Clinical Trial/Experimental Study

Medicine

OPEN

# A prospective randomized noninferiority trial comparing conventional smears and SurePath™ liquid-based cytology in endoscopic ultrasound-guided sampling of esophageal, gastric, and duodenal lesions

Jae Chang Jun, MD[a], Sang Hyub Lee, MD, PhD[a], Han Myung Lee, MD[a], Sang Gyun Kim, MD, PhD[a], Hyunsoo Chung, MD, PhD[a], Joo Seong Kim, MD, PhD[a], Namyoung Park, MD[a], Jin Ho Choi, MD, PhD[a], Yoonjin Kwak, MD, PhD[b], Soo-Jeong Cho, MD, PhD[a,*]

**Abstract**

**Background:** Several liquid-based cytology (LBC) methods are currently used, but the diagnostic accuracy of each method is not well known. We aimed to compare the diagnostic performance of SurePath™ LBC and conventional smear (CS) cytology in endoscopic ultrasound-guided fine needle aspiration (EUS-FNA) samples of esophageal, gastric, and duodenal lesions.

**Methods:** As a prospective randomized noninferiority study, patients who needed EUS-FNA due to subepithelial mass in the upper gastrointestinal tract were randomly assigned 1:1 to the LBC and CS groups. Cytologic preparation was carried out using a crossover design where 1 method was used for the first needle-pass sample and another method was used for the second needle-pass sample. The primary outcome was to compare the diagnostic performance between LBC and CS using the final diagnosis as the gold standard.

**Results:** A total of 87 patients were randomized and 60 patients were analyzed. There were no differences between LBC and CS in diagnostic accuracy (91.7% vs 86.7%, $P = .380$), sensitivity (97.7% vs 90.7%, $P = .169$), specificity (76.5% vs 76.5%, $P > .99$), negative predictive value (92.9% vs 76.5%, $P = .225$), or positive predictive value (91.3% vs 90.7%, $P = .921$). The background of LBC was less bloody than that of CSs (5.0% vs 53.3%, $P < .001$) and the sample preparation time of LBC was shorter than that of CSs (29 ± 7 seconds vs 90 ± 17 seconds, $P < .001$).

**Conclusion:** In the EUS-FNA of a subepithelial mass in the upper gastrointestinal tract, the diagnostic performance of LBC was not inferior to that of CS. The field of view was better in LBC, because the background was less bloody and necrotic. As LBC is more convenient to perform and takes shorter time, it is expected that it can replace the CS method for EUS-FNA samples.

**Abbreviations:** CS = conventional smear, EUS = endoscopic ultrasound, EUS-FNA = endoscopic ultrasound-guided fine needle aspiration, GI = gastrointestinal, GIST = gastrointestinal stromal tumor, LBC = liquid-based cytology.

**Keywords:** endoscopic ultrasound, fine needle aspiration, lymph node enlargement, subepithelial tumor, SurePath

## 1. Introduction

A subepithelial tumor is a disease found in 0.36 to 1.94% of the upper gastrointestinal (GI) endoscopy cases in Korea.[1,2] As participation in the National Cancer Screening Program and medical checkups increase, the number of subepithelial tumor cases is also increasing. The prevalence of subepithelial tumors is known to increase with age and it is expected to increase more with the aging society.[3] As subepithelial tumors are located below the epithelial cell layer, it is difficult to obtain tissue through the usual endoscopic forceps biopsy. If subepithelial tumors are

*This study was supported by grants from the National Research Foundation of Korea (#NRF-2022R1A2B5B01001430), the Korean Society of Gastrointestinal Endoscopy (2021), and the SNUH research fund (#03-2022-0140).*

*The authors have no conflict of interests to disclose.*

*The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.*

*Supplemental Digital Content is available for this article.*

*[a] Department of Internal Medicine, Liver Research Institute, Seoul National University Hospital, Seoul National University College of Medicine, Seoul, Korea, [b] Department of Pathology, Seoul National University Hospital, Seoul National University College of Medicine, Seoul, Korea.*

** Correspondence: Soo Jeong Cho, Department of Internal Medicine and Liver Research Institute, Seoul National University College of Medicine, 101 Daehak-ro, Jongno-gu, Seoul 03080, Republic of Korea (e-mail: crystal5@snu.ac.kr).*



*How to cite this article: Jun JC, Lee SH, Lee HM, Kim SG, Chung H, Kim JS, Park N, Choi JH, Kwak Y, Cho S-J. A prospective randomized noninferiority trial comparing conventional smears and SurePath™ liquid-based cytology in endoscopic ultrasound-guided sampling of esophageal, gastric, and duodenal lesions. Medicine 2023;102:29(e34321).*

*Received: 28 March 2023 / Received in final form: 21 June 2023 / Accepted: 22 June 2023*

*http://dx.doi.org/10.1097/MD.0000000000034321*

suspected by endoscopy, endoscopic ultrasound (EUS) is firstly conducted to confirm the characteristics of the subepithelial tumors.

