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담도 협착에서 종양 특성과 솔질세포검사 순서에 따른 진단 정확도

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Diagnostic Accuracy of Brush Cytology with Direct Smear and Cell-block Techniques according to Preparation Order and Tumor Characteristics in Biliary Strictures

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Background/Aims: There are few data supporting the diagnostic yield of brush cytology depending on the order of cytologic preparation method or the location or shape of tumors in biliary strictures. We investigated diagnostic yields and variations in brush cytology with direct smear and cell-block preparations according to sampling preparation sequence and tumor location and shape in biliary strictures.
Methods: Patients who had undergone ERCP with tissue sampling between August 2009 and April 2013 were analyzed retrospectively. Group A was examined using brush cytology with direct smear followed by cell-block with or without biopsy, while the reverse order was performed for group B.
Results: Among 138 enrolled patients, 92 patients (A: 36, B: 56) underwent both brush cytology with direct smear and cell-block preparations. No differences in sensitivity, specificity, or accuracy were observed according to the sampling preparation method and the location or shape of tumors in biliary strictures. The cellularity observed from brush cytology with direct smear was better than that from cell-block according to the location of the tumor ($p < 0.01$). The diagnostic yield was increased in both groups with addition of an endobiliary biopsy.
Conclusions: No difference in diagnostic accuracy was observed between the sequences of preparation for brush cytology with direct smear and cell-block techniques. Brush cytology showed better cellularity for diagnosis. **(Korean J Gastroenterol 2014;63:223-230)**

Key Words: Biliary stricture; Brush cytology; Cell-block

INTRODUCTION

ERCP-guided endobiliary biopsy is an important tissue acquisition method for diagnosis of pancreaticobiliary diseases; however, it has limitations in accurately obtaining a sufficient amount of tissue according to the location and shape of the lesion. Recently, brush cytology under fluoroscopy has often been used in combination with endobiliary biopsy for suspected lesions.[1] Brush cytology with direct smear is a simple, commonly used sampling technique. This method usually involves performance of frequent to-and-pro passing of a brush into the targeted biliary lesion under fluoroscopic guidance. The tissue and/or cells on the brush are smeared directly on slides immediately, and may also be used for cell-block

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preparation. A cell-block preparation of a tissue slice from samples of brush cytology is also a useful preparation method for increasing the diagnostic yield.[2,3]

However, reports on the diagnostic yields of these cytologic preparation methods vary, and there is a lack of data regarding which diagnostic technique should be used first, whether or not there is a difference in the diagnostic yield depending on the sequence or method, and whether or not diagnostic yields vary according to tumor location and shape. We performed a routine endobiliary biopsy combined with brush cytology with direct smear and/or cell-block at the time of ERCP in patients with biliary strictures.

In this retrospective study, we investigated the diagnostic yields and cellularities of brush cytology with direct smear and cell-block preparation with and without endobiliary biopsies with regard to sample acquisition order and the location and shape of the tumor lesion.

MATERIALS AND METHODS

1. Patients

Patients who underwent endobiliary biopsy, brush cytology with direct smear, and/or cell-block preparations for treatment of pancreaticobiliary diseases between August 2009 and April 2013 were enrolled. This retrospective study was approved by the Institutional Review Board of Soonchunhyang University Hospital Cheonan. The final diagnosis of malignant biliary stricture was made when the malignancy was surgically confirmed, when there were histopathological findings with definite proof of malignancy in patients with unresectable tumors, or when the clinical course of the disease with a minimum follow-up time of six months indicated malignancy if there were cytological or histological findings lacking proof of malignancy.

2. Methods

1) Endobiliary forceps biopsy, brush cytology with direct smear and cell-block preparations

Endobiliary forceps biopsy under fluoroscopy was performed using biopsy forceps (6 Fr, rat-tooth biopsy forceps; Olympus, Tokyo, Japan). For brush cytology, a wire-guided cytology brush (RXCytology brush; Boston Scientific, Natick, MA, USA) was inserted into the bile duct. The brush was then advanced from the catheter to a proximal point of the stricture and moved back and forth 10 times for acquisition of samples. The samples on the brush were then smeared onto six slides, immediately fixed in 95% alcohol, and stained. For the cell-blocks, a second session of brushing was performed in the same manner, after which the inside of the catheter was perfused with normal saline in order to make a preparation for the cell-block. The preparation was centrifuged and fixed in 95% alcohol, and paraffin blocks were then produced using a tissue processor (Excelsior ES®; Thermo Scientific, Waltham, MA, USA). Two sessions of brushing were performed for collection of cytological samples for brush cytology with direct smear and cell-block. In group A, smears for brush cytology were obtained from the first pass, and preparations for the cell-block were then obtained from the second pass. In group B, the sample acquisition sequence was performed in the reverse order (first pass for cell-block and second for brush cytology with direct smear). All endoscopic procedures and cytologic preparations were performed by two experienced endoscopists.

