invasion and tumor spread following s.c. injection and orthotopic transplantation of Capan-1: the impact of *SNCG* gene silencing by 2 different sequences

shRNAs	Perineural invasion model		Orthotopic transplantation model				
	Perineural invasion	Tumor size, mm	Ascites	Peritoneal dissemination	Distant metastasis		
					Liver	Lymph nodes	Lungs
sh-Control	9/11 (82%)	19 ± 5	7/7 (100%)	4/7 (57%)	4/7 (57%)	6/7 (86%)	0/7 (0%)
sh-A	3/12 (25%)*	14 ± 4†	4/9 (44%)†	1/9 (11%)	0/9 (0%)†	2/9 (22%)†	0/9 (0%)
sh-B	4/12 (33%)†	17 ± 4	7/7 (100%)	4/7 (57%)	2/7 (29%)	5/7 (71%)	0/7 (0%)

*$P < 0.01$ versus sh-Control.
†$P < 0.05$ versus sh-Control.

studies were used to differentiate protein expression profiles between pancreatic cancer tissues and normal or inflamed pancreas (32, 33), and our tumor phenotype-oriented approach may become a breakthrough to genetically tailored cancer diagnosis and therapy. In our series, 33% of patients with stage I disease showed SNCG overexpression. Several authors have detected SNCG in serum and urine samples of patients with malignant tumors (19, 20, 34) and SNCG as an indicator for early diagnosis warrants further investigation. Stratification of resected cases by SNCG status is worth considering to customize postoperative multidisciplinary approach.

In conclusion, this is the first report of *in vivo* evidence that SNCG overexpression is the key biological marker of increased malignant potential and is closely involved in perineural invasion and liver/lymph node metastasis in pancreatic cancer. In surgically resected cases, SNCG is a significant prognostic factor. SNCG may serve as a novel molecular target of early diagnosis as well as antimetastatic therapy.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

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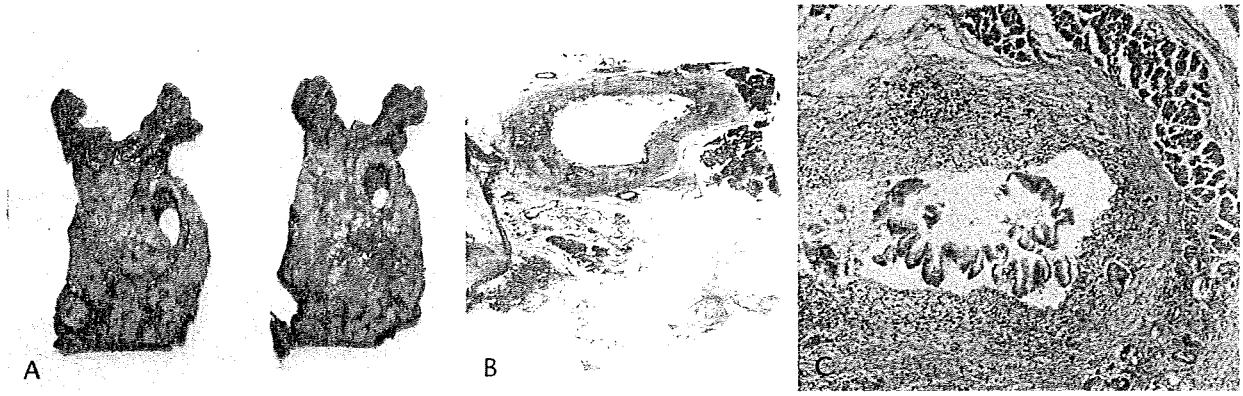

FIGURE 1. Resected specimen including pancreatic head (A). Pathological examination of the specimen showing sclerosing cholangitis of the intrapancreatic and extrapancreatic tracts of the CBD with mild to moderate dysplasia (B), lymphocytic and eosinophilic infiltrate, and multiple aspects of pancreatic intraepithelial neoplasia of 1-2-3 grades (C).

the gallbladder, and the CBD, were examined. Histopathological examination of the specimen showed sclerosing cholangitis of the intrapancreatic and extrapancreatic tracts of the CBD with mild to moderate dysplasia (Fig. 1B), eosinophilic enteritis, lymphoplasmacytic and eosinophilic infiltrate, and multiple aspects of pancreatic intraepithelial neoplasia of 1-2-3 grades (Fig. 1C). An eosinophilic infiltrate involving the gallbladder was also shown in the cholecystectomy specimen.

The main characteristics of AIP are as follows: (1) diffuse or segmental narrowing of the main pancreatic duct at imaging techniques, (2) increased levels of serum gamma-globulin or the presence of autoantibodies, and (3) the presence of fibrotic changes with lymphoplasmacytic cell infiltration around the main pancreatic duct. Diagnosis of autoimmune pancreatitis is established when criterion 1 together with criteria 2 and/or 3 are fulfilled. Clinically, however, the patient has a serrated stenosis of the third low of the CBD without evidence of major diagnostic criteria of AIP (preoperative imaging and presence of autoantibodies and hypergammaglobulinemia), and so we suspected a cancer of the lower bile duct. In addition, abnormal serum value of the tumor marker CA 19-9 was present. Even if it has been reported that approximately 50% of patients with autoimmune pancreatitis[6–8] may have abnormal serum levels of CA19-9, in our patient, the diagnosis of CBD cancer was made and we decided to operate him.

In conclusion, we have reported the first case of association of sclerosing cholangitis, autoimmune chronic pancreatitis, and situs viscerum inversus totalis.

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Slow Growth of Small Pancreatic Carcinoma With a 20-Month Follow-Up

To the Editor:

Invasive ductal adenocarcinoma of the pancreas has a poor prognosis mainly because of rapid progressive growth beyond the pancreas and a tendency to demonstrate early metastasis to the regional lymph nodes.[1] An evaluation of the growth rate of pancreatic carcinoma is essential to predict the efficacy of treatment and the prognosis. The growth rate of pancreatic carcinoma has been described in patients

with liver metastases or with far advanced stages, including unresectable patients.[2,3] However, there has so far been no report concerning the growth rate of small pancreatic cancers. In this report, we describe a small pancreatic cancer, which grew from 12 mm to 30 mm in maximum diameter serially measured by ultrasonography (US), with a follow-up period of 20 months.

