의학석사 학위논문

Comparison of Diagnostic Efficacy of Liquid-based Cytology and Conventional Smears in Diagnosing Biliary Tract Cancer

담도암 진단에서 고식적도말과 액상세포검사의 진단 효능 비교

2023년 2월

서울대학교 대학원

의학과 내과학

이 민 우

Comparison of Diagnostic Efficacy

# of Liquid-based Cytology and Conventional Smears in Diagnosing Biliary Tract Cancer

지도교수 김 용 테

이 논문을 의학석사 학위논문으로 제출함
2022년 10월

서울대학교 대학원
의학과 내과학
이 민 우

이민우의 의학석사 학위논문을 인준함
2023년 1월

위 원 장 _____ (인)

부위원장 _____ (인)

위 원 _____ (인)

Abstract

Background and Aims: Tissue sampling under endoscopic retrograde cholangiopancreatography (ERCP) is the primary diagnostic test for biliary tract cancer. However, it suffers from its low sensitivity. Liquid-based cytology (LBC) has been shown to improve the diagnostic efficacy of brush cytology for thyroid, cervical and pancreatic cancer. But the data on LBC in biliary tract cancer is still limited. To evaluate the diagnostic performance of LBC for biliary tract cancer, we compared it with conventional smears and forceps biopsies.

Methods: A retrospective study was conducted of all consecutive patients who underwent brush cytology under ERCP from January 2010 to April 2020. The primary outcome was the diagnostic efficacy of conventional smears and LBC. The difference between the two groups was corrected using stabilized inverse probability weighting (IPW). The secondary outcome was the sensitivity of forceps biopsy alone and forceps biopsy combined with brush cytology. The secondary outcome was evaluated in patients who underwent both methods.

Results: Among 162 patients, conventional smears were performed in 70 patients, and LBC was performed in 92 patients. In the primary analysis using stabilized IPW, the sensitivity of conventional smears and LBC was 76.00% and 92.75% respectively (P = 0.003). The accuracy was 78.46% for conventional smears and 86.67% for LBC (P = 0.178). In the secondary analysis, LBC improved sensitivity

when combined with forceps biopsy (97.06% vs 88.24%, P = 0.041). Conclusions: Liquid-based cytology demonstrated better sensitivity and accuracy than conventional smears. Moreover, LBC revealed improvement in sensitivity when combined with forceps biopsies.

Keywords: Bile duct, Biliary tract neoplasms, Biopsy, Cytology, ERCP

Student Number: 2021-29744

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Chapter 1. Introduction

Biliary strictures can occur anywhere in the biliary tract and result from a wide arrange of benign and malignant etiologies.[1] Because it is difficult to accurately diagnose biliary tract cancer by imaging alone, endoscopic evaluation and tissue acquisition are often required. Transpapillary brush cytology and/or forceps biopsy during endoscopic retrograde cholangiopancreatography (ERCP) are recommended as tissue sampling methods.[2]

The tissue diagnosis of biliary tract cancer is challenging for several reasons. Brush cytology is routinely performed to diagnose malignant strictures but is limited by its low sensitivity. In the case of forceps biopsy, it is often difficult to accurately approach and target the lesion since it is done under fluoroscopy. Even when the two sampling methods were combined, the sensitivity was about 60%, which was insufficient for a definite diagnosis.[3]

Many attempts have been made to improve the sensitivity of brush cytology such as mutation analysis, immunohistochemistry, and fluorescent in situ hybridization.[4-8] Liquid-based cytology (LBC), mainly using the filtration method, has also been evaluated, but the diagnostic efficacy was similar to that of conventional smears.[9,10] Recently, many researchers reported improved diagnostic performance of another LBC method using centrifugation in diagnosing thyroid, cervical, and pancreatic cancer.[11-13] However, there is limited data on the centrifugation method in biliary

tract cancer.

In the present study, we compared the diagnostic performance of LBC using centrifugation to that of conventional smears in diagnosing biliary tract cancer. Furthermore, we compared the synergistic effect of brush cytology when combined with forceps biopsies.

Chapter 2. Methods

2.1. Study design and population

We retrospectively reviewed 240 patients who underwent brush cytology during ERCP at Seoul National University Hospital between January 2010 and April 2020 (Fig. 1). During this period, only patients over the age of 18 with biliary stricture observed in endoscopic retrograde cholangiography were included. The exclusion criteria were as follows: 1) Failure of selective biliary cannulation, 2) Surgically altered anatomy including Billroth II anastomosis or Roux en Y anastomosis, 3) Malignancy other than biliary tract cancer. In our institution, LBC has been available since October 2018. Each of the four endoscopists started using LBCs at slightly different times, but they only used LBC once they had started. Therefore LBC has been in almost all cases since 2019.