Table 1. Baseline Characteristics

Clinical characteristics	Value
Patient	138 (100)
Male/female	83 (60.1)/55 (39.9)
Age (yr)	67.9±11.7
Clinical diagnosis	
Malignant	113 (81.8)
Cholangiocarcinoma	81 (58.6)
Pancreatic cancer	24 (17.3)
Gallbladder cancer	3 (2.1)
Hepatocellular carcinoma	2 (1.4)
Others[a]	3 (2.1)
Benign	25 (18.1)
Location of lesion (malignant/benign)	
Intrahepatic	9 (7.9)/4 (16.0)
Perihilar	40 (35.3)/1 (4.0)
Extrahepatic	62 (54.8)/17 (68.0)
Pancreatic duct	2 (1.7)/3 (12.0)
Cholangiographic appearance	
Protuberant (nodular)	16 (20.2)/4 (5.0)
Papillary protuberant	6 (7.5)/0 (0)
Sclerosed	29 (36.7)/11 (13.9)
Constricted	11 (13.9)/2 (2.5)
Laboratory finding	
ALP (IU/L)	407.0±317.7
Total bilirubin (mg/dL)	9.2±9.46
CA 19-9 (U/mL)	1,499.3±4,895
CEA (ng/mL)	13.6±77.9

Values are presented as number (%) or mean±SD.
[a]Thymic carcinoma, periampullary carcinoma.

2) Cytological and histological classification

For diagnosis by ERCP-guided endobiliary biopsy and brush cytology using two cytological preparation methods, the following diagnostic categories were used: malignancy, suspicious for malignancy, atypical, benign, and non-diagnostic. Malignancy and suspicious for malignancy were considered 'positive' diagnostic results, while atypical and benign findings were considered 'negative.' Cellularity was divided according to good, fair, and poor. For interpretation of the pathology, an experienced pathologist (H.D.C.) at our center, who was unaware of the order of tissue acquisition and the patients' baseline clinical data, performed a re-interpretation for all 138 patients.

3) Statistical analysis

For evaluation of diagnostic yield, sensitivity, specificity, positive predictive value, negative predictive value, and accuracy of the three sampling techniques, alone and in combination, were estimated. Cellularity according to cytological technique was compared among patients who were finally diagnosed with a malignancy. Fisher's exact test and the generalized estimating equation method were used for statistical tests of diagnostic yield and cellularity. A p-value < 0.05 was considered statistically significant. Statistical analyses were performed using SPSS software (ver. 14.0; SPSS Inc., Chicago, IL, USA).

RESULTS

1. Patient characteristics

A total of 138 patients with a mean age of 67.9 years were included in the retrospective analysis (Table 1). Among them, in 58 patients (36.9%), final diagnosis was confirmed histologically by surgery or definite pathology. The final diagnosis was malignancy in 113 patients (81.8%); the most frequent was cholangiocarcinoma in 81 patients (58.6%), followed by pancreatic cancer in 24 patients (17.3%), and gall bladder cancer in three patients (2.1%). An extrahepatic lesion was the most common tumor site in 79 patients (57.2%), and in

Table 2. Baseline Characteristics in Brush Cytology Depending on the Order of Cytologic Preparation Methods

Subgroup characteristics	Group A	Group B	p-value
Patient	36 (100)	56 (100)	
Male/female	21 (58.3)/15 (41.6)	30 (53.5)/26 (46.4)	0.654
Age (yr)	66.5±12.6	71.6±10.2	0.048
Clinical diagnosis			
Malignant/benign	31 (86.1)/5 (13.8)	46 (82.1)/10 (17.8)	0.615
Malignant	31 (100)	46 (100)	
Cholangiocarcinoma	23 (74.1)	30 (65.2)	
Pancreatic cancer	4 (12.9)	14 (30.4)	
GB cancer	1 (3.2)	2 (4.3)	
Others[a]	3 (9.6)	-	
Location of lesion			-
Intrahepatic	4 (11.1)	5 (8.9)	
Perihilar	11 (30.5)	20 (35.7)	
Extrahepatic	19 (52.7)	28 (50.0)	
Pancreatic duct	2 (5.5)	3 (5.3)	
Cholangiographic appearance			-
Protuberant (nodular)	5 (13.8)	10 (27.7)	
Papillary protuberant	-	3 (5.3)	
Sclerosed	25 (69.4)	39 (69.6)	
Constricted	6 (16.6)	4 (7.1)	
Laboratory finding			
ALP (IU/L)	359.0±329.4	455.0±319.0	0.169
Total bilirubin (mg/dL)	7.6±8.9	9.7±9.2	0.265
CA 19-9 (U/mL)	1,763±5,313	2,306±6,329	0.675
CEA (ng/mL)	30.6±153.1	10.8±18.98	0.459

