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Chapter 3
Natural History and Malignant Transformation of Branch Duct IPMN

Hiroyuki Maguchi and Satoshi Tanno

Abstract The number of reports published on the follow-up data of patients with BD-IPMN has been increasing. Accumulating evidence from independent 12 studies revealed that the mean frequency of morphological changes of BD-IPMN, such as increased cyst size, increased MPD diameter, and/or appearance or enlargement of MNs, was 27.4 % (range, 14.9–61.8 %) of 1,293 followed-up patients (follow-up period, 2.6–8.1 years). Surgical resection was carried out in 9.9 % (range, 0–22.2 %) of all cases. Among the resected cases, 27.3 % were diagnosed histologically as malignant. During the follow-up period, malignant transformation was observed in only 2.7 %. BD-IPMNs without MNs have a low risk for malignant transformation regardless of cyst size at the initial diagnosis. Malignant transformation is associated with signs of progression especially appearance or enlargement of MNs and/or an increase in the MPD diameter. On the other hand, PDAC develops independently in the pancreas distinct from BD-IPMN. The mean frequency of PDAC occurrence was 2.8 % (range, 1.4–8.0 %) of all cases during the follow-up.

In conclusion, careful attention should be paid to the occurrence of PDAC in the entire pancreas in addition to progression of BD-IPMN when performing follow-up examinations in patients with BD-IPMN.

Keywords BD-IPMN • BD-IPMNs without MNs • Follow-up • Guideline 2012 • Malignant transformation • MNs • Morphological changes • MPD diameter • Natural history • Pancreatic ductal adenocarcinoma (PDAC) • PDAC concomitant with IPMN • Progression

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3.1 Introductory Remarks

IPMNs can be classified into three types, i.e., main duct IPMN (MD-IPMN), branch duct IPMN (BD-IPMN), and mixed type, based on imaging study and/or the histology in the revised guidelines (Tanaka et al. 2012). The frequency of malignant BD-IPMN such as IPMN with high-grade dysplasia or noninvasive cancer and IPMN with an associated invasive cancer is lower than that of MD-IPMN and mixed type (Tanaka et al. 2012). Patients with BD-IPMN who do not have any sign of malignancy may be managed conservatively.

Although the natural history of BD-IPMN is not well established, there have been an increasing number of reports published on the follow-up data in patients with BD-IPMN.

3.2 Morphological Change of BD-IPMN During the Follow-Up Period

The published reports on the follow-up of BD-IPMN were summarized in Table 3.1 (Kobayashi et al. 2005; Lee et al. 2007; Rautou et al. 2008; Tanno et al. 2008; Guarise et al. 2008; Sawai et al. 2010; Uehara et al. 2011; Maguchi et al. 2011; Arlix et al. 2012; Bae et al. 2012; Ohno et al. 2012; Khannoussi et al. 2012). Total number of cases was 1,293. The range of mean initial cyst size and main pancreatic duct (MPD) diameter was 15–28 mm and 2.4–3.8 mm, respectively. Almost all

Table 3.1 Morphological changes of BD-IPMN during follow-up

		Initial imaging findings				
Author (year)	Number of cases	Cyst size (mm)	MPD (mm)	MN (absent/present)	Progression (%)	Follow-up period (year)
Kobayashi et al. (2005)	47	28	–	10/37	7 (14.9)	3.5
Lee et al. (2007)	45	28	–	–	10 (22.2)	3.5
Rautou et al. (2008)	121	15	–	–	33 (27.3)	2.8
Tanno et al. (2008)	82	20	3	0/82	13 (15.9)	8.1
Guarise et al. (2008)	52	17	2.8	11/41	11 (21.2)	2.6
Sawai et al. (2010)	103	18	3	–	29 (28.2)	4.9
Uehara et al. (2011)	100	21	3.8	5/95	28 (28.0)	5.1
Maguchi et al. (2011)	349	19	3	0/349	62 (17.8)	3.7
Arlix et al. (2012)	47	15	2.4	0/47	18 (38.3)	6.4
Bae et al. (2012)	152	22	–	–	94 (61.8)	2.6
Ohno et al. (2012)	142	22	2.5	61/81	35 (24.6)	3.5
Khannoussi et al. (2012)	53	–	–	–	15 (28.3)	7.0
Total	1,293				355 (27.4)	

MPD main pancreatic duct, *MN* mural nodule

cases were suspected to have a low risk of malignancy. The mean follow-up period ranged from 2.6 to 8.1 years.

