level 2a and three of 12 patients at dose level 2b having grade 3 or 4 neutropenia. On the other hand, the incidence of gastrointestinal toxicity during the first cycle and all cycles was low. Only one patient at dose level 2a had grade 4 anorexia and grade 3 nausea, one patient at dose level 2b had grade 3 anorexia.

A median number of 10 cycles were administered at dose level 1, seven cycles at dose level 2a, and four cycles at dose level 2b. However, there was no significant difference among the median number of administered cycles at every dose level. During all treatment cycles in this study, the incidence of grade 3 or 4 neutropenia at dose level 2b was 10%, at dose level 1 it was 19%, and at dose level 2a it was 33%. Consequently, only six of 61 cycles at dose level 2b needed a dose reduction of gemcitabine compared to 31 of 66 cycles at dose level 2a, which required that.

The first course of chemotherapy was conducted by hospitalisation for all patients, but the second or subsequent courses could be performed at an outpatient clinic for 19 of 21 patients. The other two patients showed early progression of the disease. Moreover, oral administration of S-1, which eliminates the cost and inconveniences of infusion pumps and catheters with their potential risks of infection and thrombosis, also contributes to fewer hospital visits during this outpatient treatment. Anticancer treatment for APC would be preferable on an outpatient rather than an inpatient basis, given the short life expectancy and quality of life considerations. In treatment for patients with APC, it is important to not only improve the prognosis of APC but also create a feasible regimen of chemotherapy that does not require hospitalization. These results indicated that the combination at the RDs selected in this study is quite feasible in the outpatient treatment setting.

In conclusion, this combination chemotherapy with gemcitabine and S-1 was well tolerated. Although this trial was only a phase I study to determine the RD and feasibility of such combination, an encouragingly high response rate has been observed. This result is very promising, but the survival benefit in comparison with gemcitabine monotherapy needs to be confirmed in future studies.

Clinical Studies

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Evaluation of vascular signal in pancreatic ductal carcinoma using contrast enhanced ultrasonography: effect of systemic chemotherapy

Evaluation of the effect of chemotherapy for pancreatic ductal cancer (PC) is generally conducted based on changes in tumour diameter using imaging modalities; however, exact measurement is often difficult because of local inflammation, fibrotic change, and desmoplastic reaction to treatment, leading to an unreliable evaluation.[1 2] PC is considered a hypovascular tumour. However, newly developed highly sensitive ultrasonic equipment has enabled the detection of vascular signals in PC; vascular signals were detected in 20–67% of cases.[3–7] We focused on changes in tumour vascularity of PC associated with chemotherapy, and attempted to apply it to evaluation of the effect of treatment and usefulness in relation to prognosis. In this study, we assessed vascular images of the tumour based on the Doppler signal (v signal) using contrast enhanced ultrasonography (CEUS).

Thirty one histopathologically confirmed consecutive patients with PC who had distant metastases were included in the study. Informed consent was obtained from all patients and the study was approved by the ethics committee. The tumour was located in the head of the pancreas in 16 patients and in the body or tail in 15. All patients were treated with a combination of S-1, an oral fluorinated pyrimidine derivative, and gemcitabine. Chemotherapy was performed every three weeks as one cycle. CEUS was performed before and after one and two cycles of treatment using a SSA-770A (Toshiba Co. Ltd, Tokyo, Japan) and a 3.75 MHz convex probe. CEUS images were obtained by Advanced Dynamic Flow mode, which is wideband Doppler sonography with a high sensitivity and resolution. The contrast agent was Levovist (SHU 508 A; Schering AG, Berlin, Germany), which was administered at a concentration of 300 mg/ml by intravenous injection of 8 ml at 1 ml/s. After injection, v signals in the tumour of the pancreas were continuously observed for 120 seconds. CEUS images showing the highest intensity of the vascular signal were selected and classified into five categories according to intensity: no signal (grade 0), spotty signals (grade 1), linear signals between grades 1 and 3 (grade 2), mosaic pattern signals (grade 3), and diffuse pattern signals (grade 4). Dynamic computed tomography (CT) was performed with a helical CT scanner (Light Speed Ultra, GE Medical Systems) which was performed every two cycles. In this study, treatment effect after two cycles of chemotherapy was examined.

The response to treatment, as determined by dynamic CT after two cycles of treatment, was as follows: partial response (PR) in five patients (16%), stable disease (SD) in 17 (55%), and progressive disease (PD) in nine (29%). A significant decrease in the v signal score was observed in PR compared with SD or PD after one cycle of treatment ($p = 0.0009$ and $p = 0.0017$, respectively). After two cycles of treatment, the decrease was conspicuous in PR ($p = 0.0022$ and $p = 0.0021$, respectively) whereas in PD a significant increase in the v signal score was observed compared with SD ($P = 0.0160$). In univariate analysis, the increase in v signal (before the second cycle) was a significant prognostic factor ($p = 0.0150$). Median survival time of patients in the non-increased v signal group ($n = 22$) after two cycles of treatment was 382 days (71–484) and for those in the increased group ($n = 9$), 176 days (68–257). Thus patients in the increased group had a significantly shorter survival than those in the non-increased group ($p = 0.0094$) (fig 1).

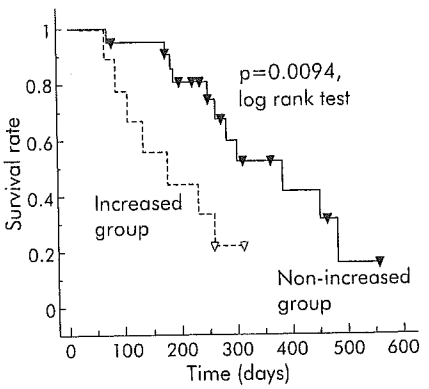

Figure 1 Cumulative survival rate according to changes in the v signal score after two cycles of treatment. Median survival time (MST) of patients in the non-increased v signal group ($n = 22$) was 382 days (range 71–484) and for those in the increased group ($n = 9$), 176 days (range 68–257). MST in the increased group was significantly shorter compared with the non-increased group (log rank test; $p = 0.0094$).

In conclusion, analysis of tumour vascularity by CEUS evaluated the effect of treatment much earlier than dynamic CT, and predicted prognosis in patients with PC.

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Smoking status in therapeutic trials in Crohn's disease

We were interested to hear the results of a number of trials of novel therapies for Crohn's disease (CD) that were presented at the 12th UEGW and reported in abstract form in *Gut*.[1–6] Many of the studies were randomised controlled trials in which the active and control groups were reported to have identical baseline characteristics. However, in all of the studies that were reported there was no mention of the smoking status of the participants, consistent with recent therapeutic trials in CD published in high profile journals.[7 8] Smoking is a well documented and universally recognised risk factor for increased CD severity as smokers are more likely to relapse and require corticosteroids, immunosuppressants, and surgery.[9 10] Furthermore, smokers are more likely to have a less favourable response to infliximab.[11] Smoking status is therefore a potential confounding factor in therapeutic trials in Crohn's disease. We urge investigators to include smoking status in the abstract, text, and analyses of all therapeutic trials of CD. Furthermore, we believe that stratification for smoking should be included at the planning stage for all randomised controlled trials in CD. Investigators may wish to re-analyse published data to ensure that results have not been confounded by smoking.

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Pancreatic Juice Cytology in the Diagnosis of Intraductal Papillary Mucinous Neoplasm of the Pancreas

Significance of Sampling by Peroral Pancreatoscopy

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BACKGROUND. The examination of pancreatic juice cytology could hypothetically contribute to the establishment of a definite diagnosis of malignant intraductal papillary mucinous neoplasm of the pancreas (IPMN), but to the authors' knowledge, its significance has not been confirmed to date. The current study was conducted to assess the diagnostic value of pancreatic juice cytology in IPMN and to examine the usefulness of peroral pancreatoscopy (POPS) in sampling pancreatic juice.

METHODS. The study subjects were comprised of 103 patients with IPMN who underwent surgical resection of pancreatic tumors (adenoma in29 patients, borderline in17 patients, carcinoma in situ in 25 patients, and invasive carcinoma in 32 patients). Pancreatic juice was collected with a catheter in 71 patients and by POPS in 32 patients. Patients with pancreatic carcinoma (n = 81) and chronic pancreatitis (n = 76) also were investigated.

RESULTS. The cytologic diagnosis was found to be of nondiagnostic value in only one patient with an IPMN, whereas it was of no diagnostic value in 14 of the patients with pancreatic carcinoma (17.3%), a difference that was statically significant ($P < 0.001$). The location of the IPMN (either in the pancreas or the pancreatic ducts) was not found to significantly affect the diagnostic value of the test. The sensitivity for IPMN was 62.2% when pancreatic juice was collected by POPS, and was 38.2% when it was collected using a catheter. In the case of pancreatic carcinoma, the sensitivity of pancreatic juice cytology was found to be 25.4%, which was significantly lower than that for IPMN when the pancreatic juice was collected by POPS ($P < 0.001$).

CONCLUSIONS. Pancreatic juice cytology was found to have better diagnostic value in the patients with IPMNs compared with those with pancreatic carcinoma. POPS was found to be useful for the collection of pancreatic juice. ***Cancer* 2005;104: 2830–6.** © *2005 American Cancer Society.*

KEYWORDS: pancreatic juice cytology, intraductal papillary mucinous neoplasm (IPMN) of the pancreas, pancreatic carcinoma, chronic pancreatitis, peroral pancreatoscopy.

