**TABLE 4. Univariate analysis of potential factors predictive of the intestinal type in 100 branch duct IPMNs**

| | Intestinal (n = 26) | Nonintestinal (n = 74) | *P* value |
|---|---|---|---|
| Sex, male/female | 20/6 | 44/30 | .154 |
| Age <65 y/≥65 y | 11/15 | 31/43 | 1.000 |
| Symptoms, yes/no | 15/11 | 21/53 | .010 |
| History of acute pancreatitis, yes/no | 4/22 | 3/71 | .073 |
| Location pancreas head/pancreas body to tail | 21/5 | 45/29 | .092 |
| Diameter of main pancreatic duct, <6 mm/≥6 mm | 11/15 | 50/24 | .035 |
| Size of cyst, <30 mm/≥30 mm | 6/20 | 21/53 | .798 |
| Mural nodule, yes/no | 11/15 | 18/56 | .130 |
| Serum CEA level, normal/high* | 20/5 | 48/24 | .311 |
| Serum CA19-9 level, normal/high† | 21/5 | 65/8 | .317 |
| Orifice of duodenal papilla, dilated/not dilated | 20/6 | 25/49 | < .001 |
| Pathologic grade, nonmalignant/malignant | 14/12 | 59/15 | .019 |

*IPMN*, Intraductal papillary mucinous neoplasm; *CEA*, carcinoembryonic antigen; *CA19-9*, carbohydrate antigen 19-9.
*Carcinoembryonic antigen was assessed in 97 patients.
†Carbohydrate antigen was assessed in 99 patients.

**TABLE 5. Multivariate analysis of potential factors predictive of the intestinal type in 100 branch duct IPMNs**

| | 95% CI | OR | *P* value |
|---|---|---|---|
| Sex, male | 0.240-4.354 | 1.016 | .983 |
| Age, ≥65 y | 0.242-3.649 | 0.921 | .904 |
| Symptom, yes | 2.766-153.952 | 15.348 | .001 |
| History of acute pancreatitis, yes | 0.124-14.683 | 1.463 | .748 |
| Location, pancreas head | 0.733-14.455 | 2.962 | .130 |
| Diameter of main pancreatic duct, ≥6 mm | 0.488-7.659 | 1.902 | .351 |
| Size of cyst, ≥30 mm | 0.253-4.536 | 1.03 | .961 |
| Mural nodule, yes | 0.503-6.171 | 1.781 | .364 |
| Serum CEA level, high | 0.140-2.500 | 0.639 | .527 |
| Serum CA19-9 level, high | 0.235-11.858 | 1.746 | .570 |
| Dilated orifice of duodenal papilla, yes | 3.430-156.864 | 17.191 | < .001 |

*IPMN*, Intraductal papillary mucinous neoplasm; *CI*, confidence interval; *OR*, odds ratio; *CEA*, carcinoembryonic antigen; *CA19-9*, carbohydrate antigen 19-9.

factors augments the likelihood of malignancy in branch duct IPMNs, predicting malignancy at higher sensitivity (96%) and specificity (71%).[24] On the other hand, an approach from the viewpoint of subtypes also may contribute to the clinical management of IPMNs. This is because intestinal type IPMNs appear to be frequently found with higher grades of dysplasia but have a better prognosis after resection, compared with nonintestinal type IPMNs, as demonstrated in our previous report.[5] Therefore, preoperative diagnosis of intestinal subtypes could be useful to shorten the surveillance interval and to recommend earlier surgical resection of IPMNs.

Intestinal type IPMN is considered to be associated with a greater amount of mucus hypersecretion, and our result that the D factor is a significant factor predictive of the intestinal IPMN would be acceptable. In multivariate analysis, in addition to the D factor, a history of acute pancreatitis in main duct IPMNs (Table 3) and the presence of

symptoms in the branch duct type (Table 5) also were factors predictive of intestinal type IPMNs. These factors might also occur as a result of hypersecretion of mucus in intestinal type IPMNs. In addition, in cases of branch duct IPMN with papillary dilation, mucin hypersecretion also would cause MPD dilation, and this might be one of the reasons for a similar rate of D+ between branch duct IPMNs with MPD dilation and main duct IPMNs.

There are some limitations to this study. First, the D factor is a subjective finding, and the validity of our definition of a diameter of the papillary orifice of more than twice that of a 5F ERCP cannula with mucus bulging from the orifice by visual inspection is unclear. This is because there have been no reports demonstrating a relationship between this factor and the histologic subtypes or epithelial mucus production. The timing of observation and endoscopic procedure such as aspiration before the observation might also affect the results of evaluation of the D factor. Therefore, a more quantitative and universal evaluation method for this factor is required. Second, there is a selection bias of the cohort in this study. Because many small branch duct IPMNs were carefully observed without surgery, they were not included in this study. The relationship between the D factor and these indolent branch duct IPMNs also should be investigated.

We previously demonstrated that a history of acute pancreatitis in IPMNs is a factor predictive of the intestinal subtype.[19] This is the only report demonstrating a relationship among IPMNs, a history of acute pancreatitis, and the histologic subtype of IPMNs. In addition to these clinical factors, molecular analyses of the pancreatic juice obtained during ERCP by using mRNAs or microRNAs might contribute to prediction of the subtype of IPMNs preoperatively. Habbe et al[27] reported a significantly lower proportion of gastric type IPMNs expressing miR-155 (4/7 cases, 57%), compared with intestinal type IPMNs expressing miR-155 (19/19 cases, 100%; $P = .01$). Papillary dilation or a history of acute pancreatitis alone could not predict the subtypes of IPMNs because the sensitivity and specificity for predicting intestinal type were relatively low. Therefore, a combination of these clinical and molecular factors might increase the sensitivity and specificity of preoperative assessment of the subtypes of IPMN.

In conclusion, the finding of a dilated orifice of the duodenal papilla proved to be a useful factor to predict intestinal type IPMN preoperatively, and this finding would be helpful for clinical management of branch duct IPMNs. More precise prediction of intestinal type IPMNs should be established by a combination of this factor and other factors, including molecular biology assessment.

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# An increase in the number of predictive factors augments the likelihood of malignancy in branch duct intraductal papillary mucinous neoplasm of the pancreas

**Takao Ohtsuka, MD,[a] Hiroshi Kono, MD,[a] Yosuke Nagayoshi, MD,[a] Yasuhisa Mori, MD,[a] Kosuke Tsutsumi, MD,[a] Yoshihiko Sadakari, MD,[a] Shunichi Takahata, MD,[a] Katsuya Morimatsu, MD,[b] Shinichi Aishima, MD,[b] Hisato Igarashi, MD,[c] Tetsuhide Ito, MD,[c] Kousei Ishigami, MD,[d] Masafumi Nakamura, MD,[a] Kazuhiro Mizumoto, MD,[a]** *and* **Masao Tanaka, MD, PhD, FACS,[a]** *Fukuoka, Japan*

***Background.*** *International consensus guidelines for the management of intraductal papillary mucinous neoplasms (IPMNs) of the pancreas provide several factors that can be used to predict which IPMNs will become malignant. The sensitivity of each factor's predictive accuracy, however, is relatively low, making it difficult to determine the appropriate treatment in individual cases. The aim of this study was to investigate whether increasing the number of predictive factors might augment the sensitivity of the established guidelines to detect malignant IPMNs.*

***Methods.*** *The medical records of 138 patients with IPMNs resected at our institution were reviewed. Possible malignant predictors were analyzed by univariate and multivariate analysis, and the effects of the number of factors and the predictive score of the pathologic results were examined. The cutoff points for the number of predictors to discriminate between malignant and nonmalignant IPMNs were established by constructing receiver operating characteristic curves.*

***Results.*** *A predictive analysis could not be carried out for the main duct IPMNs because of the high prevalence of malignancy and the small number of significant predictors associated with them. For malignant branch duct IPMNs, however, we identified 4 predictive factors that helped determine the correct diagnosis as follows: (1) the presence of a cyst ≥30 mm in diameter; (2) the presence of mural nodules; (3) a history of acute pancreatitis; and (4) atypical results of pancreatic juice cytology. An increase in the number of these factors significantly affected the sensitivity to predict malignancy. The area under the curve for the number of predictors for malignant branch duct IPMNs was 0.856, and the sensitivity and specificity were 96% and 71%, respectively, when the cutoff point was set at 2. The predictive scoring system also showed the same values of sensitivity and specificity for the number of factors.*

***Conclusion.*** *Patients with branch duct IPMNs who have 2 or more of the 4 predictive factors described above should undergo standard pancreatectomy with lymph node dissection, whereas patients who present with 0 or 1 predictive factor can be treated by minimal pancreatectomy without nodal dissection or by careful observation without resection. All patients with main duct IPMNs, therefore, should be treated with resection as suspected malignancies. (Surgery 2012;151:76-83.)*

*From the Departments of Surgery and Oncology,[a] Anatomic Pathology,[b] Medicine and Bioregulatory Science,[c] and Clinical Radiology,[d] Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan*

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Reprint requests: Masao Tanaka, MD, PhD, FACS, Department of Surgery and Oncology, Graduate School of Medical Sciences, Kyushu University, 3-1-1 Maidashi, Higashi-ku, Fukuoka 812-8582, Japan. E-mail: masaotan@med.kyushu-u.ac.jp.