Among these, the mean frequency of morphological changes on the imaging findings, such as increased cyst size, increased MPD diameter, and/or appearance or enlargement of mural nodules (MNs), was 27.4 % (335/1,293) (range, 14.9–61.8 %) of all cases during the follow-up period.

There are several reasons for varying the range in frequency. The most obvious reason may be the different definition of the progression of BD-IPMN especially in cyst size. Some authors have defined cyst size changes of 5–10 mm or greater as progression (Rautou et al. 2008; Maguchi et al. 2011) because of the difficulty in the accurate measuring of a grape-like dilated cyst. However, Bae et al. (2012) reported that 94 (61.8 %) of 152 patients showed an increase in cyst size, and the mean incremental rate of cyst size growth was 0.0038 cm/month. Arlix et al. (2012) also reported that 18 (38.3 %) of 47 patients showed an increased cyst size, and the mean enlarged size was less than 3 mm.

Other reasons include differences in the patient characteristics at the initial diagnosis, the difference of imaging modalities in each institution, and the difference of the follow-up periods.

3.3 Malignant Transformation of BD-IPMN During the Follow-Up

The number of resected cases with BD-IPMN during the follow-up was shown in Table 3.2 (Kobayashi et al. 2005; Lee et al. 2007; Rautou et al. 2008; Tanno et al. 2008; Guarise et al. 2008; Sawai et al. 2010; Uehara et al. 2011; Maguchi et al. 2011; Arlix et al. 2012; Bae et al. 2012; Ohno et al. 2012; Khannoussi et al. 2012). The mean frequency of resected cases was 9.9 % (128/1,293) (range, 0–22.2 %) of all cases. Of 128 BD-IPMNs, 35 (27.3 %) cases were diagnosed histologically as malignant (noninvasive 25 and invasive 10). Therefore, the frequency of malignant transformation was only 2.7 % (35/1,293) in total during the follow-up period, whereas the remaining patients without surgical resection may have a risk of malignant transformation in the future.

Thirty-two (91.4 %) of 35 patients with malignant BD-IPMNs exhibited obvious signs of progression such as increased cyst size, increased MPD diameter, and/or appearance or enlargement of MNs. Three (8.5 %) malignant cases having no change of cyst size or MPD diameter showed an appearance of a solid mass at the periphery of the cyst during the follow-up (Kobayashi et al. 2005).

In addition, a multicenter study in Japan (Maguchi et al. 2011) reported that all nine malignant BD-IPMN cases exhibited progression, although all seven patients with benign BD-IPMN had no progression during the follow-up period.

These findings support the notion that malignancy is associated with signs of progression.

Table 3.2 Resected cases of BD-IPMN during follow-up

Author (year)	Number of resected cases (%)	Malignancy of resected cases		Histological findings of malignancy	
		Progression	No change	Noninvasive	Invasive
Kobayashi et al. (2005)	6 (12.8)	0/3	3[a]/3		3[a]
Lee et al. (2007)	10 (22.2)	2/10		1	1
Rautou et al. (2008)	8 (6.7)	4/8		4	
Tanno et al. (2008)	7 (8.5)	1/7		1	
Guarise et al. (2008)	0				
Sawai et al. (2010)	11 (10.7)	3/8	0/3	2	1
Uehara et al. (2011)	1 (1)	1/1		1	
Maguchi et al. (2011)	29 (8.3)	9/22	0/7	8	1
Arlix et al. (2012)	5 (10.2)	0/5			
Bae et al. (2012)	18 (11.8)	3/18		2	1
Ohno et al. (2012)	30 (21.1)	9/30		6	3
Khannoussi et al. (2012)	3 (5.7)	0/3			
Total	128 (9.9)	32/115	3[a]/13	25	10

