

Triple-Tissue Sampling during Endoscopic Retrograde Cholangiopancreatography Increases the Overall Diagnostic Sensitivity for Cholangiocarcinoma

Seung June Lee*, Yoon Suk Lee*, Min Geun Lee*, Sang Hyub Lee*, Eun Shin[†], and Jin-Hyeok Hwang*

Departments of *Internal Medicine and [†]Pathology, Seoul National University Bundang Hospital, Seoul National University College of Medicine, Seongnam, Korea

Background/Aims: There are several methods for obtaining tissue samples to diagnose malignant biliary strictures during endoscopic retrograde cholangiopancreatography (ERCP). However, each method has only limited sensitivity. This study aimed to evaluate the diagnostic accuracy of a combined triple-tissue sampling (TTS) method (on-site bile aspiration cytology, brush cytology, and forceps biopsy). **Methods:** We retrospectively reviewed 168 patients with suspicious malignant biliary strictures who underwent double-tissue sampling (DTS; n=121) or TTS (n=47) via ERCP at our institution from 2004 to 2011. **Results:** Among the 168 patients reviewed, 117 patients (69.6%) were eventually diagnosed with malignancies. The diagnostic sensitivity for cancer was significantly higher in the TTS group than the DTS group (85.0% vs 64.9%, respectively; p=0.022). Furthermore, the combination of brush cytology and forceps biopsy was superior to the other method combinations in the DTS group. With respect to cancer type (cholangiocarcinoma vs noncholangiocarcinoma), interestingly, the diagnostic sensitivity was higher for cholangiocarcinoma in the TTS group than the DTS group (100% vs 69.4%, respectively; p<0.001) but not for the noncholangiocarcinoma patients (57.1% vs 57.1%, respectively). **Conclusions:** TTS can provide an improved diagnostic accuracy in suspicious malignant biliary strictures, particularly for cholangiocarcinoma. (*Gut Liver* 2014;8:669-673)

Key Words: Malignant biliary stricture; Combined tissue sampling; Diagnostic accuracy; Cholangiopancreatography, endoscopic retrograde

INTRODUCTION

Imaging studies, such as computed tomography and magnetic resonance cholangiopancreatography, are useful modalities in the evaluation of biliary strictures. However, a definite diagnosis of biliary strictures can only be established by histopathological examination, which is crucial in planning the further management of this disease entity.^{1,2}

Endoscopic retrograde cholangiopancreatography (ERCP) is a highly popular diagnostic method that involves direct cholangiography and allows the use of several techniques for tissue sampling, including on-site bile aspiration cytology (aspiration cytology), brush cytology, endobiliary forceps biopsy (biopsy), and endoscopic fine-needle aspiration (FNA).³ An ideal tissue sampling method should have high sensitivity and specificity while being simple and safe. However, each available tissue sampling technique during ERCP only has low to modest sensitivity for cancer detection. Therefore, several studies have examined whether combining tissue sampling techniques (double-tissue sampling [DTS]) can improve diagnostic accuracy for biliary strictures during ERCP.⁴⁻⁶ However, considering that the diagnostic accuracy for such combined methods ranges from 61% to 77%, the usefulness of such combinations remains undetermined.

It was recently reported that a combination of brush cytology, biopsy, and endoscopic FNA had an overall sensitivity for cancer detection up to 77%.⁵ In contrast, another study failed to demonstrate the efficacy of such triple-tissue sampling (TTS) in patients with suspicious malignant biliary strictures compared to biopsy alone.⁷ Therefore, it remains unclear whether TTS can provide improved diagnostic sensitivity as compared to DTS in

Correspondence to: Jin-Hyeok Hwang

Department of Internal Medicine, Seoul National University Bundang Hospital, Seoul National University College of Medicine, 82 Gumi-ro 173beon-gil, Bundang-gu, Seongnam 463-707, Korea

Tel: +82-31-787-7017, Fax: +82-31-787-4051, E-mail: woltoong@snu.ac.kr

Received on August 7, 2013. Revised on November 29, 2013. Accepted on December 19, 2013. Published online on April 23, 2014

pISSN 1976-2283 eISSN 2005-1212 <http://dx.doi.org/10.5009/gnl13292>

© This is an Open Access article distributed under the terms of the Creative Commons Attribution Non-Commercial License (<http://creativecommons.org/licenses/by-nc/3.0>) which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

suspicious biliary strictures.