Intraductal papillary mucinous neoplasms (IPMNs) of the pancreas are often difficult to diagnose even after the exclusion of benign and malignant tumors based on findings obtained with different imaging techniques. Therefore, it is not easy to determine the best treatment option.[1–3] There have been various attempts to overcome this situation by investigating the pancreatic juice: examination of its cytology,[4,5] analysis of K-*ras* gene mutations,[6] or telomerase activity.[7] Of these,

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cytology plays a central role in the evaluation of IPMN and there have been some very promising reports,[4,8] but unfortunately, these were without confirmation in a large number of patients. Therefore, to our knowledge, the usefulness of pancreatic juice cytology for the differential diagnosis of benign IPMNs from malignant ones has not been clarified as yet.

Peroral pancreatoscopy (POPS) has been reported to be useful for the differential diagnosis between benign IPMNs and malignant ones.[9] POPS allows a direct view of the lesion and the sampling of relatively large amounts of pancreatic juice from a site close to the lesion. Uehara et al. reported the high diagnostic value of the cytologic examination of pancreatic juice collected by POPS in the case of pancreatic ductal carcinoma,[10] but to our knowledge there have been no reports concerning the usefulness of this method in the diagnosis of IPMN.

The objective of the current study was to evaluate the usefulness of pancreatic juice cytology in the diagnosis of IPMN in a large number of patients, with special reference to the role of pancreatic juice collected by POPS. In addition, the cytology results in patients with an IPMN were compared with those from patients with ordinary pancreatic carcinoma and chronic pancreatitis.

MATERIALS AND METHODS

The subjects were 103 consecutive patients with IPMN who underwent surgical resection between May 1989 and December 2004. They were 72 men and 31 women, with a mean age of 63.3 years (range, 37–86 yrs). The tumor was located in the head of the pancreas in 76 patients and in the body and/or tail of the pancreas in 27. The tumor was located in the main pancreatic duct (MPD) in 27 patients (26.2%) and in a branch duct (BD) in 76 patients (73.8%). Based on World Health Organization (WHO) criteria,[11] IPMNs were histopathologically diagnosed as adenoma in 29 patients, borderline in 17 patients, carcinoma in situ (CIS) in 25 patients, and invasive carcinoma in 32 patients. With regard to the type of IPMN, there were 3 BD-type IPMN cases of 26 adenomas, 5 BD-type cases of 12 borderline tumors, 10 BD-type cases of 15 CIS, and 9 BD-type cases of 23 invasive carcinomas. These patients were divided into two groups for the purpose of data analysis. One group was comprised of 46 patients with benign tumors (adenomas and borderline tumors) and the other group consisted of 57 patients with malignant tumors (carcinoma).

The patients initially were suspected of having an IPMN based on diagnostic imaging including ultrasonography (US), computed tomography (CT), magnetic resonance cholangiopancreatography (MRCP), and endoscopic ultrasonography (EUS). A definite diagnosis was established based on findings in combination of these images as well as those of endoscopic retrograde cholangiopancreatography (ERCP). The diagnostic criteria of IPMNs were the presence of a filling defect in the dilated ductal system, a patulous ampulla, efflux of mucin, and opacification of a mucinous substance noted on pancreatography.[2,12]

Pancreatic juice was sampled before surgical resection in all patients for the cytologic examination. In 71 patients, pancreatic juice was collected for 10–15 minutes during ERCP through a catheter measuring 1.8 mm in greatest dimension by the intravenous administration of 50 U of secretin (Eizai, Tokyo, Japan). In the remaining 32 patients, the pancreatic juice was collected using POPS (BP-30 scope; Olympus, Tokyo, Japan) through a working channel (measuring 1.2 mm in greatest dimension) while observing the lesion or from a position close to the lesion.

POPS was performed using the "mother–baby" endoscopic method. TJF10 scope (Olympus Optical Co. Ltd., Tokyo, Japan) was used as the mother endoscope and BP-30 was used as the baby endoscope that was inserted directly through the papillary orifice. When insertion was difficult, a guidewire was used through the working channel. The pancreatic duct was examined from the distal to proximal end.

The tumor was found to be located in the MPD in 8 patients and in a BD in 63 patients from the group whose pancreatic juice was collected by catheter. In the group whose pancreatic juice was sampled by POPS, the tumor was found to be located in the MPD in 19 patients and in a BD in 13 patients.

The results of cytology and the diagnosis by POPS were compared. POPS findings indicative of malignancy were as follows: a fish egg-like protruding lesion with a vascular image, a villous protruding lesion, and a vegetative protruding lesion.[9]

To improve the visualization by POPS, physiologic saline was flushed into the pancreatic ducts, and pancreatic juice mixed with saline was used for the cytologic examination. The samples were immediately centrifuged at 1000 × gravity for 10 minutes. Smears were fixed in 95% ethyl alcohol and stained by the Papanicolaou method as well as with Giemsa stain. Cytologic findings were categorized to be nondiagnostic or diagnostic (benign, atypical, and malignant).

The samples with cytology as well as histology were reviewed blindly by two pathologists who provided a consensus opinion with regard to the diagnosis without having any clinical information.

The cytological criteria used to establish the diagnosis of IPMN were the presence of mucinous epithelial cells that were either isolated or arranged in small

TABLE 1
Comparison between Cytologic and Histopathologic Diagnoses

Histopathologic diagnosis	Cytologic diagnosis			
	Nondiagnostic	Benign	Atypical	Malignant
IPMN				
Adenoma (n = 29)	0	16	13	0
Borderline (n = 17)	0	8	8	1
Carcinoma in situ (n = 25)	0	6	8	11
Invasive carcinoma (n = 32)	1	5	9	17
Pancreatic carcinoma (n = 81)	14	46	4	17
Chronic pancreatitis (n = 78)	2	70	6	0

IPMN: intraductal papillary mucinous neoplasm.

papillary clusters. Furthermore, IPMNs were graded based on these cytologic criteria and the degree of cell differentiation as follows: benign (tall columnar cells arranged in cohesive folds with mucinous hypertrophy in the apical region, and small and uniform nuclei), atypical (an increased nuclear-to-cytoplasmic ratio, nuclear hyperplasia, nuclear crowding, and stratification), and malignant (irregular projections, prominent anisonucleosis, large nuclei, nuclear irregularities, and loss of polarity, with or without neoplastic infiltrating glands).[13]

The 81 patients with pancreatic carcinoma were comprised of 56 men and 25 women with a mean age of 63 ± 9.6 years. The final diagnosis of pancreatic carcinoma was obtained by surgical resection in 32 patients, by fine-needle aspiration biopsy of the tumor in 42 patients, and by both imaging diagnosis and clinical follow-up in 7 patients. The 78 patients with chronic pancreatitis were comprised of 57 men and 21 women with a mean age of 58.1 ± 11 years. The definitive diagnosis of chronic pancreatitis was made using various imaging methods as well as by clinical follow-up for longer than 1 year (mean, 7.4 yrs;, range, 1–14 yrs); none of these patients died or developed apparent malignancy during the follow-up period. Data from consecutive patients with pancreatic carcinoma and chronic pancreatitis were collected between May 1989 and December 2004, which is the same period during which data from patients with IPMN were collected. In the group of patients with pancreatic carcinoma and chronic pancreatitis, the pancreatic juice was sampled using a catheter in the same manner as in the IPMN group, and the criteria for the cytologic diagnosis also were the same.

The sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) (percentage) of the pancreatic juice cytology in differentiating benign IPMNs from malignant IPMNs were calculated based on the histopathologic diagnosis of the resected specimens. These patients also were compared between those with malignant IPMNs and those with pancreatic carcinoma as well as between the catheter collection method and the POPS collection method.

Comparisons regarding discrete values were evaluated using the chi-square test or Fisher exact test. Statistical significance was set at a P value < 0.05.

Informed consent was obtained from all patients and the study was approved by the institutional review board of Chiba University.

RESULTS

Comparison between Cytologic Diagnosis and Histopathologic Diagnosis

The results of the cytologic diagnosis of the pancreatic juice and the histopathologic diagnosis of the resected specimen are shown in Table 1. Pancreatic juice cytology was found to be of nondiagnostic value in only one patient with an IPMN, whereas it was found to be of nondiagnostic value in 14 of the 81 patients with pancreatic carcinoma (17.3%), which is a significant difference ($P < 0.001$).

The results of the cytologic examination were analyzed in relation to the location of the tumor (Table 2). The sensitivity, specificity, PPV, and NPV according to the tumor location in the pancreas were as follows: 50%, 97.1%, 95.5%, and 61.1%, respectively, for tumors in the head of the pancreas, and 50%, 91.7%, 87.5%, and 61.1%, respectively, for tumors in the body and/or tail of the pancreas. This difference was not statistically significant between the two groups. Similarly, the same values according to the type of IPMN were as follows: 57.9%, 100%, 100%, and 50.0%, respectively, for MPD tumors and 47.4%, 94.7%, 90.0%, and 64.3%, respectively, for BD tumors (Table 3). Therefore, the sensitivity was better, but not significantly different, in the patients with MPD tumors.