Patients who were diagnosed with other neoplasms except biliary tract cancer were excluded from the analysis. Also, patients who were suspected of benign biliary stricture but whose follow-up period was less than one year were excluded. Since there are some possibility of subsequently being diagnosed as malignancy even when it is initially presumed as benign stricture. This study was approved by the Institutional Review Board of Seoul National University Hospital (IRB No. H-2007-124-1142) and was conducted in accordance with the Declaration of Helsinki. The requirement for

informed consent was exempted. In this study, we investigated the diagnostic efficacy and followed the guidelines of the Standards for Reporting of Diagnostic Accuracy (STARD) initiative.[14]

2.2. Classification of stricture location and final diagnosis

The biliary stricture locations were classified as the intrahepatic duct, hilum, common bile duct, and cystic duct. Malignant strictures resulted from intrahepatic cholangiocarcinoma, perihilar cholangiocarcinoma (Klatskin tumor), distal cholangiocarcinoma (CBD cancer), and gallbladder cancer. In contrast, benign strictures resulted from choledocholithiasis, chronic pancreatitis, primary sclerosing cholangitis, and IgG4 sclerosing cholangitis.

2.3. Diagnostic approach using ERCP and brush cytology

ERCP was performed with a standard duodenoscope (JF-240, TJF-260, Olympus Corporation, Tokyo, Japan) under conscious sedation. After bile duct cannulation, a contrast agent was injected to obtain the cholangiogram, and the location of the stricture was identified. To obtain adequate specimens, a cytology brush (Cytomax II Double Lumen Cytology Brush, Wilson-Cook Medical, Winston-Salem, NC, USA) was passed 10 to 15 times through the stricture. Later, in some cases, an additional forceps biopsy (Single-Use Radial JawTM 4 Biopsy Forceps, Boston Scientific, Natick, MA, USA) was performed without the assistance of a guide wire. When biliary

drainage was needed, a plastic or metal stent was inserted after brush cytology or forceps biopsy. All ERCP procedures were performed by four expert endoscopists who had performed more than 500 ERCP procedures.

2.4. Specimen acquisition and preparation

For conventional smears, the specimens were smeared with a quick rolling motion of the brush on a glass slide and then fixed in 95% alcohol for Papanicolaou staining. In case of LBC, the specimen was immediately suspended in an ethanol-based preservative fluid (CytoRich®) and vortexed prior to preparation. The specimen was dispersed onto a density gradient reagent and centrifuged to trap small particulates and debris. After that, the pellet was resuspended and transferred to a glass slide for Papanicolaou staining (Fig. 2).
In cases where an additional forceps biopsy was performed, the biopsy specimen was placed in a container with 10% formaldehyde solution and sent to the pathology department. After that, hematoxylin and eosin staining was performed, and immunohistochemical staining was also done if necessary.

2.5. Cytologic evaluation and final diagnosis

Cytologic and histologic assessments were performed independently by two experienced pathologists (Kim HR and Lee KB). The cytology results from either conventional smears or LBC were

classified into four categories according to the presence of malignant cells as negative, atypical, suspicious, or positive. In case of suspicious of positive in cytology, all cases were finally diagnosed as malignancy. As a result, suspicious/positive cytology could be categorized as malignant and the diagnosis was confirmed. However, in case of atypical cytology, some cases were diagnosed as malignancy by additional exam. Therefore it was not acknowledged as definite diagnosis and additional exams were needed. Taking into account these clinical aspects, we classified the negative groups as benign and the atypical, suspicious, and positive groups as malignant. The histologic assessment of forceps biopsies was done in the same manner.

The final diagnosis was based on pathologic reports of the surgical specimen or biopsy specimen of metastatic lesions. If any of these specimens were reported as suspicious or positive, the final diagnosis was a malignancy. If all of these results were atypical or inconclusive, additional tests were performed within the follow-up period including forceps biopsy, biopsy of other organs (liver or lymph node), or surgery. If malignancy was not identified by all these efforts and signs of malignancy were absent during a minimum 1-year follow-up with imaging studies, it was finally considered benign. In case of disease progression during this period, it was eventually diagnosed as malignancy.

2.6. Study data and outcome measurements

Patient demographics, endoscopic features, laboratory test results, and pathology reports were reviewed. The endoscopic features included the location of the stricture, the presence of biliary stones, or periampullary diverticulum. The laboratory test results before the ERCP procedure were used.

The primary outcome was the diagnostic efficacy of conventional smears and LBC. The secondary outcome was the diagnostic efficacy of brush cytology when combined with forceps biopsy.