This study therefore aimed to elucidate whether the TTS method could improve the diagnostic accuracy in suspicious malignant biliary strictures and whether the type of cancer had any effect on the accuracy.

MATERIALS AND METHODS

1. Patients

From August 2004 to October 2011, a total of 292 patients who underwent ERCP for suspicious malignant biliary strictures with single or more tissue samplings via on-site aspiration cytology, brush cytology, or biopsy at Seoul National University Bundang Hospital, Seongnam, Korea, were retrospectively reviewed. There were two members of faculties whose expertise in pancreatobiliary procedures and they run their procedure independently (Hwang JH and Lee SH). Also, Hwang JH acquired tissue samples with TTS and Lee SH acquired tissue samples with DTS. This study was approved by the Institutional Review Board. Among these, we included 168 patients who underwent DTS or TTS. Patients were excluded in the following cases: 1) prior histological confirmation of malignancy, 2) postoperative biliary strictures, and 3) less than 6 months of follow-up in patients with negative malignant results. The final diagnosis was established by histopathological examination of tissues obtained by endoscopic, percutaneous, or surgical means. If the histopathological diagnosis was negative for carcinoma, clinical diagnosis was made by serial radiological image findings, laboratory findings, and the clinical course over 6 months or more.

2. Intervention and techniques

ERCP was performed using the standard technique with a duodenoscope (TJF; Olympus, Tokyo, Japan). When biliary strictures were identified under radiographic guidance, endoscopic sphincterotomy was performed before tissue sampling. Brush cytology was performed using the Rx cytology brush (Boston Scientific, Boston, MA, USA) prior to aspiration cytology and biopsy. In brief, the brush was moved back and forth across the stricture once, and the brush/catheter unit was then removed. The cytology specimen was immediately transferred to a glass slide by nursing staff. The cellular material adhering to the brush was directly smeared and stained with Giemsa and Papanicolaou for routine diagnostic cytology.

The biopsy specimens were obtained by using rat tooth forceps (Olympus). First, the forceps were advanced to the distal end of the stricture as far as possible, and the forceps were then opened and the specimen was obtained. The specimen was immediately fixed in 10% formalin.

After brush cytology or biopsy, the catheter was placed into the proximal bile duct of stricture. Bile was aspirated by 10 mL syringe as much as possible, usually 30 to 50 mL or more, while the catheter was gradually pulled back toward the ampulla of

Vater. The aspirated bile was centrifuged and stained using the standard Papanicolaou technique.

3. Cytopathological interpretation

A pathologist (Shin E) blinded to the clinical information reviewed all the slides for this study. The cytological results were indicated as one of the following: 1) benign, 2) atypia (reactive), 3) atypia (indeterminate), 4) atypia (suspicious for malignancy), and 5) positive for malignancy. Atypia (reactive) was considered as negative for malignancy. Atypia (suspicious for malignancy) was considered as positive for malignancy. Atypia (indeterminate) was considered as benign, based on whether the patient's clinical course indicated malignancy.

4. Statistical analysis

Baseline characteristics were compared using the independent t-test for continuous variables and chi-square test or Fisher exact test for categorical variables. A two-sided p-value of <0.05 was considered statistically significant in all analyses. Sensitivity, specificity, positive predictive value, negative predictive value, and overall accuracy were calculated. All statistical operations were performed using the PASW software version 18.0 (SPSS Inc., Chicago, IL, USA).

