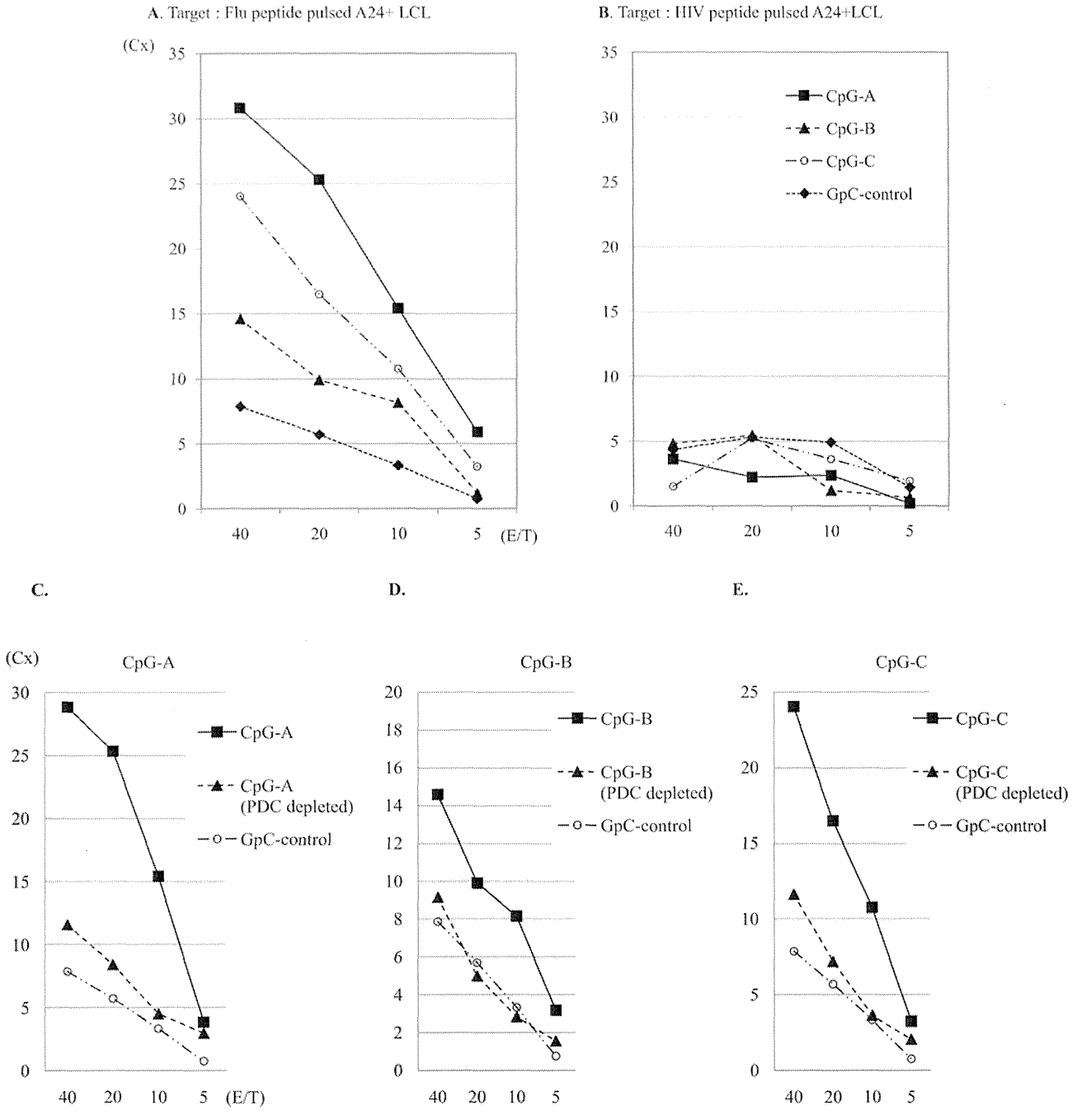

Figure 2. CpG-ODNs augments the influenza peptide-specific CTL induction in a PDC-dependent manner. PBMCs from HLA-A24-positive healthy volunteers were stimulated with the flu peptide in the presence or absence of each class of CpG-ODN (20, 5 and 5 μg/ml for CpG-A, -B and -C, respectively). After 7 days, effector cells were harvested and re-stimulated with the flu peptide pulsed on adherent cells of irradiated PBMCs. After another 7 days, the cytotoxicity of harvested cells against the HLA-A24-positive LCL-A24 cell line pulsed with the flu peptide (A) or a control HIV peptide (B) was assessed by standard ^{51}Cr release assay. Data shown are representative of three independent experiments. PDCs were depleted from PBMCs on day 0. After stimulation with peptide and CpG-ODNs, harvested cells were assessed by standard ^{51}Cr release assay in the same way (C-E).

These data indicated that the stimulation of PDCs by CpG-A augmented the expansion and activation of LY6K peptide-specific CD8$^+$ T cells.

Discussion

Recently, dozens of clinical trials of vaccine therapy for infectious diseases or cancers using CpG-ODNs as an adjuvant have been performed, and some of these trials have shown promising results (5-8). In contrast, there have been few studies that showed the augmenting effects of CpG-ODNs on acquired immunity as a vaccine adjuvant (20). Therefore, the mechanism by which CpG-ODNs augment the efficiency of a vaccine has yet to be clarified in sufficient detail.

In the present study, the efficiency of *in vitro* flu peptide-specific CTL induction by flu peptide was enhanced in the presence of each type of CpG-ODN, and CpG-A showed a more potent adjuvant effect than CpG-B and CpG-C. Moreover, the

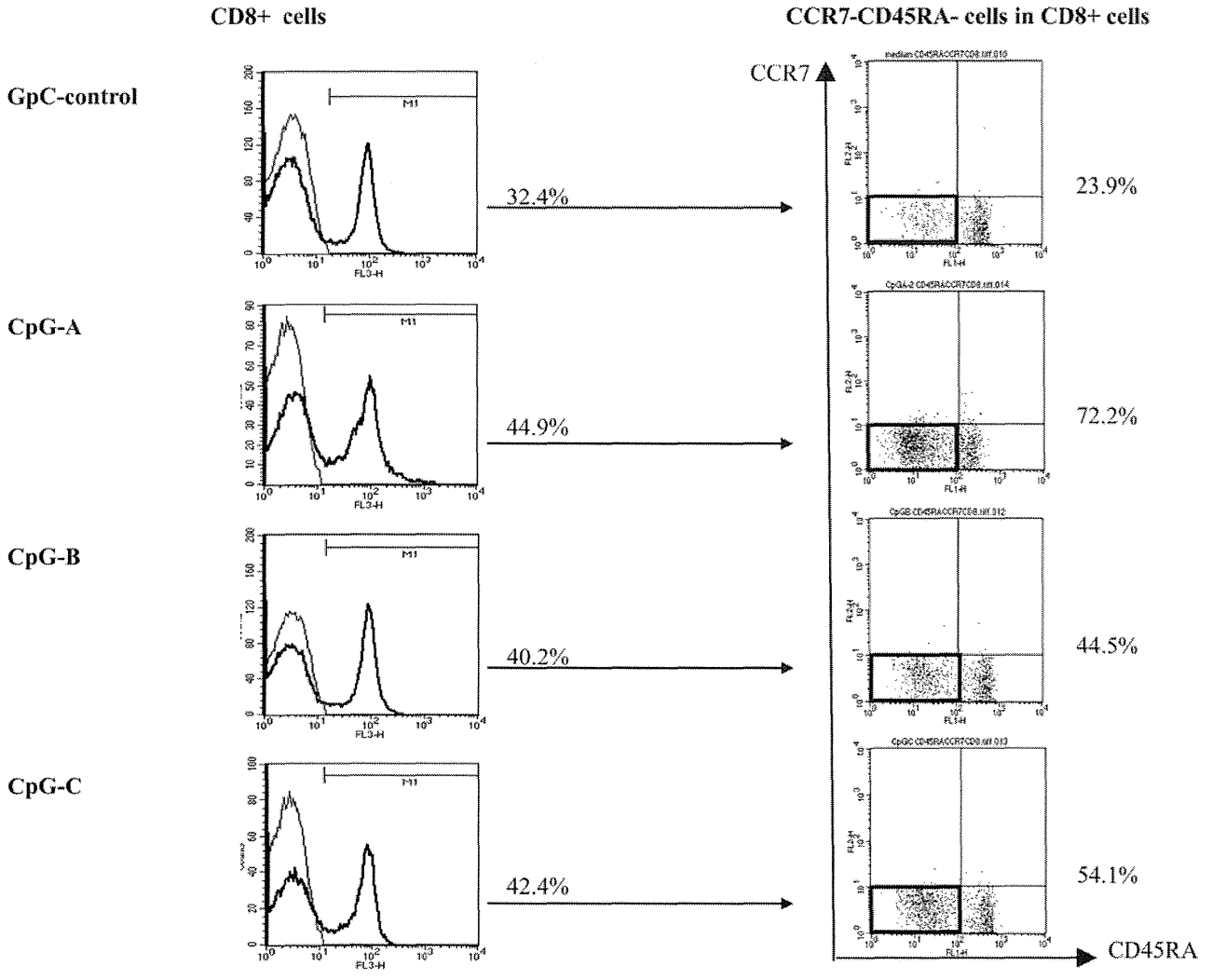

Figure 3. The subpopulation of effector-memory cells in CD8$^+$ cells was increased with CpG-ODN. Populations of CD8$^+$ cells in harvested cells and CCR7$^-$ CD45RA$^-$ effector-memory T cells among CD8$^+$ cells were assessed by flow cytometry.