[a]No change of cyst size and MPD diameter except the appearance of a solid mass at the periphery of the cyst

Table 3.3 Progression and malignancy of BD-IPMN without MN during follow-up

Author (year)	Number of cases	Progression (%)	Malignant (%)	IPMN with an associated invasive carcinoma (%)	Follow-up period (year)
Kobayashi et al. (2005)	29	0	0	0	3.5
Tanno et al. (2008)	82	13 (15.9)	1 (1.2)	0	8.1
Guarise et al. (2008)	41	4 (9.7)	0	0	2.6
Uehara et al. (2011)	95	7[a] (7.4)	2 (2.1)	1(1.1)	5.1
Maguchi et al. (2011)	349	62 (17.8)	9 (2.6)	1 (0.3)	3.7
Arlix et al. (2012)	47	18 (36.7)	0	0	6.4
Total	643	104 (16.2)	12(1.9)	2(0.3)	

[a]Appearance of MN alone

3.4 Progression and Malignancy of BD-IPMN Without MNs

The presence of MNs has been reported to be strongly suggestive of malignancy. Table 3.3 shows the follow-up data of BD-IPMN patients who had no MNs at the initial diagnosis (Kobayashi et al. 2005; Tanno et al. 2008; Guarise et al. 2008; Uehara et al. 2011; Maguchi et al. 2011; Arlix et al. 2012). The mean frequency of progression was 16.2 % (104/643) of all cases during the follow-up period. Twelve (1.9 %) cases were found to be malignant by histological examination, and only two (0.3 %) cases were IPMN with an associated invasive cancer.

These findings suggest that BD-IPMNs without MNs have a low risk of progression and malignant transformation. They are suitable for management without surgery and do not need short interval surveillance.

Table 3.4 Initial cyst size in relation to progression and malignancy

Author (year)	Number of cases (%)	Progression (%)	Malignant (%)
Tanno et al. (2008)			
<3 cm	72 (87.8)	10 (13.9)	1 (1.4)
≧3 cm	10 (12.2)	3 (30.0)	0
Maguchi et al. (2011)			
<3 cm	287 (82.2)	49 (17.1)	6 (2.1)
≧3 cm	62 (17.8)	13 (21.0)	3 (4.8)

3.5 Cyst Size in Relation to Progression and Malignancy

Cyst size >3 cm was previously thought to be one of the predictors of malignancy. Therefore, a BD-IPMN >3 cm was included in the consensus criteria for resection in the first guidelines (Tanaka et al. 2006).

Several studies have validated the safety of this guideline for surgical treatment of BD-IPMN >3 cm and revealed that the specificity is quite low (Rodriguez et al. 2007; Tang et al. 2008; Pelaez-Luna et al. 2007). These reports suggest that a BD-IPMN size of >3 cm is a weaker indicator of malignancy than the presence of MNs (Tanaka et al. 2012).

There was few number of long-term follow-up data in patients with BD-IPMN >3 cm. Table 3.4 shows the initial cyst size in relation to progress and malignancy during the follow-up (Tanno et al. 2008; Maguchi et al. 2011). Two studies demonstrated that there was no significant difference in the frequency of progression and malignancy in the resected cases between initial cyst size of less than 3 and 3 cm or greater.

With accordance to this, the revised guideline 2012 recommends that the indication for resection is more conservative (Tanaka et al. 2012). BD-IPMN >3 cm without any signs of further risk stratification can be observed without immediate resection.

3.6 Predictive Sign of Malignancy During Follow-Up

Although malignancy is associated with sign of progression of BD-IPMN during follow-up, adequate predictive signs of malignancy have not been defined. Many investigators proposed signs of malignancy as the appearance or the enlargement of MNs and/or an increase in MPD diameter (Lee et al. 2007; Tanno et al. 2008; Sawai et al. 2010; Uehara et al. 2011; Maguchi et al. 2011; Ohno et al. 2012). It is still controversial whether an increase in cyst size alone is an adequate predictive sign of malignancy (Rautou et al. 2008; Bae et al. 2012; Kang et al. 2011) or not (Lee et al. 2007; Tanno et al. 2008; Sawai et al. 2010; Uehara et al. 2011; Maguchi et al. 2011; Ohno et al. 2012).