Among subepithelial tumors, there are characteristic findings on EUS, such as lipoma, duplication cyst, and ectopic pancreas. However, with EUS alone, hypoechoic lesions, especially leiomyomas, gastrointestinal stromal tumors (GISTs), and schwannomas, are difficult to confirm, and histological diagnosis is sometimes required. In particular, GISTs are important in the differential diagnosis because they have a malignant potential[4] and often needs resection, whereas the other hypoechoic subepithelial tumors generally need to be followed only. The benefit of tissue acquisition from subepithelial tumors includes the avoidance of unnecessary resection or surgery. Accordingly, various methods are used to obtain subepithelial tumor tissues, such as EUS-guided Trucut biopsy, EUS-fine needle aspiration and biopsy, and the bite-on-bite technique. However, there is no perfect method because the diagnosis rate is not high.

In endoscopic ultrasound-guided fine needle aspiration (EUS-FNA), rapid on-site evaluation increases diagnostic efficacy, but many institutions cannot perform the procedure due to a shortage of medical resources, including a pathologist. Therefore, where rapid on-site evaluation is limited, studies have compared the diagnostic accuracy of various methods such as cell block immunohistochemistry, liquid-based cytology (LBC), and conventional smears (CSs).[5,6] The cytologic diagnosis of samples obtained by EUS-FNA is performed using LBC or CSs. In CSs, material obtained by aspiration is smeared on glass slides and placed in a 95% alcohol fixative for additional staining.[7] LBC is not a direct smear of a specimen on a slide, but a process in which the specimen is suspended in a preservative solution and then uniformly mixed by vortexing or rotation to remove unnecessary cell debris, mucus, and blood cells. After going through this process, it is made into a relatively homogeneous single-cell sample. Recently, the LBC method was introduced in the gynecologic field (Papanicolaou smears) and has been widely used including for non-gynecologic organs.

Many fine needle aspiration studies using LBC for thyroid and breast samples have been conducted, but no study has compared LBC and CSs of specimens obtained by EUS-FNA from subepithelial lesions including intra-GI wall masses and extra-GI wall masses. Therefore, we designed a prospective clinical study to evaluate the diagnostic accuracy and clinical efficacy of LBC in samples collected by EUS-FNA in the above-mentioned lesions.

## 2. Methods

### 2.1. Study design

In this randomized, prospective, comparative, single-center study, consecutive patients with a subepithelial mass or lesion, including an intra-GI wall or extra-GI wall mass, such as enlarged lymph nodes or a metastatic mass, in imaging findings or endoscopy were enrolled at Seoul National University Hospital from January 2019 to August 2022.

EUS-FNA was performed in patients who needed pathological examination among those who had subepithelial lesions from or around the upper GI tract, and the performance of CSs and LBC was compared.

Written informed consent was obtained from all participants before recruitment. Those younger than 18 years of age or who did not consent to the study were excluded. Patients who had coagulopathy, serious mental illness or clinically significant cardiopulmonary diseases were also excluded. In addition, pregnant patients or those who had difficulty with EUS due to previous esophageal, gastric, or duodenal surgery were excluded.[8]

This study was approved by the Institutional Review Board of Seoul National University Hospital (IRB number 1902-076-1011). It complied with the Declaration of Helsinki Statement. The trial was registered in ClinicalTrials.gov (Identifier: NCT05394129).

### 2.2. Diagnostic approach using EUS-FNA

The procedure was performed using a 19-gauge or 22-gauge needle (EZ Shot 3 Plus; Olympus Co., Tokyo, Japan) at the discretion of the endoscopist on a linear EUS scope (GF-UCT260; Olympus Co.). When the target lesion was identified in EUS, the target was punctured with a needle and the stylet was removed after puncturing. Three needle passes were performed, and the specimen was acquired by moving the needle back and forth 15 to 20 times for each needle pass.

Before the EUS-FNA procedure, participants were randomly assigned 1:1 to CS and LBC group by personnel not involved in this study. As for the randomization method, block randomization was performed by mixing block sizes of 4 and 6 using Microsoft Excel 2013 (Microsoft Corp., Redmond, WA). LBC was done with the first needle-pass samples in the LBC group, and the samples acquired from the second needle-pass were used for CSs. In the CS group, the CS was used for the first sample in the same manner as in the LBC group, and the second sample was used for LBC. In both groups, the third needle-pass samples were used as tissue core biopsy specimens. The sample preparation time was also compared between the groups, and it was defined as the total time from needle retrieval to sample preparation for each pass in the EUS-FNA session. Independent staff measured the time taken using a stopwatch.

### 2.3. Specimen acquisition and evaluation

In the CSs, the aspirated samples were smeared onto glass slides and fixed with 95% alcohol. For LBC, the samples were promptly suspended in an alcohol-based preservative fluid (CytoRich). The samples in SurePath (TriPath Imaging, Burlington, NC) vials were sent to the pathology department for processing with a PrepStain Slide Processor (Becton, Dickinson and Company, Franklin Lakes, NJ).[9] The core biopsy specimens were sent in 10% formalin, if there were visible solid particles.