Ly6K peptide-specific cytotoxicity was induced only with the coexistence of CpG-A, but not with CpG-B and CpG-C. It was suggested that the stimulation of CpG-B or CpG-C was insufficient to elicit Ly6K peptide-specific CTLs because the number of Ly6K peptide-specific precursor CTLs in healthy volunteers is much smaller than that of flu peptide-specific precursor CTLs. These data suggested that CpG-A might be more effective than CpG-B or CpG-C in terms of inducing peptide-specific CTLs *in vitro*.

Our data showed that this CpG-ODN-induced enhancement of cytotoxicity completely disappeared when PDCs were depleted from PBMCs, which means that PDCs were responsible for this enhancement effect. CpG-ODNs mature PDCs by up-regulating the expression of CD80, CD83 and CD86 (21). While most studies have indicated that MoDCs are better antigen-presenters than PDCs (22), many studies have demonstrated the ability of PDCs to function as APCs for both CD4- and CD8-positive cells (22-24). On the other hand, it is well known that CpG-B and CpG-C are more potent to mature PDCs than CpG-A (1,2). Because our data showed that CpG-A was superior to CpG-B and CpG-C in inducing peptide-specific CTLs, the maturation of PDCs by the stimulation of CpG-ODNs could not affect the results in view of our study design. Therefore, we considered that any cytokines produced from PDCs stimulated by CpG-ODNs must contribute to the enhancement of peptide-specific CTL induction. In addition to IFNs, PDCs also produced the pro-inflammatory cytokines TNF-α and IL-6 (data not shown). Type-1 IFN, TNF-α and IL-6 are known to drive the differentiation of DCs into mature antigen-presentation cells. Our data also showed that the supernatant of PBMCs stimulated by CpG-ODNs up-regulated the expression of CD80, 83 and 86 on monocyte-derived DCs, and the expression level was highest with CpG-A, less with CpG-C, and least with CpG-B (data not shown). However, the production levels of TNF-α and IL-6 from PBMCs stimulated by CpG-A were almost the same as those for CpG-B and CpG-C. Murine PDCs are known to produce IL-12, which induces Th1 differentiation by the stimulation of CpG-ODNs, but human PDCs do not induce IL-12 (25,26). In contrast, CpG-A produced a much higher level of IFN-α than CpG-B or CpG-C, and we considered that the reason why CpG-A has the most potent augmenting effect to induce peptide-specific CTLs is that CpG-A induces the highest level of type-1 IFN from PDCs.

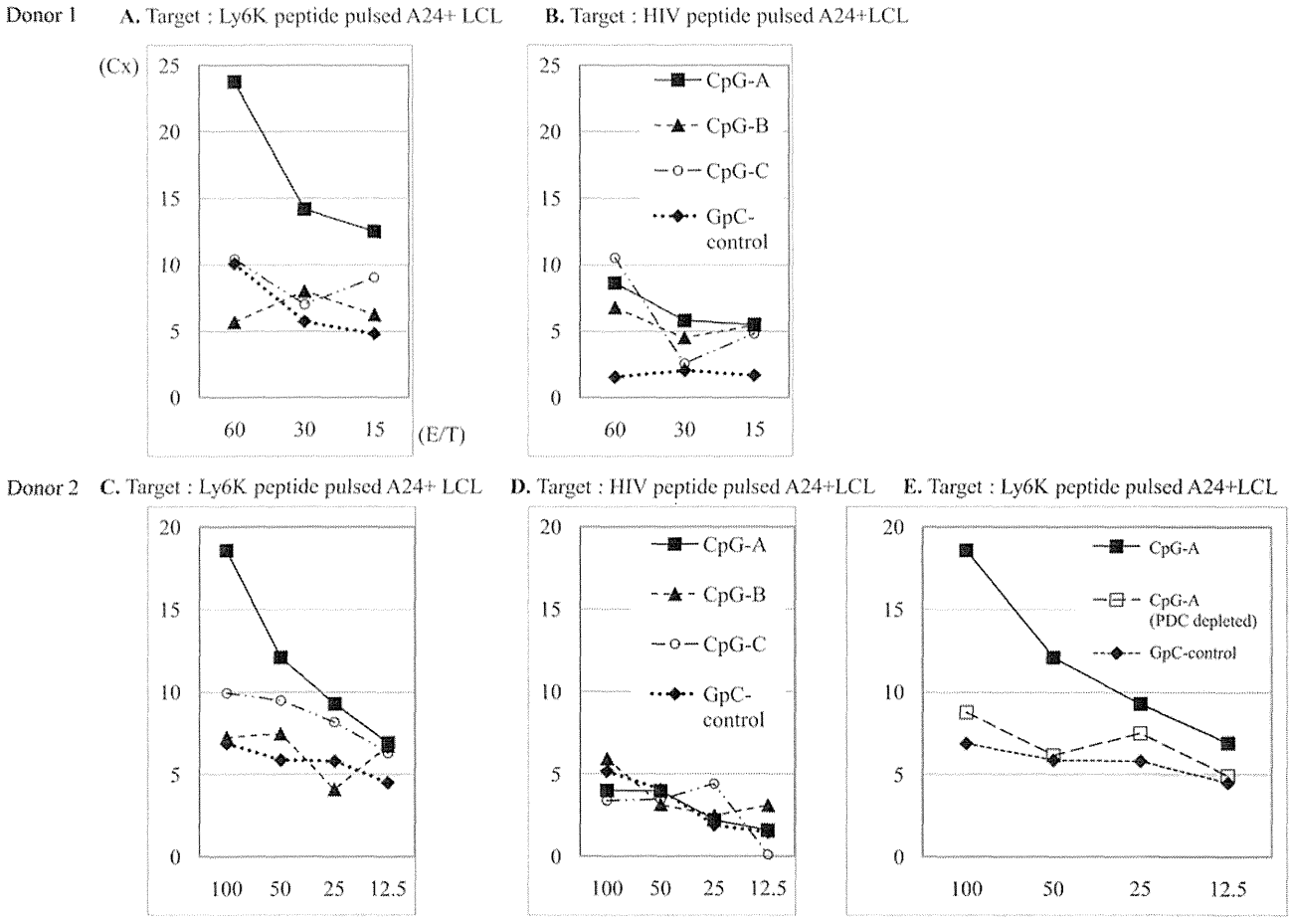

Figure 4. CpG-A augments the Ly6K peptide-specific CTL induction in a PDC-dependent manner. LY6K peptide-specific CTL was generated from 2 different donors in the presence or absence of each class of CpG-ODN (20, 5 and 5 μg/ml for CpG-A, -B and -C, respectively) as described in Materials and methods. The cytotoxicity of harvested cells against the HLA-A24-positive LCL-A24 cell line pulsed with the LY6K peptide (A and C) or a control HIV peptide (B and D) was assessed by standard ^{51}Cr release assay. PDCs were depleted from PBMCs on day 0. After stimulation with peptide and CpG-A, harvested cells were assessed by standard ^{51}Cr release assay in the same way (E).

Type-1 IFNs are known to activate NK cells (27,28) and induce activation of DCs (29-32). In murine models, it is known that type-1 IFNs promote Th1 cytokine production, effector differentiation, proliferation and contribute to the clonal expansion and formation of memory CD8$^+$ T cells (33-40).

Our data revealed that the population of CD8$^+$ cells and that of effector-memory cells in CD8$^+$ cells after induction of flu peptide-specific CTLs were increased the most with CpG-A, less with CpG-C and least with CpG-B. Memory cells persist for extended periods owing to antigen-independent homeostatic turnover and they respond rapidly upon re-encountering a pathogen (41). Two subsets of memory T cells were described on the basis of their anatomical location, expression of cell surface markers and effector functions (42). Memory T cells that express molecules such as CCR7, which allow efficient homing to lymph nodes (LN), are termed central memory cells (T_{CM}), whereas memory T cells that lack expression of these LN homing receptors and are located in no lymphoid tissues are termed effector memory cells (T_{EM}). Some studies have also shown that T_{EM} acquire effector functions, such as cytokine production and killing, more rapidly than T_{CM} (42-44). The mechanisms that contribute to the generation of memory cells are poorly understood. Previous studies suggested that infectious antigen-experienced CD8$^+$ T cells undergo programmed expansion for about 1 week after infection and then undergo programmed cell death (45-47). A majority of the daughter cells derived from antigen-experienced CD8$^+$ T cells undergo death in parallel with proliferation during the acute phase of viral infection and direct type-I IFN action rescues them from this death, thereby tilting the balance effectively toward clonal expansion (40). Therefore, the type-I IFN-mediated rescue from death during antigen-driven proliferation might be critical for the expansion of memory precursors.

In conclusion, our data showed that the stimulation of PDCs by CpG-ODN augmented the generation of effector-memory peptide-specific CTLs. Furthermore, CpG-A might be superior to CpG-B and CpG-C in augmenting the generation of human peptide-specific CTLs *in vitro*. Therefore, CpG-A could become a superior vaccine adjuvant rather than CpG-B or CpG-C in clinical application.

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OPEN

Natural History of Branch Duct Intraductal Papillary Mucinous Neoplasm With Mural Nodules

A Japan Pancreas Society Multicenter Study

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Objective: This study aimed to elucidate the natural history of intraductal papillary mucinous neoplasm (IPMN) of the pancreas with mural nodules (MNs) in branch duct IPMN (BD-IPMN).