RESULTS

1. Baseline characteristics

Among the 168 enrolled patients, 117 patients (69.6%) were confirmed to have malignant strictures—75 patients (64.1%) with cholangiocarcinoma; 28 patients (23.9%) with pancreatic cancer; four patients (3.4%) with ampulla of Vater cancer which were not exposed into the duodenum; 10 patients (8.5%) with other carcinomas—and 51 patients (30.4%) were diagnosed with benign strictures (Table 1). DTS was performed in 121 patients—aspiration cytology and brush cytology, 18 patients; brush cy-

Table 1. Patient Characteristics

Characteristic	DTS group (n=121)	TTS group (n=47)	p-value
Age, yr	62.58±11.1	63.11±12.8	0.79
Sex, male/female	79/42	33/14	0.54
Final diagnosis			
Malignant	77 (63.6)	40 (85.1)	<0.01
Cholangiocarcinoma	49	26	0.88
Pancreatic cancer	18	10	0.85
Ampullary cancer	3	1	1.00
Other cancers*	7	3	1.00

Data are presented as mean±SD or number (%).

DTS, double-tissue sampling; TTS, triple-tissue sampling.

*Primary tumors included gallbladder cancer (DTS, n=5; TTS, n=2) and hepatocellular carcinoma (DTS, n=2; TTS, n=1).

tology and biopsy, 95 patients; aspiration cytology and biopsy, eight patients, while TTS was performed in 47 patients. The demographic characteristics and clinical profiles in each group are summarized in Table 1. The TTS group had a greater number of cancer patients than that in the DTS group (85.1% vs 63.6%, respectively; $p < 0.01$). However, the proportions of patients with cancer and benign disease were similar between the two groups. Other baseline characteristics among the two groups showed no significant differences.

2. Outcomes according to tissue sampling methods: DTS versus TTS

The overall sensitivity and accuracy of each method was

42.1% and 54.8% in aspiration cytology, 58.4% and 70.6% in brush cytology, and 63.5% and 74.7% in biopsy, respectively (Table 2). DTS showed a sensitivity of 64.9%, while TTS (aspiration cytology, brush cytology, and biopsy) had a significantly improved sensitivity of up to 85.0% (Table 2). In the DTS group, the combination of brush cytology and biopsy showed a slightly higher sensitivity of 66.7% as compared to the other two combinations, although the difference was not statistically significant (Table 3). In the TTS group, the sensitivity and accuracy of each method was 45.0% and 53.2% in aspiration cytology, 65% and 70.2% in brush cytology, and 77.5% and 80.8% in biopsy (Table 4). Among nine cases with negative results by forceps biopsy, three cases were confirmed by aspiration cytology or brush cy-

Table 2. Diagnostic Efficacy of the Single or Combined Methods

	Sensitivity*, %	Specificity, %	PPV, %	NPV, %	Accuracy, %
Aspiration cytology (n=73)	42.1 (24/57)	100.0 (16/16)	100.0 (24/24)	32.7 (16/49)	54.8 (40/73)
Brush cytology (n=160)	58.4 (66/113)	100.0 (47/47)	100.0 (66/66)	50.0 (47/94)	70.6 (113/160)
Biopsy (n=150)	63.5 (66/104)	100.0 (46/46)	100.0 (66/66)	54.8 (46/84)	74.7 (112/150)
DTS (n=121)	64.9 (50/77)	100.0 (44/44)	100.0 (50/50)	62.0 (44/71)	77.7 (94/121)
TTS (n=47)	85.0 (34/40)	100.0 (7/7)	100.0 (34/34)	53.8 (7/13)	87.2 (41/47)

PPV, positive predictive value; NPV, negative predictive value; DTS, double-tissue sampling; TTS, triple-tissue sampling.
* $p < 0.05$ for TTS vs DTS.