2.7. Statistical analysis

The difference between the conventional smear group and the LBC group was assessed using the student t-test for continuous variables, and the chi-squared test and Fisher's exact test for categorical variables. Univariable and multivariable logistic regression analyses were done to identify the factors associated with the detection of malignant cells by cytological examination. This analysis included age, sex, location of the stricture, pre-procedure laboratory test results, and the presence of biliary stones or periampullary diverticulum. Only factors with a P value of less than 0.25 according to univariable analysis were considered candidates for the multivariable model, which was determined by the bi-directional stepwise selection method.

The primary outcome of this study was the diagnostic efficacy

expressed as sensitivity, specificity, positive predictive value, negative predictive value, and accuracy.[15] The outcomes were compared using stabilized inverse probability weighting (IPW) adjustment to reduce the differences in contributable factors.[16] The propensity scores used to estimate the probability that the patients would be selected for LBC were developed by logistic regression including all variables. Then, stabilized weights were used to preserve the sample size and reduce the type I error rates.[17] The balance between both groups after weighting was considered adequate if the absolute standardized differences were less than 0.2. In the secondary outcome analysis, brush cytology and forceps biopsy were paired in each group. Statistical tests of sensitivity and specificity were conducted by the McNemar test.[18]

A two-sided P value of less than 0.05 was considered statistically significant. The univariable and multivariable logistic regression results were summarized by estimating the odds ratio (OR) and the respective 95% confidence interval (CI). All statistical analyses were conducted with R 4.0.1 software (http://www.r-project.org) and IBM SPSS version 25.0 (IBM Corp., Armonk, NY, USA).

Chapter 3 Results

3.1. Baseline characteristics

A total of 162 patients with biliary stricture were analyzed in this study. The baseline characteristics are summarized in Table 1. The stricture location and the alkaline phosphatase level were significantly different between the two groups (P value < 0.05). CBD stricture was more frequent in the conventional smear group than in the LBC group, and hilar stricture showed the opposite frequency (61.4% vs 40.2% for CBD strictures; 30.0% vs 53.3% for hilar strictures, P = 0.019). The ALP level was lower in the conventional smear group (252.8 IU/L vs 360.2 IU/L, P = 0.019). Benign stricture was more frequent in the conventional smear group, and klatskin tumor was more frequent in the LBC group (34.3% vs 20.7% for benign strictures and 22.9% vs 40.2% for klatskin tumor, P = 0.104).

3.2. Factors contributing to cytologic diagnosis of malignancy

The factors contributing to the detection of malignant cells by brush cytology were assessed by logistic regression analysis (Table 2). In univariable analysis, the P values for age, female sex, CBD stricture, hilar stricture, biliary stone, total bilirubin, and ALP were less than 0.25. These factors were selected as candidates for multivariable analysis. In multivariable analysis, age (OR= 1.064; 95%

CI: 1.028 - 1.105), female sex(OR = 0.042; 95% CI: 0.209 - 0.968), hilar stricture (OR = 3.822, 95% CI: 1.838 - 8.276), biliary stone (OR = 0.154, 95% CI: 0.036 - 0.517) and ALP (OR = 1.001, 95% CI: 1.000 - 1.003) remained significant.

3.3. Follow-up of negative/atypical cytology

When cytologic examination reported as negative or atypical, additional test were performed, and follow-up was sustained. The final diagnosis was determined based on the result of further examination during follow-up. The proportion of patients finally diagnosed as malignancy was 67.7% in atypical cytology, and was 27.9% in negative cytology. Additional exams included another ERCP, percutaneous liver biopsy, and surgical resection. When there was no evidence of malignancy despite additional exams, follow-up was sustained from 408 to 3598 days. Further information about additional test and follow-up period of each group is summarized in Table S1 and Table S2.

3.4. Primary outcome

A total of 70 patients were in the conventional smear group, and there were 92 patients in the LBC group. Four patients in the conventional smear group were excluded from the analysis as inadequate specimens. All four specimens were classified as inadequate due to scant cellularity. There was no inadequate

specimen in the LBC group.

After stabilized inverse probability weighting, the population was adjusted to 70 cases of conventional smears and 90 cases of LBC. The absolute standardized differences between the two groups after weighting were less than 0.1 except for stricture of the cystic duct (Fig. 3). There were no significant differences between the two groups including age, sex, location of stricture, and laboratory test results. The cytology result and final diagnoses of the adjusted population are summarized in Table 3. The diagnostic performance without the inadequate specimens is shown in Table 4. The diagnostic sensitivity of LBC was significantly higher than that of conventional smears (76.00% vs 92.75%, P = 0.003. The accuracy was also higher in LBC, but differences was not significant(78.46% vs 86.67%, P = 0.178).