Methods: Among the 402 registered patients with BD-IPMN on long-term follow-up at 10 institutions in Japan, 53 patients with MNs of less than 10 mm in height detected by endosonography were included in this study. The morphological changes of the BD-IPMN in these patients and histologic findings of the resected specimen were investigated.

Results: The median height of the MNs at the initial diagnosis was 3 mm (range, 1–8 mm), and 12 (23%) of the 53 patients showed an increase in the height of the MNs during follow-up (mean duration, 42 months). Six patients underwent surgery because of an increase in the height of MNs, yielding high-grade dysplasia in 1 patient and low-grade dysplasia in 5 patients. No patients developed invasive carcinoma derived from IPMN, and distinct pancreatic ductal adenocarcinoma developed in 1 (2%) patient. The incidence of the development of malignancy in BD-IPMNs, including distinct pancreatic ductal adenocarcinoma, was similar to that of those without MNs.

Conclusions: In patients who have BD-IPMN with MNs of less than 10 mm in height, observation instead of immediate resection is considered to be possible.

Key Words: intraductal papillary mucinous neoplasm, natural history, follow-up, endoscopic ultrasonography, pancreatic ductal adenocarcinoma

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The authors declare no conflict of interest.

Abbreviations: BD-IPMN - branch duct intraductal papillary mucinous neoplasm, CT - computed tomography, ERCP - endoscopic retrograde cholangiopancreatography, EUS - endoscopic ultrasonography, IPMN - intraductal papillary mucinous neoplasm, MD-IPMN - main duct intraductal papillary mucinous neoplasm, MN - mural nodule, MPD - main pancreatic duct, MRCP - magnetic resonance cholangiopancreatography, PDAC - pancreatic ductal adenocarcinoma, US - ultrasonography

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According to the international consensus guidelines 2012[1] for the management of intraductal papillary mucinous neoplasms (IPMNs) and mucinous cystic neoplasms of the pancreas, main duct IPMN (MD-IPMN) and branch duct IPMN (BD-IPMN) are significantly different with regard to the prevalence of carcinoma, and therefore, the classification has prognostic implications. When MD-IPMN is diagnosed in a patient, surgical treatment should be considered. In BD-IPMN, however, it is important to differentiate low-grade dysplasia from high-grade dysplasia (carcinoma in situ) or ordinary invasive pancreatic ductal adenocarcinoma (PDAC) to avoid excessive surgery.

The presence of mural nodules (MNs) has reportedly been the most important factor for predicting malignancy and determining the indication for surgery of BD-IPMN. However, there is a paucity of data on the morphological and histologic changes in patients with BD-IPMN with MNs during follow-up. The aim of this study was to evaluate long-term follow-up results of patients with BD-IPMN who had MNs on initial imaging in a retrospective multicenter series for better management of patients with BD-IPMN.

MATERIALS AND METHODS

Patients

The working group of the Japan Pancreas Society for the investigation of the natural history of IPMN,[2] 5 university hospitals and 5 tertiary referral institutions, collected information on 417 follow-up patients for more than 1 year who had undergone endoscopic ultrasonography (EUS) at the time of initial diagnosis during the period from November 1993 to February 2008. Those patients who had been followed up due to an inoperable PDAC derived from IPMN were excluded.

The indications for follow-up were based on the suggestions described in the international consensus guidelines 2006[3]; these are as follows: BD-IPMNs with no symptoms such as abdominal pain, jaundice, or pancreatitis, MNs of less than 10 mm in height, cyst size of less than 3 cm, and main pancreatic duct (MPD) dilation of less than 10 mm. Ten patients with an initial

cyst size of 3 cm and 5 patients with that of more than 3 cm were included.

Among the 417 follow-up patients, 15 were excluded from the analysis because they did not satisfy the inclusion criteria, which are as follows: follow-up periods of less than 1 year in 4 patients, MPD dilation of more than 10 mm in 5 patients, histologically diagnosed as non-IPMN in 3 patients, MN height of more than 10 mm in 1 patient, and incomplete data in 2 patients. Accordingly, 402 patients with BD-IPMN without MNs of 10 mm or greater in height on EUS at the time of initial diagnosis who had been followed up by several surveillance imagings for 1 year or more were eligible for this study.[2] Finally, a total of 53 patients with BD-IPMN with MNs of less than 10 mm in height who underwent EUS, ultrasonography (US), and/or computed tomography (CT) at least twice, including the initial EUS during follow-up, were included.

Definitions

A diagnosis of IPMN was made by imaging when a dilated MPD or a cystically dilated branch duct was recognized in association with secretion of mucin from the major or minor papilla or mobile filling defects in the pancreatic duct on endoscopic retrograde cholangiopancreatography (ERCP) or when multilocular cystic lesions were recognized on EUS, magnetic resonance cholangiopancreatography (MRCP), and/or CT. Branch duct IPMN was defined as a condition in which the main lesion was a cystically dilated branch duct with an MPD diameter of less than 10 mm. The size of the dilated branch duct was measured en bloc in patients with multilocular cysts. The presence or absence of MNs in cystic branches was determined based on morphological features on EUS at the initial diagnosis. Color Doppler imaging or contrast-enhanced EUS was not applied in most of the cases because of the dominant use of a mechanical radial scanner. The change in the height of MNs was assessed essentially by follow-up EUS at registration, as available. In patients who underwent surgery after follow-up, the diagnosis of IPMN was confirmed histologically. Pathologic results were determined by the World Health Organization criteria published in 2010[4]; these are as follows: low-grade dysplasia ("intraductal papillary mucinous adenoma"), intermediate-grade dysplasia ("IPMN with moderate dysplasia"), high-grade dysplasia ("intraductal papillary mucinous carcinoma, noninvasive," "carcinoma in situ"), and PDAC. The highest pathologic grade was adapted when there were multifocal lesions.

Pancreatic ductal adenocarcinoma was divided into 2 types, as reported by Yamaguchi et al,[5] 1 derived from IPMN ("IPMN with an associated invasive carcinoma") showing a histologic transition between IPMN and invasive carcinoma and the other concomitant with IPMN in which invasive carcinoma developed at a site in the pancreas different from that of the IPMN, according to the radiologic images and macroscopic or microscopic findings.

Methods

In patients with evident MNs in the cystic lumen, the height of the most prominent MNs was measured by EUS. During the follow-up period, strict monitoring of BD-IPMNs was performed by EUS, US, MRCP, and/or CT at intervals of 3 to 6 months. The modality used for the monitoring of BD-IPMNs was at the discretion of each institution and on a case-by-case basis, not following a unified protocol.

The maximum diameter of cystically dilated branch ducts and MPDs was measured by EUS in combination with US, CT, and/or MRCP, as available. Morphological findings at the initial examination, including the height of MNs, size of cystic branch, diameter of MPD, and presence of multifocal lesions, were collected. The frequency of enlargement of the cystically dilated branch, progression of MPD dilation, and an increase in the height of MNs were investigated using the follow-up data. Then, the characteristics of patients with BD-IPMN showing an increase in the height of MNs during follow-up were evaluated and compared with those patients without such an increase. In patients who had undergone surgery with morphological progression during follow-up, histologic findings of the resected specimens were evaluated, and the incidence and background of the development of invasive carcinoma associated with those lesions during follow-up were investigated.

These characteristics and morphological changes in the patients with IPMN with MNs were compared with those of the patients who did not show MNs on EUS at the time of initial diagnosis. The incidences of the development of PDAC derived from IPMN and PDAC concomitant with IPMN during follow-up were compared as well. Furthermore, the factors predictive of PDAC concomitant with IPMN were investigated.

Statistical Analysis

The average age, maximum size of cystic branch, maximum diameter of the MPD, and maximum height of MNs at the initial examination were compared using Student *t* test. The differences in the incidence of sex, enlargement of cystic branch, progression of MPD dilation, progression of MNs height, and multifocal lesions were examined with the χ^2 test or Fisher exact test. *P* value of less than 0.05 was considered significant. The predictive factors for PDAC concomitant with IPMN were investigated by univariate analysis. The calculations were carried out using SPSS II for Windows (release 16.0; SPSS, Chicago, Ill).

RESULTS

Follow-up Results

The mean follow-up period of the 53 patients with BD-IPMN with MNs was 42.4 (SD, 22.2) months (range, 12–196 months). There were 28 men and 25 women, with a mean age of 66.1 (SD, 8.1) years (range, 44–83 years).

At the time of the initial diagnosis, all 53 patients underwent EUS. Computed tomography, US, MRCP, and ERCP were also carried out in 32, 30, 26, and 40 patients, respectively. At registration, EUS, CT, US, MRCP, and ERCP were carried out in 43, 25, 15, 27, and 20 patients, respectively. The mean height of MNs among the 53 patients at the start of follow-up was 3.2 (SD, 1.6) mm (median, 3 mm; range, 1–8 mm), and MN heights of less than 5 mm and those 5 to 10 mm were found in 41 (77.4%) patients and 12 (22.6%) patients, respectively. The mean maximum size of the cystically dilated branch and the mean diameter of the MPD were 2.2 (SD, 0.9) cm (range, 0.8–4.2 cm) and 3.9 (SD, 1.4) mm (range, 1–9 mm), respectively. The cystically dilated branch with the main MN was located in the head of the pancreas in 32 patients, in the body in 16 patients, and in the tail in 5 patients. Ten patients underwent surgery because of an increase in the height of the MNs (n = 6), enlargement of the dilated branches (n = 1), development of concomitant PDAC (n = 1), emergence of symptoms (n = 1), and patient's request (n = 1).