3.7 Pancreatic Ductal Adenocarcinoma in Patients with BD-IPMN

Pancreatic ductal adenocarcinoma (PDAC) may develop independently in the pancreas separately from IPMNs, especially in BD-IPMN (Tanaka et al. 2012; Yamaguchi et al. 2002). The frequency of PDAC concomitant with IPMN was 4.1–9.3 % in the resected case studies (Yamaguchi et al. 2002; Ingakul et al. 2010; Kanno et al. 2010; Tanno et al. 2010a; Yamaguchi et al. 2011).

During the last several years, an increasing number of reports for the occurrence of PDAC in follow-up patients with IPMN have been published (Sawai et al. 2010; Uehara et al. 2011; Maguchi et al. 2011; Tada et al. 2006; Uehara et al. 2008; Tanno et al. 2010b; Ikeuchi et al. 2010) (Table 3.5). The mean frequency of occurrence of PDAC concomitant with IPMN was 2.8 % (range, 1.4–8.0 %) during follow-up. It is noted that the frequency (2.8 %, 30/1,085) was similar to the frequency of malignant transformation of BD-IPMN (2.7 %, 35/1,293) during the follow-up.

These findings suggest that BD-IPMN may be an indicator for a precancerous state of the pancreas and that PDAC may have not infrequently occurred in the pancreas distinct from BD-IPMN.

The long-term prognosis of the patients with BD-IPMN is still unclear. However, a multicenter study in Japan (Maguchi et al. 2011) described that the patients with PDAC distinct from BD-IPMN had a poor prognosis, whereas patients with malignant BD-IPMNs, including noninvasive and invasive carcinomas, had a relatively better prognosis after surgical treatment.

In conclusion, special attention should be paid to the occurrence of PDAC in the entire pancreas when performing follow-up examinations in patients with BD-IPMN including postoperative status, and shorter interval surveillance is required.

Table 3.5 Occurrence of PDAC in patients with BD-IPMN during follow-up

Author (year)	Number of cases	Number of PDAC concomitant with IPMN (%)	Follow-up period (year)
Tada et al. (2006)	197[a]	5 (2.6)	3.8
Uehara et al. (2008)	60	5 (8.0)	7.3
Tanno et al. (2010b)	89	4 (4.5)	5.3
Ikeuchi et al. (2010)	145	5 (3.4)	4.6
Sawai et al. (2010)	103	2 (1.9)	4.9
Maguchi et al. (2011)	349	7 (2.0)	3.7
Ohno et al. (2012)	142	2 (1.4)	3.5
Total	1,085	30 (2.8)	

[a]Included pancreatic cyst

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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