Table 3. Comparison of the Diagnostic Efficacy between the Combined Triple- and Double-Tissue Sampling Methods

	Sensitivity, %	Specificity, %	PPV, %	NPV, %	Accuracy, %
DTS (n=121)					
Asp. cytology+Brush (n=18)	61.5 (8/13)	100.0 (5/5)	100.0 (8/8)	50.0 (5/10)	72.2 (13/18)
Brush+Biopsy (n=95)	66.7 (40/60)	100.0 (35/35)	100.0 (40/40)	63.6 (35/55)	78.9 (75/95)
Asp. cytology+Biopsy (n=8)	50.0 (2/4)	100.0 (4/4)	100.0 (2/2)	66.7 (4/6)	75.0 (6/8)
Total	64.9 (50/77)	100.0 (44/44)	100.0 (50/50)	62.0 (44/71)	77.7 (94/121)
TTS (n=47)	85.0 (34/40)	100.0 (7/7)	100.0 (34/34)	53.8 (7/13)	87.2 (41/47)

PPV, positive predictive value; NPV, negative predictive value; DTS, double-tissue sampling; Asp. cytology, bile aspiration cytology; Brush, brush cytology; TTS, triple-tissue sampling.

Table 4. Diagnostic Efficacy of Triple-Tissue Sampling

	Sensitivity, %	Specificity, %	PPV, %	NPV, %	Accuracy, %
Asp. cytology	45.0 (18/40)	100.0 (7/7)	100.0 (18/18)	24.1 (7/29)	53.2 (25/47)
Brush	65.0 (26/40)	100.0 (7/7)	100.0 (26/26)	33.3 (7/21)	70.2 (33/47)
Biopsy	77.5 (31/40)	100.0 (7/7)	100.0 (31/31)	43.8 (7/16)	80.8 (38/47)
Asp. cytology+Brush	70.0 (28/40)	100.0 (7/7)	100.0 (28/28)	36.8 (7/19)	74.5 (35/47)
Brush+Biopsy	82.5 (33/40)	100.0 (7/7)	100.0 (33/33)	50.0 (7/14)	85.1 (40/47)
Asp. cytology+Biopsy	82.5 (33/40)	100.0 (7/7)	100.0 (33/33)	50.0 (7/14)	85.1 (40/47)
TTS	85.0 (34/40)	100.0 (7/7)	100.0 (34/34)	53.8 (7/13)	87.2 (41/47)

PPV, positive predictive value; NPV, negative predictive value; Asp. cytology, bile aspiration cytology; Brush, brush cytology; TTS, triple-tissue sampling.

Table 5. Comparison of the Sensitivity between the Combined Triple- and Double-Tissue Sampling Methods in Cholangiocarcinoma and Noncholangiocarcinoma

	DTS	TTS	p-value
Cholangiocarcinoma	69.4 (34/49)	100.0 (26/26)	<0.01
Noncholangiocarcinoma	57.1 (16/28)	57.1 (8/14)	1.00

DTS, double-tissue sampling; TTS, triple-tissue sampling.

tology (33%, 3/9; one by aspiration cytology and two by brush cytology). With respect to the diagnostic yield, TTS represented a higher sensitivity than DTS (85.0% vs 64.9%, $p=0.022$). Interestingly, this significant difference was only documented in patients with cholangiocarcinoma (100% in TTS group vs 69.4% in DTS group, $p=0.002$) (Table 5). In contrast, no such difference between TTS and DTS was observed in patients with noncholangiocarcinoma malignancies (57.1% vs 57.1%) (Table 5). No serious complications related to tissue sampling occurred during ERCP.

DISCUSSION

Although several methods are available today for obtaining tissue samples during ERCP, such as aspiration cytology, endoscopic FNA, brush cytology, and biopsy, the best technique for obtaining tissue for a definite diagnosis of suspicious malignant biliary strictures remains a challenging issue. The single-tissue sampling method has a rather low sensitivity, being 6% to 32% in bile aspiration,^{2,8-10} 27% to 62% in endoscopic FNA,^{5,11,12} 30% to 57% in brush cytology,^{5,6,10,13} and 41% to 81% in forceps biopsy.^{6,10,14,15} Several studies have examined the usefulness of combining methods for tissue sampling; however, the results have not encouraged their routine use in clinical practice.^{6,7} Therefore, we conducted this study in order to determine whether TTS can enhance the diagnostic yield in malignant biliary strictures. Our data showed that the TTS method, combining aspiration cytology, brush cytology, and biopsy, could improve the diagnostic yield in suspicious malignant biliary strictures without additional risks of complications, particularly in cases of cholangiocarcinoma.