The sensitivity of pancreatic juice cytology in sam-

TABLE 2
Correlation between Cytologic and Histopathologic Diagnoses with Regard to the Location of the IPMN in the Pancreas

	Cytologic diagnosis according to the location of the tumor							
	Head				Body and tail			
Histopathologic diagnosis	Nondiagnostic	Benign	Atypical	Malignant	Nondiagnostic	Benign	Atypical	Malignant
Adenoma (n = 29)	0	11	10	0	0	5	3	0
Borderline (n = 17)	0	7	6	0	0	1	2	1
Carcinoma in situ (n = 25)	0	4	5	7	0	2	3	4
Invasive carcinoma (n = 32)	0	5	7	14	1	0	2	3

IPMN: intraductal papillary mucinous neoplasm.

TABLE 3
Correlation between Cytologic and Histopathologic Diagnosis with Regard to the Location of the IPMN in the Pancreatic Duct

	Cytologic diagnosis according to the location of IPMN							
	MPD				BD			
Histopathologic diagnosis	Nondiagnostic	Benign	Atypical	Malignant	Nondiagnostic	Benign	Atypical	Malignant
Adenoma (n = 29)	0	1	2	0	0	15	11	0
Borderline (n = 17)	0	1	4	0	0	7	4	1
Carcinoma in situ (n = 25)	0	3	2	5	0	3	6	6
Invasive carcinoma (n = 32)	0	2	1	6	1	3	8	11

IPMN: intraductal papillary mucinous neoplasm, MPD: main pancreatic duct; BD: branch duct.

ples obtained by POPS was found to be better compared with that obtained using the usual catheter collecting method in patients with IPMN, but the difference was not statistically significant. Similarly, there was no statistically significant difference noted between IPMN by catheter and pancreatic carcinoma; however, the sensitivity of POPS in patients with IPMN was found to be statistically better compared with that in patients with pancreatic carcinoma ($P < 0.001$) (Table 4).

With regard to the location of the IPMN in the pancreatic ducts, the sensitivity of pancreatic juice cytology in samples obtained by POPS also was found to be better in the case of MPD tumors compared with BD tumors, but the difference was not statistically significant (Table 5).

Results of Pancreatic Juice Cytology in Patients with Malignant IPMNs in whom Malignant Findings Could Not Be Detected in Samples Obtained by POPS

Of the 32 patients who underwent POPS, 22 were found to have malignant IPMN; no malignant findings were observed by POPS in 7 patients, of whom the results of pancreatic juice cytologic examination indicated malignancy in 4 patients (57.1%). In another 15 patients whose POPS findings met the criteria of malignancy, cytology indicated malignancy in 11 patients (73.3%). Therefore, cytology could actually compensate for the insufficient diagnosis made using the POPS examination.

TABLE 4
Comparison of the Diagnostic Value of Pancreatic Juice Cytology between Malignant IPMN and Pancreatic Carcinoma According to the Method Used to Collect Pancreatic Juice

	IPMN		
Diagnostic value	By POPS (n = 32)	By catheter (n = 71)	PC (n = 81)
Sensitivity	68.2%[a,b]	38.2%	25.4%
Specificity	100%	97.2%	100%
Positive predictive value	100%	92.9%	100%
Negative predictive value	58.8%	62.5%	60.3%

IPMN: intraductal papillary mucinous neoplasm, PC: pancreatic carcinoma; POPS: peroral pancreatoscopy.
[a] $P = 0.0002$ vs. pancreatic carcinoma.
[b] $P = 0.056$ vs. by catheter. (using the chi-square test.).

DISCUSSION

IPMNs can be of various histopathologic degrees ranging from adenoma to invasive carcinoma.[1] In addition,

TABLE 5
Diagnostic Value of Pancreatic Juice Cytology in Samples Obtained by POPS in Patients with Malignant IPMN according to their Location in the Pancreatic Ducts

	IPMN	
Diagnostic value	MPD (n = 19)	BD (n = 13)
Sensitivity	80.0%[a]	42.9%
Specificity	100%	100%
Positive predictive value	100%	100%
Negative predictive value	57.0%	60.0%

POPs: peroral pancreatoscopy, IPMN: intraductal papillary mucinous neoplasm; MPD: main pancreatic duct; BD: branch duct.
[a] P = 0.21 vs. branch duct (using the chi-square test).

they often exhibit extensive intraductal growth in the premalignant and invasive phases of their development.[14] The exact cytologic discrimination of benign from malignant tumors based on the cytologic findings of pancreatic juice is sometimes difficult. For these reasons, the usefulness of pancreatic juice cytology in the diagnosis of malignant IPMNs remains controversial.[4,5,15–17]

The accuracy of diagnosis based on pancreatic juice cytology will depend on the patients' background; that is, if the study includes a high proportion of patients with one type of cancerous lesion, then the results of the cytologic examination will be biased. The same will occur if the study involves a small number of patients with IPMNs. In the current study, there was a large number of patients and their distribution by histopathologic diagnosis was homogeneous. In addition, because the study was conducted in one institution and the cytologic examination was performed by the same pathologists, interpretation of the results was considered to have very little variation.

The results of the current study indicate that one could almost definitely diagnose malignant IPMN in approximately 50% of the patients based on cytologic findings. Cytology has the advantage of providing the final diagnosis over other diagnostic techniques. In this respect, the validity of cytology is basically different from that of imaging diagnosis.

In cytology, if the atypical diagnosis included malignancy, the sensitivity would increase to approximately 80%, which was better than that of imaging techniques.[18,19] However, at the same time, the PPV was reduced to approximately 50%, indicating that a patient could be at risk of being referred to undergo surgery for a nonmalignant IPMN. Because IPMNs may progress from adenoma to invasive carcinoma, this risk is believed to be fundamentally different from the risk of patients with noncancerous diseases such as chronic pancreatitis to be mistakenly referred to surgery for pancreatic carcinoma. In fact, some researchers have emphasized the need for an early surgical resection in patients with suspected IPMN of the pancreas because of the high frequency of invasive carcinoma and the inadequacy of preoperative imaging for assessing malignancy.[1,5,18] Similarly, because the tumors can be considered as either premalignant or already malignant, their removal would potentially prevent or eliminate a neoplastic process that could eventually kill the patient.[2]

Based on the location of the tumor in either the pancreas or the ducts, the sensitivity of the cytologic examination was better in the case of MPD tumors than in BD tumors; however, the difference was not statistically significant. This was presumably because the epithelial cells of IPMNs along the entire length of the main pancreatic duct and branch ducts, together with those lining peripheral cysts communicating with the main duct, can be collected in a transpapillary manner.[4]

Several efforts have been made to improve the sensitivity of cytology in diagnosing malignant pancreatic lesions, including IPMNs. With regard to the diagnosis of ordinary pancreatic carcinoma, some authors have attempted to collect pancreatic juice aided by POPS, as in our study,[10] but to our knowledge, the current study is the first to use POPS for the diagnosis of IPMNs. This procedure has been reported to be useful for the qualitative diagnosis of IPMNs.[9,20] In previous studies, we demonstrated that POPS had a high diagnostic value in the differential diagnosis of benign IPMNs and malignant IBMNs.[17] POPS allows us to examine the lesion directly and to collect pancreatic juice under direct vision. In addition, POPS combined with cytology would contribute to increasing the diagnostic accuracy of the procedure.

In the current study, samples collected using POPS were found to have better diagnostic value than those collected by catheter. In the case of BD tumors, POPS did not permit direct visualization of the lesion, and collecting pancreatic juice by POPS was believed to be less useful. In fact, the sensitivity of cytology in the case of BD tumors was comparatively low compared with that for MPD tumors. However, the proportion of MPD tumors to BD tumors differed between those patients in whom the pancreatic juice was collected by POPS and those in whom it was collected by catheter; therefore, we cannot draw a definite conclusion regarding the usefulness of POPS. A controlled randomized study will be needed to clarify this point.

There have been previous reports on the analysis of pancreatic juice cytology in specimens collected using EUS-guided fine-needle aspiration (EUS-

FNA).[21–25] The indication for EUS-FNA is the same as that of POPS in terms of collecting pancreatic juice and identifying the lesion; in addition, EUS-FNA may be better for patients with BD tumors. Furthermore, there is an advantage in obtaining samples directly from the mural nodule. However, thick, viscous mucin may cause some difficulties for the aspiration of pancreatic juice when using a thin needle in patients with IPMNs[8]; it occasionally is difficult to obtain an adequate amount of pancreatic juice, even with a large-bore catheter. In fact, Uehara et al. indicated the need for frequent flushing with saline through the catheter to obtain the sample of pancreatic juice.[4] Nevertheless, additional studies will be needed to assess the significance of EUS-FNA in the differential diagnosis of IPMN in a large number of patients.

There have been other attempts to distinguish benign IPMNs from malignant ones using pancreatic juice: the examination of K-*ras* mutations, telomerase activity, or carcinoembryonic antigen levels (CEA). The examination of K-*ras* mutations was found to be useless because of its considerably low specificity.[6] The examination of telomerase activity was reported to be hopeful but at the same time very complicated, requiring a special technique, making it far from useful in a clinical setting.[7] Conversely, CEA has recently been reported to have a relatively high diagnostic value.[24] However, to our knowledge, the significance of CEA values in IPMN has not been elucidated to date.

