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Prospective comparative study of endoscopic ultrasonography-guided fine-needle biopsy and unroofing biopsy

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ABSTRACT

Background and aim: Adequate tissue acquisition is important in making treatment decisions for patients with upper gastrointestinal subepithelial tumors (SETs). This study aimed to compare the outcomes of endoscopic ultrasonography-guided fine-needle biopsy (EUS-FNB) with those of the unroofing biopsy technique.

Methods: This study was a single-center, prospective comparative study conducted at Severance Hospital, Yonsei University College of Medicine. A total of 39 patients with SETs ≥15 mm were enrolled between January 2016 and August 2017.

Results: Of the 39 patients, 28 underwent biopsy with both techniques (4 underwent only unroofing and 7 underwent only EUS-FNB). Histological diagnosis was made with EUS-FNB in 64.3% and unroofing biopsy in 78.6% ($p = 0.344$), and immunohistochemical diagnosis was made with EUS-FNB in 46.4% and unroofing biopsy in 67.9% ($p = 0.180$). In the subgroup analysis (28 patients), there was no significant difference in diagnostic yield between the 2 methods The mean procedural time with EUS-FNB was shorter than that with unroofing biopsy ($p < 0.001$). The larger SET (≥ 20 mm) ($p = 0.035$) and satisfaction of procedure ($p = 0.019$) were positively associated with successful histological diagnosis by EUS-FNB.

Conclusions: There was no significant difference in the histological diagnostic yield for SETs between the EUS-FNB and unroofing biopsy techniques (CinicalTrials.gov. identifier NCT02646241).

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1. Introduction

Subepithelial tumors (SETs) are commonly detected in asymptomatic patients during routine endoscopic examination. SETs occur anywhere in the gastrointestinal tract in approximately 0.36–2.0%, and most are asymptomatic [1,2]. Endoscopic ultrasound (EUS) could further characterize and identify benign lesions; however, routine biopsy is not usually helpful for identifying benign SETs [3]. Among SETs, it is critical to exclude malignant or premalignant lesions such as gastrointestinal stromal tumors (GISTs), neuroendocrine tumors, and lymphoma. However, the accuracy of EUS in diagnosing SETs is relatively low (46–48%), especially for masses in the 3rd and 4th layers [4,5]. Therefore, tissue acquisition is required to confirm the diagnosis of SET, and to predict malignant potential and decide further management.

To overcome the limitations in diagnostic accuracy of EUS alone, advanced endoscopic techniques have been introduced for a definitive diagnosis. The options for sampling SETs include jumbo forceps or bite-on-bite biopsy, fine-needle aspiration (FNA), fine-needle biopsy (FNB), and unroofing biopsy [6]. However, it is technically difficult to achieve the correct histological diagnosis because the surface of SETs is covered with normal epithelium and the tumor is movable.

Bite-on-bite biopsy had a disappointing yield of up to 38–40% in previous studies [7,8]. EUS-guided FNA has a better diagnostic yield than bite-on-bite biopsy; however, it can be technically challenging in small, firm, and mobile SETs. Furthermore, samples are often inadequate for immunohistochemistry (IHC) and mitotic index assessment [9,10]. Recently, EUS-FNB has been used more frequently than EUS-FNA because EUS-FNB could provide better diagnostic yield and a larger sample with preserved tissue core architecture [11]. However, EUS-FNB should be performed by an expert gastroendoscopist and the diagnostic yield is affected by the tumor location and size.

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Another clinically useful biopsy technique is unroofing biopsy. If unroofing biopsy is technically successful, it could provide sufficiently large tissue samples relatively easier than EUS-guided biopsy owing to its use of a direct endoscope with flexible biopsy forceps.

Despite the advantages and disadvantages of each procedure, performing comparative studies is not easy and there is no prospective study directly comparing the diagnostic accuracy of EUS-FNB with that of unroofing biopsy in upper gastrointestinal SETs. Therefore, this study aimed to compare the diagnostic yield, ability of IHC staining, procedural time, and procedural safety of EUS-FNB with a EUS histology needle (ProCore; Cook Medical Inc., Winston-Salem, NC, USA) with those of unroofing biopsy.