Only a few studies have examined the role of bile aspiration cytology in multimodal tissue diagnosis for biliary strictures.^{7,16} Aspiration cytology is easy to perform and requires minimal effort when combined with other sampling methods. Our results revealed that aspiration cytology conferred additional diagnostic sensitivity, especially in cases of cholangiocarcinoma. These findings differ from those of a previous study, wherein the addition of aspiration cytology to tissue biopsy did not enhance the diagnostic sensitivity.⁷ Theoretically, the accuracy of aspiration cytology can be improved by scraping the stricture site prior to aspiration by exposing the subepithelial malignant tissue.^{6,16,17}

Therefore, in the present study, tissue sampling was performed sequentially, and bile aspiration was performed immediately after brush cytology and forceps biopsy. This may be the reason for the improved diagnostic sensitivity observed in our results.

In our study, the overall sensitivity of TTS was 85%, being 100% in the cholangiocarcinoma subgroup. This is considerably higher than that reported by other studies concerning TTS that comprised brush cytology, forceps biopsy, and endoscopic FNA.^{5,11} Although there were other factors that might have influenced the cancer detection rate, such as cellular adequacy, processing of samples, or histopathological interpretation,¹⁸ we consider TTS that includes bile aspiration cytology to be very useful in the diagnosis of suspicious malignant biliary strictures.

Our results demonstrated that the overall sensitivity of TTS was higher than that of DTS. Interestingly, the high sensitivity was observed in cholangiocarcinoma cases, but not in other malignancies. This could be explained by the fact that the bile aspiration cytology yield may depend on the presence of floating cancer cell nests; since pancreatic cancer or metastases from other sites may lead to the compression of the biliary tract without direct invasion of the bile duct epithelium, bile aspiration cytology in TTS would therefore not confer improved sensitivity in such cases.

Recently, new methods, such as endoscopic ultrasound-guided FNA or spyglass cholangioscopy, have been introduced for evaluating biliary strictures.¹⁹⁻²¹ However, we consider that tissue sampling, particularly TTS, would continue to be an essential procedure with its easy feasibility and safety.

This study has certain limitations. Its retrospective and non-randomized nature, along with the small sample size, may reduce the generalized applicability of our findings. However, each faculty uses different way to obtain tissues, such as DTS and TTS, with their preference and they do not affect each other for their procedure. Therefore, we thought that this study might give us some useful insights to compare the efficacy of DTS and TTS even in retrospective study. And we consider that the simple and easy tissue sampling methods included in the TTS method employed in the present study can be applied for diagnosing suspicious malignant biliary strictures, being particularly useful in cases of cholangiocarcinoma.

In conclusion, the combination of bile aspiration cytology, brush cytology, and forceps biopsy can significantly improve the diagnostic yield in cases of suspicious malignant biliary strictures, especially for cholangiocarcinoma. Further prospective studies are warranted for validating our findings.

CONFLICTS OF INTEREST

No potential conflict of interest relevant to this article was reported.