Considering the treatment strategies for IPMNs, the diagnostic value of pancreatic juice cytology is believed to be useful. Specifically, the majority of patients with malignant cytology must be referred to undergo surgical treatment for malignant IPMN. With regard to patients with atypical cytology, those whose imaging diagnosis is consistent with a diagnosis of malignant IPMN should be strongly referred to surgery; however, when the imaging diagnosis does not suggest a malignant IPMN, the treatment strategy is more complicated. Our policies are as follows: young patients, those who have symptoms associated with IPMN, or those who seriously desire aggressive treatment should be referred to surgical treatment; the patients whose imaging diagnosis and pancreatic juice cytology do not suggest a malignant IPMN can be followed without surgical treatment and they should be followed closely using imaging modalities such as US or EUS. Repeated cytology would be desirable if possible. Other patients can be followed periodically.

In the current study, pancreatic juice cytology detected approximately 50% of malignant tumors in patients with malignant IPMNs. POPS was useful for the collection of pancreatic juice in patients with malignant IPMNs, especially those with MPD tumors.

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Contrast-enhanced sonography of pancreatic carcinoma: correlations with pathological findings

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Background. We examined contrast-enhanced harmonic gray-scale sonographic findings of pancreatic carcinoma in relation to the pathological findings in resected specimens to evaluate correlations between observations made by this modality and the pathological findings. ***Methods.*** The pathological findings of surgical specimens obtained from 16 patients were examined in relation to the contrast-enhanced harmonic gray-scale sonography findings. Lesion vascularity was examined by contrast-enhanced harmonic gray-scale sonography from 20 to 50 s after the injection of Levovist (Schering, Berlin, Germany) (early phase), and lesion enhancement was also monitored at approximately 90 s after injection (delayed phase). ***Results.*** Contrast-enhanced harmonic gray-scale sonography showed positive enhancement in 12 of the 16 lesions (peripheral tumor region alone, $n = 9$; entire tumor, $n = 3$), while the other 4 lesions showed no contrast enhancement in any region. Twelve enhanced regions (9 peripheral tumor region and 3 entire tumor regions) detected by contrast-enhanced harmonic gray-scale sonography showed: (1) mild fibrosis with inflammation, in 10 regions (83%); (2) the presence of both carcinoma cells and residual acinar cells in 8 (67%); and (3) presence of relatively large arteries in 2 (17%). In contrast, 13 non-enhanced regions (4 entire tumor regions and 9 central regions) showed: (1) severe fibrosis in 10 regions (77%); (2) necrosis in 7 (54%); and (3) mucin in 4 (31%). ***Conclusions.*** Contrast-enhanced harmonic gray-scale sonographic findings of pancreatic carcinoma are influenced by interstitial histological features associated with tumor growth.

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Key words: pancreatic carcinoma, contrast-enhanced harmonic gray-scale sonography, helical CT, pathologic findings

Introduction

Pancreatic carcinoma is an aggressive and devastating disease, which is characterized by invasiveness, rapid progression, and profound resistance to treatment.[1] In general, the vascularity of a pancreatic carcinoma lesion is evaluated by helical computed tomography (CT).[2–4] Contrast-enhanced harmonic gray-scale sonography was recently introduced to evaluate the vascularity of pancreatic mass lesions,[5–9] because this modality can visualize blood perfusion in pancreatic mass lesions without motion artifacts,[7] and the technique is simple, easy, and sufficiently noninvasive to be performed on an outpatient basis. It can also be used in patients with renal failure and patients who are allergic to iodine contrast agents. We recently reported that the grade of lesion vascularity on contrast-enhanced harmonic gray-scale sonographic images correlated with the pathological grade of inflammation and correlated inversely with the grade of fibrosis associated with autoimmune pancreatitis.[10] However, there have been no formal studies evaluating any correlations between pathological findings and the findings of contrast-enhanced harmonic gray-scale sonography of pancreatic carcinoma lesions. In the present study, we examined contrast-enhanced harmonic gray-scale sonographic findings in surgically treated pancreatic carcinoma lesions in relation to the pathological findings. We also assessed the enhanced and non-enhanced regions, detected by contrast-enhanced harmonic gray-scale sonography, in relation to the pathological findings.

Subjects and methods

Subjects

Between January 2000 and November 2003, 65 patients with pancreatic carcinoma were admitted to our institutions. Sixteen of the 65 patients were treated surgically, and were the subjects of this study. The patients consisted of 6 men and 10 women, and they ranged in age from 46 to 75 years (mean, 66 years). The maximal diameter (mean ± SD) of the pancreatic mass lesions in the surgical specimens was 37 ± 14 mm. The location of the carcinoma lesion was in the pancreatic head in 12 patients, in the tail in 2, and in both the body and the tail in 2. According to the Japan Pancreas Society (JPS) classification,[11] 1 lesion was stage II, 4 were stage III, 6 were stage IVa, and 5 were stage IVb. Follow-up for all patients ranged from 133 to 1210 days; the follow-up period ended on September 30, 2004. Ten patients died; six patients died of local recurrence, peritonitis carcinomatosa, and liver metastasis; the remaining 4 died of local recurrence and peritonitis carcinomatosa. The survival time ranged from 133 to 511 days. The mean survival time was 308 ± 124 days. Six patients were alive at the end of the study period. Four patients have no recurrence, 1 has lung metastasis, and 1 has local recurrence. The survival time in these 6 patients ranges from 501 to 1180 days. The mean survival time is 925 ± 248 days. All of the patients had been previously examined by conventional and color Doppler sonography. Contrast-enhanced harmonic gray-scale sonography and helical CT examinations were performed in every patient. These two examinations were performed within 1 week of each other. Because no Institutional Review Board existed at the time the study was initiated, the study was performed according to the guidelines of the Helsinki Declaration. Informed consent was obtained from all patients.

Methods

Contrast-enhanced harmonic gray-scale sonography

Contrast-enhanced harmonic gray-scale sonography was performed with a Sonoline Elegra system (Siemens Medical Systems, Issaquah, WA, USA) and a 3.5-MHz convex probe, in all patients, as previously described.[7,10] The pancreas was scanned by native tissue harmonic gray-scale imaging (transmit, 1.6, 1.8, or 2.0 MHz; receive, 3.2, 3.6, or 4.0 MHz Fig. 1A). The pancreatic lesion was then scanned by contrast-enhanced wideband phase-inversion harmonic gray-scale sonography (transmit, 2.5 or 2.8 MHz; receive, 5.0 or 5.6 MHz) at a frame rate of 1 to 5/s, immediately before and after the intravenous injection of a 300-mg/ml concentration of a galactose/palmitic acid mixture contrast agent (Levovist; Schering, Berlin, Germany). Transmission power was 100%, and the mechanical index values were between 1.0 and 1.9. The focus position was just below the bottom of the lesion. Because a certain amount of time is probably required to completely fill pancreatic lesions with the ultrasound contrast agent, especially when extensive fibrosis is present, we decided to evaluate lesion enhancement in two phases (early and delayed) of contrast-enhanced wideband harmonic gray-scale sonography. After the bolus injection of a 7-ml dose of Levovist, at 0.5 ml/s, via a 22-gauge cannula in an antecubital vein, the patients gently inhaled and then held their breath for about 30 s (starting between 20 and 50 s after the contrast medium injection) while the lesion was examined for enhancement (early phase). After observation of the early phase, we froze the image. The images were then reviewed frame-by-frame from cine-loop memories and stored on magneto-optical disks. This procedure took approximately 15–35 s (mean, 25 s) and the time was used to allow pooling of the contrast agent within the pancreatic mass lesion. The entire lesion was then scanned and examined for enhancement about 90 s after injection of the contrast agent, while patients held their breath for a few seconds (delayed phase). The image was frozen again, and the images were then reviewed on a frame-by-frame basis with a cine-loop and stored on a magneto-optical disk for hard copy printing. The entire examination was recorded on S-VHS videotape.

We visually graded tumor enhancement of the pancreatic carcinoma lesions as "no contrast enhancement", "mild enhancement", and "marked enhancement", as compared to the preenhanced appearance on contrast-enhanced wideband phase-inversion harmonic gray-scale sonography.

If the early or delayed phase was the same as the before the finding infusion of Levovist, there was no enhancement (Fig. 2A). "Mild enhancement" was defined as enhancement during the early phase or the delayed phase that was slightly greater than that before the infusion of Levovist (Figs. 1B,C; 2B; 3A,B; 4A,B), and "marked enhancement" was defined as enhancement during the early or delayed phases that was much greater than that before the infusion of Levovist. Mildly and markedly enhanced areas were subdivided into "peripheral region" and "entire tumor". If enhancement was noted in the periphery of the tumor during the early or the delayed phase, it was judged to show peripheral enhancement; if enhancement was seen in the whole tumor during the early or the delayed phase, it was judged to show entire tumor enhancement.