Changes in the Size of Cystic Branches

During follow-up, enlargement of the cystic branch was identified in 4 (7.5%) of the 53 patients, none of whom showed an increase in the size of the MNs (Fig. 1). One patient, a 68-year-old

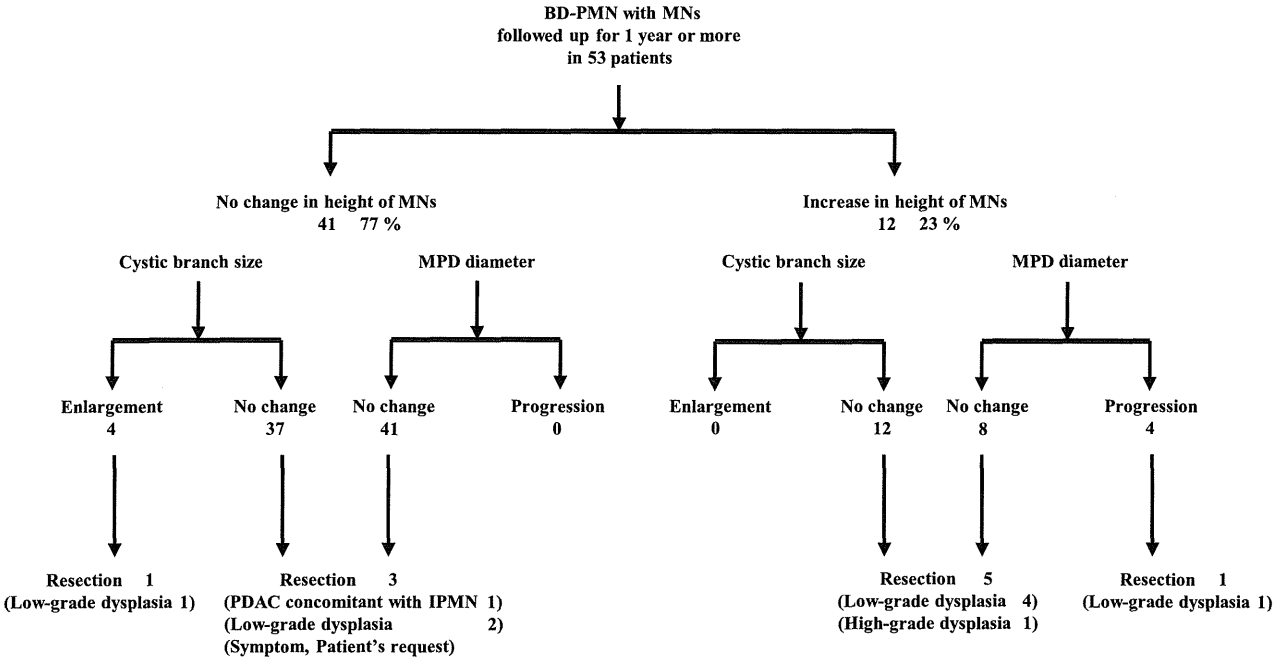

FIGURE 1. Changes in MNs, cyst size, and MPD diameter during follow-up.

man, underwent surgery because of an increase in the size of the cystic branch from 3 to 4.5 cm in 37 months, whereas the height of MN (3 mm) remained unchanged during follow-up. The histologic examination of the resected specimen verified low-grade dysplasia.

Changes in the Size of MPD

Of the 53 patients, 4 (8%) showed progression of MPD dilation during the follow-up (Fig. 1). All of these 4 patients exhibited an increase in the size of the MNs as well; these are as follows: from 3 to 6 mm, from 8 to 13 mm, from 4 to 8 mm, and from 5 to 8 mm, respectively. One patient underwent resection, leading to a pathologic diagnosis of low-grade dysplasia (patient 7; Table 1). The other patients are now under follow-up.

Changes in the Height of MNs

Of the 53 patients, 12 (23%) showed an increase in the height of the MNs during follow-up (Fig. 1). In those 12 patients, the mean size of the cystic branch, the mean diameter of the MPD, and the mean height of the MNs at the initial examination were not significantly different from those in the group without an increase in the size of the MNs during follow-up (Table 2). Furthermore, none of these 12 patients showed an enlargement of the cystic branch during follow-up. The frequency of progression of MPD dilatation during follow-up was significantly higher in the group with an increase in the height of MNs than those in the group without (33% vs 0%, $P = 0.002$). Furthermore, there was no significant difference in the frequency of enlargement of cystic branch between the groups (0% vs 10%, $P = 0.35$).

TABLE 1. Patients With BD-IPMN Showing an Increase in Height of MNs During Follow-up (n = 12)

Patient no	Age, y	Sex	Follow-up, mo	MN Size, mm	Progression of MPD Dilation, mm	Enlargement of Cystic Branch	Resection	Histologic Findings
1	58	F	60	1 → 4	—	—	−	—
2	76	F	40	1 → 5	—	—	−	—
3	74	F	32	3 → 8	—	—	−	—
4	69	F	60	3 → 6	5 → 7	—	−	—
5	75	M	74	8 → 13	6 → 12	—	−	—
6	75	M	67	4 → 8	4 → 9	—	−	—
7	61	F	82	5 → 8	6 → 10	—	+	Low-grade dysplasia
8	56	F	24	3 → 5	—	—	+	Low-grade dysplasia
9	54	M	15	1 → 3	—	—	+	Low-grade dysplasia
10	63	M	26	7 → 13	—	—	+	Low-grade dysplasia
11	60	M	91	5 → 10	—	—	+	Low-grade dysplasia
12	72	M	71	6 → 13	—	—	+	High-grade dysplasia

F indicates female; M, male.

TABLE 2. Comparison of Characteristics Between the Patients With BD-IPMN With and Without an Increase in Height of MNs During Follow-up (n = 53)

	Height of MNs		
	Increased	No Change	
	n = 12 (23%)	n = 41 (77%)	*P*
Mean (SD) age, y	66.1 (8.3)	66.1 (8.2)	0.99
Sex (male/female)	7/5	21/20	0.66
Initial average (SD) size of cystic branch, cm	2.6 (1.0)	2.1 (0.8)	0.07
Initial average (SD) diameter of MPD, mm	4.3 (1.50)	3.8 (1.4)	0.29
Initial average (SD) height of MNs, mm	3.9 (2.4)	3.0 (1.4)	0.24
Enlargement of cystic branch	0	4 (10%)	0.35
Progression of MPD dilation	4 (33%)	0	<0.01 (0.002)
High-grade dysplasia	1 (8%)	0	
Invasive carcinoma derived from IPMN	0	0	
Invasive carcinoma concomitant with IPMN	0	1 (2%)	

Six of the 12 patients showing an increase in the height of MNs underwent surgery. Histologic examination of the resected specimens verified high-grade dysplasia in 1 patient and low-grade dysplasia in 5 patients. None of them showed development of PDAC (Table 1). Among the 6 other patients who did not undergo surgery, 1 patient with MNs of 13 mm in height refused surgery and the remaining 5 patients who had MNs of less than 10 mm in height are under follow-up.

Of the 41 patients without an increase in the height of MNs, 4 underwent surgery, 1 of whom had a new appearance of a solid mass in a different portion in the pancreas. Histologic examination of the resected specimen revealed the mass to be a PDAC concomitant with IPMN and the IPMN itself was a low-grade dysplasia (Fig. 1). In the remaining 3 patients, pathologic diagnosis was all low-grade dysplasia.

Development of Malignancy Among Surgical Cases

To summarize the 10 surgical cases, 1 (2%) patient developed PDAC concomitant with IPMN without enlargement of the cystic branch, an increase in the height of MNs, or progression of MPD dilation.

In the remaining 9 patients, 1 (2%) had high-grade dysplasia and the others had low-grade dysplasia. The patient with high-grade dysplasia showed an increase in the height of the MNs from 6 to 13 mm in 71 months without enlargement of the cystic branch or progression of MPD dilation (Fig. 1).

Comparison of Morphological Changes and Histologic Findings Between Patients With BD-IPMN With and Without MNs

Size of Cystic Branches

The comparison of morphological changes and histologic findings between patients with BD-IPMN with and without MNs is shown in Table 3. At the time of the initial diagnosis, the mean maximum size of the cystically dilated branch in the patients with and without the MNs was 2.2 (SD, 0.9) cm and 2.0 (SD, 0.9) cm, respectively. There was no significant difference between the 2 groups ($P = 0.28$). Furthermore, there was no significant difference in the incidence of enlargement of cystic branch during follow-up between the groups (8% vs 9%, $P = 0.47$).

Diameter of MPD

The initial diameter of the MPD in the patients with MNs was significantly greater than that in those without (3.9 [SD, 1.4] mm vs 3.3 [SD, 1.3] mm, $P = 0.001$). On the other hand, there was no significant difference in the frequency of progression of MPD dilation during follow-up between the groups (8% vs 7%, $P = 0.52$).