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RECENT INTENSIVE INVESTIGATIONS OF THE CLINICAL, PATHOLOGIC, AND MOLECULAR CHARACTERISTICS OF INTRADUCTAL PAPILLARY MUCINOUS NEOPLASMS (IPMNs) OF THE PANCREAS have provided a better understanding of their biologic behavior.[1-11] According to international consensus guidelines,[1] all patients with main duct IPMNs and patients with branch duct IPMNs and symptoms, including abdominal pain, jaundice, the presence of mural nodules, a cyst

size ≥30 mm, dilation of the main pancreatic duct, and positive cytology results, should be considered for surgical resection because of the possibility of malignancy. The sensitivity to predict the malignant potential of IPMNs for each of these listed factors individually, however, is relatively low.[2-8] Consequently, it is difficult to determine the appropriate treatment for individual cases (ie, standard pancreatectomy with lymph node dissection for invasive IPMN, organ-preserving pancreatectomy for borderline or noninvasive IPMN, and follow-up for benign IPMN).

Although several reports[12,13] have demonstrated the utility of cytology and the molecular analyses of the pancreatic juice to evaluate the degree of malignancy of IPMNs, these assessments are not always consistent with the pathology results after pancreatectomy. Consequently, highly sensitive markers to predict the malignant potential of IPMNs are urgently needed.

The frequency of malignant IPMNs in the main duct of the pancreas has been reported to range from 60% to 90%[1,2]; therefore, there should be no hesitation about performing a standard pancreatectomy with lymph node dissection for main duct IPMNs. Yet, it seems difficult to predict the degree of histologic grade of branch duct IPMNs using the criteria provided in the consensus guidelines.[1] Although cyst size of branch duct IPMN is one of the malignant predictor[1], we have recently shown that the prevalence of malignancy in flat branch duct IPMN is 3.6%. Therefore, we considered the malignant potential to be low in flat branch duct IPMNs measuring 30 mm that do not have any other predictors for malignancy, whereas we strongly suspect the presence of cancer in branch duct IPMNs with mural nodules and the presence of atypical cells in the pancreatic juice, especially if these IPMNs are ≥30 mm in diameter.

Therefore, an increase in the number of predictive factors as described in consensus guidelines might augment the sensitivity for predicting the malignant potential of branch duct IPMNs. This type of analysis, however, has not been used to date, and the 2006 consensus guidelines[1] do not describe the effects that increasing the number of predictors could have on the sensitivity for predicting malignancy. The aim of this study was to investigate the relationship between the number of predictive factors for malignant IPMNs and the pathology results after pancreatectomy.

## PATIENTS AND METHODS

The medical records of 161 patients who underwent pancreatectomy for IPMN in the Department of Surgery and Oncology at Kyushu University Hospital between January 1990 and August 2010 were reviewed retrospectively. A total of 14 patients with synchronous distinct pancreatic ductal carcinomas and 9 without detailed pathology results were excluded; therefore, data from 138 patients were available. Among these patients, data from 126 who had undergone pancreatectomy between January 1990 and June 2009 were used in previous studies carried out by our group.[14-18]

Of the 138 patients in the current study, 83 were males and 55 were females, with a mean age of 67 years (range, 33–85). Routine preoperative examinations included the following: contrast-enhanced computed tomography, magnetic resonance cholangiopancreatography, percutaneous ultrasonography (US), and/or endoscopic ultrasonography (EUS). Endoscopic retrograde pancreatography and subsequent cytologic examination of the pancreatic juice were also assessed in all patients except for those whose duodenal papilla could not be approached because of a postgastrectomy state or those with a history of severe pancreatitis. We have not performed EUS-guided fine needle aspiration (FNA) cytology to date because of our concerns regarding the possibility of tumor cell seeding along the needle track.

The pathologic diagnosis of the IPMN was based on the 2010 World Health Organization criteria (ie, low-grade dysplasia, intermediate-grade dysplasia, and high-grade dysplasia), which is compatible for noninvasive carcinoma and invasive carcinoma.[19] IPMN was classified into 2 types: the main duct type and branch duct type based on the preoperative imaging studies, as we have described previously.[14,15]

In brief, the branch duct type is defined as IPMNs exclusively involving the branch duct and showing a grape-like collection of small cysts. The main duct type is defined as IPMNs predominantly dilating the main pancreatic duct (MPD) without a grape-like appearance of the branch duct. If the IPMNs have the grape-like appearance of the branch duct type the dilation of the MPD associated with the main duct type, but there are no findings of main duct involvement (such as the presence of mural nodules in the MPD), the dilated MPD is thought to be caused by mucin hypersecretion from the branch duct type IPMN; this presentation of IPMN is classified as the branch duct type. In this study, mixed-type IPMNs were included in the main duct type.

Based on the international consensus guidelines[1] and previous studies including our own,[3-8,17,18] a total of 13 preoperative factors that could possibly be

used to predict malignant IPMNs were evaluated. These factors included the following: sex, age (<65 years or ≥65 years), type (main duct/branch duct), size of the cyst in the branch duct type (<30 mm or ≥30 mm), diameter of the MPD in the branch duct type (<7 mm or ≥7 mm), tumor location (pancreas head/pancreas body to tail), presence or absence of mural nodules, symptoms, history of acute pancreatitis, recent deterioration of diabetes mellitus, serum carcinoembryonic antigen level (normal limit <2.3 ng/mL at our institution), serum carbohydrate antigen 19-9 level (normal limit <37 ng/mL), and the presence of atypical cells in the pancreatic juice cytology (class III, IV, and V). The presence or absence of mural nodules was evaluated based on US and/or EUS findings.

The values were expressed as the mean ± standard deviation. Comparisons between the groups were performed by the chi-square test, the Fisher exact probability test, or the Mann-Whitney *U* test. A multivariate logistic regression model was used to determine the effects of possible predictive factors on malignant IPMN, and then whether the number of these factors would affect the sensitivity to detect the malignant IPMNs was analyzed.

The predictive score for malignant IPMN was calculated as the sum of the odds ratios of the predictive factors to reflect the importance of each factor, and then the relationship between the score and the pathology results was assessed. The optimal cutoff points for the number of predictive factors and the value of the predictive score to discriminate malignant IPMNs were sought by constructing receiver operating characteristic (ROC) curves, which were generated by calculating the sensitivities and specificities at several predetermined cutoff points. A $P$ value <.05 was considered to be statistically significant.

## RESULTS

Of the 138 patients whose data were included in the current study, 39 were diagnosed with main duct IPMNs and 99 with branch duct IPMNs. Of the 138 patients' pathology reports, 24 showed cancer lesions in the main duct type patients (62%) and 22 documented cancer lesions in the branch duct type patients (22%). The diagnosis of main duct IPMN itself proved to be a significant independent predictive factor for malignancy by the univariate ($P < .01$) and multivariate (95% confidence interval [CI], 1.08–11.10; $P = .04$) analyses using the data from all 138 patients.

In the assessment of the 39 main duct IPMNs, the univariate ($P < .01$) and multivariate (95% CI, 3.58–388.91; $P < .01$) analyses revealed only an atypical result of pancreatic juice cytology to be a significant independent predictor for malignant main duct IPMNs. In terms of the aims of this study to assess the relationship between the number of predictors and malignant IPMNs, no further investigation could be carried out because of the high prevalence of malignancy and the small number of significant predictors of main duct IPMNs.

Table I shows the results of the univariate analysis of the 99 branch duct IPMNs in a comparison of the total number of patients without cancer with those patients with cancer. As demonstrated in the table, there were 3 the significant predictive factors for malignant IPMNs: (1) presence of a cyst ≥30 mm ($P = .02$); (2) the presence of mural nodules ($P < .01$); and (3) atypical results of pancreatic juice cytology ($P < .01$). The sensitivity and specificity of pancreatic juice cytology were 54% and 89%, respectively, and there were 11 false-positive and 7 false-negative findings.

As shown in Table II, multivariate analysis revealed 3 significant independent predictive factors for malignant IPMNs: (1) the presence of mural nodules ($P = .02$); (2) a history of acute pancreatitis ($P < .01$); and (3) positive pancreatic juice cytology ($P < .01$). Although the statistical value of the presence of a cyst ≥30 mm did not reach statistical significance in the multivariate analysis ($P = .06$), possibly because this study population did not include patients with small IPMNs without resection, this factor was included in the further investigation because it is 1 of the well-known predictors of malignant IPMNs.[1,2] Therefore, patients with malignant IPMNs are likely to have some or all of these 4 factors.

In this series, 17 patients did not have any of the 4 predictive factors; 39 patients had 1 of the factors; 29 patients had 2; 13 patients had 3; and 1 patient had all 4 predictive factors. As a result, we found that an increase in the number of predictive factors significantly affected the sensitivity to predict malignant branch duct IPMNs ($P < .0001$; Table III). Most of the small branch duct IPMNs that did not have any of the factors were treated during the 1990s, before publication of the consensus guidelines in 2006.[1] The mean number of factors in patients with malignant IPMNs was 2.4 ± 0.6, which was significantly greater than those with nonmalignant IPMNs (1.1 ± 0.9; $P < .01$).