2. Materials and methods

2.1. Study population

This was a prospective comparative study. A total of 39 patients aged >19 years with upper gastrointestinal SETs at least 15 mm in maximal diameter and expected to require a histological diagnosis were consecutively enrolled between January 2016 and August 2017 at Severance Hospital, Yonsei University College of Medicine. Both EUS-FNB and unroofing biopsy were performed with SETs that were diagnosed with EUS before the EUS-FNB and unroofing biopsy procedures. All patients were admitted on the day of the procedures and usually discharged on the next day. This study was approved by the institutional review board of Severance Hospital, Yonsei University, Seoul, Korea (4-2015-1033). All patients signed an informed consent form before enrollment (CinicalTrials.gov identifier NCT02646241).

2.2. EUS-FNB technique

All procedures were conducted by 1 expert gastroendoscopist with a standard linear array echoendoscope (GF-UCT260; Olympus Medical Systems, Tokyo, Japan). To perform EUS-FNB, the EUS histology needle (ProCore, Cook Medical Inc.) was advanced through the accessory channel of the endoscope. One patient was punctured with a 22-gauge needle and the rest was punctured with a 20-gauge needle. The lesion was punctured at least 2 times with real-time EUS guidance (Fig. 1**A**). When the gastroenterologist judged that there was no core tissue grossly, biopsy was performed a maximum of 4 times. After the needle has passed into the lesion, the endoscopist moved the needle back and forth in the lesion several times. Core biopsy specimens were placed onto glass slides and collected into a formalin bottle. Cytologic smear screening was done for aspirated specimens, which were then fixed in absolute alcohol solution. For all cases, the aspiration and biopsy slides were assessed based on hematoxylin and eosin staining by an experienced upper gastrointestinal pathologist (J.H.J., Fig. 1**D**–**1I**). For the differential diagnosis of SETs, IHC assays for c-kit, desmin, CD34, smooth muscle actin, and S-100 protein were performed for all lesions, and mitotic figures were counted by 1 pathologist specialized in GISTs.

2.3. Unroofing biopsy technique

After confirming that there was no bleeding after the EUS-FNB procedure, unroofing biopsy was performed. All procedures were conducted with a standard upper endoscope (GF-UCT290, Olympus Medical Systems). To perform unroofing biopsy, epinephrine in hypertonic saline solution (dilution 1:100,000) was injected into the submucosal layer of the SET. Then, the overlying mucosa was snared and endoscopic mucosal resection with a blended electrosurgical current was performed to create a 10–15-mm mucosal defect on the SET (Fig. 1**B**). The flex needle knife was introduced and a 10-mm precut incision was made (Fig. 1**C**). Thereafter, multiple endoscopic biopsy samples were obtained using forceps (FB 21K 1, 1.9 mm maximum insertion portion diameter; Olympus Medical Systems). We obtained an average of six tissues when performing unroofing biopsy procedure (mean, 6.4 times) [12]. Finally, the incision site was closed using hemoclips.

2.4. Outcome measurements

Technical failure was defined as an inability to complete any step from needle insertion into the endoscope channel to tissue procurement. The primary endpoint of this study was the diagnostic yield of EUS-FNB and unroofing biopsy. The outcomes were categorized as diagnostic if the biopsy specimens were adequate for a pathologist to make a diagnosis and nondiagnostic if the specimens were insufficient for making a diagnosis. The ability of IHC staining, procedural time, and safety outcomes were also analyzed. Benign SETs were regularly followed-up, and malignant SETs were resected according to the guidelines [1,3].

2.5. Statistical analysis

A sample size calculation was performed to compare the diagnostic yield of EUS-FNB and unroofing biopsy. Based on a literature review, we expected that 40 pairs, with a 10% dropout rate, would achieve 80% power to detect the difference between an EUS-FNB proportion of 65% and an unroofing biopsy proportion of 90% by using a two-sided McNemar test with a significance level of 0.05 [1,13].