Development of Malignancy

The number of patients who underwent surgery in each group with and without MNs was 10 of the 53 patients and 29

TABLE 3. Comparison of BD-IPMNs With and Without MNs by EUS at the Initial Examination (n = 402)

MNs by Initial EUS	Present (n = 53)	Absent (n = 349)	*P*
Mean (SD) age, y	66.1 (8.1)	65.7 (10.0)	0.79
Sex (male/female)	28/25	178/171	0.80
Initial average (SD) size of cystic branch, cm	21.7 (8.8)	20.2 (9.3)	0.28
Initial average (SD) diameter of MPD, mm	3.9 (1.4)	3.3 (1.3)	<0.01 (0.001)
Enlargement of cystic branch	4 (8%)	32 (9%)	0.47
Progression of MPD dilation	4 (8%)	24 (7%)	<0.01 (0.002)
High-grade dysplasia	1 (2%)	8 (2%)	0.66
Invasive carcinoma derived from IPMN	0	1 (0.3%)	0.87
Invasive carcinoma concomitant with IPMN	0	1 (2%)	0.72

TABLE 4. Risk Factors of PDAC Concomitant With IPMN During Follow-up by Univariate Analysis n = 402

	PDAC Concomitant With IPMN (n = 8)	Others (n = 394)	*P*
Mean (SD) age, y	69.0 (6.6)	65.7 (9.8)	0.34
Sex (male/female)	5/3	201/193	0.39
Presence of MNs	1 (13%)	52 (13%)	0.72
Initial average (SD) size of cystic branch, cm	2.0 (1.2)	2.0 (0.9)	0.81
Initial average (SD) diameter of MPD, mm	3.4 (7.4)	3.4 (1.3)	0.97
Initial average (SD) height of MNs, mm	0.3 (0.7)	0.4 (1.3)	0.69
Enlargement of cystic branch	0	36 (9%)	0.47
Progression of MPD dilation	1 (13%)	27 (7%)	0.44
Increase in height of MNs	0	38 (10%)	0.45
Multifocal lesions	2 (25%)	129 (33%)	0.49

of the 349 patients, respectively. Among the 53 patients with MNs, the PDAC derived from IPMN developed in 0 patient, the PDAC concomitant with IPMN in 1 (1.9%) patient, and high-grade dysplasia in 1 (1.9%) patient. The corresponding numbers in the group without MNs including 3 patients with unresected PDAC concomitant with IPMN were 1 (0.3%), 7 (2.0%), and 8 (2.3%). There were no significant differences in the development of malignancy—high-grade dysplasia ($P = 0.66$), PDAC derived from IPMN ($P = 0.87$), and PDAC concomitant with IPMN ($P = 0.72$)—between the 2 groups.

Factors Predictive of Development of Malignancy in BD-IPMNs During Follow-up

One patient with PDAC derived from IPMN did not show the emergence of MNs, enlargement of cystic branch, increase in the height of MNs, or multifocal lesions. However, MPD dilation progressed during the follow-up. In other patients with BD-IPMN without PDAC derived from IPMN, the frequency of progression of MPD dilation was 7%.

The factors predictive of PDAC concomitant with IPMN in the follow-up patients were investigated by univariate analysis. There was no significant difference in each morphological feature between the patients with PDAC concomitant with IPMN and those without (Table 4).

DISCUSSION

The international consensus guidelines recommend that patients with BD-IPMNs who have MNs should basically consider surgery if clinically appropriate. The subjects of the present study, however, were patients who had been observed because the height of MNs was less than 10 mm. In these patients, strict monitoring of BD-IPMNs had been performed at short intervals, which enabled investigation of the natural history of BD-IPMNs with MNs of less than 10 mm in height. Among these patients, 23% showed an increase in the height of the MNs during follow-up for over 40 months. In this group, there were no patients who showed enlargement of the cystic branch; however, all 4 patients who showed progression of MPD dilation exhibited an increase in the size of the MNs. The frequency of progression of MPD dilatation during follow-up was significantly higher in the group with an increase in the height of MNs than in the group without.

In the follow-up patients who had BD-IPMN with MNs, none developed PDAC derived from IPMN. The incidence of the development of malignancy in BD-IPMNs including a distinct PDAC was similar to that of those without MNs reported in the literature. Therefore, in those who have a BD-IPMN with MNs of less than 10 mm in height, observation instead of immediate resection is considered to be possible.

It is well known that IPMNs are characterized by slow progression and a favorable prognosis in contrast to ordinary PDAC, which is recognized as being very invasive.[6–11] Histologic studies of resected IPMNs have revealed that most IPMNs are dysplasia without parenchymal invasion such as high-grade dysplasia (carcinoma in situ), intermediate-grade dysplasia (borderline, moderate dysplasia), and low-grade dysplasia.[4] On the other hand, the presence of PDAC derived from IPMN showing parenchymal invasion has also been recognized, colloid carcinoma and tubular adenocarcinoma being its predominant histologic cell types. Therefore, the indications for surgery and determination of operative procedures based on the biologic behavior of this tumor are currently of great concern.

There are 2 opinions as to the indications for surgery in IPMN. One is that all patients with IPMN, including those with low-grade dysplasia, should undergo resection. This idea is based on the possible existence of an adenoma-carcinoma sequence in the evolution of this type of neoplasm and is also supported by the observations of oncogene activation. Yanagisawa et al[12] reported that the same point mutation was detected both in the area of carcinoma and in coexisting adenoma components. Furthermore, duct-ectatic mucinous cystic neoplasms accompany K-*ras* point mutation similar to typical exocrine pancreatic carcinomas.

On the other hand, with the increase in clinical knowledge on the progression of IPMNs, the demand for establishing surgical indications that take the biologic behavior of such neoplasms into consideration is increasing.[13–16] There are some groups who recommend surgery only in cases of high-grade dysplasia or invasive carcinoma, avoiding excessive surgery for benign conditions.

Main duct IPMN and BD-IPMN are significantly different with regard to the prevalence of carcinoma,[13–16] and therefore, the classification has prognostic implications. In the review by Tanaka et al,[1] the frequency of invasive carcinoma in MD-IPMN and in BD-IPMNs have a mean of 43% (range, 11%–81%) and 18% (range, 1%–37%), respectively.

According to the new international consensus guidelines 2012 for the management of IPMNs,[1] when MD-IPMN is diagnosed in a patient with an IPMN, surgical treatment is strongly recommended. In BD-IPMN, however, the likelihood of invasive carcinoma is substantially less compared with that in MD-IPMNs. Thus, the differentiation of low-grade dysplasia from high-grade dysplasia or PDAC derived from IPMN would enable us to avoid excessive surgery.

Among the subjects of the present study on BD-IPMN, surgical resection was indicated according mainly to the previous international guidelines 2006,[3] which are as follows: the

appearance of symptoms attributable to IPMN (eg, pancreatitis), a cyst size greater than 30 mm, and dilation of the MPD (>6 mm).[16–19] The usefulness of the previous consensus criteria for resection has been validated by many reports.[20–24] According to the international consensus guidelines 2012, because a cyst size of more than 3 cm is a weaker indicator of malignancy than the presence of MNs and positive cytology, a BD-IPMN of more than 3 cm in size without MNs or positive cytology can be observed without immediate resection, particularly in elderly patients.

Some researchers consider the measurement of the maximum height of the MNs in BD-IPMNs to be effective for the differentiation between high-grade dysplasia and low-grade dysplasia and have suggested the height of the papillary protrusion of 3 to 10 mm as a cutoff value for determining the indication for surgical treatment.[25,26] In our retrospective study on the relationship between the height of MNs on EUS and histologic findings,[26] most patients in whom the maximum height of the MNs was more than 10 mm experienced high-grade dysplasia (86%). Furthermore, among those who underwent surgery due to the presence of MNs, no patients with MNs of 5 to10 mm in maximum height as shown by EUS developed PDAC derived from the BD-IPMN. Considering the biologic behavior of this neoplasm, performing surgery only in cases of BD-IPMN with a maximum height of MNs of more than 10 mm is likely to be justified.

Unfortunately, these retrospective studies entailed selection bias, that is, only patients with BD-IPMN who had been considered to have indications for surgery due to the presence of MNs and had undergone surgery were included. To verify the appropriateness of surgical indications based on the maximum height of MNs, a better understanding of the developmental course and the process of invasion in IPMN is necessary. The investigation of the morphological and histologic changes in patients with BD-IPMN who have undergone follow-up studies before resection is thus indispensable.

In 2011, the same working group of the Japan Pancreas Society[2] reported long-term follow-up results of 349 patients who had no MNs on EUS at initial diagnosis. The results showed that the PDAC derived from IPMN and the distinct PDAC developed in 0.3% of the patients and 2.0% of the patients, respectively. In contrast, among the patients with MNs in the present multicenter study, PDAC derived from IPMN and PDAC concomitant with IPMN developed in 0% of the patients and 2% of the patients, respectively; that is, the incidence of the development of invasive carcinoma in BD-IPMNs with MNs was similar to that of those without MNs.

As previously reported, there may possibly be 2 developmental patterns of PDAC derived from IPMN, 1 with an increase in the height of MNs of more than 10 mm[10] and the other at a site that is rather flat.[26] Therefore, periodical surveillance is mandatory in BD-IPMNs regardless of the presence/absence and height of MNs.

Concerning the histologic type, most of the patients with PDAC had tubular adenocarcinomas showing few papillary growths, whereas approximately 30% of the patients with PDAC derived from IPMN had colloid carcinomas with high papillary protrusions. Colloid carcinoma derived from intestinal type[27] is deemed to show more expansive and slower progression compared with other invasive carcinomas.[11] Therefore, PDAC derived from IPMN shows a different invasive behavior from ordinary PDAC. Yamaguchi et al[5] reported that the median survival time of 122 patients with PDAC derived from IPMN was 46 months, which was significantly longer than what was reported (12 months) in 7605 patients with ordinary PDAC.