CASE REPORT

A 63-year-old woman received an annual health checkup, without any specific symptoms, in February 2006. Ultrasonography (US) revealed a 12-mm hypoechoic mass in the pancreatic head, with no dilation of the main pancreatic duct (Fig. 1A). She had no history of diabetes mellitus or alcohol abuse. The physical examination was unremarkable. The serum levels of carcinoembryonic antigen and carbohydrate antigen 19-9 (CA19-9) were within the reference ranges. Computed tomography (CT) and magnetic resonance imaging demonstrated a well-demarcated and enhanced 12-mm tumor. Contrast-enhanced CT during the late phase showed a small hyperattenuating lesion in the pancreatic head (Fig. 1B). Under a provisional diagnosis of pancreatic head cancer or islet cell tumor, a pylorus-preserving pancreaticoduodenectomy was thus strongly recommended. However, the patient refused to undergo surgical treatment without a definitive diagnosis of malignancy. Consequently, a fine-needle aspiration biopsy was obtained, and the tumor was diagnosed as suggestive of an islet cell tumor.

During the follow-up, the tumor gradually increased in maximum diameter, measuring 30 mm with 5-mm dilation of the main pancreatic duct by US (Fig. 1C) in October 2007. The value of CA19-9 also increased to 113 U/mL in September 2007. The maximum diameter of the tumor by US (Fig. 2A) and the value of CA19-9 level (Fig. 2B) were serially measured at almost the same time. A mean growth rate of 0.9 mm per month was calculated. The tumor volume doubling time (TVDT)[4] and the CA19-9 doubling time were 152 and 276 days, respectively.

In October 2007, a pancreaticoduodenectomy was performed on the diagnosis of pancreatic head carcinoma because of the progressive tumor growth. The cut surface of the resected tumor was well demarcated and grayish-white. The macroscopic appearance was a nodular type, which histopathologically measured 18 mm × 18 mm × 15 mm in size (Fig. 2C). The TVDT based on the tumor diameter was histopathologically confirmed to be 341 days. Obstructive pancreatitis was observed just distal to the tumor. Most of the tumor was confined to the pancreas, but it slightly invaded the retroperitoneal fat tissue near the gastroduodenal artery, but no invasion of the bile duct, duodenum, and adjacent large vessels was observed. The pathological examination of the tumor revealed a well to moderately differentiated tubular adenocarcinoma of the pancreas (Fig. 2D). This carcinoma formed mainly irregular papillotubular patterns accompanied by a desmoplastic stroma. The morphological structures clearly demonstrated a typical ductal adenocarcinoma of the panccreas. There was slight lymphatic and intrapancreatic nerve invasion, but no venous invasion. Node involvement was absent in the 19 harvested lymph nodes, and all of the resection margins were negative.

The postoperative course was uneventful. The patient is doing well without recurrence at 3 months after the operation.

DISCUSSION

Even with a sophisticated imaging assessment, the precise preoperative

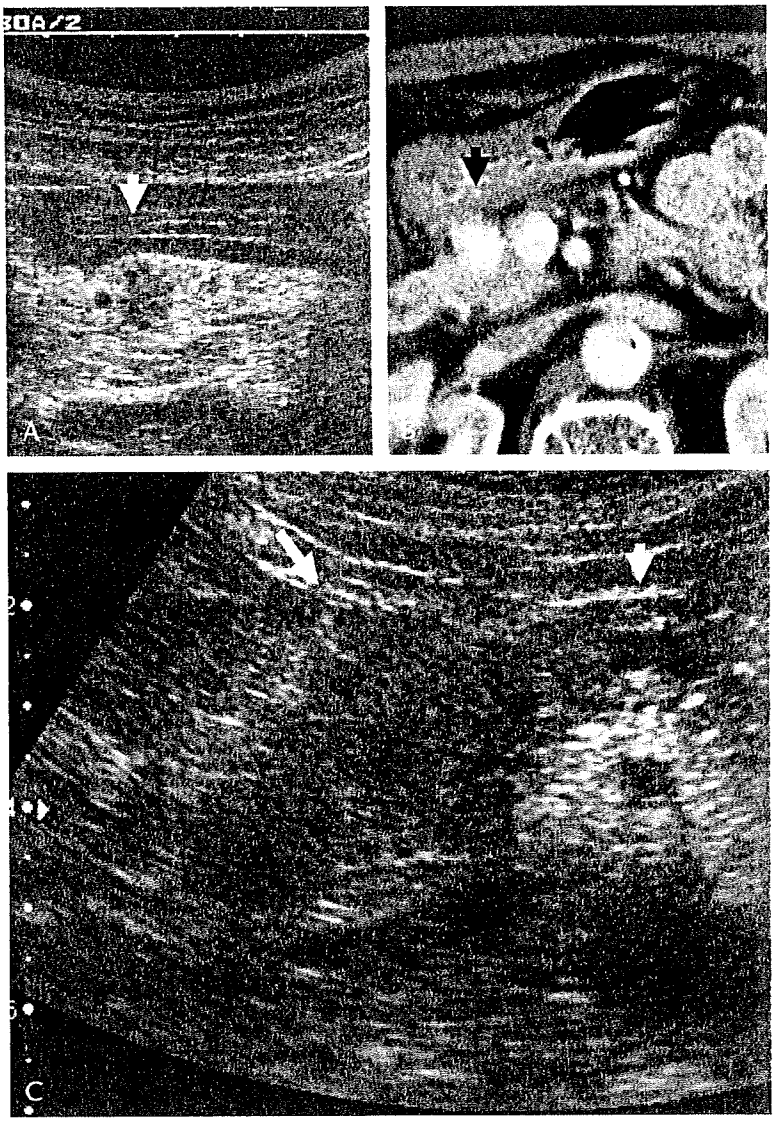

FIGURE 1. A, The US shows a small low-echoic mass measuring 12 mm in size. The white arrow indicates a small tumor. B, Enhanced CT in the late phase shows a round hyperattenuating lesion in the pancreatic head. The black arrow indicates a small tumor. C, The US shows a low-echoic mass 30 mm in diameter, with dilation of the main pancreatic duct. The white arrow indicates a tumor, and the black one the dilated main pancreatic duct.