The detailed histologic grades and the relationship between the grade and the number of factors are also shown in Table III. All of the cancer patients had some of the predictive factors; only 1 patient had only 1 factor (atypical results of pancreatic juice cytology), and this patient had high-grade dysplasia

**Table I.** Univariate analysis of preoperative factors predictive of cancer in 99 branch duct type IPMNs

| | *Noncancer* (n = 77) | *Cancer* (n = 22) | P *value* |
|---|---|---|---|
| Sex (male/female) | 44/33 | 16/6 | .19 |
| Age, y (<65 y/≥65 y) | 33/44 | 8/14 | .63 |
| Size of cyst (<30 mm/≥30 mm) | 31/46 | 3/19 | .02 |
| Diameter of main pancreatic duct (<7 mm/≥7 mm) | 31/46 | 8/14 | .79 |
| Location (pancreas head/pancreas body to tail) | 49/28 | 16/6 | .61 |
| Mural nodule (yes/no) | 25/52 | 16/6 | <.01 |
| Symptoms (yes/no) | 29/48 | 9/13 | .81 |
| History of acute pancreatitis (yes/no) | 6/71 | 4/18 | .15 |
| Recent deterioration of diabetes mellitus (yes/no) | 0/77 | 2/20 | .47 |
| Serum CEA level (normal/high)* | 63/12 | 16/4 | .74 |
| Serum CA19-9 level (normal/high)† | 70/7 | 19/3 | .69 |
| Pancreatic juice cytology (benign/atypical)‡ | 59/11 | 7/13 | <.01 |

*Carcinoembryonic antigen (CEA) was assessed in 95 patients.
†CA19-9 was assessed in all 99 patients.
‡Pancreatic juice cytology was assessed in 90 patients.
*IPMNs*, Intraductal papillary mucinous neoplasms; *CEA*, carcinoembryonic antigen; *CA19-9*, carbohydrate antigen 19-9.

**Table II.** Multivariate analysis of preoperative factors predictive of cancer in 99 branch duct type IPMNs

| | *95% Confidence interval* | *Odds ratio* | P *value* |
|---|---|---|---|
| Sex, male | 0.36–7.13 | 1.6 | .54 |
| Cyst size, ≥30 mm | 0.86–35.93 | 5.6 | .06 |
| Mural nodule, yes | 1.37–22.06 | 5.5 | .02 |
| History of acute pancreatitis, yes | 2.99–297.27 | 29.8 | <.01 |
| Recent deterioration of diabetes mellitus, yes | —* | 219756 | .98 |
| Pancreatic juice cytology, atypical | 1.84–26.90 | 7.0 | <.01 |

*Data not available.
*IPMNs*, Intraductal papillary mucinous neoplasms.

(a noninvasive cancer). However, 45% of the patients with 2 factors and 54% of the patients with 3 factors had malignant lesions, including invasive carcinomas. The 1 patient with all 4 factors had an invasive IPMN.

Based on the analysis shown in Table II, 4 predictive factors had the following respective scores: the presence of a cyst ≥30 mm had a score of 5.6, the score was 5.5 for the presence of mural nodules, 29.8 for the presence of a history of acute pancreatitis, and 7.0 for atypical results of pancreatic juice cytology. The predictive score was calculated for each patient, and ranged from 0 to 47.9. The mean score of cancer patients was 18.4 ± 10.5 (range, 7–47.9), which was significantly greater than that of noncancer patients (8.5 ± 8.9; range, 0–40.9; $P < .01$) (Fig 1, *A*). Fig 1, *B*, demonstrates the relationship between the predictive score and the detailed histologic grade, and shows the stepwise increase in the value of the score according to the histologic grade ($P = .01 \sim .05$).

Fig 2, *A* and *B*, demonstrate the ROC curves of the number of predictive factors and the value of the predictive scores, respectively. The area under the curve (AUC) for the number of factors was 0.856 (95% CI, 0.771–0.918), and the sensitivity and specificity were 96% and 71%, respectively, when the cutoff point to predict malignant branch duct IPMN was set at 2. The AUC for the predictive score for malignant branch duct IPMNs was 0.842 (95% CI, 0.753–0.908), and the sensitivity and specificity were 100% and 66%, respectively, when the cutoff point was set at 7.0. There were no significant differences in the AUC between the number of factors and the predictive score ($P = .74$).

## DISCUSSION

Our present study analyzing the relationship between the number of predictive factors for malignant IPMNs and pathology results demonstrated the following findings: (1) a predictive analysis could not be carried out for main duct IPMNs

**Table III.** Relationship between number of factors and pathology results in 99 branch duct type IPMNs

| | | | | | *Pathologic grade* | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | *Noncancer* | | *Cancer* | |
| *No. of factors* | *Noncancer* | *Cancer* | *Total* | *Sensitivity for cancer (%)* | *Low-grade dysplasia* | *Intermediate grade-dysplasia* | *High-grade dysplasia* | *Invasive cancer* |
| 0 | 17 | 0 | 17 | 0 | 14 | 3 | 0 | 0 |
| 1 | 38 | 1 | 39 | 3 | 27 | 11 | 1 | 0 |
| 2 | 16 | 13 | 29 | 45 | 10 | 6 | 7 | 6 |
| 3 | 6 | 7 | 13 | 54 | 4 | 2 | 1 | 6 |
| 4 | 0 | 1 | 1 | 100 | 0 | 0 | 0 | 1 |
| Total | 77 | 22 | 99 | | 55 | 22 | 9 | 13 |

*IPMNs,* Intraductal papillary mucinous neoplasms.

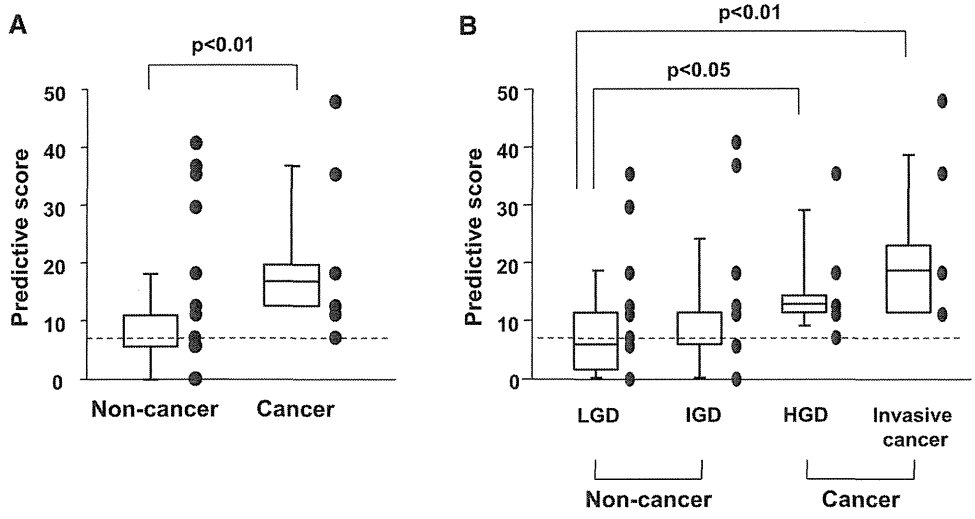


**Fig 1.** The relationship between the predictive score and the pathologic results of branch duct IPMNs. (*A*) The predictive score of malignant lesions was significantly greater than that of nonmalignant lesions. (*B*) The predictive score shows a stepwise increase according to the histologic grade. *LGD,* Low-grade dysplasia; *IGD,* intermediate-grade dysplasia; *HGD,* high-grade dysplasia.

because of the high prevalence of malignancy and the small number of significant predictors; (2) a total of 4 predictive factors for malignant IPMNs, including the presence of a cyst ≥30 mm, the presence of mural nodules, a history of acute pancreatitis, and the presence of atypical cells in pancreatic juice cytology, were determined by univariate and multivariate analysis, and an increase in the number of these factors significantly affected the sensitivity to predict malignant branch duct IPMNs; (3) the present scoring system using these 4 predictors also significantly predicted the malignant branch duct IPMNs; and (4) the values of the AUC and the sensitivity constructed using ROC curves to predict malignant branch duct IPMNs were high in both systems using the number of factors and the predictive score.

Consistent with our expectations, this study demonstrated that an increase in the number of predictive factors affected the sensitivity to predict malignant IPMNs, especially the branch duct type. Although it seems obvious that IPMNs with multiple malignant predictors would be related to a high probability for cancer, no previous reports have described the relationship between the number of predictive factors and the pathologic results.