Mean and standard deviation or median and ranges were calculated for all continuous variables, as appropriate. Categorical data were analyzed using the chi-square test or Fisher exact test. Continuous data were analyzed using Student's t-test or the Mann Whitney U-test. The diagnostic yield was assessed using the McNemar test and compared between EUS-FNB and unroofing biopsy. Univariate and multivariate logistic regression analyses were conducted to identify the independent predictors of successful EUS-FNB for gastric SETs. Variables in univariate analysis were entered into multivariate analysis if the p-value was < 0.05 (considered statistically significant). All statistical analyses were performed with the Statistical Package for Social Sciences (SPSS version 23.0; SPSS Inc., Armonk, NY, USA).

3. Results

3.1. Patient baseline characteristics

All patients underwent EUS and were diagnosed as having a gastric SET originating from the submucosal layer (3rd layer) or the muscularis propria layer (4th layer). The mean patient age was 58.3 ± 13.1 years (median, 59.0 years; range, 31–80 years), and 53.8% of them were women. The tumors had a mean size of 21.0 ± 6.6 mm (median, 19.0 mm; range, 15–45 mm), and 20 (51.3%) tumors were <20 mm in diameter. The histological diagnoses were gastrointestinal stromal tumor (n = 16), leiomyoma (n = 15), ectopic pancreas (n = 2), schwannoma (n = 1), extranodal marginal zone B-cell lymphoma (n = 1), and nondiagnostic (n = 4) (Table 1.)

EUS-FNB was technically successful in 35 of 39 (89.7%) patients and unroofing biopsy was successful in 32 of 32 (100.0%) patients. Eleven patients were excluded, of whom 4 had unroofing biopsy only (all had failed needle injection) and 7 had EUS-FNB only (3 without identifiable lesions on endoscopy, 2 with current bleeding after EUS-FNB, and 2 with severe belching) (**Supplementary** Fig.