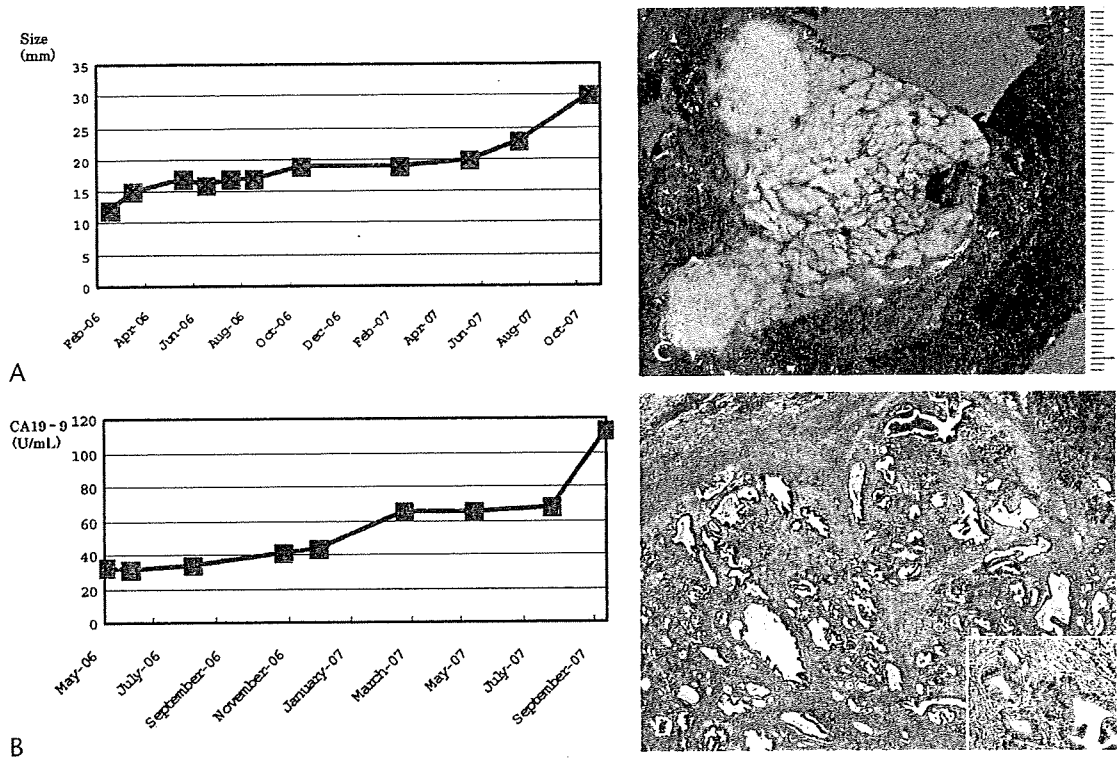

FIGURE 2. A, The maximum tumor size serially measured by US over time. B, The CA19-9 serially measured during the follow-up period. C, The macroscopic appearance shows a nodular-type tumor, histopathologically measured at 18 mm in maximum diameter. D, The histological appearance of the pancreatic lesion (hematoxylin-eosin, original magnification ×25). The morphological appearance is typical of pancreatic ductal carcinoma with irregular papillotubular patterns accompanied by a desmoplastic stroma. Inset: A higher magnification shows a well to moderately differentiated tubular adenocarcinoma of the pancreas (hematoxylin-eosin, original magnification ×100).

diagnosis of small pancreatic lesions has been challenging.[5,6] It has at times been difficult to obtain a cytological diagnosis using fine-needle biopsy in pancreatic lesions measuring less than 20 mm in diameter.[7] Fundamentally, a surgical resection should be recommended, but the present patient refused surgical treatment because of the lack of a definitive diagnosis. In the current case, the pancreatic tumor gradually increased from 12 mm up to 30 mm as measured by US, but the histopathologic measurement disclosed 18 mm in maximum diameter. The TVDT measured by US was 152 days, but that on the basis of the histopathologic measurement was 341 days. Furukawa et al[3] described the TVDT in 9 patients with primary pancreatic carcinoma to range from 64 to 255 days. This tumor, most of which was confined to the pancreas without nodal involvement, had a slow-growing nature, although the pathological findings were typical of pancreatic carcinoma. The mode of progression or growth rate of advanced pancreatic carcinomas,[2,3] intraductal papillary-mucinous tumor,[8] and other low-grade malignant lesions[9] have been previously described, but to our knowledge, the growth rate of small pancreatic carcinomas has not yet been reported. The measurement of the growth rate of a small pancreatic carcinoma might be extremely difficult because many patients with a tiny cancer have negative imaging findings, with only a dilation of the main pancreatic duct.[5]

A well-demarcated tumor with homogeneous vascularity is one of the major characteristics of the islet cell tumor.[6] On the contrary, the concomitant dilation of the main pancreatic duct is a principal finding in differentiating the small pancreatic cancer. Yang et al[6] revealed that 5patients (5/32, 15.6%) with pancreatic carcinoma showed a homogeneous enhancing pattern on contrast-enhanced US. Only 1 patient with an islet cell tumor located in the pancreatic head had ductal dilation. In the current case, imaging studies and fine-needle aspiration biopsy cytology could not provide a precise diagnosis. Regardless, a close follow-up study was indispensable when surgical treatment could not be pursued. We recommend such a patient to receive a US examination and a CA19-9 test at least every 2 to 3 months.