In the present study, we also tried to establish a scoring system using all 4 predictors, because the importance of each factor seems to be different, and our scoring system could predict each type of malignant branch duct IPMN when a cutoff point of 7.0 was used. Fortunately, the 4 predictive factors in the present study are already included as factors described in the international consensus

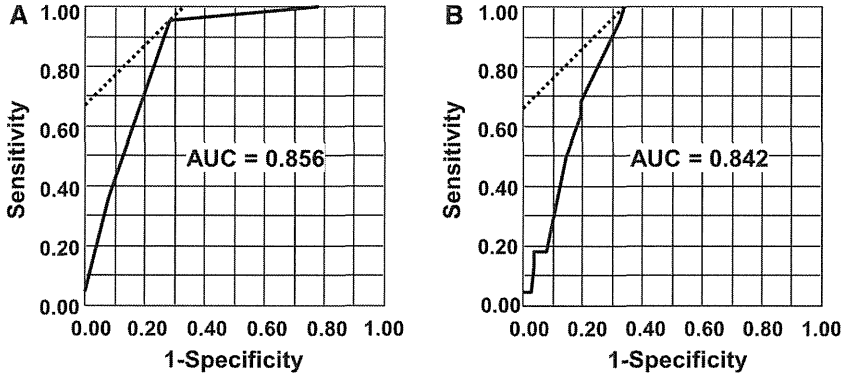


**Fig 2.** Receiver operating characteristic (ROC) curves of the number of factors and the predictive score in branch duct IPMNs. (*A*) The area under the curve (AUC) for the number of factors was 0.856, and the sensitivity and specificity were 96% and 71%, respectively, when the cutoff point to predict malignant IPMNs was set at 2. (*B*) The AUC for the predictive score for malignant IPMNs was 0.842, and the sensitivity and specificity were 100% and 66%, respectively, when the cutoff point was set as 7.0. There were no significant differences in the AUC between the number of factors and the predictive score.

guidelines,[1] and so these factors could be applied easily in clinical practice for the management of the branch duct type IPMNs.

Based on the results shown in Table III and Fig 2, *A*, we could predict 21 of 22 malignant branch duct IPMNs by using a cutoff point of 2 predictive factors. In contrast, 1 patient with 1 predictive factor had noninvasive cancer; this particular factor was the presence of atypical cells in pancreatic juice cytology. However, when the predictive score of 7.0 was used, which was the cutoff point for 100% sensitivity for malignant branch duct IPMNs, this patient was no longer included in the diagnosis of benign IPMNs.

Consequently, the recommended treatment strategy according to the number of predictive factors would be as follows: (1) patients with branch duct IPMNs without any predictive factors of malignancy could be carefully followed up without resection; (2) those patients with any 1 predictive factor — other than the presence of atypical pancreatic juice cytology — could be treated using a minimal pancreatectomy without lymph node dissection; and (3) those patients with 2 or more predictive factors should undergo standard pancreatectomy with lymph node dissection.

Our scoring system could predict all malignant branch duct IPMNs when the cutoff point was set at 7.0. Contrary to our initial expectations, however, this scoring system could not further increase our predictive ability. One of the possible reasons for this conclusion might be that the system using the number of predictors already has a high ability to predict malignant branch duct IPMNs. In clinical practice, the use of the number of predictors would be easier to handle compared with the complicated scoring system we have presented here.

Although both the system using the number of predictors and the scoring system have a high sensitivity to predict malignant branch duct IPMNs, there are still problems regarding the relatively high incidence of false-positive findings in both these systems. Further investigation will be necessary to decrease these false-positive findings. One possible way to resolve this issue might be to use molecular analysis of pancreatic juice. Recent reports[10,11] have demonstrated that several specific markers can classify the histologic grade of IPMNs by the use of a resected specimen; analysis of those markers in pancreatic juice could be applied to the prediction of pathology results.

Another way to eliminate false-positives would be to assess the cystic fluid obtained by EUS- FNA,[20,21] although this study did not use such samples because of concerns about the seeding of the tumor cells. Especially in the case of branch duct IPMNs, cystic fluid obtained by EUS-FNA might provide more reliable information than pancreatic juice during endoscopic retrograde pancreatography in terms of the assessment of molecular markers, as well as cytology.

A predictive analysis could not be carried out for main duct IPMNs because of the high prevalence of malignancy and the small number of significant predictors. The main duct type seems to be a strong independent predictor for malignant IPMNs, and therefore, all main duct IPMNs should be treated as malignancies by standard pancreatectomy with lymph node dissection, as described in the consensus guidelines.[1]

Preoperative imaging studies are crucial for predicting the malignant potential of IPMNs, and one of the limitations of the present study was that it included patients who had undergone surgery 20

years ago, because the type and resolution of imaging studies have changed dramatically during this time.[2] In addition to the assessment of the cystic size and the presence of mural nodules, recent imaging studies can provide detailed information to predict the malignant potential of IPMNs.

We recently showed that positive radiologic findings for invasive IPMNs are a good predictor for lymph node metastasis.[17] Another group[22] classified the mural nodules of IPMNs into 4 morphologic types based on the findings of EUS, and showed that type III (papillary nodules) and IV (invasive nodules) were associated with invasive carcinomas. It might be possible, therefore, to use the more reliable imaging factors to predict malignant IPMNs in the future.

The other limitation of this study is the selection bias of the study population, because many small branch duct IPMNs without any predictors that had been carefully observed without surgery were not included in this series. Most of these IPMNs were probably benign, although it is difficult to confirm the pathology in patients without resection of the small branch duct IPMNs. Potential malignancies, however, might have been missed, because these patients were not selected for surgery.

We demonstrated previously the surveillance data of 93 small branch duct IPMNs without resection and showed that there were no patients who experienced rapid progression of the disease requiring resection during a mean follow-up period of 31.6 months.[14] Although this study population included only 17 patients without any predictors, all of them had noncancerous lesions. Thus, the branch duct IPMNs without any predictors could be carefully observed rather than undergo resection without any major risk.

In conclusion, an increase in the number of predictive factors would augment the sensitivity to detect malignant branch duct IPMNs. Patients with branch duct IPMNs without any of the 4 predictors can be carefully followed up without resection. Patients with any 1 factor other than the presence of atypical pancreatic juice cytology can be treated by organ-preserving pancreatectomy without lymph node dissection, and patients with 2 or more factors should undergo standard pancreatectomy with lymph node dissection. All main duct IPMNs should be treated by resection as suspected cancers.

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Clinical Investigation: Gastrointestinal Cancer

# Concurrent Radiotherapy and Gemcitabine for Unresectable Pancreatic Adenocarcinoma: Impact of Adjuvant Chemotherapy on Survival

**Kazuhiko Ogawa, M.D.,* Yoshinori Ito, M.D.,† Naoki Hirokawa, M.D.,‡ Keiko Shibuya, M.D.,¶ Masaki Kokubo, M.D.,§ Etsuyo Ogo, M.D.,** Hitoshi Shibuya, M.D.,†† Tsutomu Saito, M.D.,‡‡ Hiroshi Onishi, M.D.,¶¶ Katsuyuki Karasawa, M.D.,§§ Kenji Nemoto, M.D.,*** Yasumasa Nishimura, M.D.†††, the Japanese Radiation Oncology Study Group Working Subgroup of Gastrointestinal Cancers**

**Department of Radiology, University of the Ryukyus, Okinawa, Japan; †Department of Radiation Oncology, National Cancer Center, Tokyo, Japan; ‡Department of Radiology, Sapporo Medical University, Sapporo, Japan; ¶Department of Radiation Oncology and Image-Applied Therapy, Kyoto University, Kyoto, Japan; §Department of Radiation Oncology, Institute of Biomedical Research and Innovation Hospital, Kobe, Japan; **Department of Radiation Oncology, Kurume University, Kurume, Japan; ††Department of Radiology, Tokyo Medical and Dental University, Tokyo, Japan; ‡‡Department of Radiation Oncology, Nihon University Itabashi Hospital, Tokyo, Japan; ¶¶Department of Radiology, Yamanashi University, Yamanashi, Japan; §§Department of Radiation Oncology, Tokyo Metropolitan Komagome Hospital, Tokyo, Japan; ***Department of Radiation Oncology, Yamagata University, Yamagata, Japan; and †††Department of Radiation Oncology, Kinki University School of Medicine, Osaka, Japan*

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**Summary**

This retrospective study looked at patients with unresectable pancreatic cancer treated with different combinations of chemotherapy and radiation. When concurrent chemo-radiotherapy using gemcitabine was used, a relatively favorable local control rate was seen. When adjuvant chemotherapy was given,

**Purpose:** To retrospectively analyze results of concurrent chemoradiotherapy (CCRT) using gemcitabine (GEM) for unresectable pancreatic adenocarcinoma.

**Methods and Materials:** Records of 108 patients treated with concurrent external beam radiotherapy (EBRT) and GEM were reviewed. The median dose of EBRT in all 108 patients was 50.4 Gy (range, 3.6–60.8 Gy), usually administered in conventional fractionations (1.8–2 Gy/day). During radiotherapy, most patients received GEM at a dosage of 250 to 350 mg/m$^2$ intravenously weekly for approximately 6 weeks. After CCRT, 59 patients (54.6%) were treated with adjuvant chemotherapy (AC), mainly with GEM. The median follow-up for all 108 patients was 11.0 months (range, 0.4–37.9 months).