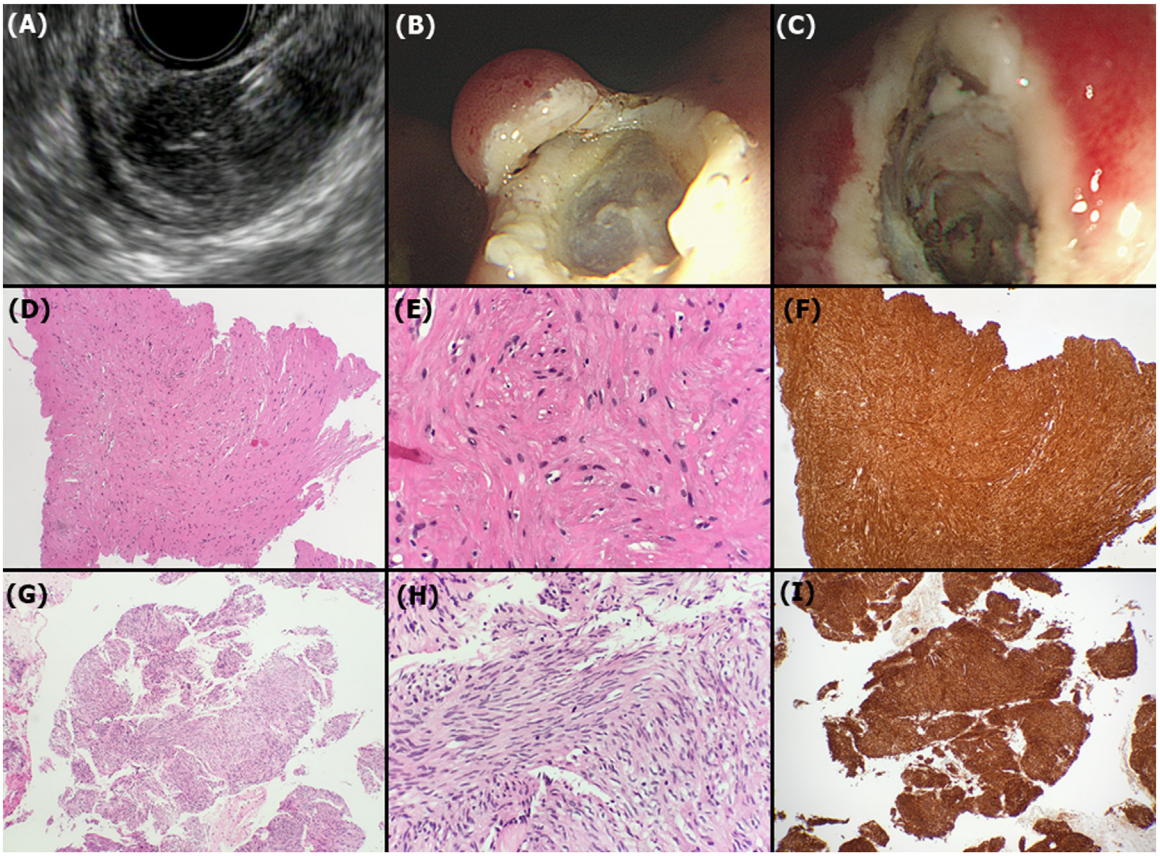

Fig. 1. EUS-FNB and unroofing biopsy technique and representative histological findings of gastric subepithelial tumors. (A) EUS-FNB of a hypoechoic lesion in the muscularis propria layer. (B) The overlying mucosa was snared and endoscopic mucosal resection with a blended electrosurgical current was performed to create a 10–15-mm mucosal defect on the SET. (C) Precut incision was made using a flex needle knife. (D) Leiomyoma in the unroofing biopsy specimen (100×, H&E), (E) (400×, H&E). (F) The tumor cells are positive for actin. (G) Gastrointestinal stromal tumor in the EUS-FNB specimen (100×, H&E), (H) (400×, H&E). (I) The tumor cells are positive for c-kit. H&E, hematoxylin and eosin; EUS-FNB, endoscopic ultrasonography-guided fine-needle aspiration biopsy.

Table 1
Summary of the 39 patients with subepithelial tumors who underwent endoscopic ultrasonography-guided fine needle biopsy and/or unroofing biopsy.

Variables	
Age (years)	58.3 ± 13.1
Male: Female	18:21
Tumor location	
Esophagus	6 (15.4%)
Cardia	9 (23.1%)
Fundus	5 (12.8%)
Body	15 (38.5%)
Antrum	4 (10.3%)
Tumor size on EUS (mm)	21.0 ± 6.6
Growth pattern	
Intraluminal	24 (61.5%)
Extraluminal	15 (38.5%)
Histological diagnosis of SETs sampled by biopsy	
Gastrointestinal stromal tumor	16 (41.0%)
Leiomyoma	15 (38.5%)
Ectopic pancreas	2 (5.1%)
Schwannoma	1 (2.6%)
Extranodal marginal zone B cell lymphoma, MALT type	1 (2.6%)
Not diagnosed	4 (10.3%)
Biopsy method	
Both EUS-FNB and unroofing biopsy	28 (71.8%)
Only EUS-FNB	7 (17.9%)
Only unroofing biopsy	4 (10.3%)

Variables are expressed as mean ± standard deviation or n (%).
EUS, endoscopic ultrasonography; SET, subepithelial tumor; MALT, mucosa-associated lymphoid tissue; FNB, fine needle biopsy.

1). Finally, 28 patients were analyzed to compare the outcomes between EUS-FNB and unroofing biopsy.