The precise tumor size measurement before resection is difficult because the boundary between the pancreatic carcinoma and the noncancerous parenchyma is usually irregular and indistinct because of the cancer's invasive growth. The tumor size measured by US was extensively larger than the tumor size confirmed by the histopathologic examination. The reason might be because of an inflammatory reaction around the tumor, with concomitant pancreatitis caused by the progressive increase of the tumor. Furukawa et al[10] recommended tumor size measurement by dynamic CT because there was a significant correlation between the tumor size as determined by dynamic CT and that on the histopathologic examination, especially in small tumors.

The current case disclosed an unexpected slow growth, but no specific clinical factors that could explain the slow growth of the tumor were observed besides a well-demarcated structure with a homogeneous hyperattenuating nature. The growth rate of small pancreatic carcinomas

is a great clinical concern because it is important to collect enough cases to evaluate the relationship between the growth speed of the small pancreatic carcinoma and the screening and diagnostic procedures, pathological factors, and prognosis.

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A Case of Squamoid Cyst of Pancreatic Ducts

To the Editor:

Detection of cystic lesions in the pancreas has increased because of the widespread use of high-resolution diagnostic imaging techniques. Therefore, cystic lesion of the pancreas constitutes an increasingly important category with a challenging differential diagnosis.[1] Squamoid cyst of pancreatic ducts is a recently recognized type of cystic lesion in the pancreas in which cystically dilated ducts are lined by nonkeratinized squamous epithelium.[2] Clinically, it is important to distinguish this generally benign cystic lesion from the potentially malignant mucinous cyst-forming neoplasias, especially mucinous cystic neoplasms (MCNs) and intraductal papillary mucinous neoplasms (IPMNs). We report the first case of squamoid cyst of pancreatic ducts in Japan.

CASE REPORT

An 80-year-old man underwent an abdominal computed tomography (CT) for routine follow-up postrectal cancer surgery; a 2.0-cm cystic lesion was detected in the body of the pancreas. At 16 months of follow-up, the size of the cystic lesion had increased to 3.0 cm and the patient was admitted to our hospital for further examinations. Inpatient evaluation revealed no symptoms related to the lesion. The patient's abdomen was soft and flat, and no pressure pain was observed. Complete blood count, electrolytes, and liver function tests were all normal. There were no data to indicate pancreatitis. Both serum carbohydrate antigen 19-9 and carcinoembryonic antigen levels were normal.

A contrast-enhanced multidetector-row CT showed a well-demarcated, low-attenuating cystic lesion in the body of the pancreas. The lesion measured 3.1 × 2.4 cm and had several small calcifications. There were no indications of invasion into the surrounding tissues or dilatation of the main pancreatic duct (Fig. 1A). Magnetic resonance cholangiopancreatography revealed a 3-cm round cystic lesion in the pancreatic body, which did not communicate with the main pancreatic duct. The main pancreatic duct was not dilated (Fig. 1B). Abdominal ultrasound (US) showed a cystic lesion with clear margins and a smooth surface that contained several echogenic solid processes and small intraluminal calcifications (Fig. 1C). Endoscopic US revealed that the cystic lesion was divided into several cysts by thin septa and that the lesion contained several 6- to 12-mm solid processes within the lumina of the cysts (Fig. 1D).

We diagnosed the cystic lesion as MCN and performed laparotomy. Intraoperatively, the cystic lesion, approximately 3 cm in diameter, was palpated arising from the body of the pancreas without any infiltration into the surrounding tissues. Most of the lesion was intrapancreatic, and its border was well defined. There was no peripancreatic lymph node swelling. Therefore, we performed a central pancreatectomy. The patient had an uneventful postoperative course and was discharged 17 days after surgery. The patient quickly returned to his normal activities.

Macroscopically, the cystic lesion of the pancreas (3.1 × 2.4 cm) was multilocular with thin septa. There were several elastic, hard, white lumps within the lumina, and the central portions of the relatively large lumps were particularly hard (Fig. 1E). The cystic lesion contained serous clear fluid. Histologic examination revealed that the cysts had variable linings ranging from flat squamoid cell to transitional, to stratified squamous without keratinization. No tall columnar mucinous cells were identified. No associated lymphoid or splenic tissue was present. The cyst walls were composed of relatively thin fibrous tissue. None of the lesions displayed ovarianlike stroma. The white

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SURGERY TODAY

Roux-en-Y Reconstruction Using Staplers During Pancreaticoduodenectomy: Results of a Prospective Preliminary Study

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Abstract

Purpose. The aim of this study was to reveal the utility of alimentary reconstruction using staplers during pancreaticoduodenectomy (PD), focusing on the occurrence of delayed gastric emptying.

Methods. Between 2003 and 2007, 72 PDs with alimentary reconstruction were performed by a single surgeon. Since August 2006, the new Roux-en-Y reconstruction methods using staplers were applied in 26 of the patients. We compared their clinical outcomes with those of the 46 patients who underwent PD using the conventional hand-sewn reconstruction methods.

Results. The results of upper gastrointestinal study showed improvement within 10 postoperative days (PODs; $P = 0.03$): the patients resumed eating their regular diet sooner (13 vs 6 days, $P < 0.001$), and both the incidence of delayed gastric emptying (43% vs 19%, $P = 0.04$) and the hospital stay (27 vs 21 days, $P = 0.008$) were reduced significantly in patients with stapled reconstruction. Despite the fact that operative costs were significantly higher for patients with stapled reconstruction ($P = 0.009$), hospital costs were significantly lower ($P = 0.049$) for those who underwent the conventional method.

Conclusions. Our retrospective analysis shows that stapled reconstructions might reduce the incidence of delayed gastric emptying; however, further study will be necessary to evaluate the utility of this new method.