3.5. Secondary outcome

Forty-four of 70 patients in the conventional smear group and 82 of 92 patients in the LBC group underwent both cytology specimen collection and forceps biopsies. The final diagnoses and the results of forceps biopsy combined with brush cytology and forceps biopsy alone are summarized in Table 5. The sensitivity of forceps biopsy plus conventional smear was higher than that of forceps biopsy alone but did not showed significant differences.(89.19% vs 81.08%, P = 0.248). However, the sensitivity of forceps biopsy plus LBC revealed significant improvement in sensitivity (97.06% vs 88.24%, P = 0.041)

Chapter 4 Discussion

Biliary tract cancer is a highly aggressive tumor and is often diagnosed in the advanced stage with a dismal prognosis.[19,20] Most patients unsuitable for surgery are treated with gemcitabine-based chemotherapy, which requires pathologic confirmation of malignancy.[21] However, diagnosing biliary tract cancer is challenging because of its highly desmoplastic, paucicellular nature and difficulty in anatomical access. Especially in the left intrahepatic duct, forceps biopsy is almost impossible due to the stiffness of forceps. Brush cytology has been used instead, but it is limited due to its low sensitivity.[3] To overcome these difficulties, liquid-based cytology has been gradually used in diagnosing biliary tract cancer. Most studies comparing LBC with conventional smear dealt with the filtration method.[22,23] In two studies, the LBC using centrifugation showed better sensitivity and accuracy when compared with aspiration cytology or forceps biopsy. But these studies only included 76 and 57 patients, respectively, and did not cover both intrahepatic and extrahepatic cholangiocarcinoma.[24,25]

In this retrospective comparative study, we evaluated the diagnostic efficacy of LBC using centrifugation in biliary tract cancer. Other malignancies were excluded from the analysis. Especially pancreatic cancer was excluded, although endoscopic sampling during ERCP was reported as a useful method.[26] This is because the primary diagnostic test for pancreatic cancer is an endoscopic

ultrasound-guided approach rather than ERCP. In addition, malignant cells could not be identified by brush cytology or forceps biopsy when there was only extrinsic compression without bile duct invasion. Therefore, we analyzed diagnostic efficacy only for biliary tract cancer. In this study, LBC showed significantly better sensitivity than conventional smears. In addition, the diagnostic efficacy of forceps biopsy was significantly improved when combined with LBC.

The results of this study are quite different from those of previous studies. Many researchers have pointed out that insufficient cellularity, air-drying artifact, obscuring material, and thick smears resulted in misdiagnoses by conventional smears.[27] Liquid-based cytology methods (both filtration and centrifugation) have overcome these shortcomings of conventional smears using collection tubes, preservative fluid, and a semi-automated transfer technique.[28-30] However, in previous studies comparing conventional smears and the filtration method, the improvement in sensitivity was modest.[9,10,22,23,29]

In this study, the centrifugation method showed higher sensitivity than previous studies using the filtration method. We assume that the sample preparation technique of the centrifugation method enabled this result. In the filtration method, the collection device is discarded and followed by a filtration process. However, in the centrifugation method, the collection device is retained, followed by density gradient centrifugation without the loss of malignant cells.[31] Although there was no significant difference between the two LBC methods in diagnosing cervical intraepithelial neoplasm, the

centrifugation method showed better diagnostic performance in cervical glandular neoplasm/adenocarcinoma than the filtration method.[32,33] Since biliary tract cancer is mainly adenocarcinoma, the filtration method might also be useful.

Our study had several limitations in patient selection and cytologic interpretation. First, as a retrospective study, the two cytology method groups had different distributions of the factors that affected the outcomes, such as stricture sites and the presence of biliary stones. To eliminate differences in baseline characteristics, it is necessary to compare two tests performed simultaneously on the same patient. However, we were not able to use both methods because the Korean National Insurance Service covered either of them and prohibited using both. Second, there was a time lag in each method since LBC was a more recently used method than conventional smears; conventional smears in 2010–2018, and LBC in 2019–2020. We considered that changes in endoscopic procedures or accessories during that period were insignificant. However, this time lag might be associated with the proportion of CBD stricture. The CBD stricture, especially benign, was more frequent in conventional smear group (28.6%) than that of LBC group (14.1%). As MR cholangiopancreatography and EUS were more commonly used, the frequency of "diagnostic" ERCP and brush cytology for the differential diagnosis of CBD stricture was gradually decreased. It might have resulted in these differences in stricture sites. Third, cytomorphologic features such as background material, cellular array, and nuclear pleomorphism were not analyzed in this

study. Also we could not evaluate the amount of epithelial cells quantitatively. Instead, we estimated the cellularity and the presence of artifacts semi-quantitatively. Both acellular slide and artifacts were more frequent in the conventional smear group: acellular slide; 4(5.26%) in conventional smears vs 0 in LBC, artifacts; 6(7.89%) in conventional smears vs 1 (1.11%) in LBC. Lastly, we only compared the diagnostic efficacy of targeted specimens such as brush cytology and forceps biopsy. Further study is needed including non-targeted specimens such as aspiration cytology.

