Internal Medicine
The Japanese Society of Internal Medicine
doi: 10.2169/internalmedicine.7889-21
Intern Med 61: 935-942, 2022
http://internmed.jp

【 ORIGINAL ARTICLE 】

Endoscopic Ultrasonography-guided Fine-needle Aspiration Cytology Combined with a Cell-block Method for Gastrointestinal Subepithelial Lesions

Takeshi Shimizu[1], Shinsuke Koshita[1], Tetsuya Ohira[1], Yoshihiro Harada[1], Yoshihide Kanno[1], Takahisa Ogawa[1], Taku Yamagata[1], Hiroaki Kusunose[1], Toshitaka Sakai[1], Takashi Tsuchiya[2], Masaya Oikawa[2], Yutaka Noda[1,3], Takashi Sawai[3] and Kei Ito[1]

Abstract:

Objective The diagnostic accuracy of an endoscopic ultrasound-guided fine-needle aspiration cytology/biopsy combined with a cell-block method (FNA-CB) for gastrointestinal subepithelial lesions (GI-SELs) has not been fully studied.

Methods A total of 109 patients (with 110 GI-SELs) were evaluated to clarify the rate of obtaining evaluable histology specimens using FNA-CB. In addition, we investigated the following: 1) the accuracy for determining the histology, 2) effects of the number of cell clusters obtained via FNA-CB, 3) correlation of the Ki67 labelling index (Ki67LI) of the gastrointestinal stromal tumor (GIST) lesions between FNA-CB and resected specimens, and 4) clinical courses for patients followed up after FNA-CB.

Results Of the 110 GI-SELs for which FNA-CB was performed, 95 (86%) were able to be histologically evaluated using the first FNA-CB. For the 70 resected GI-SELs, the accuracy of FNA-CB to determine histology was 96%, remaining at 90% even when only a few cell clusters were obtained. The concordance rate of the risk-grouping of GIST (high-risk, Ki67LI ≥8; low-risk, <8) between FNA-CB and resected specimens was 84%. Of the 29 patients followed up after the first FNA-CB, 12 with benign GI-SELs determined using the first FNA-CB showed no obvious increases in their GI-SEL sizes.

Conclusion Since FNA-CB can be used to determine the histology and reproductive activity of GI-SELs accurately, not only preoperative histological confirmation but also reliable information to determine clinical plans, such as follow-up without surgery or neoadjuvant chemotherapy, can be obtained.

Key words: EUS-FNA, subepithelial lesion, cell-block method, Ki67 labeling index, GIST

(Intern Med 61: 935-942, 2022)
(DOI: 10.2169/internalmedicine.7889-21)

Introduction

Since gastrointestinal subepithelial lesions (GI-SELs) include various diseases, ranging from neoplastic to non-neoplastic lesions, tissue acquisition using an endoscopic ultrasonography-guided fine-needle aspiration cytology/biopsy (EUS-FNA) is often needed to create clinical plans for those lesions. Although a meta-analysis of the pooled accuracy of EUS-FNA for determining the histology of GI-SELs was shown to be inadequate (59.4%) (1), it was suggested that the diagnostic accuracy might be able to be improved using a cell-block method. Because almost all specimens obtained via EUS-FNA can be used for histocytological evaluations with this method (2-5), this approach may be useful when specimens obtained via EUS-FNA are small in size or involve a relatively large volume of blood.

In addition, various immunostaining approaches can be

[1]Department of Gastroenterology, Sendai City Medical Center, Japan, [2]Department of Surgery, Sendai City Medical Center, Japan and [3]Department of Pathology, Sendai City Medical Center, Japan
Received: May 3, 2021; Accepted: July 25, 2021; Advance Publication by J-STAGE: September 11, 2021
Correspondence to Dr. Shinsuke Koshita, skoshita@openhp.or.jp

Table 1. Baseline Characteristics of 109 Subjects.