Key words Pancreaticoduodenectomy · Delayed gastric emptying · Stapled reconstruction · Roux-en-Y reconstruction · Hospital stay

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Introduction

Alimentary reconstruction using staplers during gastric and colorectal surgery is widely accepted. The use of circular staplers in esophagojejunostomy is more convenient and safer than hand-sewn suturing.[1] Moreover, colorectal anastomoses using a double stapling technique have also become popular,[2] especially since the advent of laparoscopic surgery.[3] However, to our knowledge, mechanical reconstruction using staplers during pancreatectomy has never been documented.

One of the most common complications of pancreaticoduodenectomy (PD) is delayed gastric emptying (DGE), otherwise known as "gastroparesis,"[4] which is not fatal but results in prolonged hospital stay and increased hospital costs. Delayed gastric emptying is defined as nasogastric decompression after postoperative day (POD) 10 or a failure to tolerate a regular diet after POD 14. The incidence of DGE has been reported to range from 5% to 72%.[5–13] We hypothesized that the hand-sewn, two-layered, or continuous suture could induce anastomotic edema, which is one of the causes of DGE. Mechanical alimentary reconstruction can prevent anastomotic edema and may keep the oral intake stable. Therefore, in August 2006, we introduced a new Roux-en-Y reconstruction method, which uses circular or linear staplers during PD. We report the preliminary results of our new method.

Patients and Methods

Between August 1, 2003 and September 30, 2007, 302 patients underwent PD in our institute. These operations were performed by one or more of five surgeons, so to maintain consistency we evaluated the surgical outcomes of the 76 PDs performed by a single surgeon (Y.S.). Between August 2003 and July 2006, 50 patients underwent PD with alimentary reconstruction using the

conventional hand-sewn method. The new stapled Roux-en-Y reconstruction method was introduced in August 2006, and 26 patients underwent mechanical reconstruction in the final year of the study. Among the 50 patients operated on during the former 3 years, the following four patients were excluded from the analysis: two who had undergone previous gastrojejunostomy, one who had undergone previous total gastrectomy, and one who underwent PD concomitant with total gastrectomy.

The underlying diseases were as follows: invasive pancreatic cancer in 41 patients, bile duct cancer in 12 patients, ampullary cancer in 7 patients, intraductal papillary mucinous tumor in 3 patients, neuroendocrine tumor in 2 patients, metastatic cancers in 2 patients, duodenal cancer in 1 patient, and other noncancerous diseases in 4 patients. The surgical procedures consisted of standard Whipple procedure (SW) in 22 patients and pylorus-preserving pancreaticoduodenectomy (PPPD) in 50 patients. Twenty patients (28%) underwent combined portal vein resection. One patient underwent concomitant extended right hemihepatectomy, and another underwent concomitant distal pancreatectomy.

The surgical outcomes of PD, including the occurrence of DGE and other surgical complications, were compared between the 46 patients who underwent PD using conventional reconstruction and the 26 patients who underwent the new Roux-en-Y stapled reconstruction. The backgrounds and surgical procedures of each group are summarized in Table 1.

Surgical Technique of PD

The details of the standard procedure for PD have been described elsewhere.[14] After removal of the pancreatic head, we wrapped the stump of the gastroduodenal artery using the falciform ligament to prevent the bleeding caused by pancreatic leakage.[15] A jejunal loop was lifted and pancreaticojejunostomy was performed by duct-to-duct anastomosis using 5-0 polydioxanone (PDS). The anterior and posterior pancreatic walls were tightly affixed to the jejunal serosa using interrupted 4-0 PDS sutures. A hepaticojejunostomy was then done using interrupted 5-0 PDS sutures.

Conventional Reconstruction

In the 46 conventional PDs, we performed an antecolic gastrojejunostomy and duodenojejunostomy during the standard Whipple procedure (SW, $n = 16$) and pylorus-preserving pancreaticoduodenectomy (PPPD, $n = 30$), respectively. These anastomoses were done on antecolic routes, by the Albert–Lembert ($n = 38$), layer to layer ($n = 6$), or Gambee ($n = 2$) methods. A Braun jejunojejunostomy was done to prevent direct exposure of the anastomotic site to pancreatic and bile juice. Gastric tubes ($n = 43$) and jejunal feeding tubes ($n = 45$) were pulled out through the afferent loop between the duodeno- or gastrojejunostomy and Braun anastomosis. External drainage of the pancreatic and biliary ducts was performed in all of the patients.

Roux-en-Y Reconstruction Using a Circular Stapler During Pylorus-Preserving Pancreaticoduodenectomy (PPPD)

An antecolic duodenojejunostomy was performed by Roux-en-Y reconstruction using a circular stapler in 20 PPPDs (Proximate ILS 29 or 25 mm, Ethicon Endo-Surgery, Cincinnati, OH [$n = 19$], EEA circular stapler,

Table 1. Clinical characteristics of patients who underwent conventional versus Roux-en-Y stapled reconstruction

	Conventional ($n = 46$)	Roux-en-Y, stapled ($n = 26$)	*P* value
Sex			
Male	29	15	0.66
Female	17	11	
Age (years)	68 (18–82)	66 (47–80)	0.80
Body mass index	21 (15–28)	22 (17–27)	0.25
Diseases			
Pancreatic cancer	26	15	0.81
Bile duct cancer	7	5	
Vater or duodenal cancer	7	2	
Others	6	4	
Procedure			
SW	16	6	0.30
PPPD	30	20	
Portal vein resection			
Performed	14 (30%)	6 (23%)	0.50
Operative time (min)	560 (400–842)	570 (374–790)	0.77
Blood loss (ml)	850 (215–2360)	710 (130–2420)	0.19