**Results:** Initial responses after CCRT for 85 patients were partial response: 26 patients, no change: 51 patients and progressive disease: 8 patients. Local progression was observed in 35 patients (32.4%), and the 2-year local control (LC) rate in all patients was 41.9%. Patients treated with total doses of 50 Gy or more had significantly more favorable LC rates (2-year LC rate, 42.9%) than patients treated with total doses of less than 50 Gy (2-year LC rate,

Reprint requests to: Dr. Kazuhiko Ogawa, Department of Radiology, University of the Ryukyus, 207 Uehara, Nishihara-cho, Okinawa, 903-0215, Japan. Tel: 81-98-895-3331 (ext. 2401); Fax: 81-98-895-1420; E-mail: kogawa@med.u-ryukyu.ac.jp

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a small survival benefit became evident. Adjustments in the sequencing of chemotherapy and radiation thus have the potential to improve outcomes.

29.6%). Regional lymph node recurrence was found in only 1 patient, and none of the 57 patients with clinical N0 disease had regional lymph node recurrence. The 2-year overall survival (OS) rate and the median survival time in all patients were 23.5% and 11.6 months, respectively. Patients treated with AC had significantly more favorable OS rates (2-year OS, 31.8%) than those treated without AC (2-year OS, 12.4%; $p < 0.0001$). On multivariate analysis, AC use and clinical T stage were significant prognostic factors for OS.

**Conclusions:** CCRT using GEM yields a relatively favorable LC rate for unresectable pancreatic adenocarcinoma, and CCRT with AC conferred a survival benefit compared to CCRT without AC. © 2012 Elsevier Inc.

**Keywords:** Chemotherapy, Gemcitabine, Pancreatic neoplasms, Radiotherapy, Unresectable

## Introduction

Pancreatic cancer is one of the leading causes of cancer death worldwide. The prognosis for patients with this disease remains extremely poor, with a 5-year survival rate after diagnosis of less than 5% (1, 2). Most patients with pancreatic cancer already have advanced disease at the time of diagnosis, and among patients with unresectable pancreatic cancer, nearly half of patients have advanced but localized disease (2).

In the 1980s, the Gastrointestinal Tumor Study Group reported the survival benefit of 5-fluorourcil (5-FU)-based concurrent chemoradiotherapy (CCRT) over that of external beam radiotherapy (EBRT) alone in patients with unresectable pancreatic cancer (3). Until recently, CCRT has been the standard approach to treating surgically unresectable, localized disease. More recently, therapy using the drug gemcitabine (GEM), a nucleoside analogue, has been reported to confer marginally superior clinical benefit and survival compared with that with 5-FU (4). GEM has also been shown to be a potent radiosensitizer in pancreatic cancer (5). Therefore, concurrent radiotherapy and GEM may be a promising strategy for treating unresectable localized pancreatic cancer. However, optimal management of concurrent EBRT and GEM for unresectable disease has not been fully investigated.

In the current study, we reviewed a retrospective and multi-institutional series of 108 patients with nonmetastatic unresectable pancreatic cancer, who were treated with concurrent radiotherapy using GEM, and evaluated the efficacy and safety of this treatment for these tumors.

## Methods and Materials

The Japanese Radiation Oncology Study Group (JROSG) conducted a nationwide questionnaire survey of patients with non-metastatic pancreatic adenocarcinoma who were treated with radiotherapy. The questionnaire elicited detailed information regarding patient characteristics, treatment characteristics, and outcomes of treatments. Details of the JROSG survey have been described elsewhere (6–8). Briefly, 34 radiation oncology centers belonging to the JROSG agreed to participate in this survey, and detailed information for 870 patients was accumulated. Of these patients, 223 patients with unresectable disease were treated with concurrent EBRT and GEM. Histology finding for 108 patients was adenocarcinoma; 3 patients had other histological findings, such as anaplastic carcinoma and undifferentiated carcinoma; and 112 patients had no histological information. These last 115 patients were excluded from this study, and the remaining 108 patients with histological diagnosis of adenocarcinoma were the subjects of the current study. Their tumors were judged to be unresectable by the respective physicians at each institution. Of these 108 patients, there were 3 patients with inoperable cancer, who were not fit for surgery, and the remaining 105 patients had unresectable tumors at presentation.

Patient and treatment characteristics for all 108 patients are shown in Table 1. The median age of patients was 63 years old (range, 40–83 years old), and the Eastern Cooperative Oncology Group (ECOG) performance status (PS) ranged from 0 to 3 (median, 1). We used the tumor staging system devised by the Union Internationale Contre le Cancer (9). The median maximum tumor size was 3.9 cm (range, 1.4–10.0 cm), and the median serum concentration of carbohydrate antigen 19-9 (CA19-9) was 511 U/mL (range, 0–57,300 U/mL). Total doses of EBRT ranged from 3.6 to 60.8 Gy (median, 50.4 Gy), with a single fraction of 1.8 to 2 Gy given 5 days per week in most patients. On the other hand, 11 patients (10.2%) were treated with a single fraction of 2.2 to 2.5 Gy.

Chemotherapy schedules are described in Table 2. During radiotherapy, 8 patients received a dosage of 1,000 mg/m$^2$ GEM weekly for 3 weeks with a 1-week rest period, depending on their response and toxicity (using the standard dosage of GEM). The remaining 100 patients received GEM at a dosage of 250 to 350 mg/m$^2$ intravenously weekly during radiotherapy for approximately 6 weeks (low-dose GEM). After radiotherapy, 59 of 108 patients (54.6%) were treated with adjuvant chemotherapy (AC). Fifty-three of 59 patients (89.8%) received GEM maintenance chemotherapy, usually given at 1,000 mg/m$^2$ weekly for 3 weeks with a 1-week rest period, until disease progression or unacceptable toxicity was reached. Six patients received intravenous bolus infusions of 300 to 500 mg/m$^2$ 5-FU, until disease progression or unacceptable toxicity was reached. For 5 patients, a combination compound of tegafur, 5-chloro-2, 4-dihydroxypyridine, and oteracil potassium (S-1) was administered orally, and S-1 doses ranged from 50 to 80 mg/m$^2$.

In the current study, there were no definitive treatment policies for pancreatic cancer during the survey period; thus, treatment was determined by the respective physicians at each institution. We assigned 108 patients to two groups (patients treated with AC and those without AC treatment) and determined whether the AC influenced patient characteristics, such as age, tumor size, and clinical stage. There were no significant differences in age, gender, tumor site, tumor size, or clinical T stage and clinical N stage, except for CA19-9 levels, which varied according to the AC used (data not shown). Concerning PS, there were no significant differences according to the AC used, and 56 of 58 patients with

**Table 1** Patient and disease characteristics

| Characteristic | No. of patients | % of total |
|---|---|---|
| Age (median, 63 years old) | | |
| <70 | 84 | 77.8 |
| ≥70 | 24 | 22.2 |
| Gender | | |
| Female | 50 | 46.3 |
| Male | 58 | 53.7 |
| Primary site | | |
| Head | 55 | 50.9 |
| Body | 48 | 44.4 |
| Tail | 4 | 3.7 |
| Unknown | 1 | 0.9 |
| Maximum tumor size (median, 3.9 cm) | | |
| <4.0 cm | 48 | 44.4 |
| ≥4.0 cm | 54 | 50.0 |
| Unknown | 6 | 5.6 |
| ECOG performance status scale | | |
| 0 | 28 | 25.9 |
| 1 | 70 | 64.8 |
| 2 | 5 | 4.6 |
| 3 | 1 | 0.9 |
| Unknown | 4 | 3.7 |
| CA19-9 (U/ml) (median, 248.2 U/ml) | | |
| <1,000 | 56 | 51.9 |
| ≥1,000 | 43 | 39.8 |
| Unknown | 9 | 8.3 |
| Clinical T stage (UICC 2002) | | |
| 2 | 3 | 2.8 |
| 3 | 15 | 13.9 |
| 4 | 90 | 83.3 |
| Clinical N stage (UICC 2002) | | |
| 0 | 57 | 52.8 |
| 1 | 49 | 45.4 |
| Unknown | 2 | 1.8 |
| EBRT total radiation dose (Gy) (median, 50.4 Gy) | | |
| <40 | 6 | 5.6 |
| 40≤ to <50 | 9 | 8.3 |
| 50≤ to <60 | 89 | 86.4 |
| ≥60 | 4 | 3.7 |
| Dose per fraction (Gy) | | |
| 1.8–2 | 97 | 89.8 |
| 2.2–2.5 | 11 | 10.2 |
| Radiation field | | |
| Primary plus LN | 65 | 60.2 |
| Primary only | 43 | 39.8 |
| CT-based treatment planning | | |
| Yes | 106 | 98.1 |
| No | 2 | 1.9 |
| Conformal therapy | | |
| Yes | 91 | 84.3 |
| No | 17 | 15.7 |
| GEM dose during EBRT | | |
| Low dose (250–350 mg/m$^2$/week) | 100 | 92.6 |
| Standard dose (1,000 mg/m$^2$/week)* | 8 | 7.4 |
| Adjuvant chemotherapy use | | |
| Yes | 59 | 54.6 |
| No | 49 | 45.4 |

*Abbreviations*: CA19-9 = carbohydrate antigen 19-9; CT = computed tomography; EBRT = external beam radiotherapy; ECOG = Eastern Cooperative Oncology Group; GEM = gemcitabine; LN = lymph nodes; UICC = Union Internationale Contre le Cancer.