Age, median (IQR)	63 (53-72)
Sex (male:female)	60:40
Lesion size, median (IQR), mm	27 (21-34)
Location of lesions, n (%)	
Esophagus	11 (10)
Stomach [U/M/L]	87 (79) [44/24/19]
Duodenum	10 (9)
Rectum	2 (2)
Surgical or endoscopic resection, n (%)	70 (63)
Details of needles mainly used for EUS-FNA, n (%) (total number of lesions: 114)	
Type of needles	
Conventional FNA needles	91 (80)
Echo Tip (Cook Medical)	18 (16)
EXPECT (Boston Scientific)	43 (37)
EZshot3 (Olympus)	24 (21)
EZshot2 (Olympus)	2 (2)
Sono Tip (Medico's Hirata)	4 (4)
FNB needles	10 (9)
Acquire (Boston Scientific)	8 (7)
Echo Tip ProCore (Cook Medical)	2 (2)
N/A	13 (11)
Gauge sizes	
19G	9 (8)
20G	1 (1)
22G	82 (72)
25G	10 (9)
N/A	12 (10)

IQR: interquartile range, U: upper third, M: middle third, L: lower third, EUS-FNA: endoscopic ultrasound-guided fine-needle aspiration cytology/biopsy, FNB: fine-needle biopsy, N/A: not applicable, G: gauge

used with this method. GI-SELs include several diseases for which a conclusive histological diagnosis can be obtained with immunostaining, including gastrointestinal stromal tumors (GISTs) (6, 7). Therefore, these GI-SELs can be histologically confirmed even when the pieces of tissue obtained from EUS-FNA are very small. Furthermore, immunostaining may be useful for the classification of risk groups of GISTs, as the Ki67 labelling index (Ki67LI) can be evaluated to determine the risk of GISTs more easily than a mitotic count can.

The combination of the cell-block method and immunostaining may be a promising method for histological evaluations using EUS-FNA specimens of GI-SELs. However, the diagnostic accuracy of EUS-FNA combined with the cell-block method (FNA-CB) has not been fully studied. Therefore, we conducted a retrospective study to clarify the clinical implications of this method for GI-SELs.

Materials and Methods

Study population

The Sendai City Medical Center institutional review board approved this study (registration number: 2018-0012). Indications for EUS-FNA for GI-SELs at our facility were as follows: 1) a diameter of ≥2 cm determined by using imaging studies, 2) an increase in the size of GI-SELs during surveillance, and 3) endoscopic findings indicative of malignant GI-SELs, including an ulcerative formation. A total of 109 patients with GI-SELs (comprising 110 GI-SELs) who underwent FNA-CB at our hospital from March 2009 to March 2020 were included in this study (Table 1).

Outcome measures

We determined the rate of obtaining the definitive histology from FNA-CB specimens as a primary outcome measure in this study. In addition, we investigated the following secondary outcome measures: 1) the accuracy of FNA-CB for determining the histology of GI-SELs among patients who underwent surgery for their GI-SELs, 2) the effects of the number of cell clusters obtained from FNA-CB, 3) the correlation of the Ki67LI between the FNA-CB specimens and the resected ones in patients found to have GISTs using resected specimens, and 4) the clinical courses of patients who underwent surveillance without undergoing surgery just after initial FNA-CB.

Endoscopic procedures

All endoscopic procedures were performed in accordance with the Declaration of Helsinki. The echoendoscopes used for EUS-FNA were GF-UC240P-AL5 and GF-UCT260 (Olympus, Tokyo, Japan). For processing images from EUS, the EU-ME1 and EU-ME2 (Olympus) ultrasonographic systems were used.

A 22-G needle was mainly used for EUS-FNA (Table 1). For each FNA needle pass, about 20 strokes with a negative pressure applied using a 20-mL syringe were made. Although three FNA needle passes were usually carried out, additional passes were made when the volumes of the specimens obtained with three FNA needle passes were macroscopically inadequate.

Of the 109 subjects, 17 who were admitted to our hospital from March 2009 to January 2013 underwent EUS-FNA combined with a rapid on-site cytologic evaluation (FNA-ROSE) using Diff-Quick™ and Papanicolaou staining only for their specimens obtained with the first FNA pass. All EUS-FNA specimens obtained from the 109 subjects, excluding specimens subjected to ROSE, were pushed out from the inside of the FNA needles into a centrifuge tube containing 5 mL of a 10% formalin solution using a small volume of saline or a needle stylet. The specimens were processed using the cell-block method.