SW, standard Whipple procedure; PPPD, pylorus-preserving pancreaticoduodenectomy

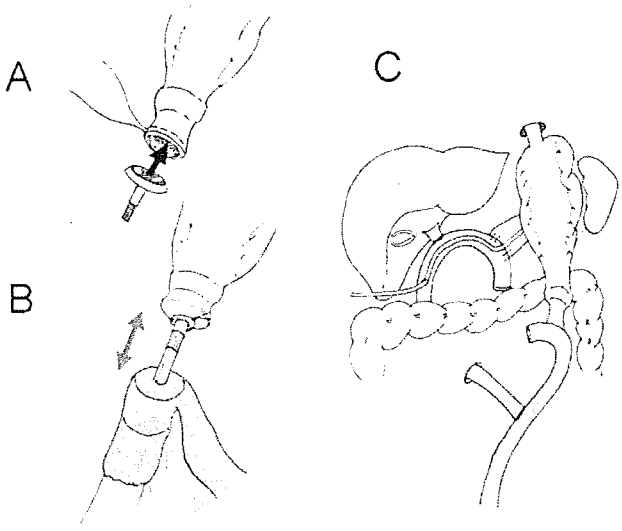

Fig. 1A–C. Schematic illustration of Roux-en-Y duodenojejunostomy using a circular stapler. **A** A purse-string suture was placed around the duodenal stump, and an anvil was placed within. **B** Duodenojejunostomy was accomplished using a circular stapler. **C** Pancreaticojejunostomy, hepaticojejunostomy, and Roux-en-Y duodenojejunostomy with an external pancreatic drainage

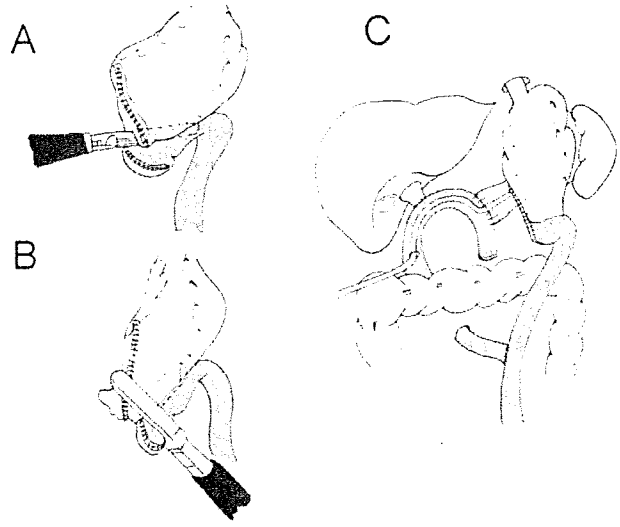

Fig. 2A–C. Schematic illustration of Roux-en-Y gastrojejunostomy using linear staplers. **A** Gastrojejunostomy using a linear stapler. A side-to-side anastomosis was made between the posterior wall of the stomach and the side wall of the jejunum. **B** The edges of the stomach and the jejunal loop were divided using a linear stapler. **C** Pancreaticojejunostomy, hepaticojejunostomy, and Roux-en-Y gastrojejunostomy with an external pancreatic drainage

28 mm, US Surgical, Norwalk, CT [n = 1]) (Fig. 1). The duodenum was divided 3–4 cm anal of the pylorus ring. A segmental jejunum was sacrificed and resected, preserving the mesojejunum, and the anal jejunum was used for the duodenojejunostomy. An anvil device was inserted into the duodenal stump, a circular stapler was inserted into the jejunal loop, and the mechanical anastomosis was completed. The stump of the jejunal loop was closed using a linear stapler.

Roux-en-Y Reconstruction Using a Linear Stapler During Standard Whipple Procedure (SW)

In four SWs an antecolic gastrojejunostomy was performed by Roux-en-Y reconstruction using a linear stapler (Endo-GIA Reticulator 60, US Surgical) (Fig. 2). A segmental jejunum was removed, preserving the mesojejunum, and the anal jejunum was used for the gastrojejunostomy. A small incision was made on the posterior wall of the stomach, near the stump on the greater curvature. A linear stapler was inserted into the remnant stomach and the jejunum to adjust the posterior wall of the stomach and the side wall of the jejunum. A mechanical anastomosis was made, and the stumps of the stomach and the jejunum were closed using another linear stapler.

In the remaining two PDs, a circular stapler was used to make a gastrojejunostomy on the posterior wall of

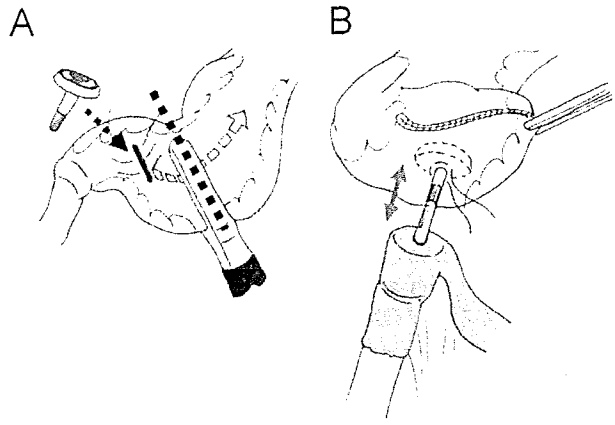

Fig. 3A,B. Schematic illustration of Roux-en-Y gastrojejunostomy using a circular stapler. **A** The anvil was inserted through a small incision in the antrum into the fornix of the stomach. The incision was closed with interrupted sutures. **B** The handle of the anvil was pulled out though the posterior wall of the stomach, and the root of the handle was closed using a purse-string suture. Thereafter, a gastrojejunostomy was accomplished using the circular stapler

the stomach (Fig. 3). Before dividing the stomach during SW the anvil was inserted into the fornix of stomach through a small incision in the antrum, which was then sutured. The handle of the anvil was pulled out of the posterior wall of the remnant stomach, and a mechani-

cal anastomosis was completed. The stump of the jejunum was closed using another linear stapler.