Another problematic issue in patients with BD-IPMNs is that a distinct PDAC may develop in patients with IPMN, either synchronously or metachronously. Ohtsuka et al[28] reported that the incidence of synchronous and metachronous multifocal occurrence of IPMNs in the remnant pancreas during follow-up evaluation after pancreatectomy for IPMNs was 20% and that of distinct PDAC was 9.9%. Izawa et al[29] stated the possibility of multicentric development of cancer in IPMN, based on the observation that hyperplasia developed multifocally in different branch ducts with a different frequency of K-*ras* point mutation. In this study, we could not detect any significant predictive factors for the development of PDAC concomitant with IPMN.

The present study has several limitations. First, because of the retrospective nature of this study, the modality used for monitoring of BD-IPMN at intervals of 3 to 6 months was at the discretion of each institution, not following a unified protocol. Second, the presence of MNs was determined based solely on morphological features on EUS without color Doppler imaging, which may have resulted in inclusion of mucus nodules. However, the requirement in the inclusion criteria to have undergone EUS, US, and/or CT at least twice is thought to have minimized this risk. Third, the number of patients with MNs included in this study was not large because the presence of MNs is considered to be the most important factor for the surgical indication of BD-IPMN regardless of its height. However, the subjects were patients who had been followed up despite having MNs, and the number is the largest in the literature to date owing to multicenter cooperation. Fourth, patients with BD-IPMN who underwent surgery and histologic examination of resected specimens included not only patients showing an increase in the MN height to more than 10 mm but also those with no change or a change within 10 mm in height of MNs during follow-up. Furthermore, there is no way to investigate the incidence of high-grade dysplasia in the 43 patients who had not had surgical resection.

In summary, no PDAC derived from BD-IPMN developed in patients with MNs of less than 10 mm in height during follow-up for over 40 months. Furthermore, the incidence of the development of malignancy in BD-IPMNs including a distinct PDAC was similar to that of those without MNs. In patients who have BD-IPMN with MNs of less than 10 mm in height, observation instead of immediate resection is considered to be possible.

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ORIGINAL ARTICLE

Validation of a Nomogram for Predicting the Probability of Carcinoma in Patients With Intraductal Papillary Mucinous Neoplasm in 180 Pancreatic Resection Patients at 3 High-Volume Centers

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AQ2 Objective: We previously published a nomogram for prediction of carcinoma in patients with intraductal papillary mucinous neoplasm (IPMN). The objective of the current study was to validate this nomogram in an external cohort of patients at multiple institutions.

Methods: The clinical details of 180 patients with IPMN who underwent a pancreatic resection at 3 hospitals were collected. Four significant predictive factors (sex, lesion type, nodule height, and pancreatic juice cytology) were analyzed.

Results: Of the 180 patients, 66 (36.7%) had a main pancreatic duct–type IPMN and 114 (63.3%) had a branch pancreatic duct–type IPMN. The final pathological diagnosis was benign IPMN in 95 (52.8%) patients and malignant IPMN in 85 (47.2%) patients. The area under the receiver operating characteristic curve for the model was 0.760. The area under the receiver operating characteristic curve of the IPMN nomogram for prediction of malignancy was 0.747 in main pancreatic duct–type IPMN and 0.752 in branch pancreatic duct–type IPMN. The sensitivity and specificity of the model were 80.0% and 57.9%, respectively, when the predictive probability of less than 10% was used to indicate the presence of carcinoma.

Conclusions: This nomogram for predicting the probability of carcinoma in patients with IPMN was accurate in an external validation patient cohort.

Key Words: IPMN, nomogram, external validation, multicenter

(*Pancreas* 2014;00: 00–00)

In 1982, Ohashi et al[1] first described intraductal papillary mucinous neoplasms (IPMNs) of the pancreas as mucin-secreting tumors. The number of patients diagnosed with IPMN has increased with increasing awareness and advances in diagnostic imaging. In 2006, the international consensus guidelines for the management of IPMN were published.[2] However, application of these guidelines led to resection in many cases of IPMN adenoma (IPMA).[3] Many reports have attempted to identify the prognostic factors that might guide the management of patients with IPMN,[4–6] but there is no consensus with regard to the operative indications. In the revised international consensus guidelines of 2012,[7] resection is recommended for all main pancreatic duct (MPD) IPMN. In branch pancreatic duct (BPD) IPMN, the indications for resection are more conservative and "worrisome feature" that can be observed without immediate resection has been proposed.

We constructed a nomogram to predict carcinoma on the basis of a test cohort of 81 patients who had undergone IPMN resection before December 2008 at the Aichi Cancer Center Hospital (ACC). The area under the receiver operating characteristics curve (AUC) of this nomogram was 0.903 for prediction of carcinoma.[8] External validation of any diagnostic tool is important to determine whether the diagnostic accuracy reported in the original study can be reproduced outside the original cohort. In this study, we validated the IPMN nomogram in an external cohort of patients who underwent pancreatic resection at multiple institutions using standardized preoperative examination modalities, shared definitions of lesion types, and standardized pathological diagnostic criteria.

MATERIALS AND METHODS

Study Population AQ3

The study population was 281 patients with IPMN who underwent pancreatic resection at Wakayama Medical University (WMU) and Teine Keijinkai Hospital (TKH) between January 1996 and March 2011 or at ACC between January 2009 and March 2011 (Table 1). Fifty-nine cases in which endoscopic ultrasonography (EUS) was not performed preoperatively and 42 cases in which pancreatic juice cytology was not performed preoperatively were excluded. We therefore included 180 patients for validationof the IPMN nomogram. The following features were evaluated: age at the time of operation, sex, presence or absence of symptoms, preoperative laboratory values (serum amylase, carcinoembryonic antigen [CEA], and carbohydrate antigen 19-9 [CA19-9] level), imaging findings (tumor location, size of mural nodules, diameter of MPD, cyst size of BPD, type of lesion), operative procedure, and pathological findings. T1

Endoscopic ultrasonography and computed tomography (CT) were considered to be essential preoperative investigations for all patients. The height of any mural nodule(s) was determined through EUS. For MPD diameter and cyst size, CT measurement values were used.

The lesions were classified as MPD IPMN, Mix-IPMN, and BPD IPMN as per recently reported criteria.[9] With MPD IPMN, the lesions exist in the MPD and there is no cystic formation of 10 mm or greater in the surrounding branches. Cases with cystic dilatation of BPD are classified as Mix-IPMN or BPD IPMN, when the MPD diameter is 10 mm or greater or less than

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The authors declare no conflict of interest.

AQ9 **TABLE 1.** Patients of External Validation Cohort

Institute	Operation Period	No. Patients	EUS(+)	Cytology(+)
WMU	January 1996–March 2011	179	120	97
TKH	January 1996–March 2011	78	78	59
ACC	January 2009–March 2011	24	24	24
Total		281	222	180

Cytology, pancreatic juice cytology.

10 mm, respectively. In this study, both MPD IPMN and Mix-IPMN were analyzed as MPD-type IPMN (Table 2). T2

Four factors of sex, type of lesion, size of nodules, and pancreatic juice cytology were scored with the IPMN nomogram, as reported previously (Fig. 1). To use the nomogram, points are assigned on a scale of 0 to 100 for each predictor and are added together for the final score. This value is located on the "total points" axis with a vertical ruler, and the ruler is followed down to read predicted cancer probability. The nomogram was used for overall prediction analysis of all 180 patients as well as the subsets of MPD type and BPD types (Table 2). F1

Pancreatic juice cytology was classified on levels I to V in accordance with the grade of structural and cytologic dysplasia.[10] Class I indicates completely benign and nonneoplastic epithelium of no or slight dysplasia, class II indicates regenerative or neoplastic epithelium of slight dysplasia, class III indicates neoplastic epithelium of mild dysplasia corresponding to adenoma, class IV indicates neoplastic epithelium of moderate dysplasia highly suggestive of adenocarcinoma, and class V indicates unequivocal malignant epithelium corresponding to adenocarcinoma.

According to the World Health Organization[11] (WHO) histological classification of IPMN, pathological diagnosis was classified as IPMA, borderline IPMN (IPMB), as well as noninvasive and invasive IPMN carcinoma (IPMC). *Invasive IPMC* is defined as a histological transition that is clearly present between IPMN and pancreatic ductal adenocarcinoma.[12]

Cytological and pathological diagnosis was performed by pathologists at the 3 hospitals (WMU, TKH, and ACC), and the central review of pathological diagnosis was done by A.Y. at Kyoto Prefectural University of Medicine in the cases of IPMB as well as noninvasive and invasive IPMC. All patients were categorized as benign (IPMA and IPMB) or malignant (noninvasive and invasive IPMC) on the basis of the pathological diagnosis after resection.

Statistical Analysis

Continuous variables were compared using the Student *t* test, and discrete variables were examined using the χ^2 test. All of the *P* values presented were 2 sided, and a *P* value of less than 0.05 was considered to be significant. A receiver operating characteristics curve[13,14] was used to measure the predictive accuracy of the nomogram for malignant IPMN.

On the basis of the nomogram, we selected a cutoff value for the predicted probability of malignant IPMN. The cutoff value was selected to provide high sensitivity while, at the same time, reducing the number of resections of benign IPMN. The JMP 7.0.1 statistical software (SAS Institute, Incorporation, Cary, NC) was used in the analysis.