Histocytological evaluations

All histocytological diagnoses for FNA-CB specimens and resected specimens were prospectively made via the consensus of two or more pathologists (YN, TS, MU, and FF). To assess the histology of the FNA-CB specimens, cell-block sections were prepared using a sodium alginate method and subjected to hematoxylin and eosin (HE) stain. Immunostaining was performed when the following situations occurred: 1) GI-SELs for which immunostaining would be diagnostic (e.g., GISTs, leiomyoma, schwannoma, etc.) were suspected based on HE staining, and 2) HE staining afforded an indeterminate histological diagnosis. The antibodies used for immunostaining were as follows: KIT (CD117; DAKO, Glostrup, Denmark), CD34 (NU-4A1; Nichirei, Tokyo, Japan), DOG1 (K9; Novocastra, Newcastle, UK), Desmin (D33; DAKO), αSMA (1A4; Enzo Life Sciences, Farmingdale, USA), Ki67 (MIB-1; Immunotech, Marseilles, France), P53 (DO-7; DAKO), MUC1 (Ma695; Novocastra), chromogranin A (chromogranin; Nichirei), and synaptophysin (27G12; Novocastra).

For resected specimens, HE staining and several immunostainings were performed using the method for FNA-CB specimens. When histological diagnoses for resected specimens could be made using HE staining, immunostaining was not performed.

Indications for surgery and surveillance methods for patients without undergoing surgery

When FNA-CB afforded a diagnosis of neoplastic GI-SELs with malignant potential, such as GISTs, neuroendocrine neoplasms (NENs), malignant lymphoma, adenocarcinoma, etc., surgery (or systemic chemotherapy) was recommended. When patients with those GI-SELs had a high risk for surgery, they did not undergo further surveillance or were monitored for their GI-SELs by imaging every three to six months. Patients with benign GI-SELs diagnosed using FNA-CB underwent surveillance by imaging every 6-12 months. Patients with an indeterminate diagnosis using FNA-CB were scheduled to undergo a re-examination of FNA-CB or underwent careful monitoring for their GI-SELs by imaging every three to six months.

Definition of the concordance of Ki67LI

To clarify the relationship of the Ki67LIs of FNA-CB specimens and resected specimens for patients who were found to have GISTs by using the resected specimens, we firstly performed correlation analysis. The value of Ki67LI was obtained from our clinical and pathological databases prospectively registered. On the basis of previous report (8), we classified patients who underwent surgery, followed by the diagnosis of GISTs, into the following two groups: 1) patients whose GISTs had a Ki67LI ≥8% (high-risk group) and 2) patients whose GISTs had a Ki67LI <8% (low-risk group). The concordance of Ki67LI was defined as follows: when the FNA-CB specimens and resected specimens were separately classified into each of the two risk groups, both of those specimens were classified into the same group.

Statistical analyses

All statistical analyses were performed by using SPSS software version 24 (SPSS, Chicago, USA). Pearson's χ^2 test or Fisher's exact test was used for the categorical variables. The Mann-Whitney U test was used for the continuous data [distribution of variables shown using the interquartile range (IQR)]. A p value of less than 0.05 was considered statistically significant.

Results

Baseline characteristics of patients (Table 1)

Among the 109 patients, there were 69 men and 40 women. The median age at the time of first FNA-CB was 63 (IQR: 53-72) years old. Of the 110 target GI-SELs for FNA-CB (1 patient had 2 GI-SELs), 87 (79%) were in the stomach. The median size of GI-SELs was 27 (IQR: 21-34) mm. A total of 70 patients (63%) underwent surgical or endoscopic resection, and 61 of those (87%) were histologically diagnosed with GISTs.

Rate of obtaining definitive histology using the first FNA-CB and histological results of the first FNA-CB procedure

Histological diagnoses determined using the first FNA-CB procedure are shown in Table 2. For 95 of the 110 GI-SEL

Table 2. Histological Diagnoses Determined Using the First FNA-CB Procedure.

	n (%)
Determinate histology	95 (86)
GIST	66 (60)
adenocarcinoma	4 (4)
NEN	2 (2)
malignant lymphoma	1 (1)
leiomyoma	9 (8)
gastric aberrant pancreas	6 (5)
schwannoma	4 (4)
submucosal heterotopic gastric mucosa	2 (2)
hamartoma	1 (1)
Indeterminate histology	15 (14)

FNA-CB: endoscopic ultrasound-guided fine-needle aspiration cytology/biopsy combined with a cell-block method, GIST: gastrointestinal stromal tumor, NEN: neuroendocrine neoplasm

Table 3. The Rate of Obtaining the Definitive Histology Using the First FNA-CB Procedure Specimens.

For total lesions	86% (95/110)
For each site	
esophagus	91% (10/11)
stomach	89% (77/87)
duodenum	70% (7/10)
rectum	50% (1/2)

FNA-CB: endoscopic ultrasound-guided fine-needle aspiration cytology/biopsy combined with a cell-block method

lesions (86%), histological diagnoses were able to be made using the first FNA-CB procedure specimens. The rate of obtaining the definitive histology using the first FNA-CB procedure was thus 86%. For 66 of the 95 lesions, the diagnoses of GISTs were able to be made using the first FNA-CB procedure.