In both the mechanical Roux-en-Y reconstructions during PPPD and SW, the anal jejunojejunostomy was created by serosa-to-serosa continuous suture. Gastrostomy was not done in any of the patients. In the pancreaticojejunostomy, an internal stent (n = 5) or an external drainage tube (n = 19) was placed in 24 patients. In the hepaticojejunostomy, an internal stent (n = 16) or external drainage tube (n = 2) was placed in 18 patients. In 23 patients a jejunal drainage tube (n = 8) to decompress the anastomotic loop or a jejunal feeding tube (n = 15) was inserted through the right-sided jejunal loop. The nasogastric tube was removed on POD 1 in all 26 patients. Two closed drains were inserted beside the pancreaticojejunostomy, and intermittent suction was applied 24 h a day for 4–6 days in the former term. In the latter 2 years, intermittent suction was not used. Patients were discharged home when they were able to resume eating about half the amount of their regular diet and had only minimal output from one abdominal drain.

Definition of Outcome Measures

The results of the upper gastrointestinal (UGI) study were evaluated within 10 PODs according to the degree of passage of the contrast medium. Grade A showed good passage of the medium without any dilatation of the stomach; grade B showed mild dilatation of the remnant stomach or formation of the niveau in the stomach, and passage of the medium could be maintained when the patient leaned forward; and grade C showed severe dilatation of the remnant stomach or no passage of the contrast medium.

Delayed gastric emptying was defined as failure to start a regular diet within 14 PODs according to previous reports.[5,6,9–11,13] Postoperative pancreatic fistula was graded according to the definitions proposed by an international study group on pancreatic fistula,[16] namely, when the amylase concentration of the drain fluid obtained on, or after POD 3 was greater than three times the serum amylase concentration. Pancreatic fistulas were classified into grades A, B, and C according to severity; grade A was a "transient fistula," not associated with a delay in hospital discharge; a grade B fistula led to a delay in discharge, with persistent drainage for more than 3 weeks; and a grade C fistula was usually associated with major complications. Grades B and C fistula were considered to be major complications.

Comparison of the Two Reconstruction Methods

We compared the operative times, blood loss, results of the UGI study, morbidity and mortality, and operative and hospital costs between the patients who underwent conventional reconstruction and those who underwent the stapled Roux-en-Y reconstruction.

Statistical Analysis

Statistical analysis was done using the chi-square test or Fisher's exact test for univariate analysis. We used the Mann–Whitney U-test to compare the variables between the two groups. Data are expressed as medians and ranges. A P value of less than 0.05 was considered significant.

Results

There was no in-hospital mortality (0%) and the overall morbidity rate was 63%. Pancreatic fistula developed in 33 (46%) patients (grade A in 12, grade B in 20, and grade C in 1) and was clinically significant in 29%. Twenty-five (35%) patients suffered DGE. Major morbidity included grade C pancreatic fistula in one patient, who suffered sepsis and required percutaneous drainage of the abdominal fluid under computed tomography (CT) guidance. Another major morbidity was anastomotic bleeding after stapled reconstruction, which resulted in shock status of the patient on POD 16. Three other patients suffered bleeding from the stapled anastomotic site: on POD 1 in one and on POD 2 in two. The bleeding was indicated by decreased hematocrit levels as the patients' general condition was stable. All three underwent endoscopic clipping of the bleeding points, and recovered conservatively. None of the patients suffered arterial bleeding associated with the pancreatic fistula.

There were no remarkable differences in the patients' backgrounds, operative parameters, incidence of pancreatic fistula, or overall morbidity rates between the patients who underwent conventional reconstruction and those who underwent Roux-en-Y stapled reconstruction (Table 1). Gastrostomy was not done in the stapled reconstruction group patients, but none of these patients required reinsertion of a nasogastric tube. The results of the UGI study were significantly better in the 26 patients with stapled reconstruction than in the 43 patients who underwent conventional reconstruction (P = 0.03). The exception was the median duration between surgery and the UGI study, which was significantly longer in the conventional reconstruction group than in the stapled reconstruction group (6 days vs 5 days, $P < 0.001$). The duration between surgery and start of oral intake, the incidence of DGE, and the hospital stay were significantly reduced in the stapled reconstruction group vs. the conventional reconstruction group ($P <$ 0.001, P = 0.04, and P = 0.008, respectively). The opera-

Table 2. Operative outcomes of patients who underwent conventional reconstruction versus Roux-en-Y stapled reconstruction

	Conventional ($n = 46$)	Roux-en-Y, stapled ($n = 26$)	P value
Removal of nasogastric tube or closure of gastrostomy (POD)	6	1	<0.001*
Results of UGI study ≤10 PODs			
Grade A	22	20	0.03*
Grade B or C	21	6	
Day of UGI study	6 (4–10)	5 (3–9)	<0.001*
Resumption of regular diet (POD)	13 (5–62)	6 (4–20)	<0.001*
DGE			
Absent	26	21	0.04*
Present	20	5	
Pancreatic fistula			
None, Grade A	34	17	0.44
Grade B or C	12	9	
Placement of drains	13 (6–72)	11 (2–65)	0.08
Minor complications	27	16	0.81
Major complications	1	1	0.68
Hospital stay (days)	27 (14–89)	21 (10–37)	0.008*
Operative costs ($)	8000	8700	0.009*
Hospital costs ($)	18000	17000	0.049*
Mortality	0	0	ND

UGI, upper gastrointestinal; POD, postoperative days; DGE, delayed gastric emptying; ND, not determined
*$P < 0.05$

tive costs were US$700 higher ($P = 0.009$), but the hospital cost was significantly ($1000) lower ($P = 0.049$) in the stapled reconstruction group than in the conventional group (Table 2).