TABLE 2. Classification of Type of Lesion in Patients With IPMN

Nomogram Lesion Type	Classification[9]	MPD	BPD Dilation
MPD	MPD IPMN	Lesions exist	None or <10 mm
	Mix-IPMN	Diameter ≥10 mm	+
BPD	BPD IPMN	Diameter <10 mm	+

RESULTS

Characteristics of Patients in External Validation Cohort

The details of the patients and their imaging, tumor location, surgical procedures, as well as pathological findings are given in Table 3. Sixty-six (36.7%) patients had an MPD-type IPMN and 114 (63.3%) patients had a BPD-type IPMN. Higher grades of dysplasia in pancreatic juice cytology were found in MPD-type lesions (Table 3). The size of mural nodules was also significantly larger in MPD-type IPMN than in BPD-type IPMN. There were no significant differences in sex, presence of symptoms, preoperative laboratory values (serum amylase, CEA, and CA19-9 level), tumor location, or pathological findings between T3

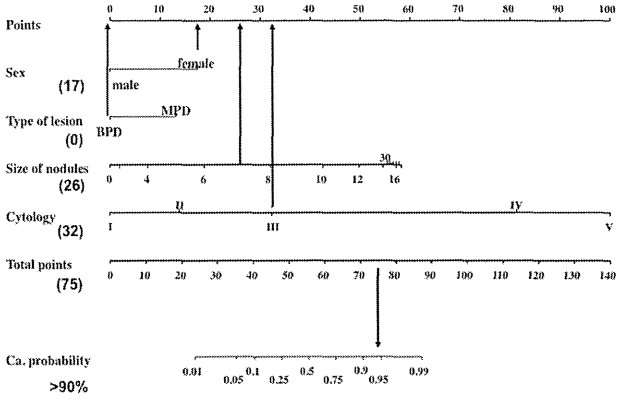

FIGURE 1. Nomogram for the detection of IPMC. Sex, BPD or MPD IPMN, size of mural nodules, and grade of pancreatic juice cytology for the individual patient were used. A line is drawn in the upward direction to indicate the number of points in each category. These points are totaled and then a line is drawn downward to indicate the patient's risk for IPMC. For example, in a case of a female with a BPD-type IPMN, a 7-mm nodule size, and a cytology class III, the patient's total score of 75 corresponds to more than a 90% likelihood of IPMC. Figure adapted from Shimizu et al.[8] Adaptations are themselves works protected by copyright. So in order to publish this adaptation, authorization must be obtained both from the owner of the copyright in the original work and from the owner of copyright in the translation or adaptation. AQ8

TABLE 3. Characteristics of Patients With IPMN Who Underwent Pancreatic Resection (n = 180)

No. Patients	Total (N = 180)	MPD Type (n = 66)	BPD Type (n = 114)	*P*
Background				
Age at pancreatectomy, y*	68.0 (9.2)	69.9 (7.8)	67.0 (9.2)	0.0387
Sex, n (%)				0.5578
Male	106 (58.9)	37 (56.0)	69 (60.5)	
Female	74 (41.1)	29 (44.0)	45 (39.5)	
Symptom, n (%)	57 (31.7)	20 (30.3)	37 (32.5)	0.7644
Laboratory data*				
AQ10 Amylase level, IU/la	121.0 (127.4)	124.8 (140.4)	118.8 (120.0)	0.7612
CEA level, ng/mla	2.6 (2.5)	2.7 (1.8)	2.5 (2.9)	0.7054
CA19-9 level, U/mla	40.9 (154.4)	49.5 (230.9)	35.9 (83.9)	0.5692
Pancreatic juice cytology				
I/II/III/IV/V	52/100/19/4/5	12/39/8/3/4	40/61/11/1/1	0.0236
Image findings				
Tumor location, n (%)				0.4344
Head	112 (62.2)	42 (63.6)	70 (61.4)	
Body	54 (30.0)	21 (31.8)	33 (29.0)	
Tail	14 (7.8)	3 (4.6)	11 (9.6)	
Size of mural nodules, mm*	8.3 (8.2)	10.3 (9.6)	7.2 (7.0)	0.0138
Diameter of MPD, mm*	8.8 (8.2)	15.2 (10.4)	5.0 (2.2)	<0.0001
Cyst size of BPD, mm*	25.3 (16.5)	17.7 (1.9)	29.8 (12.7)	<0.0001
Operative procedure				
AQ11 PD, PpPD/DP, MP, PR/TP, n (%)	114/56/10 (63.3/31.1/5.6)	44/13/9 (66.7/19.7/13.6)	70/43/1 (61.3/37.7/1.0)	0.0002
Pathology				
Benign IPMN, n (%)	95 (52.8)	29 (43.9)	66 (57.9)	0.0705
Malignant IPMN, n (%)	85 (47.2)	37 (56.1)	48 (42.1)	
Non./Inv.	61/24	26/11	35/13	

*Values are presented as mean (SD).

DP, distal pancreatectomy; Inv., invasive; MP, middle pancreatectomy; Non., noninvasive; PD, pancreatoduodenectomy; PpPD, pylorus-preserving pancreatoduodenectomy; PR, partial resection of the pancreas; TP, total pancreatectomy.

the patients with MPD-type IPMN and the patients with BPD-type IPMN.

Mural nodules were detected in 134 (74.4%) of the 180 patients, including 53 (80.3%) of the 66 patients with MPD-type IPMN and 81 (71.1%) of the 114 patients with BPD-type IPMN. Ten (11.8%) of the 85 patients with malignant IPMN had no nodules. In 4 patients with MPD-type IPMN and 6 patients with BPD-type IPMN, the pathological findings were noninvasive carcinoma in 9 patients and invasive carcinoma in 1 patient (data not shown).

External Validation of IPMN Nomogram

For the entire cohort of patients with IPMN, the AUC of the IPMN nomogram was 0.760 for predicting the presence of carcinoma. The AUC was similar for the patients recruited at the

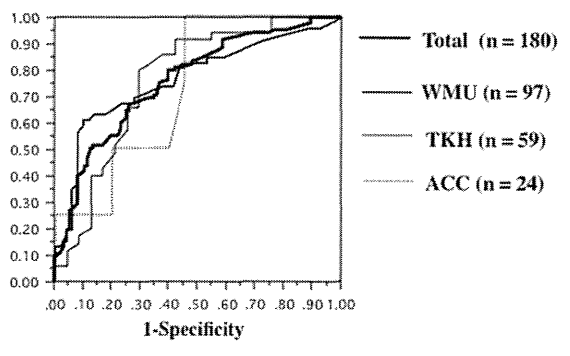

FIGURE 2. Receiver operating characteristics curve of nomogram for predicting the probability of malignant IPMN in extra validation cohort (n = 180). The AUC is 0.760. With each of the 3 centers of WMU, TKH, and ACC, the AUC was 0.768, 0.767, and 0.731, respectively.

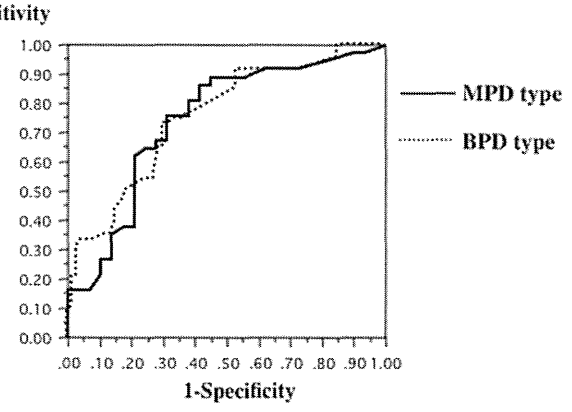

FIGURE 3. Receiver operating characteristics curve of nomogram for predicting the probability of malignant IPMN in MPD-type IPMN (n = 66) and BPD-type IPMN (n = 114). The AUC is 0.745 and 0.752, respectively.

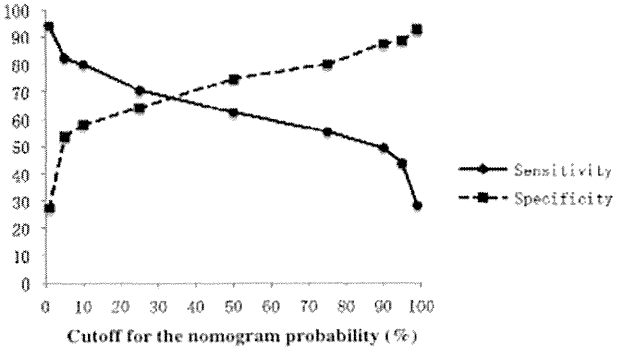

FIGURE 4. *Sensitivity and specificity are estimated on the basis of the validation data set (n = 180) as a function of a cutoff point for the malignant IPMN predicted probability.*

different centers (0.768, 0.767, and 0.731 for WMU, TKH, and ACC, respectively; Fig. 2). For the subset of MPD- and BPD-type IPMN, the AUC of the IPMN nomogram was 0.747 and 0.752, respectively (Fig. 3). There was no difference in the result between the first half period (January 1996–December 2003) and the second half period (January 2004–March 2011), with the AUC being 0.750 and 0.749, respectively (data not shown).

Using this nomogram, if only those patients with 10% or higher predicted probability of pancreatic carcinoma underwent surgery, then the model would capture 80% (68/85) of all patients with malignant IPMN (sensitivity) while sparing 57.9% (55/95) of the patients without malignancy from undergoing an unnecessary surgical procedure (specificity). The PPV and NPV of the nomogram were 63.0% (68/108) and 76.4% (55/72), respectively (Fig. 4, Table 4). There were 17 patients with malignant IPMN who had less than 10% predicted probability of pancreatic carcinoma on IPMN nomogram. In these 17 patients, the pathological findings were noninvasive carcinoma in 13 patients and invasive carcinoma in 4 patients. Three of the 4 patients with invasive carcinoma had minimally invasive carcinoma.[15,16]

The IPMN nomogram could predict carcinoma in the 66 patients with MPD-type IPMN, with a 91.9% (34/37) sensitivity, a 31.0% (9/29) specificity, a 63.0% (34/54) PPV, and a 75.0% (9/12) NPV. Applied to the 114 patients with BPD-type IPMN, the IPMN nomogram had a 70.8% (34/48) sensitivity, a 69.7% (46/66) specificity, a 63.0% (34/54) PPV, and a 76.7% (46/60) NPV (Tables 5, 6).