Values are presented as n (%) or mean±SD.
GB, gall bladder.
Group A, brush cytology with direct smear followed by cell-block; group B, brush cytology with cell-block followed by direct smear.
[a]Thymic carcinoma, periampullary carcinoma.

fluoroscopic images, a sclerotic appearance was most commonly observed in 40 patients (50.6%). No significant difference with regard to the different order of brush cytology with direct smear and cell-block preparation was observed between the two groups, except for age (p=0.048) (Table 2).

2. Diagnostic yields of single or combined techniques

Sensitivity, specificity, positive and negative predictive values, and accuracy were estimated for each technique, except for non-diagnostic cases. Among the 138 patients, 83 patients underwent ERCP-guided endobiliary biopsy, 92 underwent both brush cytology with direct smear and cell-block preparation, and 46 underwent all three techniques (Tables 3, 4). The sensitivities of endobiliary biopsy, brush cytology with direct smear, and cell-block preparation were 48.8%, 60.8%, and 56.4%, the specificities were 92.6%, 100%, and 88.8%, and the diagnostic accuracies were 55.1%, 67.5%, and 60.5%, respectively. Groups A and B, which were divided

Table 3. Diagnostic Yield of Endobiliary Forceps Biopsy, Brush Cytology with Direct Smear and Cell-block

Variable	Endobiliary forceps biopsy[a]	Brush cytology	
		Direct smear[b]	Cell-block[c]
Sensitivity	32/66 (48.4)	59/97 (60.8)	35/62 (56.4)
Specificity	11/12 (91.6)	20/20 (100)	8/9 (88.8)
PPV	32/33 (96.9)	59/59 (100)	35/36 (97.2)
NPV	11/45 (24.4)	20/58 (34.4)	8/35 (22.8)
Accuracy	43/78 (55.1)	79/117 (67.5)	43/71 (60.5)

Values are presented as n/total (%).
PPV, positive predictive value; NPV, negative predictive value.
[a]Non-diagnostic cases (5 of 83), [b]non-diagnostic cases (6 of 123), [c]non-diagnostic cases (21 of 92).

Table 4. Diagnostic Yields of Brush Cytology with or without Endobiliary Forceps Biopsy

Variable	Both techniques[a]			Triple techniques[b]		
	Group A[c]	Group B[d]	p-value	Group A	Group B	p-value
Sensitivity	18/31 (58.0)	30/46 (65.2)	0.525	10/13 (76.9)	18/29 (62.0)	0.485
Specificity	5/5 (100)	9/10 (90.0)	>0.999	1/1 (100)	3/3 (100)	-
PPV	18/18 (100)	30/31 (96.7)	>0.999	10/10 (100)	18/18 (100)	-
NPV	5/18 (27.7)	9/25 (36.0)	0.570	1/4 (25.0)	3/14 (21.4)	>0.999
Accuracy	23/36 (63.8)	39/56 (69.6)	0.566	11/14 (78.5)	21/32 (68.7)	>0.999

Values are presented as n/total (%).
PPV, positive predictive value; NPV, negative predictive value.
[a]Brush cytology with direct smear and cell-block preparation.
[b]Endobiliary forceps biopsy, brush cytology with direct smear and cell-block preparation.
[c]Non-diagnostic cases (14 of 36), [d]non-diagnostic cases (9 of 56).