Helical CT

Helical CT was performed with a Proceed SE system (General Electric Medical Systems, Milwaukee, WI,

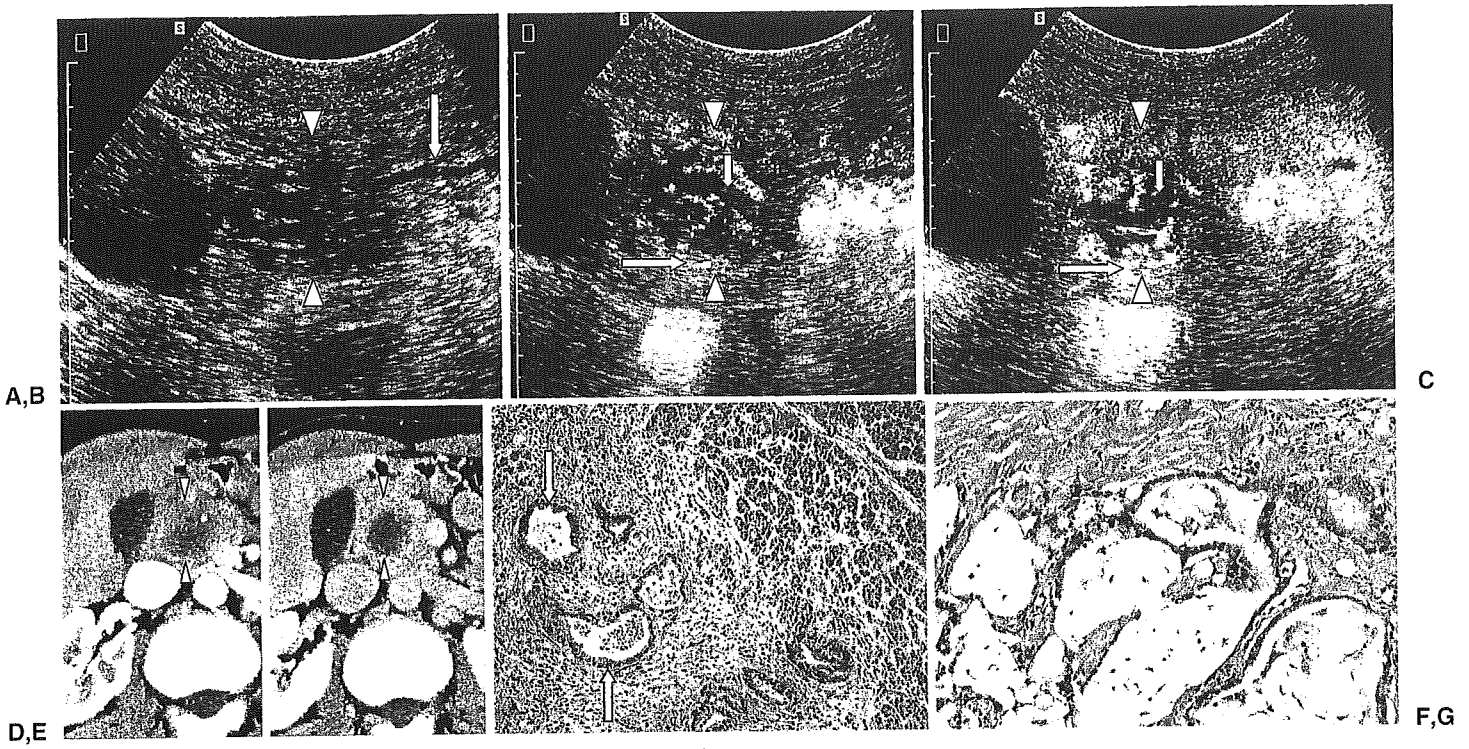

Fig. 1A–G. Findings in a 62-year-old man with mucinous carcinoma in the pancreatic head. **A** Transverse scan pre-enhanced appearance on contrast-enhanced harmonic gray-scale sonogram. *Arrowheads* in **A**, **B**, and **C**, indicate tumor margin. Dilatation of the main pancreatic duct is seen (*arrow*). **B** Early-phase contrast-enhanced harmonic gray-scale sonogram, showing mild enhancement in the peripheral region of the tumor (*long arrow*). *The long* and *short arrows* in **B** and **C** indicate the location from which the histological pictures were taken, corresponding to **F** and **G**, respectively. **C** Delayed phase contrast-enhanced harmonic gray-scale sonogram, showing mild enhancement in the peripheral region of the tumor (*long arrow*). Enhancement of nontumorous parenchyma adjacent to the tumor is seen. **D** Early phase contrast-enhanced computed tomography (CT) scan shows a hypovascular tumor (*arrowheads*). **E** Delayed-phase contrast-enhanced CT scan, showing a mostly hypovascular tumor with isovascularity in a small peripheral region of the tumor; note difference from tumor shown in **D** (*arrowheads*). **F** Surgically obtained histological specimen from the peripheral region of the tumor, corresponding to the enhanced region on both the early and delayed phases of contrast-enhanced harmonic gray-scale sonography, showing the presence of both cancer cells (*arrows*) and residual acinar cells. **G** Surgically obtained histological specimen from the central region of the tumor, corresponding to the non-enhanced region on contrast-enhanced harmonic gray-scale sonography, showing cancer cells, mucin production, and severe fibrosis. **F** H&E, ×100; **G** H&E, ×100

USA) in all patients. A dual-phase study was obtained in each patient, as follows. First, an unenhanced helical sequence through the pancreas and liver was obtained. Next, after the intravenous infusion of 100 ml of iohexol (Omnipaque; Sanofi Winthrop Pharmaceuticals, New York, NY, USA) into an antecubital vein, at a rate of 3 ml/s, an early-phase sequence was obtained after a delay of 25 s, followed by a delayed-phase sequence beginning 80 s after starting the contrast medium infusion. All images were obtained in helical mode, with a 5- or 7-mm collimation and 5- or 7-mm/s table-feed speed. Images were reconstructed at 5- or 7-mm intervals.

We classified the vascularity of pancreatic carcinoma lesions into three grades: "hypovascular", "isovascular", and "hypervascular". Lesions were classified as "hypovascular" if they appeared to be less dense than the surrounding pancreas during the early phase or the delayed phase (Fig. 1D), "isovascular" if they appeared to have the same density as the surrounding pancreas during the early or the delayed phase, and "hypervascular" if they appeared denser than the surrounding pancreas during the early or the delayed phase. Enhanced areas were subclassified into "peripheral region" and "entire tumor". If enhancement was noted in the periphery of the tumor during the early or the delayed phase, it was judged to show peripheral enhancement; if enhancement was noted in the whole tumor during the early or the delayed phase, it was judged to show entire tumor enhancement.

The helical CT findings and the contrast-enhanced harmonic gray-scale sonographic findings were reviewed in a blind fashion. Each image was evaluated by two radiologists and two sonographers, all of whom were unaware of the other study results.