Discussion

The findings of the present study suggest that the new Roux-en-Y reconstruction, using circular or linear staplers during PD, might improve the early passage of duodenojejunostomy and gastrojejunostomy, and thereby reduce the incidence of DGE. The stapled anastomosis felt very neat to the touch, which might contribute to preventing postoperative anastomotic edema. In addition, Roux-en-Y reconstruction would eradicate bile reflux and position the stomach tube vertically; thereby potentially assisting in advancing the stomach contents.[11] The incidence of anastomotic bleeding in this initial study (15%) was unexpectedly higher than that after gastric or colorectal surgery. Anastomotic bleeding was a major drawback in stapled reconstruction, so we strongly recommend confirming hemostasis of the anastomotic site via the jejunal loop and administering proton pump inhibitors postoperatively. Furthermore, we hope that the quality of staplers will improve in the future.

The passage of contrast medium in the UGI study and the recommencement of oral intake were improved significantly ($P = 0.03$ and < 0.001, respectively) and the incidence of DGE was reduced significantly ($P = 0.04$) by the introduction of stapled reconstruction (Table 2). Delayed gastric emptying has been reported to be affected by several other factors including gastric dysrhythmias due to intra-abdominal complications,[5,6] gastric atony after duodenal resection in response to the reduction of motilin,[7,17] pylorospasm secondary to vagotomy,[8] and angulation of the reconstructed alimentary tract.[18] A prospective randomized trial showed that erythromycin,[7] cyclic enteral feeding,[9] and antecolic reconstruction[10] all reduced DGE, whereas a retrospective study showed that ante-mesenteric reconstruction,[6] vertical reconstruction,[11] and straight-lined antecolic reconstruction[12] improved the oral intake. However, to our knowledge, the present study is the first to show the possible advantages of stapled Roux-en-Y reconstruction during PD.

This study is a retrospective analysis of one surgeon's experience. During the initial 3 years, oral intake was resumed very late, partly because we feared early bleeding, which might evoke pancreatic fistula. Since the introduction of stapled reconstruction in August 2006, the incidence of pancreatic fistula has been gradually decreasing, probably as a result of the improved surgical skill in pancreaticojejunostomy during PD. Fortunately, since the introduction of internal stents in pancreaticojejunostomy and in hepaticojejunostomy, external drainage is no longer needed. This may contribute to early discharge from hospital. Gastrostomy was placed in 97% of the patients who underwent the conventional

method, but it was not required in any of those who underwent stapled reconstruction. This strongly affected the duration of gastric decompression. The difference in hospital stay between the two groups may be reflected not only by the methods of alimentary reconstruction, but also by the placement of drainage or feeding tubes, and management of drainage tubes. A multi-institutional prospective randomized trial will be necessary to evaluate the efficiency of this stapled reconstruction during PD.

The possible advantages of the Roux-en-Y stapled reconstruction we described are as follows: standardization of reconstruction, irrespective of the attending surgeon; easy reconstruction; possible prevention of anastomotic edema and subsequent stricture, brought about by continuous two-layer anastomosis; and a complete diversion of the bile and pancreatic juice from the anastomosis. On the other hand, its disadvantages are as follows: higher cost; a risk of bleeding at the anastomotic site; and mass production of industrial waste. It is noteworthy that the operative costs were higher in the stapled group, but the overall hospital costs were higher in the conventional method group. This is most likely due to the reduced costs for hospital stay and nutritional support required in the stapled group.

In conclusion, our retrospective analysis shows that this new method of Roux-en-Y reconstruction using staplers during PD might reduce the incidence of delayed gastric emptying vs. conventional hand-sewn reconstruction. However, this study is a historical cohort analysis of one surgeon's experience. The improvement in the surgeon's skills, the change in drainage-tube placement, and the early return to a normal diet may have created bias. Thus, further study will be necessary to evaluate the utility of this new method.

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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.

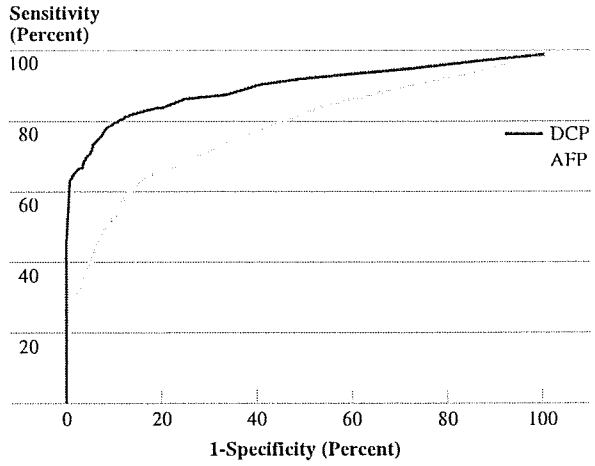

FIG. 1 ROC curves for AFP and DCP. The yellow line represents AFP and the blue line represents DCP. The AUROC (95% CI) for AFP and DCP were 0.79 (0.76–0.81) and 0.91 (0.89–0.92), respectively ($P < 0.001$)

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

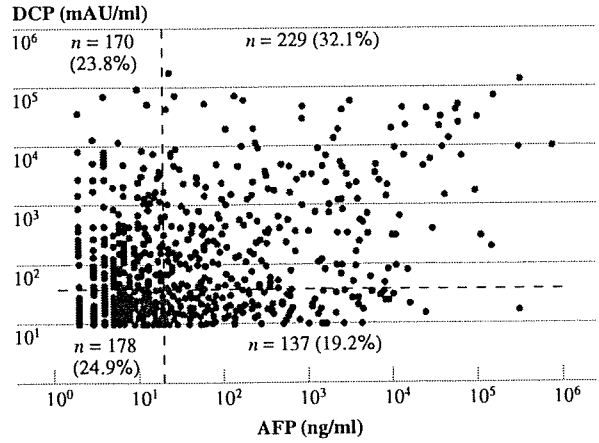

FIG. 2 Correlation between AFP and DCP values in 714 patients. No correlation was found between the two markers ($rs = 0.23$, $P < 0.0001$). Dotted line represents cutoff values, i.e., 20 ng/ml for AFP and 40 mAU/ml for DCP. Patients were placed into four categories: either positive or negative for AFP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