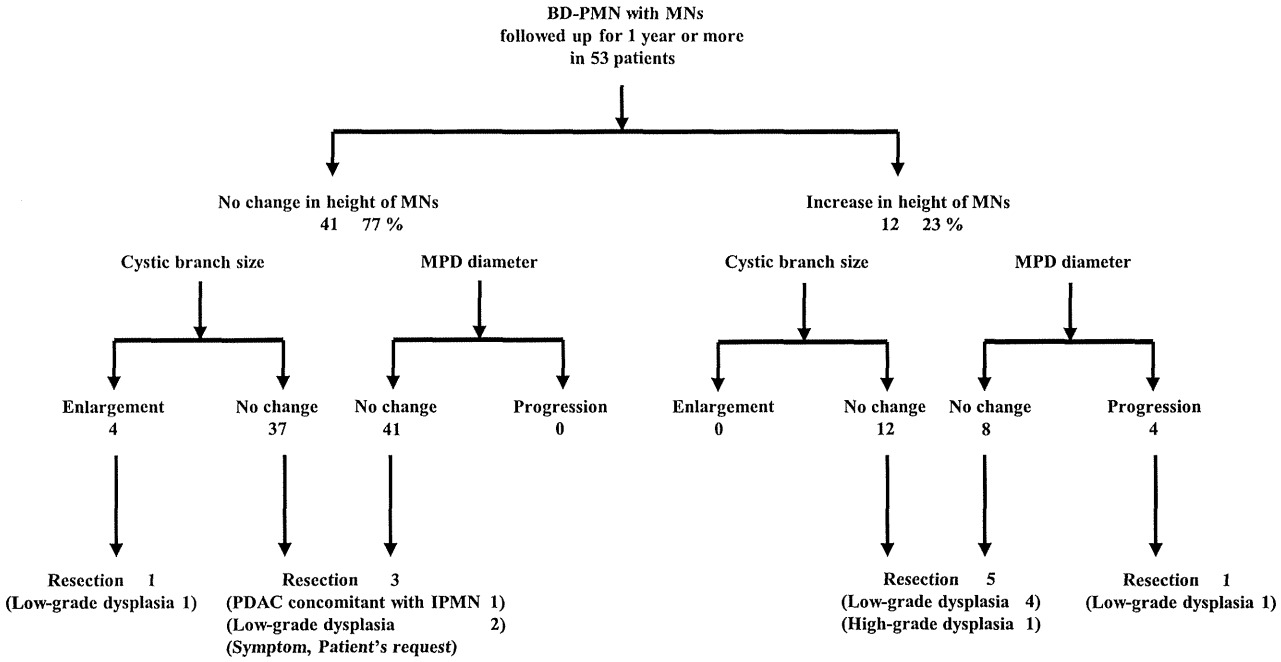

FIGURE 1. Changes in MNs, cyst size, and MPD diameter during follow-up.

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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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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(footnotes continued on last page of article)

EUS-guided FNA (EUS-FNA) biopsies were first reported by Vilmann et al[1] in 1992 and have a high diagnostic accuracy (range, 70%-98%).[2] In most cases, a cytological assessment is sufficient for the diagnosis of a pancreatic tumor. However, it is sometimes difficult to make a differential diagnosis by cytological data alone.[3] In such cases, evaluation of tissue architecture and morphology, namely, a histological diagnosis, is required for an accurate pathological diagnosis.

The success of puncture is important for tissue acquisition and is thus a crucial factor in EUS-FNA performance. A higher technical success rate is achievable with a 25-gauge needle than with a 22- or 19-gauge needle; however, the specimen obtained with the 25-gauge needle is less adequate for histological diagnosis compared with that obtained with the other needles.[4] Two studies have indicated that EUS-FNA approaches by using high negative pressure (HNP) suction to aspirate tissue enable acquisition of adequate tissue.[5,6] However, these studies only used the 22- and 19-gauge needles, and no studies thus far have evaluated the efficacy of 25-gauge needles for EUS-FNA in combination with HNP.

Therefore, we hypothesize that a 25-gauge needle for EUS-FNA with HNP may enable us to obtain sufficient tissue material with a high success rate. We conducted a multicenter, prospective, randomized, controlled trial to determine the accuracy of this hypothesis.

Take-home Message

- The use of the high negative pressure suction technique is superior to normal negative pressure suction in terms of the amount of sufficient material for histological diagnosis obtained via EUS-FNA.
- A high diagnostic accuracy is achievable by using a 25-gauge needle and high negative pressure suction when performing EUS-FNA on pancreatic lesions.

METHODS

Patients

Between July 2011 and April 2012, patients with solid pancreatic masses, as detected by US, CT, or magnetic resonance imaging, were consecutively enrolled in this study. Seven GI tertiary referral centers, where more than 100 EUS-FNAs are performed yearly, were considered eligible for this study. Patients with the following conditions were excluded: European Cooperative Oncology Group performance status of 4, a serious underlying disorder, American Society of Anesthesiologists classes III to IV, those taking oral anticoagulants, prothrombin time/international normalized ratio more than 1.5, platelet count less than 50,000/mm^3, pregnancy, GI obstruction, and refusal or inability to provide informed consent. The study was approved by the institutional review board of each institution and was registered with the University Hospital Medical Information Network Clinical Trials Registry (number UMIN000005939).