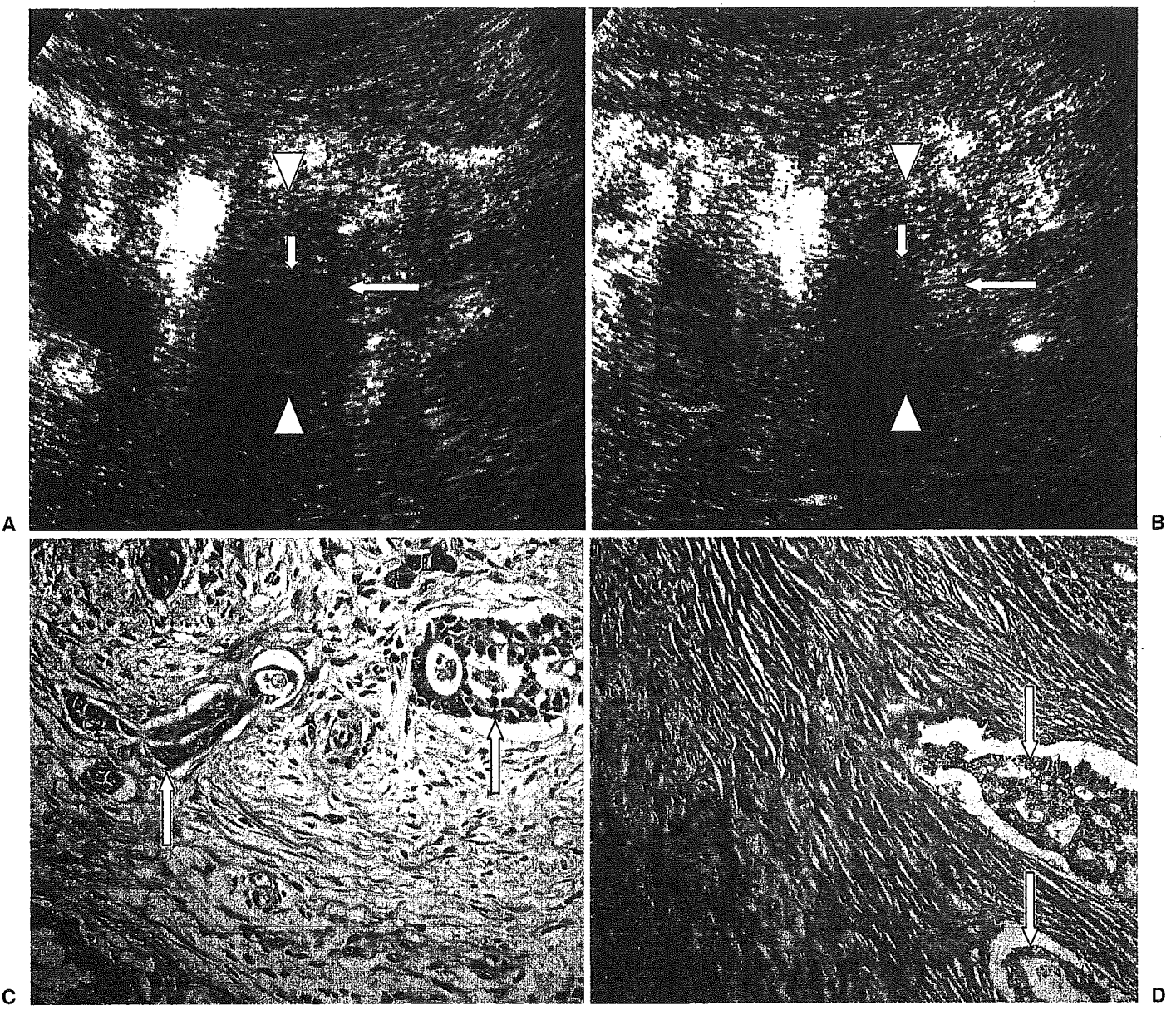

Fig. 2A–D. Findings in a 67-year-old woman with moderately differentiated adenocarcinoma in the pancreatic head. **A** Longitudinal scan early-phase contrast-enhanced harmonic gray-scale sonogram, showing no contrast enhancement in the tumor. *Arrowheads* in **A** and **B** indicate tumor margin. The *long and short arrows* in **A** and **B** indicate the location from which the histological pictures were taken, corresponding to **C** and **D**, respectively. **B** Delayed-phase contrast-enhanced harmonic gray-scale sonogram, showing mild enhancement in the peripheral region of the tumor (*long arrow*). **C** Surgically obtained histological specimen from the peripheral region of the tumor, corresponding to the enhanced region on delayed-phase contrast-enhanced harmonic gray-scale sonography, showing mild interstitial fibrosis with inflammation surrounding cancer cells (*arrows*). **D** Surgically obtained histological specimen from the central region of the tumor, corresponding to the non-enhanced region on contrast-enhanced harmonic gray-scale sonography, showing severe fibrosis and cancer cells (*arrows*). **C** H&E, ×200; **D** H&E, ×200

Pathological findings

The resected specimens were immediately fixed in 10% buffered formalin, serially cut into 4-mm-thick sections, processed routinely, and embedded in paraffin. The paraffin sections were cut into 4-μm-thick sections and were stained with hematoxylin and eosin. Two pathologists, unaware of the results of contrast-enhanced gray-scale sonography or helical CT, interpreted the pathological findings in conference. The lesions were evaluated for the presence or absence of mild fibrosis with inflammation, severe fibrosis, necrosis, mucin, the presence of both carcinoma cells and residual acinar cells in the same lesion, and the presence of relatively large arteries exceeding 500μm in diameter. Fibrosis of

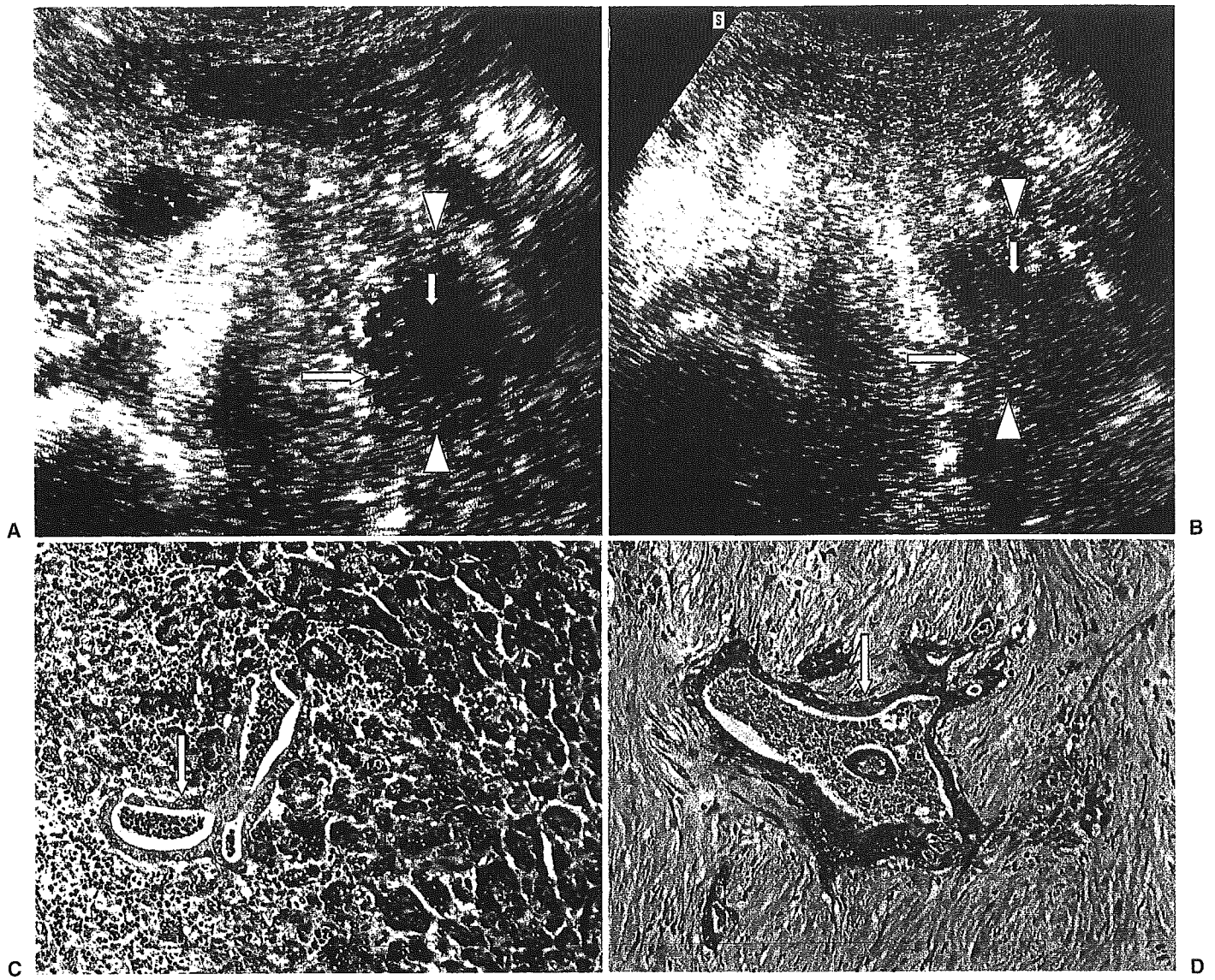

Fig. 3A–D. Findings in a 55-year-old woman with well-differentiated adenocarcinoma in the pancreatic tail. **A** Transverse scan early-phase contrast-enhanced harmonic gray-scale sonogram, showing mild enhancement in a small peripheral region of the tumor (*long arrow*). The *long* and *short arrows* in **A** and **B** indicate the location from which the histological pictures were taken, corresponding to **C** and **D**, respectively. *Arrowheads* in **A** and **B** indicate tumor margin. **B** Delayed phase contrast-enhanced harmonic gray-scale sonogram, showing mild enhancement in the peripheral region of the tumor (*long arrow*). Non-tumorous parenchyma adjacent to the tumor is enhanced. **C** Surgically obtained histological specimen from the peripheral region of the tumor, corresponding to the enhanced region on both the early and dealyed phases of contrast-enhanced harmonic gray-scale sonography, showing the presence of both cancer cells (*arrow*) and residual acinar cells. **D** Surgically obtained histological specimen from the central region of the tumor, corresponding to the non-enhanced region on contrast-enhanced harmonic gray-scale sonography, showing severe fibrosis and cancer cells (*arrow*). **C** H&E, ×150; **D** H&E, ×150

the tumor was graded as "severe" or "mild", based on the severity of the fibrotic changes. Sonographic-pathological correlations were assessed on the basis of the detection of tumor enhancement by contrast-enhanced gray-scale sonography.

Statistical analysis

Data values are expressed as means ± SDs. Relationships between categorical variables were analyzed using the χ^2 test. A P value of less than 0.05 was considered to be statistically significant.

Results

Contrast-enhanced harmonic gray-scale sonography

All lesions were initially detected by conventional sonography. Early-phase contrast-enhanced harmonic