Despite these limitations, this study demonstrated the diagnostic efficacy of LBC in a relatively rare disease, biliary tract cancer. In this study, we identified factors that could affect the diagnostic efficacy by multivariable logistic regression and used stabilized IPW analysis to minimize the selection effect of these factors. By using stabilized IPW analysis instead of propensity score matching, we maintained statistical power in a relatively small number of patients. After stabilized IPW adjustment, the covariate imbalance between the two groups was well-balanced the selection bias was minimized. Even in this way, residual confounding is still possible. So, further studies comparing the two cytology methods in the same specimen are needed.

In conclusion, LBC using density gradient centrifugation demonstrated better sensitivity than conventional smears. Moreover, when combined with forceps biopsies, the sensitivity was improved. The results of this study support the usefulness of LBC in diagnosing biliary tract cancer.[34]

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Abstract

배경 및 목적: 내시경적역행성담췌관조영술을 이용한 조직검사는 담도암 진단의 주요 검사이다. 하지만 검사의 민감도가 낮아 진단에 어려움을 겪는다. 액상세포검사는 갑상선암, 자궁경부암 및 췌장암에 대한 브러쉬 세포검사의 진단 효능을 향상시키는 것으로 나타났다. 하지만 액상세포검사의 담도암에 대한 진단 성능은 보고된 바가 적다. 담도암에 대한 액상세포검사의 진단 성능을 평가하기 위해 액상세포검사를 고식적도말검사, 겸자생검과 비교하였다.

방법: 2010년 1월부터 2020년 4월까지 내시경적역행성담췌관조열술을 통해 세포검사를 받은 모든 환자를 대상으로 후향적 연구를 수행하였다. 주요결과변수는 고식적도말검사와 액상세포검사의 진단 능력이었다. 두 그룹 간의 차이는 역확률가중치를 이용하여 보정되었다. 2차결과변수는 겸자생검만 시행한 경우와 겸자생검과 브러쉬세포검사를 결합한 경우의 민감도이었다. 2차결과변수는 두 가지 조직검사방법을 모두 시행한 환자를 대상으로 평가되었다.

결과: 162명의 환자 중, 70명의 환자에서 고식적도말검사, 92명의 환자에서 액상세포검사를 실시하였다. 역확률가중치를 이용한 분석에서 고식적도말검사의 민감도는 76.00%, 액상세포검사의 민감도는 92.75%(P = 0.009)였다. 정확도는 고식적도말검사의 경우 78.46%, 액상세포검사의 경우 86.67%(P = 0.178)였다. 2차 분석에서 액상세포검사는 겸자생검과 결합하였을 때 겸자생검만 시행한 경우에 비해 민감도의 향상을 보여주었다.(88.24% vs 97.06%, P=0.041)

결론: 액상세포검사는 고식적도말검사보다 더 높은 민감도와 정확도를 보였다. 또한, 액상세포검사는 겸자생검과 함께 시행하였을 때 민감도의 향상을 보여주었다.

Table 1. Baseline Characteristics

	Conventional smear (N=70)	Liquid-based cytology (N=92)	*P* value
Age (Y, 95% CI)	64.8 [54.2 - 75.4]	67.9 [56.3 - 79.5]	0.085
Sex (%)			0.100
Male	50 (71.4%)	53 (57.6%)	
Female	20 (28.6%)	39 (42.4%)	
Stricture			0.019
Intrahepatic duct	6 (8.6%)	5 (5.4%)	
Hilum	21 (30.0%)	49 (53.3%)	
Common bile duct	43 (61.4%)	37 (40.2%)	
Cystic duct	0	1 (1.1%)	
Biliary stone	13 (18.6%)	11 (12.0%)	0.342
Periampullary diverticulum	9 (12.9%)	11 (12.0%)	1.000
Laboratory tests			
WBC (x$10^3/\mu$L)	7.04 ± 2.84	7.01 ± 2.77	0.944
T.bil (mg/dL)	4.8 ± 6.9	5.1 ± 6.7	0.751
ALP (IU/L)	252.8 ± 250.3	360.2 ± 327.0	0.019
AST (IU/L)	109.6 ± 171.3	130.7 ± 217.7	0.490
ALT (IU/L)	159.3 ± 281.5	153.8 ± 266.1	0.899
Final Diagnosis			0.104
Intrahepatic CC	4 (5.7%)	8 (8.7%)	
Klatskin tumor	16 (22.9%)	37 (40.2%)	
Common bile duct cancer	20 (28.6%)	20 (21.7%)	
Gallbladder cancer	6 (8.6%)	8 (8.7%)	
Benign stricture	24 (34.3%)	19 (20.7%)	