Regarding the results of the first FNA-CB procedure by the sites at which GI-SELs developed, the rate of obtaining the definitive histology using the first FNA-CB procedure specimens obtained from esophageal, gastric, duodenal, and rectal GI-SELs were 91% (10/11), 89% (77/87), 70% (7/10), and 50% (1/2), respectively (Table 3).

Regarding the 16 patients who underwent FNA-ROSE at the time of the first EUS-FNA, ROSE was able to be used to obtain a cytologic diagnosis of neoplastic disease for just 3 patients (19%), whereas the cell-block method afforded conclusive histological diagnoses for 12 of the 16 patients (75%).

Clinical plans after first FNA-CB procedure

A flowchart of the clinical plans for GI-SELs after the first FNA-CB procedure is shown in Fig. 1. Regarding the clinical plans for the 94 patients (95 GI-SEL lesions) with a determinate histological diagnosis at the first FNA-CB procedure, 63 patients (64 GI-SEL lesions) underwent surgery, 5 underwent systemic chemotherapy, and 26 did not undergo clinical treatments for their GI-SELs (surveillance, 18; no surveillance, 8).

The first FNA-CB procedure was unable to be used to obtain a histological diagnosis for 15 patients (15 GI-SELs) due to an indeterminate histological diagnosis on FNA-CB or an inadequate volume of FNA-CB specimens. Among those patients, one underwent a second FNA-CB procedure just after the results of the first procedure were obtained. Of the remaining 14 patients, 3 underwent surgery just after the first FNA-CB procedure despite lacking histological confirmation, and 11 underwent surveillance. After surveillance, 3 of the 11 patients underwent a second FNA-CB procedure due to an increase in the size of their GI-SEL.

Accuracy of FNA-CB for determining the histology of the resected GI-SELs

A total of 70 GI-SELs of 69 patients who underwent surgical or endoscopic resection for their GI-SELs were used to clarify the accuracy of the first FNA-CB procedure for determining the histology. The histological diagnoses of the resected specimens are shown in Table 4. The accuracy of the first FNA-CB procedure in determining the histology was 96% (67/70). Three patients with inadequate specimens obtained from the first FNA-CB procedure were able to be histologically diagnosed using their resected specimens (GIST, 1; schwannoma, 1; cyst, 1).

For the 61 GISTs histologically diagnosed using resected specimens, the accuracy of the first FNA-CB procedure in determining GISTs was 93% (57/61). When the second FNA-CB procedure was included in the analysis, the accuracy increased to 98% (60/61).

Influence of the number of cell clusters obtained from FNA-CB (Table 5)

Using first FNA-CB procedure, the rate of obtaining histologically evaluable specimens was 100% when cell clusters of ≥5 were obtained and remained high (90%) even when the number of cell clusters was 1-4. Regarding the 70 resected GI-SELs, the accuracy of the first FNA-CB procedure for diagnosing the histology was 100% when the number of cell clusters was ≥5, and it remained high (95%) even when the number was in the range of 1-4.

Correlation of the value of Ki67LI between FNA-CB and resected specimens in patients with resected GISTs

A total of 42 patients with resected GIST for whom the Ki67LI was evaluated for both the FNA-CB and resected specimens were included in this investigation. These 42 patients showed no significant differences in the Ki67LI of GIST lesions between the two types of specimens [p=0.48; median: FNA-CB 2.0% (IQR: 1.0-3.0%) vs. resected 2.0%