All pathological examinations were judged by 1 pathologist, and the cytologic examination results were classified as malignant, suspicious, atypical, benign, or inadequate. For statistical analysis in this study, atypical, suspicious, and malignant findings were considered malignant.[10,11] In this study, the following parameters were investigated in both the LBC and CS methods. In terms of sample adequacy, if the sample was sufficient for cytologic diagnosis and obtained from the target lesion, the sample was considered adequate; otherwise, it was deemed inadequate. During slide preparation, whether the background was clean, bloody, mucinous, inflammatory, or necrotic was evaluated, as was the presence of dry artifacts. Regarding the architectural pattern, single-cell predominance, 3-dimensional clusters, and 2-dimensional monolayer sheets were examined. Cellularity was evaluated as acellular without cells, sparsely cellular for fewer than 3 clusters, moderately cellular for 3 to 10 clusters, highly cellular for 10 to 20 clusters, and very highly cellular for more than 20 clusters.

The final diagnosis was made comprehensively through the EUS-FNA core biopsy, LBC, CSs, and specimens obtained after surgery or endoscopic enucleation. If the initial diagnosis was benign, the final diagnosis was concluded through imaging studies and additional biopsies during a follow-up period of at least 6 months. Samples identified as malignant, suspicious, or atypical were finally diagnosed as malignant. If confirmed as benign, it was finally diagnosed as benign.

### 2.4. Outcomes

The final diagnosis was regarded as the gold standard and used to compare the diagnostic performance of the 2 methods, which was the primary endpoint of this study. For diagnostic performance, specificity, sensitivity, accuracy, positive predictive value, and negative predictive value were compared. The secondary endpoint was a comparison of the cytomorphologic features and time taken for each method.

### 2.5. Statistical analysis

The noninferiority principle was used for calculating sample size. The diagnostic accuracy of LBC and CSs was 92.3% and 89.4%, respectively, in a previous report comparing the 2 methods.[12] The number of participants required to show a significant difference between the 2 groups with a type I error of 0.05 and 80% power was 146 when the noninferiority margin (−Δ) was set to −10% and the drop-out rate was 15%. If the lower bounds of the 95% confidence interval of the proportion difference in diagnostic accuracy between LBC and CS were higher than −10% of the noninferior margin, noninferiority would be declared.

Fisher exact test or the chi-squared test was used to analyze categorical variables, and the Student *t* test was used to analyze continuous variables. If the *P* value was <.05 (2-sided), it was considered statistically significant. SPSS version 22.0 (IBM Corp., Armonk, NY) was used for the statistical analyses.

## 3. Results

### 3.1. Baseline characteristics of the study population

Patient recruitment started in January 2019. However, trial recruitment was stopped prematurely in August 2022 due to a low recruitment rate. A total of 87 patients with a subepithelial mass or lesion, including an intra-GI wall mass or extra-GI wall mass, were enrolled. Twenty-seven patients were excluded. Four cases without LBC and 3 with inadequate sampling for both LBC and CS were excluded. In 8 cases, no lesion entity was confirmed by cytology, biopsy, or surgery, so a final diagnosis could not be made. Twelve cases showing an indeterminate nature such as atypical lymphoid hyperplasia were excluded. The clinical data of the remaining 60 patients were analyzed (Fig. 1).

Among the target lesions, subepithelial tumors from intra-GI wall were 50.0%, and extra-GI wall masses were 50.0%. In the extra-GI wall masses, lymph nodes accounted for 76.7%, and metastatic masses for 23.3%. Leiomyomas accounted for most of the benign lesions with 9 cases, and in the malignant lesions, metastatic cancer accounted for 21 cases, followed by GIST with 15 cases. The baseline characteristics between the 2 groups are presented in Table 1. The mean age of the patients was 60.7 years (range, 24–85 years). The study included 28 (46.7%) males and 32 (53.3%) females. The mean size of the target lesion was 3.65 cm (range, 1.4–13.0 cm). A 22-gauge needle was used in 96.7% (58/60) of the cases, and a 19-gauge needle was used in 3.3% (2/60). The needle was passed twice in 3.3% (2/60) of the participants, 3 times in 78.3% (47/60), and 4 times in 18.3% (11/60). The 2 groups showed no statistically significant differences in age, sex, size of the target lesion, needle gauge, or the number of needle passages.

### 3.2. Comparison of diagnostic performance

The comparison of CS and LBC results with final diagnoses is summarized in Table 2. Among the malignant lesions, 90.7% (39/43) were correctly diagnosed by CSs, and 97.7% (42/43) by LBC. Although the cytological results were reported as benign, the final diagnosis was malignant in 23.5% (4/17) of the CSs and 7.1% (1/14) of the LBC samples. Table 3 shows the results of comparing the diagnostic performance between the 2 methods. Accuracy, sensitivity, specificity, and positive and negative predictive values were not different between LBC and CSs. The difference in diagnostic accuracy between the 2 groups was 5% (LBC 91.7%, CS 86.7%), and the lower bounds of the 95% confidence interval were above the −Δ margin (Fig. 2). These outcomes showed that the diagnostic performance of LBC was noninferior compared to that of CSs.

The diagnostic performance of LBC and CSs according to needle gauge is described in Table S1, Supplemental Digital Content, http://links.lww.com/MD/J267. There was no statistically significant difference according to needle gauge, but the overall good diagnostic efficacy of 19-gauge compared to 22-gauge needles was consistent with previous reports.[13,14] Sample preparation time of LBC was shorter than CSs (LBC, 29 ± 7 seconds; CS, 90 ± 17 seconds; $P < .001$).