Plus-minus values are means ± SD; categorical values are absolute numbers and percentages. WBC, white blood cell count; T.bil, total bilirubin; ALP, alkaline phosphatase; AST, aspartate aminotransferase; ALT, alanine aminotransferase; Intrahepatic CC, intrahepatic cholangiocarcinoma

Table 2. Logistic Regression Analysis of Malignant Cytology Results

	Univariable		Multivariable	
	OR (95% CI)	*P* value	OR (95% CI)	*P* value
Age	1.059 (1.027−1.094)	<.001	1.064 (1.028−1.105)	0.001
Female sex	0.381 (0.194−0.731)	0.004	0.454 (0.209−0.968)	0.042
Str*_CBD	0.385 (0.202−0.720)	0.003		
Str_Hilum	3.722 (1.947−7.294)	<.001	3.822 (1.838−8.276)	<.001
Str_IHD	0.197 (0.029−0.797)	0.042		
Biliary stone	0.154 (0.043−0.432)	0.001	0.154 (0.036−0.517)	0.005
Periampullary diverticulum	0.773 (0.295−1.981)	0.592		
WBC (X$10^3/\mu l$)	1.000 (1.000−1.000)	0.240		
T.bil (mg/dL)	1.066 (1.015−1.129)	0.017		
ALP (IU/L)	1.001 (1.000−1.003)	0.023	1.001 (1.000−1.003)	0.045
AST (IU/L)	1.001 (0.999−1.003)	0.348		
ALT (IU/L)	1.000 (0.999−1.002)	0.468		

Str, stricture

Table 3. Final Diagnoses and Cytology Results After Stabilized IPW

Cytology	The final diagnosis in Conventional Smears			The final diagnosis in LBC		
	Benign	Malignant	Total	Benign	Malignant	Total
Positive	0	19	19	0	40	40
Suspicious	0	9	9	0	14	13
Atypical	2	10	12	7	10	18
Benign	13	12	25	14	5	19
Inadequate	2	3	5	0	0	0
Total	17	53	70	21	69	90

Table 4. Comparison of Diagnostic Efficacy After Stabilized IPW

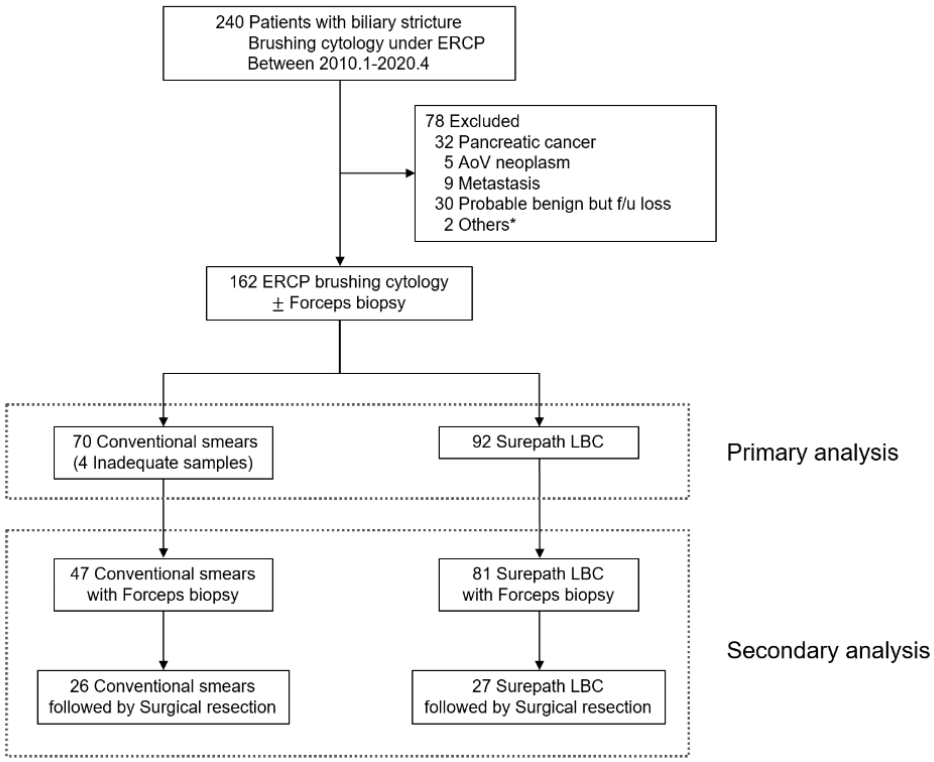
	Conventional smear (N=65)	Liquid-based cytology (N=90)	Difference (95% CI)	*P* value
Sensitivity	76.00% (61.83-86.94)	92.75% (83.89-97.61)	16.75% (5.37-28.99)	0.003
Specificity	86.67% (59.54-98.34)	66.67% (43.03-85.41)	20.00% (6.38-31.99)	0.005
PPV*	95.00% (83.82-98.59)	90.14% (83.26-94.38)	4.86 (-4.58-13.39)	0.268
NPV†	52.00% (38.90-64.83)	73.68% (53.32-87.28)	21.68 (6.34-36.03)	0.006
Accuracy	78.46% (66.51-87.69)	86.67% (77.87-92.92)	8.21 (-3.67-20.91)	0.178