* Usually administered weekly for 3 weeks with a 1-week rest period.

AC therapy (96.6%) and 42 of 46 patients without AC (91.3%) had PS of 0 to 1 ($p = 0.2543$).

The median follow-up for all 108 patients was 11.0 months (range, 0.4–37.9 months). In the current study, local failure was defined as apparent primary tumor progression detected by computed tomography (CT) scans after CCRT. Assessment of initial response by CCRT was based on CT scans that were obtained within 3 months after CCRT. In the current study, complete response was defined as the complete disappearance of all visible tumor, and partial response (PR) was defined as a reduction of 50% to 99% in the product of the perpendicular diameters of the contrast-enhancing tumor. Progressive disease was defined as an increase of more than 25% in the product of the perpendicular diameters of the contrast-enhancing tumor or any new tumor seen on CT scans, and all other situations were defined as no change (NC). Overall survival (OS), progression-free survival (PFS), and local control (LC) rates were calculated actuarially according to the Kaplan-Meier method (10) and were measured starting from the day of initial treatment. Differences between groups were estimated using the chi-square test, Student's *t* test, and the generalized Wilcoxon test (11). Multivariate analysis was performed using the Cox regression model (12). A probability level of 0.05 was chosen for statistical significance. Statistical analysis was performed using SPSS software (version 11.0; SPSS, Inc., Chicago, IL). Acute and late adverse effects were graded in accordance with the National Cancer Institute-Common Terminology Criteria (NCI-CTC) version 3.0.

## Results

Data regarding initial responses after CCRT were available for 85 patients (Table 3). Of the 3 patients with inoperable tumors, 1 patient had a response of NC, and there was no information regarding tumor responses for the remaining 2 patients. At the time of this analysis, 95 patients (88.0%) had disease recurrence (local only in 29 patients; regional lymph nodes only in 1 patient; liver only in 24 patients; peritoneum only in 27 patients; other distant metastases, such as at bone or lung, only in 4 patients; and multiple sites in 10 patients). Among the 10 patients with multiple recurrences, 6 patients had simultaneous local recurrences. Therefore, local recurrences occurred in a total of 35 patients (32.4%). The 2-year actuarial LC rate for all 108 patients was 41.9%. Figure 1 shows the LC curves according to the total radiation dose. Patients treated with a total dose of 50 Gy or more

**Table 2** Agents and chemotherapy schedules

| Drug | No. of patients receiving a drug* | |
|---|---|---|
| | During RT | After RT |
| GEM | 108 | 53† |
| 5-FU | – | 6† |
| S-1 | – | 5† |

*Abbreviations:* 5-FU = 5-fluorouracil; GEM = gemcitabine; RT = radiotherapy; S-1 = combination of tegafur, 5-chloro-2, 4-dihydroxypyridine, and oteracil potassium.

* A total of 108 patients (100%) received a drug during RT, and 59 patients (54.6%) received a drug after undergoing RT.

† When combination chemotherapy was used, each drug in the combination was counted.

had a significantly more favorable LC rate (2-year LC rate, 42.9%) than patients treated with a total dose of less than 50 Gy (2-year LC rate, 29.6%; $p = 0.0292$). Concerning the regional lymph node recurrence, all 57 patients with clinical stage N0 disease had no regional lymph node recurrence, and only 1 of 49 patients with clinical N1 disease had regional lymph node recurrence.

Eighty-seven of 108 patients (84.5%) died during the period of this analysis. Of these 87 patients, 85 patients died of pancreatic cancer, and the remaining 2 patients died without any sign of clinical recurrence (both of these patients died of intercurrent disease). The 2-year actuarial PFS rate and the median time to progression for all 108 patients were 8.2% and 6.0 months, respectively. Concerning AC use, the 2-year PFS rates for patients treated with AC (10.8%) were significantly higher than those for patients treated without AC (7.8%; $p = 0.0187$). Univariate analysis showed that AC used, clinical T stage, and CA19-9 levels had a significant impact on PFS outcomes, and multivariate analysis showed that AC use and clinical T stage were significant prognostic factors (data not shown).

The 2-year actuarial OS rate and median survival time (MST) in all 108 patients were 23.5% and 11.6 months, respectively. Concerning AC use, 2-year OS rates for patients treated with AC (31.8%) were significantly higher than those for patients treated without AC (12.4%; $p = 0.0022$) (Fig. 2). Univariate analysis showed that AC use, clinical T stage, and CA19-9 levels had a significant impact on OS outcomes (Table 4). However, when we excluded patients with hyperbilirubinemia (more than 2 mg/dl), CA19-9 concentration was not a significant factor for OS, and the 2-year OS rate was 27.4% in patients with CA19-9 concentrations $<$1,000 U/ml and 24.8% in patients with CA19-9 concentrations $\geq$1,000 U/ml ($p = 0.7104$). Multivariate analysis showed that the use of AC (relative risk, 2.475; 95% confidence interval [CI], 1.564–3.917; $p < 0.001$) and clinical T stage (relative risk, 0.374; 95% CI, 0.202–0.692; $p = 0.002$) were significant prognostic factors. Other factors, such as CA19-9 level, tumor size, and total radiation dose did not influence OS outcomes.

In the current study, there were significant differences in the frequencies of AC use according to the initial response ($p < 0.0001$) (Table 3), and patients with favorable responses had more frequently received AC than those with unfavorable responses. Therefore, we conducted subgroup analyses of OS according to initial responses. Concerning patients with an NC response, there was a significant survival benefit with AC use. On the other hand, patients with PR and those with progressive disease response had no significant survival benefit with AC use (Table 3).

Concerning adverse acute effects, 46 patients (42.6%) had Grade 3 to 4 leukopenia, 38 patients (35.2%) had Grade 3 to 4 appetite loss, and 16 patients (14.8%) had Grade 3 to 4 vomiting. Late adverse effects of Grade 3 or higher were observed in 1 patient (1.0%; Grade 3 gastrointestinal bleeding). Total radiation dose given to this patient was 50 Gy.

## Discussion

The current study indicated that CCRT using GEM yields noticeably favorable LC for unresectable pancreatic cancer, with a 2-year LC rate of 41.9%. Concerning initial responses of the 85 available patients, 27 patients (31.8%) had PR, 50 patients (58.8%) had NC response, and only 8 patients (9.4%) had progressive disease response. Several other reports also have indicated the efficacy of EBRT plus GEM therapy for LC (13, 14). Mattiucci *et al.* (13) treated 40 patients with unresectable pancreatic cancer with CCRT using GEM (1,000 mg/m2), and the 2-year LC rate was 39.6% (13). Yamazaki *et al.* (14) indicated that locoregional progression was observed in only 5 of 13 patients with unresectable tumors treated with EBRT plus GEM (14). These results indicate that CCRT using GEM produces relatively favorable LC for patients with unresectable tumors.

Although the efficacy of CCRT using GEM produces relatively favorable LC, optimal use of EBRT, that is, factors such as total radiation doses and radiation field, has not been clarified. National Comprehensive Cancer Network (NCCN) guidelines have recommended that for primary definitive chemoradiotherapy, total doses of 50 to 60 Gy (1.8–2.0 Gy/day) should be administered (15). Several investigators report using total doses of approximately 50 Gy for these tumors when GEM is combined with radiotherapy (13, 14, 16). In the current study, patients treated with total doses of

**Table 3** Comparisons of initial responses and overall survival according to AC use

| Initial response | Total no. of patients | No. of patients | | | 2-year OS rate (%) | | |
|---|---|---|---|---|---|---|---|
| | | AC (+) | AC (–) | *p* value | AC (+) | AC (–) | *p* value* |
| PR | 26 | 25 | 1 | <0.0001 | 25.3 | 0 | 0.3560 |
| NC | 51 | 24 | 27 | | 34.3 | 12.1 | **0.0251** |
| PD | 8 | 2 | 6 | | 0 | 0 | 0.7423 |
| Unknown | 23 | 8 | 15 | | – | – | – |
| Total | 108 | 59 | 49 | | – | – | – |

*Abbreviations:* AC (+) = with adjuvant chemotherapy; AC (–) = without adjuvant chemotherapy; NC = no change; OS = overall survival; PD = progressive disease; PR = partial response.

* *p* value in boldface type indicates significant difference.