Procedural technique

Patients were placed in the left lateral decubitus position and were administered conscious sedation. A curvilinear echoendoscope (GF-UCT240-AL5; Olympus Medical Systems, Tokyo, Japan) was used, and EUS-FNA was performed by using a 25-gauge needle (Echo Tip Ultra; Cook Japan, Tokyo, Japan). After the needle was advanced into the target lesion, the stylet was withdrawn. A 10-mL syringe with 10-mL negative pressure (normal negative pressure [NNP]) or the Alliance II inflation system (Boston Scientific Japan, Tokyo, Japan) by using a 60-mL syringe with 50-mL HNP was attached to the proximal end of the needle, as appropriate, for the randomized protocol. The needle was then moved back and forth 10 to 20 times while performing suction. We performed EUS-FNA by using jabbing movements under continuous suction. We also used the fanning technique during EUS-FNA for pancreatic lesions if the endoscopist was able to perform the maneuver. Four EUS-FNA procedures were performed in the following order in the NNP and HNP groups, respectively: NNP-HNP-NNP-HNP and HNP-NNP-HNP-NNP. Obtained samples were categorized according to group (NNP or HNP) and fixed with formalin for histological examination. A portion of each sample, obtained by the first and second punctures, was sent for cytological examination. The remaining tissue was instantly fixed in 10% neutral-buffered formalin solution for histological examination. The EUS-FNA procedure was performed by using NNP with a 25-gauge needle or HNP with a different 25-gauge needle. On-site modified Giemsa staining (Diff-Quik; Kokusai Shiyaku, Kobe, Japan) was performed at all institutions. If an endoscopist considered samples obtained during 4 attempts at EUS-FNA insufficient for pathological diagnosis, an additional puncture was permitted. An additional puncture was performed if (1) the cytopathologist could not identify any material on the glass slide or (2) the cytopathologist could not macroscopically identify any whitish material on the glass slide. For additional punctures, any FNA procedure (needle/suction) could be performed.

Method of assignment of NNP and HNP groups

A computer-generated sequence was used to randomize patients into the NNP or HNP group. Randomized groups were stratified by institutions.

Outcome measurements

The primary outcome of this study was to determine the adequacy of tissue acquisition by the EUS-FNA/HNP combined technique and to determine the accuracy of histological diagnoses achievable by using this technique. The

secondary outcome of this study was to assess the quality and quantity of obtained tissue and the potential for adverse events arising from the use of this procedure.

Pathological assessment of samples obtained in this study

Cytological and histological analyses were performed separately. The cytological analysis was performed in on-site pathology facilities available at each hospital. Cell-block techniques were not performed for all patients in this study. The histological analysis was performed by a single expert pathologist (T.M.) based on hematoxylin and eosin staining. This pathologist evaluated the quantity and quality of each specimen and determined a histological diagnosis while blinded to clinical information, cytology, and final diagnoses.

The quantity of samples was assessed by the scoring system described by Gerke et al.[6] This scoring system is as follows: 0 indicates a sample with no material, 1 indicates that the sample contains sufficient material for limited cytological interpretation but is probably not representative, 2 indicates that the sample contains sufficient material for adequate cytological interpretation but is insufficient for histological information; 3 indicates sufficient material for limited histological interpretation; 4 indicates sufficient material for adequate histological interpretation, but a low-quality sample (total material is within a ×10 power field in length); 5 indicates sufficient material for adequate histological interpretation and a high-quality sample (total material is more than a ×10× power field in length). Figure 1 shows representative examples. In our study, a sample with a score of 3 or higher was defined as adequate for histological diagnosis. A sample with a score of 2 or lower was defined as inadequate for histological diagnosis.

The degree of contamination (eg, GI mucosa) in the specimens was categorized into 4 grades: 0, no contamination; 1, contamination present in less than 25% of the slide; 2, contamination present in 25% to 50% of the slide; 3, contamination present in more than 50% of the slide. The degree of the amount of blood in the specimens was categorized into 3 grades: 0, mild; 1, moderate; or 2, significant.