REFERENCES

1. Kurzawinski T, Deery A, Dooley J, Dick R, Hobbs K, Davidson B. A prospective controlled study comparing brush and bile exfoliative cytology for diagnosing bile duct strictures. *Gut* 1992;33:1675-1677.
2. Kurzawinski TR, Deery A, Dooley JS, Dick R, Hobbs KE, Davidson BR. A prospective study of biliary cytology in 100 patients with bile duct strictures. *Hepatology* 1993;18:1399-1403.
3. Byrne MF, Jowell PS. ERCP: methods of tissue sampling. *Tech Gastrointest Endosc* 2003;5:27-34.
4. Pugliese V, Conio M, Nicolò G, Saccomanno S, Gatteschi B. Endoscopic retrograde forceps biopsy and brush cytology of biliary strictures: a prospective study. *Gastrointest Endosc* 1995;42:520-526.
5. Jailwala J, Fogel EL, Sherman S, et al. Triple-tissue sampling at ERCP in malignant biliary obstruction. *Gastrointest Endosc* 2000;51(4 Pt 1):383-390.
6. Ponchon T, Gagnon P, Berger F, et al. Value of endobiliary brush cytology and biopsies for the diagnosis of malignant bile duct stenosis: results of a prospective study. *Gastrointest Endosc* 1995;42:565-572.
7. Lo SK, French S, Chang L, et al. Tissue diagnosis for neoplastic biliary strictures (NBS): a prospective study comparing forceps biopsy and cytology. *Gastrointest Endosc* 1995;41:405.
8. Mansfield JC, Griffin SM, Wadehra V, Matthewson K. A prospective evaluation of cytology from biliary strictures. *Gut* 1997;40:671-677.
9. Foutch PG, Kerr DM, Harlan JR, Kummet TD. A prospective, controlled analysis of endoscopic cytotechniques for diagnosis of malignant biliary strictures. *Am J Gastroenterol* 1991;86:577-580.
10. Yagioka H, Hirano K, Isayama H, et al. Clinical significance of bile cytology via an endoscopic nasobiliary drainage tube for pathological diagnosis of malignant biliary strictures. *J Hepatobiliary Pancreat Sci* 2011;18:211-215.
11. Howell DA, Parsons WG, Jones MA, Bosco JJ, Hanson BL. Complete tissue sampling of biliary strictures at ERCP using a new device. *Gastrointest Endosc* 1996;43:498-502.
12. Howell DA, Beveridge RP, Bosco J, Jones M. Endoscopic needle aspiration biopsy at ERCP in the diagnosis of biliary strictures. *Gastrointest Endosc* 1992;38:531-535.
13. Macken E, Drijkoningen M, Van Aken E, Van Steenberghe W. Brush cytology of ductal strictures during ERCP. *Acta Gastroenterol Belg* 2000;63:254-259.
14. Kubota Y, Takaoka M, Tani K, et al. Endoscopic transpapillary biopsy for diagnosis of patients with pancreaticobiliary ductal strictures. *Am J Gastroenterol* 1993;88:1700-1704.
15. Sugiyama M, Atomi Y, Wada N, Kuroda A, Muto T. Endoscopic transpapillary bile duct biopsy without sphincterotomy for diagnosing biliary strictures: a prospective comparative study with bile and brush cytology. *Am J Gastroenterol* 1996;91:465-467.
16. Curcio G, Traina M, Mocciano F, et al. Intraductal aspiration: a promising new tissue-sampling technique for the diagnosis of suspected malignant biliary strictures. *Gastrointest Endosc* 2012;75:798-804.
17. Leung JW, Sung JY, Chung SC, Chan KM. Endoscopic scraping biopsy of malignant biliary strictures. *Gastrointest Endosc* 1989;35:65-66.
18. De Bellis M, Sherman S, Fogel EL, et al. Tissue sampling at ERCP in suspected malignant biliary strictures (Part 1). *Gastrointest Endosc* 2002;56:552-561.
19. DeWitt J, Misra VL, Leblanc JK, McHenry L, Sherman S. EUS-guided FNA of proximal biliary strictures after negative ERCP brush cytology results. *Gastrointest Endosc* 2006;64:325-333.
20. Rösch T, Hofrichter K, Frimberger E, et al. ERCP or EUS for tissue diagnosis of biliary strictures? A prospective comparative study. *Gastrointest Endosc* 2004;60:390-396.
21. Williamson JB, Draganov PV. The usefulness of SpyGlass™ choledochoscopy in the diagnosis and treatment of biliary disorders. *Curr Gastroenterol Rep* 2012;14:534-541.