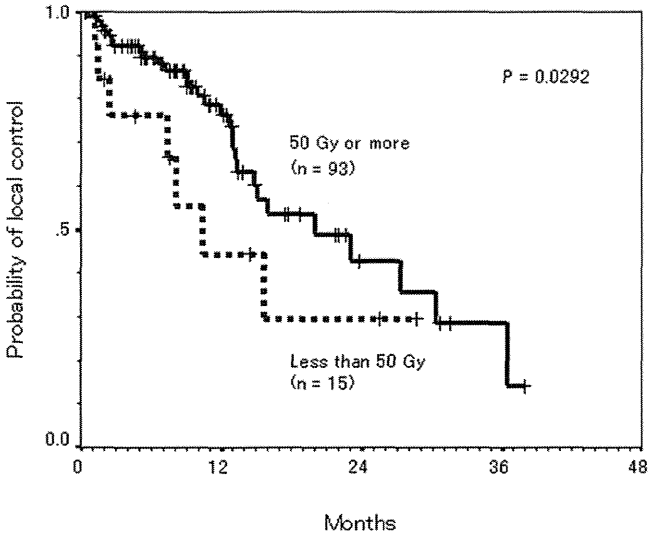


**Fig.1.** LC curves derived according to the total radiation dose in patients with unresectable pancreatic cancer are shown.

50 Gy or more had a significantly favorable LC rate (2-year LC rate, 42.9%) compared to patients treated with total doses of less than 50 Gy (2-year LC rate, 29.6%). These results suggest that doses of 50 Gy or more are appropriate for these tumors.

Concerning radiation fields, NCCN practice guidelines have also recommended that when 5-FU-based chemoradiotherapy is used, treatment volumes should include the primary tumor location and regional lymph nodes (15). When GEM is added, some authors have used the radiation field encompassing the primary tumor along with regional lymph nodes for treating these tumors (13, 16). Recently, other investigators have tried to irradiate only the primary tumor site in order to reduce radiation volume, especially to the intestine (14, 17). Murphy *et al.* (17) indicated that in conjunction with full-dose GEM, the use of conformal fields encompassing only the gross tumor volume (GTV) does not result in marginal failures. In the current study, regional lymph node recurrence was found in only 1 patient (0.9%), and none of the 57 patients with clinical N0 disease had regional lymph node recurrence. Therefore, when GEM is combined with radiation therapy, the treatment of choice may be to irradiate only the field of the primary tumor, especially for patients with stage N0 tumors. Further studies are required to confirm whether radiation only to the primary tumor field would be sufficient when CCRT with GEM is used.

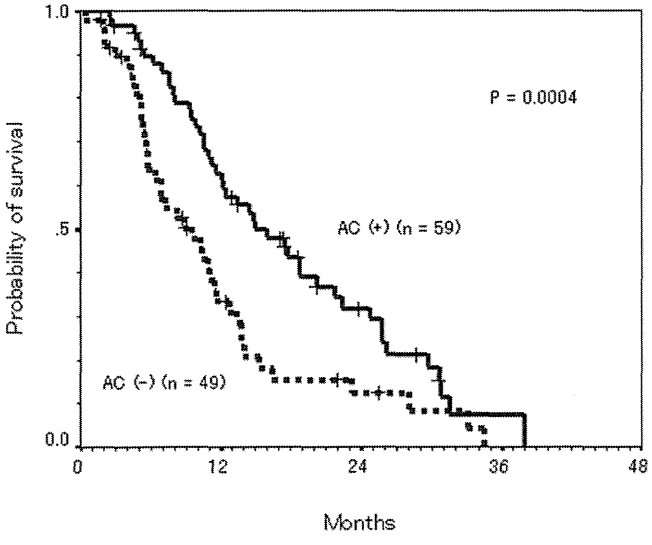


**Fig.2.** Actuarial OS curves according to administration of AC in patients with unresectable pancreatic cancer are shown.

When GEM is used as a single agent for treating patients with advanced cancer, the standard weekly dosage is approximately 1,000 mg/m$^2$, and this dosage is regarded as necessary to control occult distant metastases (4). Therefore, considering both the metastasis-prone and the radio-resistant nature of pancreatic cancer, CCRT using full-dose radiotherapy (50 Gy or more) and full-dosage GEM (1,000 mg/m$^2$ weekly) appears to produce the best outcome. Yamazaki *et al.* (14) indicated that when limited-field 50-Gy radiotherapy was applied, concurrent administration of 1,000 mg/m$^2$ GEM was safe for these patients. Murphy *et al.* (17) indicated that when conformal fields encompassing only the GTV were applied, CCRT with 1,000 mg/m$^2$ GEM was safe (17). On the other hand, several reports have pointed out that CCRT with 1,000 mg/m$^2$ GEM may be too toxic in clinical practice (18, 19). Crane *et al.* (18) indicated that patients receiving GEM-based CCRT developed significantly more severe acute toxicity during treatment than patients receiving 5-FU-based CCRT. Therefore, in order to reduce severe acute toxicity, several researchers conducted studies of CCRT using low-dose GEM (15, 18, 20–22). Shibuya *et al.* (19) conducted a phase II trial of radiotherapy (54 Gy in 28 fractions) with weekly administration of GEM (250 mg/m$^2$) and reported safe and promising results with a median survival time of 16.6 months and an acceptable level of toxicity (19). Huang *et al.* treated 55 patients with unresectable pancreatic cancer with concurrent 50.4-Gy EBRT and GEM, 400 mg/m$^2$ weekly, and found that this regimen can be safely administered (20). Further studies are required to investigate the optimal use of GEM for unresectable tumors.

Although CCRT using GEM provides relatively favorable LC rates, the role of this treatment in survival for these patients remains controversial. Several reports have indicated that when CCRT with GEM was administered, the 2-year OS rates and MSTs ranged from 11% to 25% and 10 to 16.6 months, respectively (13–20). In the current study, the 2-year actuarial OS rate and the median MST for all 108 patients were 23.5% and 11.6 months, respectively. These results indicate that despite the use of GEM, treatment outcomes are generally unfavorable for patients with these tumors. Therefore, it is important to investigate possible factors affecting the prognosis for patients treated with CCRT using GEM.

Several previous studies have suggested potential prognostic factors associated with PS and CA19-9 levels when CCRT is combined with GEM (20, 21). Recently, changes in CA19-9 levels after CCRT have emerged as a predictor for OS in patients with unresectable tumors (22). In the current study, we could not analyze changes in CA19-9 levels after CCRT due to limited information; however, it will be worthwhile to investigate more detailed analysis of CA19-9 levels in future studies. Our results indicated that AC use and clinical T stage were independent prognostic factors for OS. Several phase studies have used AC as a part of GEM-based CCRT (14, 20), and NCCN guideline recommend that (GEM-based) AC should be considered for patients with locally advanced disease who are receiving CCRT (15). Our results also indicated that CCRT with GEM-based AC conferred a survival benefit compared to CCRT without AC, and

**Table 4** Analysis of prognostic factors for OS in patients with unresectable pancreatic cancer treated with CCRT

| Factor | No. of patients | Univariate analysis 2-y OS rate (%) | Univariate analysis $p$ value[†] |
|---|---|---|---|
| Age (years) | | | |
| <70 | 84 | 22.8 | 0.9265 |
| ≥70 | 24 | 27.1 | |
| Gender | | | |
| Female | 50 | 28.1 | 0.7141 |
| Male | 58 | 18.7 | |
| Primary site | | | |
| Head | 55 | 30.3 | 0.8527 |
| Body/tail | 52 | 16.0 | |
| Maximum tumor size | | | |
| <4.0 cm | 48 | 31.0 | 0.6200 |
| ≥4.0 cm | 54 | 23.0 | |
| ECOG performance status scale | | | |
| 0–1 | 98 | 21.6 | 0.7728 |
| 2–3 | 6 | 33.3 | |
| CA19-9 level (U/ml) | | | |
| <1,000 | 56 | 24.5 | **0.0135** |
| ≥1,000 | 43 | 20.8 | |
| Clinical T stage (UICC 2002) | | | |
| 2–3 | 18 | 41.0 | **0.0044** |
| 4 | 90 | 20.0 | |
| Clinical N stage (UICC 2002) | | | |
| 0 | 57 | 22.9 | 0.1377 |
| 1 | 49 | 22.9 | |
| EBRT dose (Gy) | | | |
| <50 | 15 | 17.8 | 0.1624 |
| ≥50 | 93 | 24.6 | |
| Radiation field | | | |
| Primary plus LN | 65 | 20.5 | 0.4224 |
| Primary only | 43 | 27.1 | |
| GEM dose during EBRT | | | |
| Low dose (250–350 mg/m$^2$/week) | 100 | 24.3 | 0.3199 |
| Standard dose (1,000 mg/m$^2$/week*) | 8 | 0 | |
| Adjuvant chemotherapy used | | | |
| Yes | 59 | 31.8 | **0.0004** |
| No | 49 | 12.4 | |

*Abbreviations:* CA19-9 = carbohydrate antigen 19-9; CCRT = concurrent chemoradiotherapy; EBRT = external beam radiotherapy; ECOG = Eastern Cooperative Oncology Group; GEM = gemcitabine; LN = lymph nodes; OS = overall survival; UICC = Union Internationale Contre le Cancer.

* Usually administered weekly for 3 weeks with a 1-week rest period.