3.2. Technical outcomes of EUS-FNB and unroofing biopsy

The mean number of samplings required to obtain EUS-FNB specimens was significantly fewer than that for unroofing biopsy (2.2 times vs. 6.4 times, $p < 0.001$). The mean procedural time needed to obtain samples with EUS-FNB was shorter than that with unroofing biopsy (6.8 min vs. 14.4 min, $p < 0.001$) (Table 2). Of 35 cases, two cases showed mild bleeding after the EUS-FNB procedure, which were completely managed using only one hemoclip or epinephrine injection. Of 32 cases, three cases showed mild bleeding during the unroofing biopsy procedure, which was completely managed using hemoclips. The mean number of hemoclippings for closing the mucosa was 5.4 times (median, 5 times; range, 1–9 times) [14]. There was no fever, or peritonitis after both procedures.

3.3. Diagnostic yields of EUS-FNB and unroofing biopsy

The rates of definitive histological diagnosis were 64.3% (18 of 28) with EUS-FNB and 78.6% (22 of 28) with unroofing biopsy, with a nonsignificant difference ($p = 0.344$; Table 2). Of 28 cases, 15 (53.6%) SETs were histologically diagnosed with both methods, 3 (10.7%) were diagnosed with only EUS-FNB, 7 (25.0%) were diagnosed with only unroofing biopsy, and 3 (10.7%) were nondiagnostic with both methods. Immunohistochemical diagnosis was possible with EUS-FNB in 46.4% (13 of 28) and with unroofing biopsy in 67.9% (19 of 28) ($p = 0.180$). Including the 11 patients who were initially excluded,

Table 2
Procedural outcomes obtained with endoscopic ultrasonography-guided fine needle biopsy and unroofing biopsy (n = 28).

	Type of procedure		*p* Value*
	EUS-FNB	Unroofing biopsy	
Sampling number (times)	2.2	6.4	<0.001
Procedure time (minutes)	6.8	14.4	<0.001
Histological diagnosis	18/28 (64.3)	22/28 (78.6)	0.344
Immunohistochemical staining	13/28 (46.4)	19/28 (67.9)	0.180
Tumor location, long axis			
Esophagus	4/5 (80.0)	5/5 (100.0)	1.000
Cardia	3/6 (50.0)	4/6 (66.7)	1.000
Fundus	1/2(50.0)	2/2 (100.0)	1.000
Body	9/13 (69.2)	9/13 (69.2)	1.000
Antrum	1/2 (50.0)	2/2 (100.0)	1.000
Tumor location, short axis			
Lesser curvature	2/3 (66.7)	2/3 (66.7)	1.000
Greater curvature	1/2 (50.0)	2/2 (100.0)	1.000
Anterior wall	7/9 (77.8)	7/9 (77.8)	1.000
Posterior wall	3/7 (42.9)	4/7 (57.1)	1.000
Lesion size			
≥20 mm	11/13 (84.6)	10/13 (76.9)	1.000
<20 mm	7/15 (46.7)	12/15 (80.0)	0.125
Growth pattern			
Intraluminal	12/17 (70.6)	14/17 (82.4)	0.687
Extraluminal	6/11 (54.5)	8/11 (72.7)	0.625

Variables are expressed as n (%).
EUS-FNB, endoscopic ultrasonography-guided fine-needle biopsy.
* *p* Value for comparing the diagnostic yield between EUS-FNB and unroofing biopsy with the McNemar test.

histological diagnosis was made with EUS-FNB in 68.6% (24 of 35) and with unroofing biopsy in 78.1% (25 of 32).

We also evaluated the specimen size that was obtained from EUS-FNB technique. The size of specimen was more than 10 mm in 16 cases, 5–10 mm in three cases, and less than 5 mm in nine cases.

Additionally, we performed subgroup analysis according to the location, size, and growth pattern of SETs. In the ≥20 mm SET group, 11 (84.6%) patients had a successful diagnosis with EUS-FNB and 10 (76.9%) patients with unroofing biopsy ($p = 1.000$). In the <20 mm SET group, 7 (46.7%) patients had a successful diagnosis with EUS-FNB and 12 (80.0%) patients with unroofing biopsy ($p = 0.125$). Furthermore, in the intraluminal SET group, 12 (70.6%) patients had a successful diagnosis with EUS-FNB and 14 (82.4%) patients with unroofing biopsy ($p = 0.687$). In the extraluminal SET group, 6 (54.5%) patients had a successful diagnosis with EUS-FNB and 8 (72.7%) patients with unroofing biopsy ($p = 0.625$).