### 3.3. Comparison of cytomorphologic features

The results of comparing the differences in the cytomorphologic features of LBC and CSs are shown in Table 4. In LBC preparation, the presence of a bloody background was less than

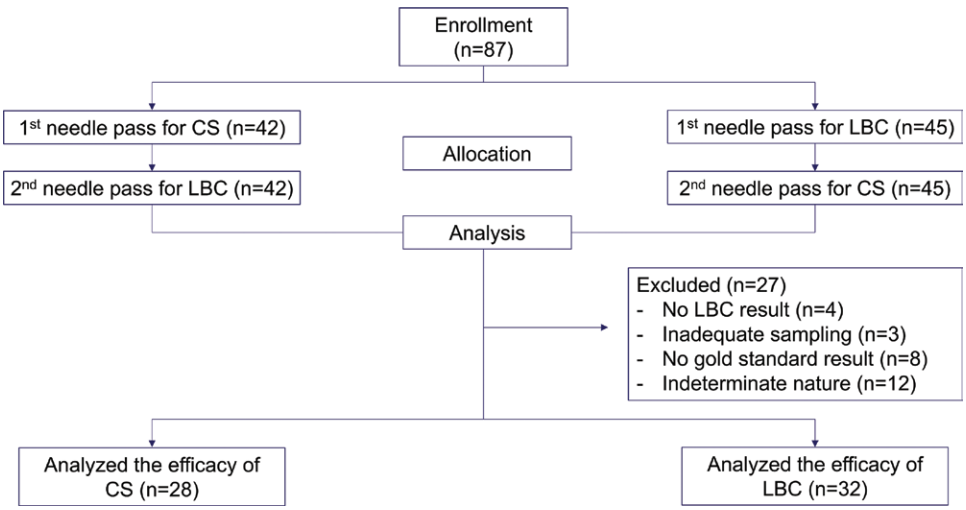


**Figure 1.** Flowchart of patients throughout the randomized trial. CS = conventional smear, LBC = liquid-based cytology.

**Table 1**

**Baseline characteristics of the study population.**

| Variable | Total | Conventional smear group | Liquid-based cytology group | *P* value |
|---|---|---|---|---|
| Number of patients | 60 | 28 | 32 | |
| Age (yr), mean ± SD | 60.7 ± 12.8 | 62.7 ± 11.5 | 58.9 ± 13.8 | .255 |
| Sex, n (%) | | | | .316 |
| Male | 28 (46.7) | 15 (53.6) | 13 (40.6) | |
| Female | 32 (53.3) | 13 (46.4) | 19 (59.4) | |
| Size (cm), mean ± SD | 3.65 ± 2.11 | 4.12 ± 2.60 | 3.24 ± 1.50 | .108 |
| Needle gauge, n (%) | | | | >.99 |
| 22 gauge | 58 (96.7) | 27 (96.4) | 31 (96.9) | |
| 19 gauge | 2 (3.3) | 1 (3.6) | 1 (3.1) | |
| Number of needle passages, n (%) | | | | .582 |
| 2 | 2 (3.3) | 0 (0.0) | 2 (6.3) | |
| 3 | 47 (78.3) | 22 (78.6) | 25 (78.1) | |
| 4 | 11 (18.3) | 6 (21.4) | 5 (15.6) | |
| Target lesion, n (%) | | | | >.99 |
| Intra-GI wall mass | 30 (50.0) | 14 (50.0) | 16 (50.0) | |
| Extra-GI wall mass | 30 (50.0) | 14 (50.0) | 16 (50.0) | |
| Adverse events, n (%) | 0 (0.0) | 0 (0.00) | 0 (0.0) | - |
| Final diagnosis, n (%) | | | | .267 |
| Benign | 17 (28.3) | 6 (21.4) | 11 (34.4) | |
| Leiomyoma | 9 (15.0) | 3 (10.7) | 6 (17.1) | |
| Tb lymphadenopathy | 2 (3.3) | 0 (0.0) | 2 (6.3) | |
| Heterotopic pancreas | 2 (3.3) | 0 (0.0) | 2 (6.3) | |
| Granulomatous lymphadenopathy | 1 (1.7) | 0 (0.0) | 1 (3.1) | |
| Lymphoid lesion | 1 (1.7) | 1 (3.6) | 0 (0.0) | |
| Benign neurogenic tumor | 1 (1.7) | 1 (3.6) | 0 (0.0) | |
| Benign cystic lesion | 1 (1.7) | 1 (3.6) | 0 (0.0) | |
| Malignant | 43 (71.7) | 22 (78.6) | 21 (65.6) | |
| Metastatic cancer | 21 (35.0) | 11 (39.3) | 10 (31.3) | |
| GIST | 15 (25.0) | 9 (32.1) | 6 (18.8) | |
| Neuroendocrine tumor | 2 (3.3) | 1 (3.6) | 1 (3.1) | |
| Malignant mesothelioma | 1 (1.7) | 0 (0.0) | 1 (3.1) | |
| Leiomyosarcoma | 1 (1.7) | 1 (3.6) | 0 (0.0) | |
| Glomus tumor | 1 (1.7) | 0 (0.0) | 1 (3.1) | |
| Paraganglioma | 1 (1.7) | 0 (0.0) | 1 (3.1) | |
| Pheochromocytoma | 1 (1.7) | 0 (0.0) | 1 (3.1) | |

GI = gastrointestinal, GIST = Gastrointestinal stromal tumor, SD = Standard deviation, Tb = Tuberculosis.