Pancreatic carcinomas, neuroendocrine tumors, lymphomas, and solid pseudopapillary neoplasms were defined as malignant diseases. Pancreatitis and non-neoplastic pancreatic tissue were defined as nonmalignant diseases. Malignancy and suspicious for malignancy were defined as positive for malignancy. Atypical cells and benign were defined as negative for malignancy. Because immunohistochemical studies could not be performed for all specimens in this study, the pathologist judged a sample to be malignant or benign based on hematoxylin and eosin staining alone. An accurate diagnosis was defined as follows: (1) positive for malignancy, with a final diagnosis of malignant disease such as carcinoma, neuroendocrine tumor, and solid pseudopapillary neoplasm (true positive); (2) negative for malignancy, with the condition ultimately being diagnosed as a nonmalignant disease, such as pancreatitis and non-neoplastic pancreatic tissue (true negative).

Diagnostic accuracy was defined as the ratio between the sum of true positive and true negative values, divided by the total number of samples. The adequacy rate was calculated by the following formula: number of adequate samples divided by total number of samples.

Clinical diagnostic methodology used for ultimate diagnosis of patients

Malignant disease was ultimately identified in patients by (1) diagnosis at autopsy after death caused by pancreatic cancer, (2) diagnosis based on histopathological analyses of surgically resected specimens, (3) radiological or clinical data indicating evidence of disease progression, or (4) diagnosis based on histopathological analyses of nodules in other organs demonstrating metastatic progression. In this study, benign disease was defined as a decrease or no change in pancreatic mass and no change in clinical data obtained for at least 6 months.

Adverse events

An adverse event was defined as any event that required the patient to stay in the hospital for a longer duration than expected or to undergo other unplanned interventions. For detailed reporting of adverse events, we referred to the Practice Committee of the American Society for Gastrointestinal Endoscopy guidelines.[7]

Sample size

The study was designed such that the sample size was large enough to obtain differences in the adequacy of samples needed for histological diagnosis.

It has been reported that a sample acquisition rate of 45.8% can be achieved by using a 25-gauge needle in pancreatic tumors.[1] We estimated that 50% and 65% of specimens obtained in the NNP and HNP groups, respectively, would be adequate for histological diagnoses. By using the McNemar test of equality of paired proportions and assuming 25% discordant pairs and a 10% dropout rate, each subject was assumed to have 1 pancreatic lesion. It was evaluated that 90 patients would be required to enable statistical analyses by using a 2-tailed test with a 5% significance level and 80% statistical power.

Statistical analysis

All statistical tests were performed by using dedicated software (JMP software version 8; SAS Institute, Cary, NC). The McNemar test was applied to adequacy, accuracy, and quality data gathered from tissue samples. $P < .05$ was considered statistically significant.