3.4. Factors affecting the diagnostic yield of EUS-FNB and unroofing biopsy

We performed logistic regression analysis to analyze the independent factors affecting the diagnostic yield of EUS-FNB (Table 3). In multivariate analysis, larger SETs (≥20 mm) were positively associated with a successful histological diagnosis with EUS-FNB than smaller SETs (<20 mm) (odds ratio [OR], 8.043; 95% confidence interval [CI], 1.164–55.572; $p = 0.035$). The patients' satisfaction with the procedure was also positively associated with a successful historical diagnosis with EUS-FNB (OR, 9.863; 95% CI, 1.452–66.972; $p = 0.019$). There were no statistically meaningful factors affecting the diagnostic yield of unroofing biopsy (**Supplementary** Table 1).

3.5. Correlation between EUS-FNB, unroofing biopsy, and surgical pathology in patients with GISTs

Of 9 patients diagnosed as having GIST with EUS-FNB, 5 patients underwent laparoscopic wedge resection, 1 patient underwent endoscopic submucosal dissection (ESD), and 3 patients refused the therapeutic procedure. We counted mitosis in EUS-FNB, unroofing biopsy, and corresponding surgical resection specimens in 6 patients who underwent resection for GIST (Table 4). The number of observed high-power fields (HPFs) for mitotic count examination ranged from 0 to 2 in the specimen obtained with both EUS-FNB and unroofing biopsy. We found mitosis in 6 patients, with the mitotic index ranging from 2 to 9 per 50 HPF in the corresponding surgical specimen. No case showed consistency between the biopsy and surgical resection reports.

3.6. Change in the impression of SET according to biopsy results

Of the 39 patients, 24 (61.5%) had the same histological diagnosis as expected with EUS. In 11 (28.2%) patients, the impression of SET changed according to biopsy results (Table 5). Of 9 patients initially considered as having GIST based on the EUS images before biopsy, 6 were finally diagnosed as having leiomyoma after EUS-FNB or unroofing biopsy, 1 as having MALT, 1 as having an ectopic pancreas, and 1 as having schwannoma. Therefore, unnecessary resection was avoided in 9 patients. A 19-mm SET was diagnosed as extranodal marginal zone B-cell lymphoma, mucosa-associated lymphoid tissue (MALT) type with unroofing biopsy. This patient underwent ESD.

The other SET, which was expected to be diagnosed as leiomyoma with EUS, was diagnosed as GIST with both EUS-FNB and unroofing biopsy, and the patient underwent laparoscopic wedge resection.

4. Discussion

Tissue sampling is critical to enable the diagnosis and characterization of the malignant potential of SETs [15]. As SETs are often movable and have an overlying mucosal layer, it is often difficult to target the lesion, especially when the size is not sufficiently large.

Table 3
Logistic regression analysis of factors affecting the diagnostic yield of endoscopic ultrasonography-guided fine needle biopsy for gastric subepithelial tumors (n = 35).

Variables	Univariate analysis			Multivariate analysis		
	OR	95% CI	*p* Value*	OR	95% CI	*p* Value*
Age	1.052	0.994–1.113	0.078			
Sex (male)	0.595	0.141–2.507	0.479			
Tumor size (≥20 mm)	**9.000**	**1.562–51.870**	**0.014**	**8.043**	**1.164–55.572**	**0.035**
Tumor location, long axis	1.000	0.574–1.741	1.000			
Tumor location, short axis	0.632	0.265–1.508	0.301			
Number of sampling (≥2 times)	4.125	0.579–29.392	0.157			
Procedure time (<7 min)	2.400	0.558–10.324	0.240			
Specimen size (≥10 mm)	3.500	0.786–15.578	0.100			
Satisfaction of procedure	**10.929**	**1.867–63.968**	**0.008**	**9.863**	**1.452–66.972**	**0.019**
Growth pattern (extraluminal)	0.600	0.139–2.581	0.493			

OR, odds ratio; CI, confidence interval.
* *p* Value for comparing the diagnostic group and the nondiagnostic group in endoscopic ultrasonography-guided fine needle biopsy for gastric subepithelial tumors.