**Table 2**

**Comparison of final diagnoses and results of the cytologic tests.**

| | Final diagnosis | | | |
|---|---|---|---|---|
| | Conventional smear, n (%) | | Liquid-based cytology, n (%) | |
| Diagnosis | Benign | Malignant | Benign | Malignant |
| Malignant | 0 (0.0) | 19 (44.2) | 0 (0.0) | 23 (53.5) |
| Suspicious | 0 (0.0) | 13 (30.2) | 0 (0.0) | 8 (18.6) |
| Atypical | 4 (23.5) | 7 (16.3) | 4 (23.5) | 11 (25.6) |
| Benign | 13 (76.5) | 2 (4.7) | 13 (76.5) | 1 (2.3) |
| Inadequate | 0 (0.0) | 2 (4.7) | 0 (0.0) | 0 (0.0) |
| Total | 17 | 43 | 17 | 43 |

**Table 3**

**Diagnostic efficacy of conventional smear versus liquid-based cytology.**

| | Conventional smear (95% CI) | Liquid-based cytology (95% CI) | Difference (95% CI) | *P* value |
|---|---|---|---|---|
| Accuracy, % | 86.7 (75.4–94.1) | 91.7 (81.6–97.2) | 5.0 (–6.7 to 16.8) | .380 |
| Sensitivity, % | 90.7 (77.9–97.4) | 97.7 (87.7–99.9) | 7.0 (–4.3 to 19.4) | .169 |
| Specificity, % | 76.5 (50.1–93.2) | 76.5 (50.1–93.2) | 0.0 (–27.5 to 27.5) | >.99 |
| Positive predictive value, % | 90.7 (80.5–95.9) | 91.3 (81.7–96.1) | 0.6 (–12.3 to 14.0) | .921 |
| Negative predictive value, % | 76.5 (55.2–89.6) | 92.9 (64.8–98.9) | 16.4 (–11.7 to 40.8) | .225 |

CI = confidence interval.

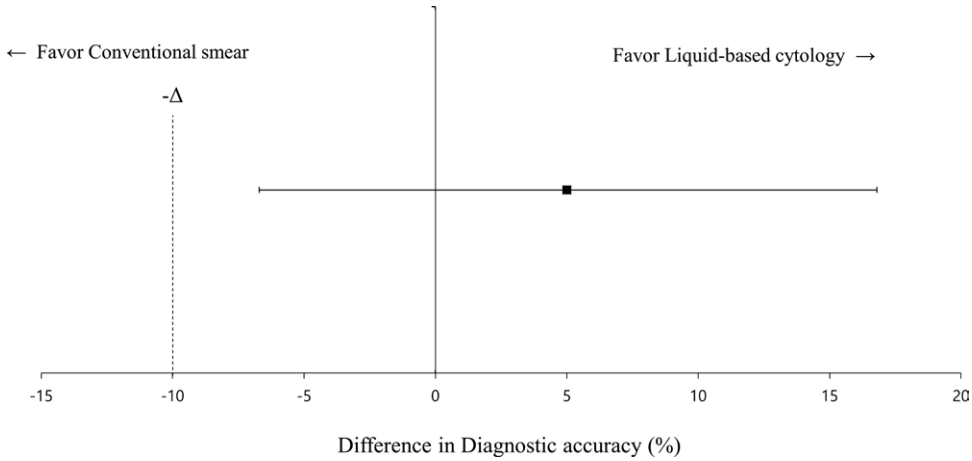


**Figure 2.** Analysis of noninferiority of liquid-based cytology compared to conventional smears.

**Table 4**

**Comparison of cytomorphologic features between conventional smear and liquid-based cytology.**

| Cytomorphologic features Quality and background | Conventional smear, n/N (%) | Liquid-based cytology, n/N (%) | *P* value |
|---|---|---|---|
| Dry artifact | 4/60 (6.7) | 0/60 (0) | .119 |
| Background | | | |
| Clean | 17/60 (28.3) | 54/60 (90.0) | <.001 |
| Bloody | 32/60 (53.3) | 3/60 (5.0) | <.001 |
| Mucinous | 0/60 (0) | 0/60 (0) | - |
| Inflammatory | 1/60 (1.7) | 0/60 (0) | .317 |
| Necrotic | 10/60 (16.7) | 3/60 (5.0) | .041 |
| Cell characteristics | | | |
| Cellularity | | | |
| Acellular | 4/60 (6.7) | 2/60 (3.3) | .404 |
| Sparsely cellular | 15/60 (25.0) | 14/60 (23.3) | .832 |
| Moderately cellular | 27/60 (45.0) | 32/60 (53.3) | .363 |
| Highly cellular | 10/60 (16.7) | 10/60 (16.7) | >.99 |
| Very highly cellular | 4/60 (6.7) | 2/60 (3.3) | .404 |
| Monolayer sheets present | 36/60 (60.0) | 46/60 (76.7) | .050 |
| Three-dimensional clusters present | 30/60 (50.0) | 21/60 (35.0) | .097 |
| Single-cell predominance | 20/60 (33.3) | 20/60 (33.3) | >.99 |

in CSs (CS, 53.3%; LBC, 5.0%; $P < .001$), and the presence of a necrotic background was the same (CS, 16.7%; LBC, 5.0%; $P = .041$). A clean background was seen in 90.0% of LBC, whereas in only 28.3% of the CSs ($P < .001$).