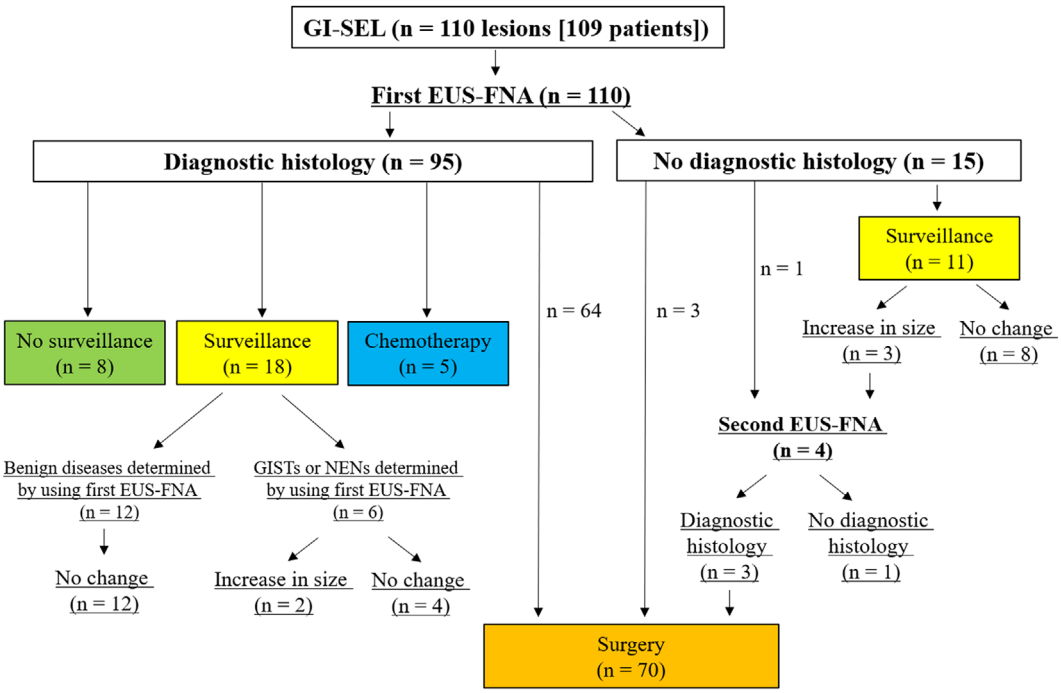

Figure 1. **Flowchart of the clinical plans for 110 GI-SELs after first FNA-CB procedure. FNA-CB: endoscopic ultrasound-guided fine-needle aspiration cytology/biopsy combined with a cell-block method, EUS-FNA: endoscopic ultrasonography-guided fine-needle aspiration cytology/biopsy, GI-SELs: gastrointestinal subepithelial lesions, GIST: gastrointestinal stromal tumor, NEN: neuroendocrine neoplasm**

Table 4. **Histological Diagnoses of the Resected Specimens of 70 Patients who Underwent Surgical or Endoscopic Resection for Their GI-SELs.**

Total lesions	96% (67/70)
For each site	
Esophagus	100% (1/1)
Stomach	97% (59/61)
Duodenum	86% (6/7)
Rectum	100% (1/1)
For each disease	
GIST	98% (60/61)
Adenocarcinoma	100% (2/2)
NEN	100% (1/1)
Leiomyoma	100% (1/1)
Schwannoma	66% (2/3)
Hamartoma	100% (1/1)
Cyst	0% (0/1)

GI-SELs: gastrointestinal subepithelial lesions, GIST: gastrointestinal stromal tumor, NEN: neuroendocrine neoplasm

(IQR: 1.0-4.3%)]. The correlation of the Ki67LIs of the GIST lesions between the two specimens was statistically significant (Fig. 2).

When the 42 patients were classified into high-risk (Ki67 LI ≥8) and low-risk groups (Ki67LI <8), the concordance rate of the 2 risk groups between the 2 types of specimens was 86% (36/42). For the six patients with discordance in risk groups between the two types of specimens, the first FNA-CB procedure overestimated the risk based on the Ki 67LI for two patients and underestimated the risk for the other four. Among the possible factors related to mismatched results of Ki67 intensities between the two types of specimens, such as the lesion size, location, and number of cell clusters, only the lesion size significantly differed between the patients whose pre- and post-operative risk groups were matched and the patients whose the groups were mismatched (median: matched risk groups 27 mm vs. mismatched risk groups 39 mm, p=0.014). The cut-off value of the lesion size to predict discordance of risk groups between the 2 types of specimens was calculated to be 35 mm using a receiver operating characteristic (ROC) curve (area under the curve: 0.82), and the sensitivity and specificity of a lesion size of ≥35 mm to predict that were 67% and 91%, respectively.

Clinical outcomes for patients who underwent surveillance without undergoing surgery after the first FNA-CB procedure

After the first FNA-CB procedure, 29 patients (29 GI-SELs) underwent surveillance for their GI-SELs by receiving imaging at regular intervals. Of those, 6 patients indicated for surgery after the first FNA-CB procedure (GIST, 5; NEN, 1) underwent surveillance without surgery due to an advanced age or the presence of comorbidities. For 12 of the 29 patients, the GI-SELs were histologically shown to

Table 5. Relationship between the Number of Cell Clusters Obtained from FNA-CB and Its Histological Efficacy.