Table 4
Mitotic counts from endoscopic ultrasonography-guided fine needle biopsy, unroofing biopsy, and surgical specimens among gastrointestinal stromal tumor samples.

Patient	EUS-FNB specimen		Unroofing biopsy specimen	Surgical specimen		
	Tumor size on EUS (mm)	Mitosis/HPF	Mitosis/HPF	Specimen size (mm)	Mitosis/HPF	Risk stratification
1	16.0	0/30	1/16	15.0	7/50	Intermediate
2	30.0	2/14	0/23	38.0	5/50	Low
3	15.0	1/44	0/39	22.0	2/50	Low
4	27.0	1/50	0/38	28.0	2/50	Low
5	15.0	0/34	Non-diagnostic	17.0	6/50	Intermediate
6	29.0	1/100	Severe crushing artifact	40.0	9/50	Intermediate

EUS-FNB, endoscopic ultrasonography-guided fine needle biopsy; HPF, high-power field.

Table 5
Change of impression of subepithelial tumors according to biopsy results.

Patient	Size (mm)	Location	EUS impression	Diagnosis from EUS-FNB	Diagnosis from unroofing biopsy	Impact on decision making
1	19	LB/GC	GIST	None	MALToma	Plan for endoscopic submucosal dissection
2	26	Angle/AW	GIST	Leiomyoma	Leiomyoma	Unnecessary resection avoided
3	19	Antrum/AW	GIST	None	Leiomyoma	Unnecessary resection avoided
4	17	Cardia/AW	GIST	Leiomyoma	Leiomyoma	Unnecessary resection avoided
5	16	Esophagus	Neurogenic tumor	Leiomyoma	Leiomyoma	Unnecessary resection avoided
6	20	Cardia/AW	GIST	Leiomyoma	None	Unnecessary resection avoided
7	17	Esophagus	GIST	Leiomyoma	Leiomyoma	Unnecessary resection avoided
8	29	UB/AW	Leiomyoma	GIST	GIST	Plan for surgery
9	20	Angle/PW	GIST	None	Ectopic pancreas	Unnecessary resection avoided
10	24	LB/LC	GIST	Schwannoma	None	Unnecessary resection avoided
11	22	UB/GC	GIST	Leiomyoma	None	Unnecessary resection avoided

EUS-FNB, endoscopic ultrasound-fine needle biopsy; LB, lower body; GC, great curvature; GIST, gastrointestinal stromal tumor; MALT, mucosa-associated lymphoid tissue; AW, anterior wall; UB, upper body; PW, posterior wall; LC, lesser curvature.

A recent study demonstrated that the overlying mucosa removed using a conventional snare provided sufficient specimen to assess the malignancy risk (92.7%; 95% CI, 80.4–100.0%) [16]. Another study reported that the jumbo biopsy unroofing technique is safer and more effective for the diagnosis of SETs than EUS-FNA (92% vs. 58%) [17]. The unroofing biopsy technique has the advantages of providing a relatively high diagnostic yield and large-sized tissue samples for diagnosing SETs. Moreover, it is technically easier to perform and safer, regardless of the location of SETs, than EUS-guided biopsy. However, unroofing biopsy is a time-consuming procedure than EUS-FNB procedure.