Among the cell architecture, monolayer sheets were better maintained in LBC than in CSs (CS, 60.0%; LBC, 76.7%; $P = .050$). Cytomorphologic features such as cellularity, the presence of 3-dimensional clusters, and single-cell predominance were not significantly different between LBC and CSs. Cytomorphologic features according to needle gauge were not different between the LBC and CS groups (Table S2, Supplemental Digital Content, http://links.lww.com/MD/J268).

## 4. Discussion

This study confirmed that the diagnostic performance of LBC for EUS-FNA of subepithelial masses, including intra-GI wall and extra-GI wall masses, such as an enlarged lymph node or a metastatic mass, was noninferior to that of CSs. And in the case of LBC, bloody and necrotic backgrounds were less common than in CSs, so the cytologic examination was easier.

Among LBC methods, ThinPrep2000 (Hologic Co., Marlborough, MA) and SurePath are the most commonly used for processing cytologic samples. Many recent reports have compared the use of LBC and CSs for pancreatic lesions among non-gynecologic specimens. CS was more accurate than LBC when determining malignancy in EUS-FNA samples of the pancreas.[10,15] (LeBlanc, ThinPrep 75.5% vs CS 95.7%; de Luna R., ThinPrep 67% vs CS 84%) However, 2 previous studies using ThinPrep LBC and a recent meta-analysis of pancreatic lesions reported that ThinPrep LBC underperformed CSs.[16] In ThinPrep LBC, sample loss occurred during the collection process in up to 38% of the samples, so the diagnostic rate was lower than that of SurePath.[17]

Other studies showed that SurePath LBC was more accurate than CSs for biliary tract cancer or pancreatic lesions.[18,19] And another study showed that the diagnostic accuracy of SurePath LBC was non-inferior to that of CSs.[19] (SurePath accuracy 88% vs CS 83.8%) Based on these results, SurePath LBC is widely used for the diagnosis of pancreatic lesions. Also, for subepithelial lesions from or around the upper GI tract, the diagnostic accuracy of SurePath LBC in our study was 91.7%, which is not inferior to the 86.7% accuracy of CSs, so LBC may be substituted for CSs for these lesions in the future. A significant number of subepithelial masses were related to malignancies, and 71.7% of the cases in this study were ultimately malignant cases. Malignancy is a factor that greatly affects a patient's quality of life, and it is important to accurately diagnose malignancy for setting up a treatment plan. Among the diagnostic

performance measures of LBC, specificity was the same as that of CSs, and the accuracy, sensitivity, and positive and negative predictive values were not statistically significant but tended to be higher than those for CSs. Moreover, more time could be saved by using the LBC method compared to CSs. Therefore, the LBC method may help to obtain tissue samples from subepithelial masses in the upper GI tract.

A few studies have compared CSs and LBC for samples in the upper GI tract except for pancreatic lesions. And although some studies have described the efficiency of LBC in the FNA of axillary and cervical lymph nodes,[20–22] lymph nodes around the GI tract have not been studied. Our study conducted a comparison of SurePath LBC and CSs for various EUS-FNA-accessible lesions, including subepithelial masses from intra-GI wall, lymph nodes, and metastatic masses. To the best of our knowledge, this was the first study to compare LBC and CSs of tissue samples from subepithelial lesions in the GI tract including intra-GI wall and extra-GI wall masses, such as enlarged lymph nodes or metastatic masses.

Consistent with previous studies,[11,23,24] our study found that the number of inadequate specimens was lower in LBC compared to CSs, and the proportion of clean backgrounds was significantly higher in LBC. EUS-FNA of the upper GI tract showed relatively limited cellularity compared to superficial organs such as the uterine cervix and thyroid,[7] and sufficient cellularity, as well as the background, is one of the important requirements for pathologic diagnosis. In our study, the rate of moderate to very high cellularity was 73.3% for LBC, which was slightly higher than 68.3% for CSs but not statistically significant ($P = .549$). Thus, no problem with cellularity was found when using LBC for samples from the GI tract. In addition to high cellularity, cluster formation was well-maintained, so cytologic evaluation was more efficient and easier in LBC method.

In our study, 50% of the subepithelial masses from intra GI wall were GIST, and immunohistochemical staining was done in all GIST cases except for the case where an additional percutaneous biopsy was performed because the diagnosis was not made by EUS-FNA. There is a limit to diagnosing GIST by cytologic examination alone, so immunohistochemical tests are essential for confirmation.[25] In addition to the advantage of a clean background, specimens used for LBC can be stored at 15°C to 30°C for up to 6 weeks, so multiple slides can be made, and additional special staining or immunohistochemical tests can be performed.[26,27]

According to a survey study of the global clinical setting of EUS-guided sampling, nearly all United States respondents used rapid on-site evaluation (98 %), while only half of the European (48 %) and Asian (55 %) respondents did. In many places, a rapid on-site evaluation is difficult due to limited pathology staff, high costs, and other factors.[28] Therefore, a method with good diagnostic performance without rapid on-site evaluation is needed, and this study showed that LBC could be an alternative.