Count of cell clusters obtained from FNA-CB Median (IQR): 11 (3-23)	110 GI-SELs for which first FNA-CB was performed		70 resected GI-SELs	
	Number of lesions, n (%)	Rate of obtaining adequate FNA-CB specimens for which histological evaluations could be performed (%)	Number of lesions, n (%)	Accuracy for the diagnosis of histology using FNA-CB for resected GI-SELs (%)
>50	15 (14)	100	7 (10)	100
20-49	21 (19)	100	15 (21)	100
10-19	20 (18)	100	14 (20)	100
5-9	16 (15)	100	12 (17)	100
1-4	29 (26)	90	20 (29)	95
0	9 (8)	0	2 (3)	0

FNA-CB: endoscopic ultrasound-guided fine-needle aspiration cytology/biopsy combined with a cell-block method, GI-SELs: gastrointestinal subepithelial lesions, IQR: interquartile range

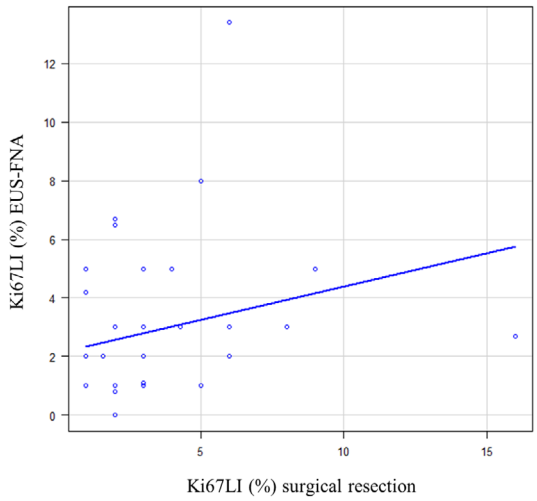

Figure 2. The correlation of the Ki67 labelling index (Ki67LI) of FNA-CB and resected specimens for patients with resected GIST (Spearman's rank correlation coefficient: 0.34, p=0.027). FNA-CB: endoscopic ultrasound-guided fine-needle aspiration cytology/biopsy combined with a cell-block method, GIST: gastrointestinal stromal tumor

be benign diseases according to the first FNA-CB procedure (leiomyoma, 5; gastric aberrant pancreas, 5; schwannoma, 1; submucosal heterotopic gastric mucosa, 1), and no obvious increase in the size of their GI-SELs was observed during a mean surveillance period of 71±11 (range: 6-12) months. Among the 11 patients with an indeterminate diagnosis using the first FNA-CB procedure, no marked changes in the size of the GI-SELs were observed for 8 patients during a mean surveillance period of 66±16 (range: 6-127) months, and an increase in the size of GI-SELs was observed for the remaining 3 patients. For 2 of those 3 patients, a second FNA-CB procedure was performed at 79 and 115 months after the first FNA-CB, resulting in diagnoses of GISTs. However, the remaining patient failed to obtain a determinate histological diagnosis, even after a second FNA-CB procedure.

Adverse events related to endoscopic procedures

For all endoscopic procedures performed on the 109 patients with GI-SELs, no adverse events, such as bleeding, perforation, peritonitis, and cardiopulmonary issues, were observed.

Discussion

This study demonstrated the excellent utility of FNA-CB for obtaining evaluable histology specimens from GI-SEL lesions (86%). The diagnostic ability of FNA-CB for determining the histology of resected GI-SELs was quite high (96%). These results may be due to the effects of immunostaining on the histological evaluations using FNA-CB specimens. The reasons for these findings are as follows: 1) immunostaining is a strong determinant for confirming histocytological diagnoses of GI-SELs, 2) no histological diagnoses for FNA-CB specimens obtained from the GISTs could be confirmed using only HE staining, and 3) FNA-CB proved extremely useful for determining the histology, even when a few cell clusters were obtained. Therefore, combining the cell-block method and immunostaining may be effective when a small volume of tissues is obtained. In addition, the ability of FNA-CB to determine the histocytological diagnosis tended to be superior to that of FNA-ROSE (75% vs. 19%), despite the investigation being carried out using a small sample size.