[†] $p$ values in boldface type indicate significant difference.

subgroup analysis indicated that patients with a response of NC had significant clinical benefit with AC use. The possible reason for the clinical benefit of AC may be that AC delays the progression of residual primary tumor and/or development of distant metastasis. Therefore, from our results, AC should be administered after GEM-based CCRT, especially for patients with a response of NC. In the current study, 53 of 59 patients (89.8%) received GEM maintenance chemotherapy, usually given at 1,000 mg/m$^2$ weekly for 3 weeks with a 1-week rest period, and this regimen may be an attractive regimen for AC!! therapy. Further studies are required to investigate the optimal regimen of AC for these tumors.

## Conclusions

In conclusion, our results indicated that CCRT using GEM had a relatively favorable LC rate for unresectable pancreatic adenocarcinoma. Our results also indicated that CCRT in addition to AC conferred survival benefit compared to CCRT without AC. Because CCRT using GEM can achieve relatively favorable LC and the addition of AC increased the OS, CCRT using GEM combined with AC appears to be an attractive strategy for treating patients with unresectable tumors. However, this study is a retrospective study with various treatment modalities, and further prospective studies are required to confirm our results.

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ORIGINAL ARTICLE

# Evaluation of pancreatic intraepithelial neoplasia and mucin expression in normal pancreata

**Masato Matsuyama · Fukuo Kondo · Takeshi Ishihara · Taketo Yamaguchi · Ryu Ito · Toshio Tsuyuguchi · Katsunobu Tawada · Osamu Yokosuka**

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**Abstract**
*Background/purpose* It has been suggested that pancreatic ductal adenocarcinoma (PDAC) and pancreatic intraepithelial neoplasia (PanIN) are closely related, but several reports indicate PanIN lesions can also be found in normal pancreata (normal PanINs). We examined differences in mucin expression between normal PanIN lesions and PanINs in PDACs (PDAC PanINs).
*Methods* We examined 54 autopsied normal pancreata and eight autopsied PDACs for PanIN lesions; graded the pancreata specimens as PanIN-1A (non-papillary hyperplasia), PanIN-1B (papillary hyperplasia), PanIN-2 (atypical hyperplasia) or PanIN-3 (carcinoma in situ); and tested the PanIN lesions for expression of MUC1 (pan-epithelial membrane-associated mucin) and MUC5AC (gastric secretory mucin) which were both previously detected in PDACs.
*Results* In normal PanIN-1A, PanIN-1B and PanIN-2 specimens, MUC1 was expressed in 2.8, 10.5 and 9.1%, respectively, compared to 19.1, 27.6 and 13.0% in PDAC PanIN-1A, PanIN-1B and PanIN-2 specimens, respectively. MUC5AC was expressed in 41.0, 65.7 and 36.4% of normal PanIN-1A, PanIN-1B and PanIN-2 specimens, respectively, and in 80.9, 75.8 and 78.3% of PDAC PanIN-1A, PanIN-1B and PanIN-2 specimens, respectively. Differences in the frequency of MUC1 expression were significant between normal and PDAC PanIN-1A ($p < 0.0001$) and PanIN-1B ($p < 0.05$); and differences in the frequency of MUC5AC expression were significant between normal and PDAC PanIN-1A ($p < 0.0001$) and PanIN-2 ($p < 0.05$).
*Conclusions* Normal PanIN and PDAC PanIN lesions differed in the rates of MUC1 and MUC5AC expression.

**Keywords** Pancreatic intraepithelial neoplasia · Mucin · Pancreas · Pancreatic carcinoma · Immunohistochemistry

M. Matsuyama (✉)
Department of Gastroenterology, Cancer Institute Hospital, 3-8-31 Ariake, Koto-ku, Tokyo 135-8550, Japan
e-mail: mahsanmahsan2000@yahoo.co.jp

F. Kondo
Department of Pathology, School of Medicine, Teikyo University, Tokyo, Japan

T. Ishihara · T. Tsuyuguchi · K. Tawada · O. Yokosuka
Department of Medicine and Clinical Oncology, Graduate School of Medicine, Chiba University, Chiba, Japan

T. Yamaguchi
Department of Gastroenterology, Chiba Cancer Center, Chiba, Japan

R. Ito
Department of Gastroenterology, Sanmu Medical Center, Sanmu, Chiba, Japan

## Introduction

The term "pancreatic intraepithelial neoplasia" (PanIN) was proposed by Hruban et al. [1] to refer to putative precursor lesions of pancreatic cancer PanIN lesions present microscopically as papillary or squamous noninvasive epithelial neoplastic lesions that are classified according to both cellular and structural dysplasia [2]. It has been observed that the prevalence of genetic changes increases with the severity of PanIN cytological and architectural atypia, which strongly suggests that such lesions are precursors of pancreatic ductal adenocarcinoma (PDAC) [1, 2].

Mucins are glycoproteins with a high molecular weight and their protein backbone peptides, which contain Ser/

Thr/Pro-rich regions, have many tandem repeats consisting of amino acids that constitute potential *O*-glycosylation sites [3–5]. At least 20 different mucins have been identified so far using molecular cloning techniques [6–9]. Overexpression of MUC1, a pan-epithelial membrane-associated mucin, has been detected in normal pancreatic ductal cells and PDACs, while overexpression of MUC5AC, a gastric secretory mucin, has been detected in PDACs [10–12].

Some investigators have reported that dysplasia of the pancreatic duct can be seen in clinically and macroscopically normal pancreata [13, 14]. Although there are morphological similarities between PanIN lesions in normal pancreata and those found in pancreata with PDACs, few reports have been published on PanIN lesions in normal pancreata. The aim of this study was to examine and compare expression of MUC1 and MUC5AC in PanIN lesions in clinically and macroscopically normal pancreata (normal PanINs) with PanIN lesions in pancreata with PDAC (PDAC PanINs).

## Materials and methods

### Specimen selection and identification of pancreatic intraepithelial neoplasia

We defined a normal pancreas as having both (1) the absence of obvious clinical and pathological signs of pancreatitis and necrosis; and (2) the absence of carcinoma invasion of the pancreas and lymph nodes located near the pancreas. In this study, we examined normal pancreas specimens from 54 autopsied cases. The specimens came from 37 men and 17 women with a mean age of 71 years (range 20 to 80 years) at the time of death and were obtained between January 2001 and October 2005 from the Department of Pathology at Funabashi Central Hospital in Funabashi, Japan. Thirty of the 54 specimens came from individuals >71 years of age. We also examined the pancreata from eight autopsied cases [four men and four women; mean age of 64 years (range 54–79 years)] with PDAC. In every case, written informed consent was obtained from relatives authorizing participation in this study.

In the normal pancreas, the pancreatic specimen was divided into five parts: head, neck, body, body-tail and tail. In the cases with PDAC, five tissue sections of non-cancerous pancreas remote from the carcinoma (or PDAC) lesion were made, and the sections were separated by at least 5 mm from each other. Paraffin-embedded tissue sections were prepared.

In order to exclude non-cancerous areas with necroinflammatory and fibrotic changes, macroscopic findings of the sections were carefully assessed. Histological findings were also carefully examined. When severe necroinflammatory changes were found, the specimen was excluded from the study and a new section was made.

PanIN lesions were identified and graded by two pathologists (FK and RI). Each PanIN lesion was classified according to the criteria established during the International Conference held at Johns Hopkins Hospital in Baltimore, Maryland in August 2003 [1, 15].

### Immunohistochemistry

Tissue blocks were cut into 4-μm-thick serial sections. Each section was deparaffinized and rehydrated using routine techniques. The sections were treated with 3% hydrogen peroxide in methanol and non-specific binding was blocked with 10% nonimmune goat serum. The tissues were then incubated at room temperature for 24 h with a primary antibody against MUC1 (Ma695mAbs; dilution 1:50) or MUC5AC (CLH2mAbs; dilution 1:100), sequentially incubated with a biotinylated secondary antibody, streptavidin-peroxidase conjugate, and 3,3-diaminobenzidine substrate (Vectastatin® ABC Kit, Vector Laboratories, Inc., Burlingame, CA, USA), and then counterstained with Mayer's hematoxylin solution. The sections were washed three times with phosphate-buffered saline after each step [14]. The two antibodies were obtained from Novocastra Laboratories Ltd. (Newcastle-upon-Tyne, UK).

### Analysis of immunohistochemical data

Two pathologists (FK and RI) independently evaluated immunohistochemical staining of MUCs in each tissue preparation. The results were semiquantitatively evaluated for the percentage and staining intensity of positively stained cells. Intensity values of 0, 1, 2 and 3 indicated no, weak, moderate or strong staining of cells, respectively. If positive cells accounted for less than 10% of the total number of cells and/or the staining intensity value was 0 or 1, the result of immunohistochemical staining was considered to be negative [14]. In 3% of the cases, the evaluations by the two pathologists differed so the immunohistochemical-stained slides were reviewed again by the pathologists working together and a consensus was reached between them.

We graded normal PanIN lesions as PanIN-1A (non-papillary hyperplasia), PanIN-1B (papillary hyperplasia), PanIN-2 (atypical hyperplasia) or PanIN-3 (carcinoma in situ) and examined MUC1 and MUC5AC expression in each grade. In addition, we compared MUC expression between normal PanIN and PDAC PanIN lesions. The normal pancreas cases were divided into two age groups, i.e., individuals ≥71 and those <71 years of age at the time