PPV, positive predictive value; NPV, negative predictive value

Table 5. Final Diagnoses and Results of the Forceps Biopsy Alone and Forceps Biopsy Combined with Brush Cytology

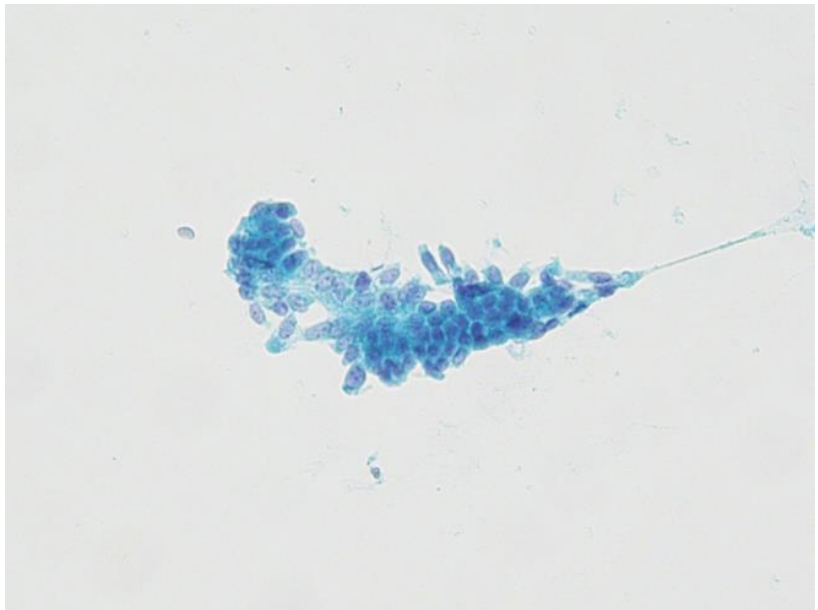
Cytology with Forceps biopsy		Final diagnosis – malignant Forceps biopsy		Final diagnosis – benign Forceps biopsy	
		Malignant	Benign	Malignant	Benign
Forceps biopsy + Conventional smear (N=44)	Malignant	30	3	0	1
	Benign	0	4	0	6
Forceps Biopsy + LBC (N=82)	Malignant	60	6	1	7
	Benign	0	2	0	6