Table 5. Cellularity of Brush Cytology and Preparation Methods (Group A and B) according to Location of Malignant Lesion

Location of lesion	Cellularity	Brush cytology		p-value	Group A	Group B	p-value
		Direct smear	Cell-block				
Total	Good	51 (50.0)	17 (22.1)	<0.01	19 (61.3)	27 (58.7)	>0.999
	Fair	34 (33.3)	29 (37.7)		10 (32.3)	15 (32.6)	
	Poor	17 (16.7)	31 (40.3)		2 (6.5)	4 (8.7)	
Intrahepatic	Good	2 (25.0)	1 (16.7)	0.391	2 (66.7)	1 (33.3)	0.400
	Fair	4 (50.0)	2 (33.3)		0 (0)	2 (66.7)	
	Poor	2 (25.0)	3 (50.0)		1 (33.3)	0 (0)	
Perihilar	Good	24 (60.0)	9 (30.0)	<0.01	6 (54.6)	15 (79.0)	0.081
	Fair	11 (27.5)	10 (33.3)		5 (45.5)	2 (10.5)	
	Poor	5 (12.5)	11 (36.7)		0 (0)	2 (10.5)	
Extrahepatic	Good	24 (46.2)	7 (18.0)	<0.01	10 (62.5)	11 (47.8)	0.778
	Fair	18 (34.6)	16 (41.0)		5 (31.3)	10 (43.5)	
	Poor	10 (19.2)	16 (41.0)		1 (6.3)	2 (8.7)	
Pancreatic duct	Good	1 (50.0)	0 (0)	-	1 (100)	0 (0)	-
	Fair	1 (50.0)	1 (50.0)		0 (0)	1 (100)	
	Poor	0 (0)	1 (50.0)		0 (0)	0 (0)	

Values are presented as n (%).
Group A, brush cytology with direct smear followed by cell-block; group B, brush cytology with cell-block followed by direct smear.

Table 6. Diagnostic Yield of Preparation Methods (Group A and B) according to Location of Lesion

Location of lesion	Yield	Group A	Group B	p-value
Intrahepatic	Sensitivity	2/3 (66.6)	3/3 (100)	>0.999
	Specificity	1/1 (100)	1/2 (50.0)	>0.999
	PPV	2/2 (100)	3/4 (75.0)	>0.999
	NPV	1/2 (50.0)	1/1 (100)	>0.999
	Accuracy	3/4 (75.0)	4/5 (80.0)	>0.999
Perihilar	Sensitivity	8/11 (72.7)	13/19 (68.4)	>0.999
	Specificity	-	1/1 (100)	-
	PPV	8/8 (100)	13/13 (100)	-
	NPV	-	1/7 (14.2)	>0.999
	Accuracy	8/11 (72.7)	14/20 (70.0)	>0.999
Extrahepatic	Sensitivity	8/16 (50.0)	13/23 (56.5)	0.752
	Specificity	3/3 (100)	5/5 (100)	-
	PPV	8/8 (100)	13/13 (100)	-
	NPV	3/11 (27.2)	5/15 (33.3)	>0.999
	Accuracy	11/19 (57.8)	18/28 (64.2)	0.763
Pancreatic duct	Sensitivity	-	1/1 (100)	>0.999
	Specificity	1/1 (100)	2/2 (100)	-
	PPV	-	1/1 (100)	-
	NPV	1/1 (100)	2/2 (100)	-
	Accuracy	1/2 (50.0)	3/3 (100)	0.400

Values are presented as n/total (%).
Group A, brush cytology with direct smear followed by cell-block; group B, brush cytology with cell-block followed by direct smear.
PPV, positive predictive value; NPV, negative predictive value.

according to order of sample preparation, had sensitivities of 56.2% and 65.2%, specificities of 100% and 90%, and accuracies of 63.8% and 69.6%, respectively. Group B showed slightly better sensitivity and accuracy, although the difference was not statistically significant ($p > 0.56$) (Table 4). When endobiliary biopsy was also performed in the two groups, the sensitivities were 76.9% and 62%, the specificities 100% and 100%, and the accuracies 78.5% and 68.7%, respectively. An improvement in diagnostic yield with addition of endobiliary biopsy was more evident in group A (brush cytology with direct smear first); however, the difference was not statistically significant ($p > 0.99$) (Table 4).

3. Diagnostic yield and cellularity according to the location and shape of the lesion

Differences in diagnostic yield and cellularity between group A and group B according to the location and shape of the lesion were compared. Brush cytology with direct smear had superior overall cellularity compared to cell-blocks, independent of the location of the lesion, and it also showed statistically greater cellularity, depending on the location of the lesion, with the exception of the pancreatic duct and intrahepatic lesions ($p < 0.01$) (Table 5). However, no statistically significant difference was observed in cellularity and diagnostic yield regardless of the location or the shape of the lesion when both brush cytology with direct smear and cell-block techniques were performed in reverse (group A and B) (Tables 5-8).