Apart from being noninferior to CSs in terms of diagnostic performance, LBC is more convenient to perform, and the sample preparation time is shorter. Considering patients quality of life as well as endoscopists efforts, saving time has benefits. In CS, an endoscopist or medical staff nurse smears the aspirated specimen onto multiple slides, whereas in LBC, the sample is simply put into a collection vial containing a fixative solution and sent to the pathology department for automated processing.

There were some limitations in this study. First, it was planned to recruit and analyze 146 participants, but additional recruitment took time, so 87 participants were recruited and recruitment was terminated early. Although the number of subepithelial masses detected has increased recently, tissue sampling is not actually required in many cases, so recruiting was very difficult. In particular, if the size is large or GIST is suspected, surgical resection is performed without tissue confirmation. Most of the subepithelial tumors are small, and in these cases, only regular follow-up is required, so it was difficult to recruit the desired number of people.

Second, depending on the clinical situation, the endoscopist selected the needle gauge and performed the EUS-FNA procedure. So, not all needle gauges were the same, but 22-gauge was mostly used. And in each crossover trial case, the same needle was used for both the LBC and CS methods. In subgroup analysis, the diagnostic performance of the 19-gauge needle seemed to be high, but this was because only 2 of 60 cases used the 19-gauge needle, and there were no statistically significant differences in diagnostic performance or cytomorphologic features depending on the needle types (Table S1, Supplemental Digital Content, http://links.lww.com/MD/J267, Table S2, Supplemental Digital Content, http://links.lww.com/MD/J268). Therefore, in this study, different needle gauges did not affect the results.

Third, regarding cost, LBC costs $103, which is more expensive than a CS, which is $73, resulting in $30 of additional cost, but less manpower and time,[29] suggesting that the additional cost may be offset by reducing the number of inadequate smears.[30]

Despite the above limitations, our study had the strength of a prospective randomized crossover study in which experienced endoscopists and pathologists participated, and it was the first study comparing LBC and CS with EUS-FNA samples for subepithelial masses or lesions, including intra-GI wall or extra-GI wall masses.

## 5. Conclusion

According to this study, the diagnostic performance of LBC for a subepithelial mass from or around the upper GI tract was noninferior to that of CSs. In LBC, the cytomorphologic features of the cells were maintained, and there was an advantage in observing the cells due to reductions in bloody and necrotic backgrounds, so LBC can be an alternative to CSs for processing EUS-FNA samples.

## Author contributions

**Conceptualization:** Soo-Jeong Cho.
**Data curation:** Yoonjin Kwak.
**Formal analysis:** Jae Chang Jun, Sang Hyub Lee, Han Myung Lee, Sang Gyun Kim, Hyunsoo Chung, Joo Seong Kim, Namyoung Park, Jin Ho Choi, Yoonjin Kwak.
**Funding acquisition:** Soo-Jeong Cho.
**Methodology:** Soo-Jeong Cho.
**Writing – original draft:** Jae Chang Jun, Soo-Jeong Cho.
**Writing – review & editing:** Sang Hyub Lee, Han Myung Lee, Sang Gyun Kim, Hyunsoo Chung, Joo Seong Kim, Namyoung Park, Jin Ho Choi, Yoonjin Kwak, Soo-Jeong Cho.

## References

[1] Song JH, Kim SG, Chung SJ, et al. Risk of progression for incidental small subepithelial tumors in the upper gastrointestinal tract. Endoscopy. 2015;47:675–9.
[2] Papanikolaou IS, Triantafyllou K, Kourikou A, et al. Endoscopic ultrasonography for gastric submucosal lesions. World J Gastrointest Endosc. 2011;3:86–94.
[3] Lim YJ, Son HJ, Lee J-S, et al. Clinical course of subepithelial lesions detected on upper gastrointestinal endoscopy. World J Gastroenterol. 2010;16:439–44.
[4] Landi B, Palazzo L. The role of endosonography in submucosal tumours. Best Pract Res Clin Gastroenterol. 2009;23:679–701.
[5] Lee JK, Choi ER, Jang TH, et al. A prospective comparison of liquid-based cytology and traditional smear cytology in pancreatic endoscopic ultrasound-guided fine needle aspiration. Acta Cytol. 2011;55:401–7.
[6] Qin S-y, Zhou Y, Li P, et al. Diagnostic efficacy of cell block immunohistochemistry, smear cytology, and liquid-based cytology in endoscopic