Figure 1. Study Population

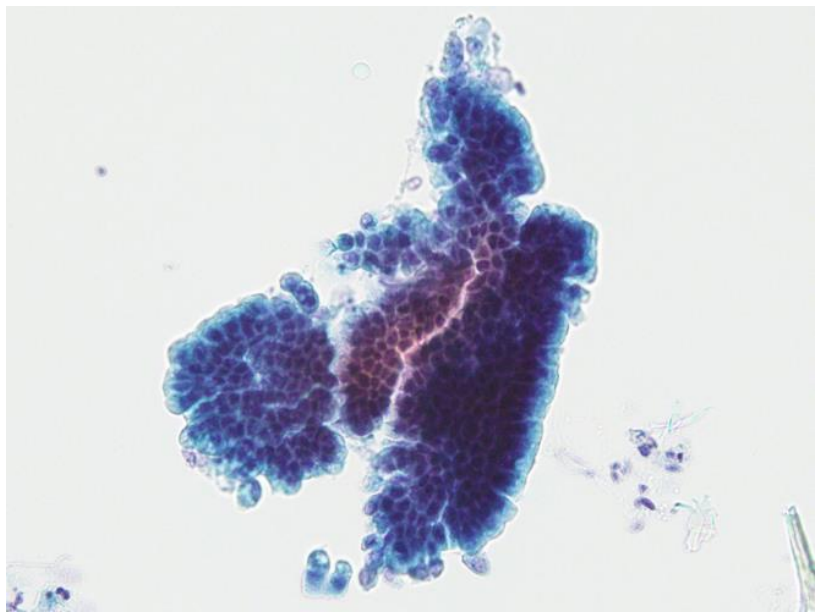


Others: one mixed neuroendocrine neoplasm, one lymphoma recurrence

Figure 2. Specimen preparation of conventional smear and liquid-based cytology



(a) The slide of conventional smear shows low cellularity (orig. mag., X200).



(b) On the other hand, the slide of liquid based cytology shows high cellularity (orig, mag., X200)

Figure 3. Change in Absolute Standardized Differences After IPW*
Str, stricture; Diverticulum, periampullary diverticulum

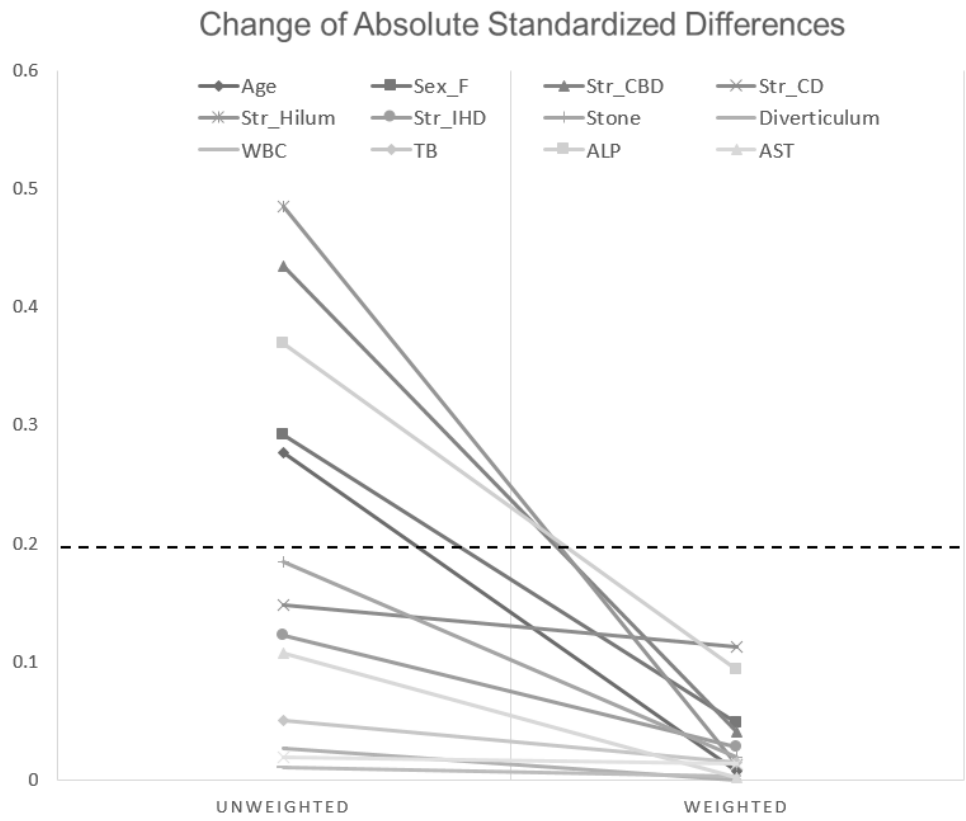


Table S1. Further examination and final diagnosis of atypical cytology

Final diagnosis	Atypical cytology in conventional smear (N=12)	Atypical cytology in Liquid-based cytology (N=19)
Malignant	9	12
Forceps biopsy of ERCP	4	6
Percutaneous liver biopsy	2	3
Percutaneous lymph node biopsy	1	
Surgery	2	3
Benign (follow-up duration, days)	3 (915-3500)	7 (595-1500)

Table S2. Further examination and final diagnosis of benign cytology

Final diagnosis	Negative cytology in conventional smear (N=28)	Neative cytology in Liquid-based cytology (N=15)
Malignant	9	3
Forceps biopsy of ERCP	1	1
Percutaneous liver biopsy	1	1
Ascites cytology	1	
EUS-FNA	1	
Surgery	4	1
Benign (follow-up duration, days)	19 (408-3598)	12 (379-1027))