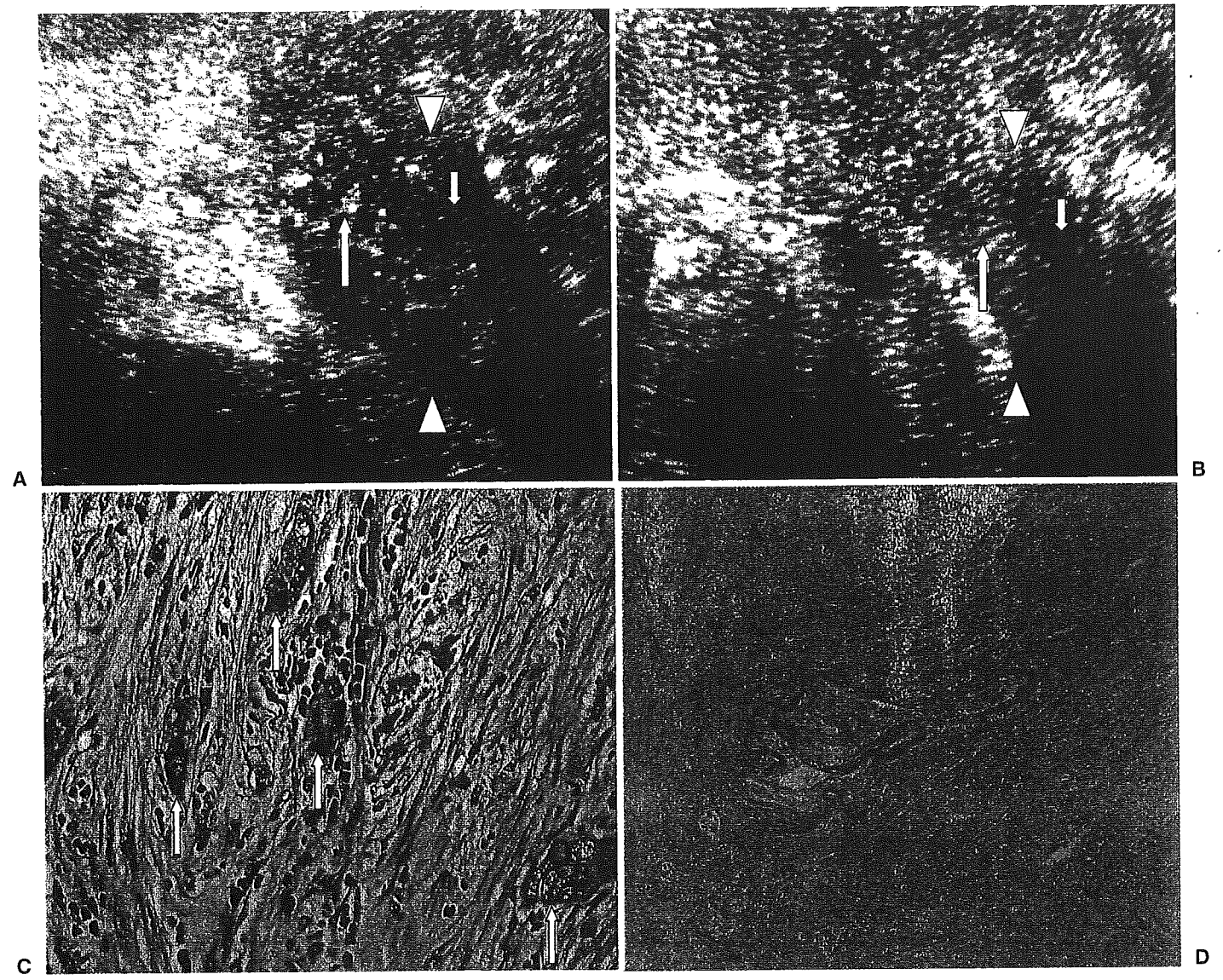

Fig. 4A–D. Findings in a 70-year-old man with poorly differentiated adenocarcinoma extending from the pancreatic body to the tail. **A** Transverse scan early-phase contrast-enahnced harmonic gray-scale sonogram, showing mild enhancement in the peripheral region of the tumor (*long arrow*). *Arrowheads* in **A** and **B** indicate tumor margin. The *long and short arrows* in **A** and **B** indicate the location from which the histological pictures were taken, corresponding to **C** and **D**, respecitvely. **B** Delayed-phase contrast-enhanced harmonic gray-scale sonogram, showing mild enhancement in the peripheral region of the tumor (*long arrow*). **C** Surgically obtained histological specimen obtained from the peripheral region of the tumor, corresponding to the enhanced region on both the early and delayed phases of contrast-enhanced harmonic gray-scale sonography, showing mild interstitial fibrosis with inflammation and an abundance of small vessels surrounding cancer cells (*arrows*). **D** Surgically obtained histological specimen from the central region of the tumor, corresponding to the non-enhanced region on contrast-enhanced harmonic gray-scale sonography, showing necrosis. **C** H&E, ×200; **D** H&E, ×100

gray-scale sonography showed mild enhancement (Figs. 1B, 3A, 4A) in 10 (63%) of the 16 lesions while the other 6 lesions (38%) showed no contrast enhancement. The delayed phase of contrast-enhanced harmonic gray-scale sonography showed mild or marked enhancement (Figs. 1C, 2B, 3B, 4B) in 12 (75%) of the 16 lesions, while the other 4 lesions (25%) showed no contrast enhancement (Table 1).

Helical CT

In the early phase of helical CT, all (100%) pancreatic carcinoma lesions were hypovascular (as in example in Fig. 1D), whereas in the delayed phase, 7 (44%) of the 16 pancreatic carcinomas appeared hypovascular and the other 9 (56%) appeared isovascular in the peripheral region of the tumor (as in example in Fig. 1E; Table 1).

Table 1. Presence or absence of enhancement on contrast-enhanced harmonic gray-scale sonography and helical CT images in patients with pancreatic carcinoma ($n = 16$)

	Contrast-enhanced harmonic gray-scale sonography			Helical CT		
	Marked enhancement	Mild enhancement	No contrast enhancement	Hypervascular	Isovascular	Hypovascular
Early phase	0 (0%)	10 (63%)	6 (38%)	0 (0%)	0 (%)	16 (100%)
Entire tumor	—	3 (19%)	—	—	—	—
Peripheral region	—	7 (44%)	—	—	—	—
Delayed phase	2 (12%)	10 (63%)	4 (25%)	0 (0%)	9 (56%)	7 (44%)
Entire tumor	1 (6%)	2 (12%)	—	—	0 (0%)	—
Peripheral region	1 (6%)	8 (50%)	—	—	9 (56%)	—

Table 2. Correlation between contrast-enhanced harmonic gray-scale sonography and pathological findings or CT findings in patients with pancreatic carcinoma treated surgically ($n = 16$)

Pattern of contrast enhancement/patient no. and histological diagnosis	Pathological findings						CT
	Severe fibrosis	Necrosis	Mucin	Mild fibrosis with inflammation	Presence of carcinoma and residual acinar cells	Presence of relatively large artery	
No contrast enhancement							
1. Well	○	○	—	—	—	—	Negative
2. Moderately	○	○	—	—	—	—	Negative
3. Moderately	○	—	—	—	—	—	Negative
4. Well	○	—	○	—	—	—	Negative
Peripheral enhancement							
5. Moderately[a]	○	○	—	●	—	—	Peripheral
6. Moderately[a]	○	—	○	●	—	—	Peripheral
7. Poorly[b]	—	○	—	●	●	—	Peripheral
8. Poorly[b]	—	○	—	●	●	—	Peripheral
9. Adenosquamous[b]	—	○	—	—	●	—	Peripheral
10. Moderately[b]	○	—	—	—	●	●	Peripheral
11. Well[b]	○	—	○	●	●	—	Peripheral
12. Mucinous[b]	○	—	○	●	●	—	Peripheral
13. Well[b]	○	○	—	●	●	—	Negative
Entire tumor enhancement							
14. Well[b]	—	—	—	●	—	●	Negative
15. Anaplastic[b]	—	—	—	●	—	—	Peripheral
16. Poorly[b]	—	—	—	●	●	—	Negative

Open circles, no contrast-enhanced region; closed circles, enhanced region; well, well-differentiated adenocarcinoma; moderately, moderately differentiated adenocarcinoma; poorly, poorly differentiated adenocarcinoma; adenosquamous, adenosquamous cell carcinoma; mucinous, mucinous carcinoma; anaplastic, anaplastic carcinoma; negative, negative enhancement; peripheral, peripheral enhancement in the delayed phase of helical CT

[a] Delayed phase alone

[b] Both early and delayed phases

Comparison between contrast-enhanced harmonic gray-scale sonographic findings and helical CT findings

The detection rates for positive enhancement of pancreatic carcinoma lesions in the delayed phase of contrast-enhanced gray-scale sonography (75%) and those for isovascular lesions in the delayed phase of helical CT (56%) were not significantly different. Three patients showed hypovascularity in the delayed phase on helical CT; however, these lesions showed positive enhancement in the delayed phase of contrast-enhanced harmonic gray-scale sonography (Table 2). Two of these three patients showed enhancement of the entire tumor and the remaining one showed peripheral enhancement in the delayed phase of contrast-enhanced harmonic gray-scale sonography. However, the detection rate for positive enhancement of pancreatic carcinoma lesions in the early phase of contrast-enhanced gray-scale

sonography (63%) and that for enhancing lesions in the early phase of helical CT (0%) were significantly different, as demonstrated with the χ^2 test ($P < 0.01$).

Correlation between enhanced or non-enhanced regions on contrast-enhanced harmonic gray-scale sonography and pathological findings

In the four pancreatic carcinomas with no contrast enhancement anywhere in the tumor by contrast-enhanced harmonic gray-scale sonography, the regions examined showed severe fibrosis ($n = 4$), necrosis ($n = 2$), and mucin production ($n = 1$). In the nine tumors with mild enhancement in the peripheral region alone, the central regions without contrast corresponded to severe fibrosis ($n = 6$; Figs. 1G, 2D, 3D), necrosis ($n = 5$; Fig. 4D), and mucin production ($n = 3$; Fig. 1G), while the enhanced peripheral tumor regions showed mild fibrosis with inflammation ($n = 7$; Figs. 2C, 4C), the presence of both carcinoma cells and residual acinar cells ($n = 7$; Figs. 1F, 3C), and the presence of a relatively large artery ($n = 1$). In three tumors with enhancement of the entire tumor, the regions examined showed mild fibrosis with inflammation ($n = 3$), the presence of both carcinoma cells and residual acinar cells ($n = 1$), and the presence of a relatively large artery ($n = 1$; Table 2). Therefore, 12 enhanced regions (9 peripheral regions and 3 regions encompassing the entire tumor) detected by contrast-enhanced harmonic gray-scale sonography showed mild fibrosis with inflammation, in 10 regions (83%); the presence of both carcinoma cells and residual acinar cells, in 8 (67%); and the presence of relatively large arteries, in 2 (17%). In contrast, 13 non-enhanced regions (4 entire-tumor regions and 9 central regions) showed severe fibrosis, in 10 regions (77%); necrosis, in 7 (54%); and mucin, in 4 (31%).