ultrasound-guided fine-needle aspiration of pancreatic lesions: a single-institution experience. PLoS One. 2014;9:e108762.
[7] Kim H, Jang K-T. Pathologic interpretation of endoscopic ultrasound-guided fine needle aspiration cytology/biopsy for pancreatic lesions. J Pathol Transl Med. 2020;54:367–77.
[8] Erickson RA. EUS-guided FNA. Gastrointest Endosc. 2004;60:267–79.
[9] Hoda RS, VandenBussche C, Hoda SA. Liquid-based specimen collection, preparation, and morphology. Diagnostic Liquid-Based Cytology: Berlin Heidelberg: Springer; 2017. 1–12.
[10] LeBlanc J, Emerson R, Dewitt J, et al. A prospective study comparing rapid assessment of smears and ThinPrep for endoscopic ultrasound-guided fine-needle aspirates. Endoscopy. 2010;42:389–94.
[11] Yeon MH, Jeong HS, Lee HS, et al. Comparison of liquid-based cytology (CellPrepPlus) and conventional smears in pancreaticobiliary disease. Korean J Intern Med. 2018;33:883–92.
[12] Hafez NH, Shaaban HM. SurePath liquid-based cytology versus conventional smears for interpretation of serous effusion fluids: a study of 104 cases. Egypt J Pathol. 2018;38:154–61.
[13] Song TJ, Kim JH, Lee SS, et al. The prospective randomized, controlled trial of endoscopic ultrasound-guided fine-needle aspiration using 22G and 19G aspiration needles for solid pancreatic or peripancreatic masses. Am J Gastroenterol. 2010;105:1739–45.
[14] Li C, Mi J, Gao F, et al. Comparison of endoscopic ultrasound-guided fine needle aspiration with 19-gauge and 22-gauge needles for solid pancreatic lesions. Int J Gen Med. 2021;14:10439–46.
[15] de Luna R, Eloubeidi MA, Sheffield MV, et al. Comparison of ThinPrep and conventional preparations in pancreatic fine-needle aspiration biopsy. Diagn Cytopathol. 2004;30:71–6.
[16] Chandan S, Mohan BP, Khan SR, et al. Comparison of EUS-guided conventional smear and liquid-based cytology in pancreatic lesions: a systematic review and meta-analysis. Endosc Int Open. 2020;8:E1611–22.
[17] Bigras G, Rieder MA, Lambercy J-M, et al. Keeping collecting device in liquid medium is mandatory to ensure optimized liquid-based cervical cytologic sampling. J Low Genit Tract Dis. 2003;7:168–74.
[18] Lee MW, Paik WH, Lee SH, et al. Usefulness of liquid-based cytology in diagnosing biliary tract cancer compared to conventional smear and forceps biopsy. Dig Dis Sci. 2022;1:10.
[19] Chun JW, Lee K, Lee SH, et al. Comparison of liquid-based cytology with conventional smear cytology for EUS-guided FNA of solid pancreatic masses: a prospective randomized noninferiority study. Gastrointest Endosc. 2020;91:837–846.e1.
[20] Rossi ED, Martini M, Capodimonti S, et al. BRAF (V600E) mutation analysis on liquid-based cytology-processed aspiration biopsies predicts bilaterality and lymph node involvement in papillary thyroid microcarcinoma. Cancer Cytopathol. 2013;121:291–7.
[21] Schiettecatte A, Bourgain C, Breucq C, et al. Initial axillary staging of breast cancer using ultrasound-guided fine needle aspiration: a liquid-based cytology study. Cytopathology. 2011;22:30–5.
[22] Garbar C, Remmelink M, Mascaux C. Fine needle aspiration cytology of lymph node: experience of 2 university hospitals with conventional smears and liquid-based cytology. Acta Cytol. 2008;52:418–23.
[23] Hashimoto S, Taguchi H, Higashi M, et al. Diagnostic efficacy of liquid-based cytology for solid pancreatic lesion samples obtained with endoscopic ultrasound-guided fine-needle aspiration: propensity score-matched analysis. Dig Endosc. 2017;29:608–16.
[24] Itonaga M, Murata S-I, Hatamaru K, et al. Diagnostic efficacy of smear plus liquid-based cytology for EUS-FNA of solid pancreatic lesions: a propensity-matched study. Medicine (Baltimore). 2019;98:e15575.
[25] Tamura T, Yamashita Y, Ueda K, et al. Rapid on-site evaluation by endosonographers during endoscopic ultrasonography-guided fine-needle aspiration for diagnosis of gastrointestinal stromal tumors. Clin Endosc. 2017;50:372–8.
[26] Gerhard R, Schmitt FC. Liquid-based cytology in fine-needle aspiration of breast lesions: a review. Acta Cytol. 2014;58:533–42.
[27] Domínguez-Vigil IG, Barajas-Olmos VH, Gallardo-Alvarado L, et al. Comparison of automated and manual DNA isolation methods of liquid-based cytology samples. Biopreserv Biobank. 2019;17:591–7.
[28] van Riet PA, Cahen DL, Poley J-W, et al. Mapping international practice patterns in EUS-guided tissue sampling: outcome of a global survey. Endosc Int Open. 2016;4:E360–70.
[29] Ilter E, Midi A, Haliloğlu B, et al. Comparison of conventional and liquid-based cytology: do the diagnostic benefits outweigh the financial aspect? Turk J Med Sci. 2012;42:1200–6.
[30] Denton KJ. Liquid based cytology in cervical cancer screening. British Medical Journal Publishing Group; 2007. 1–2.