DISCUSSION

The risk for malignancy is higher in MPD IPMN and is relatively low in BPD IPMN.[2,17–20] However, there is no consensus with regard to operative indications in individual cases. In the new international consensus guidelines revised in 2012,[7] resection is recommended for all MPD IPMN, whereas, in BPD IPMN, the indications for resection are more conservative and cyst size of BPD of greater than 30 mm without "high-risk stigmata" can be observed without immediate resection. The BPD IPMN cyst size of greater than 30 mm and MPD dilation of 5 to 9 mm are classified as worrisome features, and EUS observation is recommended to decide a treatment strategy.

Nomograms have been widely used to develop treatment and follow-up strategies for various neoplasms, such as prostate and colorectal cancer.[21–25] In 2004, Brennan et al[26] reported creation of a nomogram that predicted outcome after resection of pancreatic cancer. However, there was no similar model to predict malignancy in IPMN. In response to this problem, we previously created a cancer prediction nomogram in patients with IPMN and reported its utility.[8] This nomogram is based on 4 predictive factors (sex, lesion type, nodule height, and pancreatic juice cytology data) and provides an outstanding cancer prediction capability, with an AUC of 0.903.[8]

In the present study, we validated this nomogram in an external validation cohort of patients with IPMN who underwent pancreatic resection at the 3 institutes. In this cohort of patients, we standardized preoperative examination modalities, used common definitions for the type of lesions, and conducted a central review of pathological findings, as we reported recently.[9] The newer (2010) WHO classification uses the terms *low-grade*, *intermediate-grade*, and *high-grade dysplasia* in place of adenoma, borderline, and noninvasive carcinoma. However, in this study, the subjects were 180 patients who underwent pancreatic resection at the 3 hospitals between January 1996 and March 2011. Pathologists at these 3 hospitals (WMU, ACC, and TKH) diagnosed the lesions as IPMA (mild, moderate, severe) or IPMC (noninvasive, invasive) in accordance with the classification of pancreas carcinoma of the Japan Pancreas Society.[15,16] We used the WHO (2000) histological classification of IPMN, in which pathological diagnosis is classified as IPMA, IPMB, or noninvasive and invasive IPMC.

When creating the nomogram, lesion type was classified into 2 groups: MPD type and BPD type[8] (Fig. 1). All lesions in the MPD measuring 10 mm or greater were classified as MPD-type IPMN. In this validation study, therefore, patients with Mix-IPMN of our classifications[9] were classified as MPD-type IPMN and a total of 66 patients with MPD-type IPMN and 114 patients

TABLE 4. Diagnostic Ability of Nomogram (n = 180)

	Pathological Diagnosis	
Nomogram	Malignant IPMN (n = 85)	Benign IPMN (n = 95)
Positive (n = 108)	68	40
Negative (n = 72)	17	55

Malignancy probability 10% cutoff value.

TABLE 5. Diagnostic Ability of Nomogram in MPD-Type IPMN (n = 66)

	Pathological Diagnosis	
Nomogram	Malignant IPMN (n = 37)	Benign IPMN (n = 29)
Positive (n = 54)	34	20
Negative (n = 12)	3	9

Malignancy probability 10% cutoff value.

TABLE 6. Diagnostic Ability of Nomogram in BPD-type IPMN (n = 114)

	Pathological Diagnosis	
Nomogram	Malignant IPMN (n = 48)	Benign IPMN (n = 66)
Positive (n = 54)	34	20
Negative (n = 60)	14	46

Malignancy probability 10% cutoff value.

with BPD-type IPMN were investigated (Tables 2, 3). The AUC of the receiver operating characteristics analysis was 0.760 in all 180 patients and showed good diagnostic performance even in the subset analyses of the 3 different institutions (Fig. 2). With a cutoff score of 40 points (equivalent to 10% cancer probability), we found a good diagnostic ability (sensitivity, 80.0%; specificity, 57.9% for prediction of malignancy; Fig. 4, Table 4). Invasive carcinoma with less than 10% predicted probability of pancreatic carcinoma on IPMN nomogram was present in only 4 patients. Pathologically, there was a massive invasion of the pancreatic parenchyma in only 1 patient and a minimally invasive carcinoma in 3 patients, for which prognosis seems to be comparable with that of noninvasive carcinoma.[15,16] Hence, an invasive carcinoma was missed in only 4 (2.2%) of the 180 patients.

We found good AUC values of 0.747 and 0.752 for MPD-type IPMN and BPD-type IPMN, respectively (Fig. 3). If the 66 patients with MPD-type IPMN, in whom resection is recommended based on the existing guidelines,[7] were treated on the basis of our nomogram, 9 patients without malignancy would avoid an unnecessary operation, whereas 3 patients with malignant IPMNs (noninvasive carcinomas) would have been missed (Table 5). Particularly in BPD-type IPMN, for which operative indications are controversial, using a carcinoma probability cutoff level of 10%, we are able to predict a benign IPMN by a specificity of 69.7% while maintaining a sensitivity of 70.8%, showing a high rate of diagnostic accuracy (Table 6). Although a few cancers will be missed using this approach, the nomogram seems to be a valid adjuvant tool for the clinicians to assess an individual's risk for malignant IPMN.

Sadakari et al[27] reported that, among cases of BPD IPMN with no nodules, 6 (8.2%) of 73 patients who underwent pancreatic resection had carcinoma. Recently, we reported that the size of mural nodules observed through EUS was a significant predictor of malignancy, but there were 15 patients (15/160 [9.4%]) who had carcinoma with no nodules.[9] Even in the present investigation, 10 (11.8%) of the 85 patients with cancer had no nodules. The combination of cytology and diameter of MPD[3,27] or pancreatic juice CEA measurements[28] are reported to be effective in identifying patients with carcinoma among patients with IPMN without nodules. It is difficult to predict malignant IPMN on the basis of a single parameter, and the use of a nomogram is a more reliable tool because it takes multiple factors into consideration.

Our IPMN nomogram was based on 4 significant predictive factors (sex, lesion type, nodule height, and pancreatic juice cytology data). There are some limitations to our model. Because our analysis includes the fact that pancreatic juice was obtained for cytology during endoscopic retrograde cholangiopancreatography for all patients, the nomogram may be applicable only to potential candidates for surgery rather than all patients diagnosed with IPMN. However, as for the application to a follow-up strategy in patients with IPMN, we recently reported the ability of our nomogram.[29] We recommended the risk assessment using the nomogram at the initial evaluation of IPMN and then decided follow-up schedule through CT and/or EUS. Our results indicated that annual follow-up would be appropriate for scores of less than 35, indicating an extremely low risk for cancer development within 3 years at least. Meanwhile, 3 to 6 months of close follow-up would be recommended for scores of 35 or higher; it indicates high potential for malignant transformation. Because of the retrospective nature of our study design, we plan to prospectively validate the applicability of our nomogram to management strategies for patients with IPMN.

In conclusion, we have validated this nomogram for predicting the probability of carcinoma in patients with IPMN and it may be applicable to a diverse population treated at multiple centers.

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High and low negative pressure suction techniques in EUS-guided fine-needle tissue acquisition by using 25-gauge needles: a multicenter, prospective, randomized, controlled trial

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Background: EUS-guided FNA (EUS-FNA) has a high diagnostic accuracy for pancreatic diseases. However, although most reports have typically focused on cytology, histological tissue quality has rarely been investigated. The effectiveness of EUS-FNA combined with high negative pressure (HNP) suction was recently indicated for tissue acquisition, but has not thus far been tested in a prospective, randomized clinical trial.

Objective: To evaluate the adequacy of EUS-FNA with HNP for the histological diagnosis of pancreatic lesions by using 25-gauge needles.

Design: Prospective, single-blind, randomized, controlled crossover trial.

Setting: Seven tertiary referral centers.

Patients: Patients referred for EUS-FNA of pancreatic solid lesions. From July 2011 to April 2012, 90 patients underwent EUS-FNA of pancreatic solid masses by using normal negative pressure (NNP) and HNP with 2 respective passes. The order of the passes was randomized, and the sample adequacy, quality, and histology were evaluated by a single expert pathologist.

Intervention: EUS-FNA by using NNP and HNP.

Main Outcome Measurements: The adequacy of tissue acquisition and the accuracy of histological diagnoses made by using the EUS-FNA technique with HNP.

Results: We found that 72.2% (65/90) and 90% (81/90) of the specimens obtained using NNP and HNP, respectively, were adequate for histological diagnosis ($P = .0003$, McNemar test). For 73.3% (66/90) and 82.2% (74/90) of the specimens obtained by using NNP and HNP, respectively, an accurate diagnosis was achieved ($P = .06$, McNemar test). Pancreatitis developed in 1 patient after this procedure, which subsided with conservative therapy.

Limitations: This was a single-blinded, crossover study.

Conclusion: Biopsy procedures that combine the EUS-FNA with HNP techniques are superior to EUS-FNA with NNP procedures for tissue acquisition. (Clinical trial registration number: UMIN000005939.) (Gastrointest Endosc 2014;80:1030-7.)

Abbreviations: CI, confidence interval; EUS-FNA, EUS-guided FNA; HNP, high negative pressure; NNP, normal negative pressure.

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