Correlation between contrast-enhanced harmonic gray-scale sonographic findings and pathological findings in patients with enhanced peripheral region on contrast-enhanced harmonic gray-scale sonography

All seven (100%) tumors with mild enhancement only in the peripheral region in both the early and the delayed phase of contrast-enhanced harmonic gray-scale sonography showed the combined presence of carcinoma cells and residual acinar cells, five (71%) had mild fibrosis with inflammation and one (14%) had a relatively large artery. By contrast, both (100%) tumors with mild enhancement limited to the peripheral region only in the delayed phase of contrast-enhanced harmonic gray-scale sonography showed mild fibrosis with inflammation.

Correlation between contrast-enhanced harmonic gray-scale sonography findings and histological differentiation

The detection rates for positive enhancement in well- ($n = 5$) or moderately differentiated adenocarcinoma ($n = 5$) was 60%, whereas in poorly differentiated adenocarcinoma ($n = 3$) and other histological lesions ($n = 3$), the rate was 100%.

Correlation between helical CT and pathological findings in operated patients with pancreatic carcinoma

In both the early and the delayed phase of helical CT, seven pancreatic carcinoma lesions were hypovascular as compared to the non-cancerous parenchyma. Five (71%) of these seven non-enhanced regions showed severe fibrosis, three (43%) showed necrosis, and one (14%) showed mucin production. In only the delayed phase of helical CT, nine pancreatic carcinomas appeared isovascular in the peripheral regions of the tumors. Seven (78%) of these nine enhanced regions showed mild fibrosis with inflammation, seven (78%) showed coexistence of carcinoma cells and residual acinar cells, and one (11%) showed the existence of a relatively large artery.

Discussion

In the present study, we examined the contrast-enhanced harmonic gray-scale sonographic findings of pancreatic carcinoma in relation to the pathological findings of resected specimens. Enhanced regions on contrast-enhanced harmonic gray-scale sonography corresponded to mild fibrosis with inflammation or the presence of both pancreatic carcinoma cells and residual acinar cells in the same lesion, whereas non-enhanced regions corresponded to areas of severe fibrosis, necrosis, or mucin.

Pancreatic carcinoma appears most often as a hypovascular mass in the early phase of contrast-enhanced CT.[2–4, 12,13] Presumably, the blood supply to tumors is generally different from that to the normal pancreas, and the difference accounts for the relative tumor hypoattenuation.[14] The rates of isovascular tumor appearance in the early phase of contrast-enhanced CT have been approximately 4%–5% in previous studies.[3, 13] In the present study, we found that 64% of pancreatic carcinoma lesions exhibited some positive enhancement during the early phase of contrast-enhanced harmonic gray-scale sonography, whereas all lesions showed hypovascularity in the early phase on helical CT. During the delayed phase, three lesions showed hypovascularity on helical CT; however,

these lesions showed positive enhancement on contrast-enhanced harmonic gray-scale sonography. Contrast-enhanced harmonic gray-scale sonography is a sensitive modality to evaluate vascular-rich areas of pancreatic carcinoma, and this modality is superior to helical CT in indicating slightly enhanced areas of pancreatic carcinoma lesions more obviously.

As possible explanations for the differences between the results of these imaging techniques; first, the ultrasound contrast agent may remain within the blood vessels, whereas the CT contrast agent is distributed throughout the tissues,[15] i.e., the kinetics of the passage of CT and ultrasound contrast agents are different. Second, we compared the pre-enhancement state and the presence or absence of positive enhancement of the tumor itself during the early and delayed phases of contrast-enhanced gray-scale sonography, whereas we compared the vascularity of pancreatic carcinoma lesions with that of the surrounding pancreas in the early and delayed phases of helical CT images.[7,10] Third, contrast-enhanced harmonic gray-scale sonography permits repeated observations of the same plane, enabling tumor enhancement to be visualized more clearly. This modality detects flow motion as well as bubble disruption, by deliberately adjusting the interval between the transmit pulses.[16]

Demachi et al.[3] reported that the appearance of pancreatic carcinoma on contrast-enhanced CT scans was influenced by the histological features associated with tumor cells. Hypovascular areas in both the early and the late phases of contrast-enhanced CT corresponded to mucin and / or necrosis within the tumor histologically; hypovascular areas in the early phase and iso- or hypervascular areas in the late phase corresponded to dense fibrosis within the tumor; and isovascular areas in both the early and late phases corresponded to increased tumor cellularity and loose interstitial fibrosis or the combined presence of acinar tissue and tumor cells within the tumor.[3] Based on the results from our surgical patients, seven lesions with mild enhancement in the peripheral region of the tumor in both the early and delayed phases of contrast-enhanced harmonic gray-scale sonography showed the combined presence of carcinoma cells and residual acinar cells. Residual acinar cells may exhibit positive enhancement in the early phase of contrast-enhanced harmonic gray-scale sonography, as does the normal pancreatic parenchyma. By contrast, two lesions with mild enhancement in the peripheral regions of the tumor only in the delayed phase of contrast-enhanced harmonic gray-scale sonography showed mild fibrosis with inflammation and these lesions had no residual acinar cells. Johnson and Outwater[17] demonstrated that the region with fibrosis showed gradual enhancement on dynamic magnetic resonance (MR) imaging. Contrast-enhanced harmonic gray-scale sonography may also show gradual enhancement of regions with mild fibrosis. The entirely enhanced tumors detected by this modality were attributable to large areas of mild interstitial fibrosis with inflammation or the presence of a large artery within the tumor. Inflammatory change usually requires blood flow, and such inflamed areas are usually highly vascular.[18] Park et al.[19] reported a large feeding artery to possibly be a factor in the positive enhancement of pancreatic carcinoma lesions. Nagase et al.[9] counted the number of vessels within the most viable tumor regions of pancreatic carcinoma lesions and classified each vessel as either patent or occluded. No signficant relationship between the enhancement effects of contrast-enhanced harmonic gray-scale sonography examinations and the total number of vessels was observed. Conversely, a significant correlation was found between the ratio of patent vessels to total vessels and the gray-scale enhancement.[9]

Invasive carcinomas, such as pancreatic adenocarcinoma, do not exist in isolation. Pancreatic adenocarcinoma is composed of infiltrating carcinoma cells surrounded by a predominance of dense fibrous stroma, which, itself, contains proliferating fibroblasts, small endothelial-lining vessels, inflammatory cells, and trapped residual atrophic parechymal components.[20,21] Well-differentiated adenocarcinoma grows in tubular and glandular patterns and produces abundant dense stroma and mucin. In poorly differentiated adenocarcinoma, the glandular pattern is more bizarre, and epithelial anaplasia is prominent, with mucin production and the desmoplastic response being reduced.[22]

Therefore, severe fibrosis and mucin production are observed more frequently in patients with well-differentiated adenocarcinoma than in those with poorly differentiated adenocarcinoma. On the contrary, interstitial inflammation or residual acinar cells may more frequently be observed in patients with poorly differentiated adenocarcinoma than in those with well-differentiated adenocarcinoma. This may explain why the detection rate of positive enhanced regions on contrast-enhanced harmonic gray-scale sonography in patients with poorly differentiated adenocarcinoma was higher than that in the patients with well- or moderately differentiated adenocarcinoma among our 16 patients with pancreatic carcinoma. We consider the contrast-enhanced harmonic gray-scale sonographic appearance of pancreatic carcinoma to mainly be influenced by interstitial histological features, such as inflammation, fibrosis, residual acinar cells, mucin, and necrosis. These histological features may change according to tumor extension.

If contrast-enhanced harmonic gray-scale sonographic images are obtained only during the early phase, peripheral regions with mild interstitial fibrosis

and inflammation may be missed, as demonstrated in this study. Moreover, enhanced regions in the delayed phase are more obvious than those in the early phase. Thus, a three-phase approach is as important with contrast-enhanced gray-scale sonography as it is with helical CT. Observation with the three-phase approach may be useful in detecting mild interstitial fibrosis and inflammation. Thus, contrast-enhanced harmonic gray-scale sonography may be a useful tool for evaluating the vascularity of pancreatic carcinomas, and it has the ability to visualize vascular areas not detected by helical CT.

Angiogenesis has been shown to be associated with the growth of pancreatic carcinoma lesions, according to the evaluation of microvessels within a pancreatic tumor by immunohistochemical analysis with an anti-CD 34 antibody, as an endothelial cell marker.[23] Therefore, it may be useful to observe the tumor vascularity of pancreatic carcinomas during therapy to evaluate therapeutic efficacy. However, dynamic CT or angiography cannot fully evaluate the tumor vascularity of pancreatic carcinoma. On the contrary, contrast-enhanced harmonic gray-scale sonography is highly sensitive in evaluating the tumor vascularity of pancreatic carcinomas, and it may be useful for evaluating therapeutic efficacy after chemotherapy, radiation therapy, or a combination of the two.

In conclusion, contrast-enhanced harmonic gray-scale sonography may allow the assessment of pathological findings of pancreatic carcinoma lesions. Further study with large numbers of lesions may be needed to establish a correlation between the pathological findings and those seen on contrast-enhanced gray scale sonographic images of pancreatic carcinomas.

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