In addition, the present results indicate that follow-up visits for patients with benign GI-SELs determined using FNA-CB should be conducted. All 12 patients who did not undergo surgery after the diagnosis of benign GI-SELs using FNA-CB showed good clinical courses without an increase in the size of their GI-SELs. Based on recent clinical studies (9-11), surveillance appears feasible for small GI-SELs (<2 cm), as GI-SELs that increase in size were reported to be rare. However, few studies have examined the clinical

courses of benign GI-SELs determined using EUS-FNA. FNA-CB can be used to obtain supportive information for creating clinical plans to follow patients when GI-SELs are determined to be benign using FNA-CB, although a validation study using a larger number of patients with GI-SELs is needed.

Furthermore, the present results indicate a strong correlation between the Ki67LI of resected specimens and that of FNA-CB specimens. In a recent report among resected GI-SELs, high-risk GI-SELs, classified according to the National Institutes of Health (NIH) risk classification system for GISTs, tended to have a high Ki67LI (8, 12-15). Therefore, the Ki67LI of FNA-CB specimens may be useful for the preoperative risk classification of GISTs, which may aid in determining whether or not neoadjuvant systemic chemotherapy should be performed in order to prevent postoperative local or distant metastasis of GISTs (16). In this study, the rate of discrepancy of the risk groups was relatively low (<15%). However, since there is heterogeneity in the distribution of Ki67-positive cells within the lesion of GISTs (17), discrepancy of the Ki67LI values between sampling specimens obtained from EUS-FNA and resection, including entire GIST lesions, is sometimes observed. We therefore additionally investigated the mismatch between the Ki67 intensities of a sample and the whole tissue, with the lesion size of GISTs found to be related to the false findings of Ki67 intensity determined using EUS-FNA. In particular, based on the present findings, a GIST lesion size of ≥35 mm seems to be related to the mismatch of risk-grouping between the 2 types of specimens. For large GISTs likely to have a heterogenous distribution of Ki67-positive cells, the following may improve the accuracy of EUS-FNA for determining the Ki67 intensity: 1) multiple needle passes made within the GIST lesions in different directions and 2) using the fanning technique when to-and-fro strokes with an EUS-FNA needle within the lesions are performed. In the future, if the findings of EUS, including contrast-enhanced EUS, specific to hot spots of Ki67-positive cells within the lesion of GISTs can be identified, the mismatched results of pre- and post-operative Ki67LIs may therefore have been minimized by targeting the part of the lesion showing the EUS findings.

Several limitations associated with the present study warrant mention. First, this was a retrospective study with a small sample size conducted at a single medical center. Second, immunostaining for factors such as KIT, CD34, and Ki 67 was not carried out on all resected specimens of GISTs. Third, the procedures for EUS-FNA, including the selection of needles, number of punctures, and suction method, were not consistent, as they were left to the physicians' discretion. Fourth, because the study period was relatively long, some advances in the EUS-FNA procedure and the processing of obtained tissues may have influenced the diagnostic results of FNA-CB, depending on the era. However, the main outcome did not differ markedly between the earlier (April 2009-December 2014) and later (January 2015-April 2020) study period (89% vs. 83%, respectively). Of note, the kind of EUS-FNA needle was found to differ depending on the era (p=0.005), and so-called "EUS-fine-needle biopsy (EUS-FNB) needles" were recently used in our hospital. Fifth, despite FNA-CB demonstrating an excellent ability to obtain adequate evaluable histology specimens and determine the histology for patients with GI-SELs, FNA-CB failed for some of those patients. For 15 patients without evaluable histology specimens obtained from the first FNA-CB procedure and 3 with resected GI-SELs for which the first FNA-CB procedure could not determine the histology preoperatively, the number of cell clusters in their specimens obtained from the first FNA-CB procedure was ≤1 (0-1). Newly-designed EUS-FNB needles, including the Franseen needle and fork-tip needle (18), may be useful for improving the number of cell clusters obtained from EUS-FNA.

Despite these limitations, the study results suggest that FNA-CB may be used to aid in the selection of appropriate clinical plans for GI-SELs considered suitable for surgery based on imaging findings.

Conclusion

FNA-CB can be used to determine the histology and reproductive activity of GI-SELs with considerable accuracy. Therefore, this combined method can provide not only preoperative histological confirmation but also reliable histological information to determine appropriate clinical plans, such as follow-up without surgery or neoadjuvant chemotherapy, for patients with GI-SELs indicated for surgery.

The authors state that they have no Conflict of Interest (COI).