Table 7. Cellularity of Preparation Methods (Group A and B) according to the Cholangiographic Appearance of Extrahepatic Malignancy

Cholangiographic appearance	Celularity	Group A	Group B	p-value
Total	Good	10 (62.5)	11 (47.8)	0.778
	Fair	5 (31.3)	10 (43.5)	
	Poor	1 (6.3)	2 (8.7)	
Protuberant	Good	2 (66.7)	3 (42.9)	>0.999
	Fair	1 (33.3)	3 (42.9)	
	Poor	0 (0)	1 (14.3)	
Papillary	Good	-	0 (0)	-
	Fair	-	2 (66.7)	
	Poor	-	1 (33.3)	
Sclerosed	Good	5 (62.5)	8 (66.7)	>0.999
	Fair	3 (37.5)	4 (33.3)	
	Poor	0 (0)	0 (0)	
Constricted	Good	3 (60.0)	0 (0)	0.500
	Fair	1 (20.0)	1 (100)	
	Poor	1 (20.0)	0 (0)	

Values are presented as n (%).
Group A, brush cytology with direct smear followed by cell-block; group B, brush cytology with cell-block followed by direct smear.

Table 8. Diagnostic Yield of Preparation Methods (Group A and B) according to the Cholangiographic Appearance

Cholangiographic appearance	Yield	Group A	Group B	p-value
Protuberant	Sensitivity	3/5 (60.0)	3/7 (42.8)	>0.999
	Specificity	-	2/3 (66.6)	-
	PPV	3/3 (100)	3/4 (75.0)	>0.999
	NPV	-	2/6 (33.3)	>0.999
	Accuracy	3/5 (60.0)	5/10 (50.0)	>0.999
Papillary	Sensitivity	-	1/3 (33.3)	-
	Specificity	-	-	-
	PPV	-	1/1 (100)	-
	NPV	-	-	-
	Accuracy	-	1/3 (33.3)	-
Sclerosed	Sensitivity	13/21 (61.9)	25/33 (75.7)	0.362
	Specificity	4/4 (100)	6/6 (100)	-
	PPV	13/13 (100)	25/25 (100)	-
	NPV	4/12 (33.3)	6/14 (42.8)	0.701
	Accuracy	17/25 (68.0)	31/39 (79.4)	0.378
Constricted	Sensitivity	2/5 (40.0)	1/3 (33.3)	>0.999
	Specificity	1/1 (100)	1/1 (100)	-
	PPV	2/2 (100)	1/1 (100)	-
	NPV	1/4 (25.0)	1/3 (33.3)	>0.999
	Accuracy	3/6 (50.0)	2/4 (50.0)	>0.999

Values are presented as n/total (%).
Group A, brush cytology with direct smear followed by cell-block; group B, brush cytology with cell-block followed by direct smear.
PPV, positive predictive value; NPV, negative predictive value.

DISCUSSION

Endobiliary biopsy for definitive diagnosis of malignancy is essential in biliary strictures; however, obtaining sufficient tissue samples may be technically difficult, depending on the tumor location and the shape of the lesion.[4] Recent studies have reported varying sensitivity of endobiliary biopsy, from 32-58%. Fluoroscopy-guided endobiliary biopsy during ERCP is limited by the narrow anatomical structure of the biliary tract, restrictions in access due to the type, location, and/or shape of the lesion, size and stiffness of biopsy forceps, need for additional procedures (e.g., endoscopic sphincterotomy), long procedure time, and risk of complications, including hemorrhage and perforation.[5-7] Diagnostic sensitivity of endobiliary forceps biopsy may be improved depending on the size of the forceps, number of passes, and location of the tumor. Accuracy of endobiliary forceps biopsy can be increased by performance of four or more biopsies regardless of the size of the forceps, and sensitivity can be improved by performing a biopsy at the tip of a papillary lesion and at the margin inside the stenosis for nodular or infiltrating lesions.[8-10]

Since introduction of brush cytology in 1975 by Osnes et al.,[11] it has been evaluated for diagnostic yield in combination with endobiliary biopsy.[5,12] In recent studies, significant differences in sensitivity were reported among operators, ranging from 43-84%,[8] likely due to inability to obtain adequate samples. Obtaining samples can be particularly difficult for metastatic carcinoma, lymphoma, hepatocellular carcinoma, and some bile duct carcinomas, which may grow submucosally with normal epithelium, or strictures caused by external pressure in pancreatic cancer.[13] On the other hand, brush cytology may be technically simpler than endobiliary biopsy, allows easier access to the intrahepatic duct, takes less time, and is associated with fewer complications. As a widely used diagnostic technique today, improved sensitivity and accuracy have been reported when brush cytology is combined with biopsy.[7] The cell-block preparation method, which involves preparation of a paraffin block with cytological samples, is also used; however, data on its diagnostic yield and utility are limited.[14]