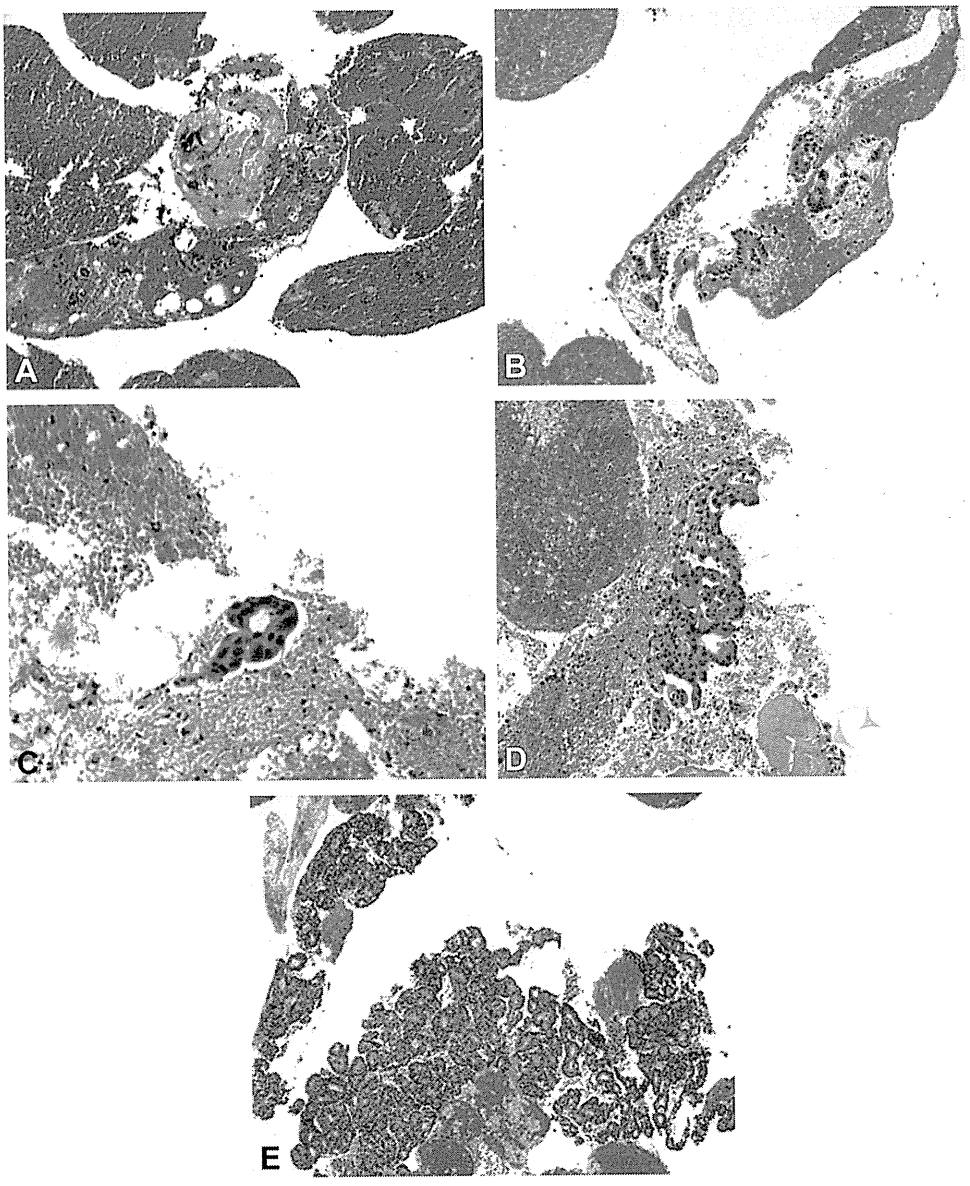

Figure 1. Representative images of specimens obtained by using EUS-guided FNA reveal differences between samples in terms of adequacy for histological diagnosis. **A**, In this sample with a score of 1, only a few cells are recognizable (hematoxylin and eosin stain, magnification ×200). This sample is inadequate for histological or cytological diagnosis. **B**, This is a sample that received a score of 2. This sample is inadequate for histological diagnosis, but might possibly be suitable for cytological diagnosis. **C**, This specimen (score of 3) is recognizable as a small tissue cluster. Evaluation of a part of tissue architecture and limited histological interpretation is possible. **D**, In this sample (score of 4), there is sufficient material for adequate histological diagnosis, and tissue architecture can be evaluated. The area of tissue on the prepared slide is within ×10 power field in length. **E**, In this sample (score of 5), there is sufficient material for adequate histological diagnosis, and tissue architecture can be evaluated. The area of tissue on the prepared slide is more than ×10 power field in length.

RESULTS

During the study period, 52 men and 38 women (90 patients) were enrolled in this study. The median age of patients was 67 years. All lesions were visible by EUS. Thirty-four patients had a lesion in the pancreas head (10 patients had lesions in the uncinate process), 40 patients in the body, and 16 patients in the tail. Fifty-six successful EUS-FNA procedures were performed through the gastric wall, whereas the remaining 34 procedures were performed through the duodenal wall. The median size of lesions was 28.2 mm (range 7.2–63.9 mm) (Table 1).

All EUS-FNA procedures were performed with on-site cytopathology evaluation. In this study, additional punctures were performed. Among these 5 patients, 2 underwent EUS-FNA with NNP by using a 22-gauge needle, 2 underwent EUS-FNA with NNP by using a 19-gauge needle, and 1 underwent EUS-FNA with HNP by using a 25-gauge needle. The definitive diagnostic procedures for a pancreatic lesion were as follows: 25 lesions were