Acknowledgments

We would like to thank Fumiyoshi Fujishima, MD, PhD, Department of Pathology, Tohoku University School of Medicine, and Miwa Uzuki, MD, PhD, Department of Nursing, Faculty of Medical Science and Welfare, for the histocytological evaluations using cytological specimens and resected specimens from all subjects, and all staff in the Department of Pathology at Sendai City Medical Center for performing immunohistochemical staining in this study. In addition, we are very grateful to Brian K. Breedlove, Associate Professor, Tohoku University School of Science, for the English proofreading.

References

1. Zhang XC, Li QL, Yu YF, et al. Diagnostic efficacy of endoscopic ultrasound-guided needle sampling for upper gastrointestinal subepithelial lesions: a meta-analysis. Surg Endosc **30**: 2431-2441, 2016.

2. Lopes CV, Rigon P, Zettler CG, Hartmann AA. Differential diagnosis of mesenchymal neoplasms of the digestive tract by cell block and immunohistochemistry. Cytopathology **29**: 531-536, 2018.

3. Noda Y, Fujita N, Kobayashi G, et al. Diagnostic efficacy of the cell block method in comparison with smear cytology of tissue samples obtained by endoscopic ultrasound-guided fine-needle as-

piration. J Gastroenterol **45**: 868-875, 2010.
4. Noda Y, Fujita N, Kobayashi G, et al. Prospective randomized controlled study comparing cell block method and conventional smear method for pancreatic juice cytology. Dig Endosc **24**: 168-174, 2012.
5. Nathan NA, Narayan E, Smith MM, Horn MJ. Cell block cytology: improved preparation and its efficacy in diagnostic cytology. Am J Clin Pathol **114**: 599-606, 2000.
6. Miettinen M, Lasota J. Gastrointestinal stromal tumors: review on morphology, molecular pathology, prognosis, and differential diagnosis. Arch Pathol Lab Med **130**: 1466-1478, 2006.
7. Rubin BP, Heinrich MC, Corless CL. Gastrointestinal stromal tumour. Lancet **369**: 1731-1741, 2007.
8. Zhao WY, Xu J, Wang M, et al. Prognostic value of Ki67 index in gastrointestinal stromal tumors. Int J Clin Exp Pathol **7**: 2298-2304, 2014.
9. Sekine M, Imaoka H, Mizuno N, et al. Clinical course of gastrointestinal stromal tumor diagnosed by endoscopic ultrasound-guided fine-needle aspiration. Dig Endosc **27**: 44-52, 2015.
10. Ye LS, Li Y, Liu W, Yao MH, Khan N, Hu B. Clinical course of suspected small gastrointestinal stromal tumors in the stomach. World J Gastrointest Surg **12**: 171-177, 2020.
11. Koizumi S, Kida M, Yamauchi H, et al. Clinical implications of doubling time of gastrointestinal submucosal tumors. World J Gastroenterol **22**: 10015-10023, 2016.
12. Zhou Y, Hu W, Chen P, et al. Ki67 is a biological marker of malignant risk of gastrointestinal stromal tumors: a systematic review and meta-analysis. Medicine **96**: e7911, 2017.
13. Di Vizio D, Demichelis F, Simonetti S, et al. Skp2 expression is associated with high risk and elevated Ki67 expression in gastrointestinal stromal tumours. BMC Cancer **8**: 134, 2008.
14. Fujimoto Y, Nakanishi Y, Yoshimura K, Shimoda T. Clinicopathologic study of primary malignant gastrointestinal stromal tumor of the stomach, with special reference to prognostic factors: analysis of results in 140 surgically resected patients. Gastric Cancer **6**: 39-48, 2003.
15. Hasegawa T, Matsuno Y, Shimoda T, Hirohashi S. Gastrointestinal stromal tumor: consistent CD117 immunostaining for diagnosis, and prognostic classification based on tumor size and MIB-1 grade. Hum Pathol **33**: 669-676, 2002.
16. Hedenström P, Nilsson B, Demir A, et al. Characterizing gastrointestinal stromal tumors and evaluating neoadjuvant imatinib by sequencing of endoscopic ultrasound-biopsies. World J Gastroenterol **23**: 5925-5935, 2017.
17. Yasui H, Konishi E, Urasaki K, Yasukawa S, Yanagisawa A. Intratumoral heterogeneity of MIB-1 labelling index in gastric gastrointestinal stromal tumor (GIST). Gastric Cancer **9**: 223-228, 2006.
18. de Moura DTH, McCarty TR, Jirapinyo P, et al. EUS-guided fine-needle biopsy sampling versus FNA in the diagnosis of subepithelial lesions: a large multicenter study. Gastrointest Endosc **92**: 108-119, 2020.