In our study, we made touch smears on slide and paraffin blocks from samples collected in two sessions of brush cytology for comparison of the diagnostic yields, alone and in combination with a routine endobiliary biopsy, and to investigate differences in diagnostic yields according to the order of sample preparation of brush cytology with direct smear and cell-block preparation. We also sought to determine whether cellularity might differ depending on the features of malignant pancreaticobiliary diseases, such as the location of the lesion or cholangiographic appearance, and any influence on diagnostic yield.

In this study, endobiliary biopsy, brush cytology with direct smear, and cell-block techniques showed sensitivities of 48.8%, 60.8%, and 56.4% and specificities of 91.6%, 100%, and 88.8%, respectively, comparable to results reported in other recent studies.[3,8] When the final diagnosis was malignancy, the number of false-negative cases, excluding atypical findings, was eight with brush cytology with direct smear and two with cell-block preparation (9.2% *vs.* 3.2%), indicating a lower false negative rate with the latter. Among studies on false negativity with brush cytology, the most frequently suggested causes were sampling errors, such as hypocellular sample errors, and sample preparation errors, such as air drying artifacts. In addition, some studies have also suggested repeated brushing, rapid on-site cytopathological

examination, use of a grasping basket, reclassification of atypical diagnoses, and improvement of sample preparation methods and cytopathologists' diagnostic techniques for reduction of the false-negative rate and for enhancement of sensitivity for pancreatobiliary malignancies.[13,15-18]

When brush cytology with direct smear and cell-block were performed sequentially, no statistically significant difference in sensitivities was observed between the two groups, regardless of the sample acquisition order.

While a direct comparison is not available, our results are comparable to those of recent studies reporting sensitivities of 48-74% for simultaneous brush cytology and endobiliary biopsy, and the increase in sensitivity using both methods was also comparable to our findings.[5,12,19] Both groups received a benefit from the additional endobiliary biopsy, regardless of the sample acquisition order. Sensitivity was the highest when the three techniques were performed simultaneously, again, regardless of the order. Brush cytology with direct smear or cell-block alone seems to be capable of providing a similar diagnostic yield when an endobiliary biopsy or sufficient tissue acquisition is not an option.

Regarding cellularity, an important factor for accurate diagnosis in cytological techniques, our results showed that brush cytology with direct smear has a superior overall cellularity for biliary malignancies ($p < 0.01$) as well as a greater cellularity depending on the location of the lesion, with the exception of pancreatic duct and intrahepatic lesions. However, no difference in cellularity and diagnostic yield of pancreatobiliary malignancies was observed between tissue preparation order (group A and B) according to the location of the lesions or cholangiographic appearance.

Regarding limitations of this study, because brush cytology with direct smear and cell-block were performed prospectively at a certain time point and the analysis was then performed retrospectively, this study is limited by the lack of randomization, the relatively small number of cases, and low statistical power due to uneven proportion of characteristic variables, including disease entity, location, and shape in compared groups. Diseases such as pancreatic malignancy were not evenly distributed, which might affect the diagnostic yield. According to our results, group B included more patients with pancreatic cancer and showed a difference of sensitivity, even though there was no statistical difference. It is thought that there is a margin of error due to limited validation. In addition, endoscopic ultrasound-guided fine-needle aspiration and biopsy, widely used nowadays, were not included in this study. Conduct of further larger-scale, randomized studies will be required using other devices and techniques.

In summary, our results showed that brush cytology with direct smear had the highest diagnostic accuracy, compared with endobiliary biopsy and cell-block. Accuracy could be enhanced by combining brush cytology with an endobiliary biopsy. Brush cytology with direct smear alone had the highest cellularity for malignant biliary strictures, excluding pancreatic duct and intrahepatic lesions. Brush cytology with direct smear and cell-block showed identical cellularity, regardless of the location and shape of the lesion or the order of sample acquisition. Brush cytology with direct smear alone may be beneficial if sufficient sample acquisition is difficult or not an option. However, conduct of further larger-scale studies using diverse devices and techniques will be necessary in order to enhance the diagnostic yield for difficult biliary strictures.

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