EUS-FNB was designed to overcome of the limitation of EUS-FNA, and to allow the assessment of tissue architecture and cell morphological changes. The diagnostic yield of EUS-FNB has been reported differently in each study, ranging from sixties to eighties percent [18,19]. EUS-FNB is superior to tissue acquisition, although it has clinical limitations because it requires technical skill and shows a difference in tissue acquisition rate depending on the size and location of SETs

Overall, the unroofing biopsy and EUS-FNB techniques are more effective than EUS-FNA; however, no study has prospectively compared EUS-FNB with unroofing biopsy. In this study, we reported the technical success rate, diagnostic yield, ability of IHC staining, procedure time, and safety outcomes of the 2 methods. We demonstrated that unroofing biopsy showed a 100% technical success rate and EUS-FNB showed an 89.7% success rate. However, once both biopsies were successful, the yield of EUS-FNB was not different from that of unroofing biopsy for diagnosing upper gastrointestinal SETs in our study (64.3% vs. 78.6%, $p = 0.344$). In the subgroup analysis according to the location, size, and growth pattern of SETs, there was no significant difference in diagnostic yield between the 2 methods.

The strength of this study lies on its prospective design with a direct comparison of the EUS-FNB and unroofing biopsy techniques including the ability of IHC staining. As the 2 procedures were performed sequentially in the same SET, we could compare the differences between them without any confounding factors. In terms of technical outcome, EUS-FNB took significantly less time than unroofing biopsy and required significantly fewer samplings ($p < 0.001$).

In fact, the management of incidental SETs <20 mm remains controversial because most small gastric SETs were considered benign. The European Society for Medical Oncology guidelines recommend surgical resection when a small SET is diagnosed as GIST [20]. A decision of either medical observation or surgery is reasonable; however, before that, a histological diagnosis for a correct decision is crucial even if the tumor is small [21,22]. Although most gastric GISTs <20 mm were known to follow a benign clinical course, a previous report demonstrated that 23.0% (10 of 43) of patients with a GIST <20 mm who had undergone surgery had a moderate-risk mitotic index (>5/50 HPF) [21]. Recently, GIST is considered to have a malignant potential regardless of its size; thus, it is necessary to perform tissue confirmation in SETs.

In our study, there was no difference in diagnostic yield between the 2 methods regardless of the tumor size. However, in the multivariate analysis with patients who received only EUS-FNB, the size of the tumor was a positively associated factor affecting the diagnostic yield. In the unroofing biopsy only group, there was no correlation between the size and diagnostic yield. Although there were no statistically differences in diagnostic yield of both techniques, EUS-guided tissue sampling might be technically difficult when target SETs are small and firm.

To assess whether the risk of GIST can be measured through sampling, we determined the mitotic count from the EUS-FNB, unroofing, and surgical specimens. The mitotic index reported from both EUS-FNB and unroofing biopsy had a poor correlation with that of the subsequent surgical specimen. Although EUS-FNB or unroofing biopsy may provide a relatively larger sample with a preserved tissue architecture, GIST is a very heterogeneous tumor not suitable for predicting risk with some tissue pieces [23]. Therefore, the purpose of EUS-FNB or unroofing biopsy is to aid in diagnostic decision making for therapeutic approaches, but not for risk assessment. In addition, when deciding the biopsy method from the two techniques, the SMT removal process in the future should also be

considered. Unroofing biopsy could affect endoscopic treatment such as ESD due to fibrosis. When it comes to endoscopic resection, it is necessary for the gastroenterologist to determine which method should be performed while considering these points.

This study has several limitations. First, the sample size was small and drawn from a single institution, and the dropout rate was slightly higher than expected because of cases in which only 1 modality was performed. Nevertheless, our study is the first to prospectively compare EUS-FNB with the unroofing biopsy technique. Moreover, there was no technical difference related to the discretion of the attending gastroendoscopist because 1 expert gastroendoscopist performed all examinations. Second, our sample might not be representative of the whole population of patients with SET because tissue sampling is usually performed in suspicious malignant tumors identified with EUS. The SETs included in our study were mostly located in the muscularis propria layer. Therefore, this study is highly relevant to real-life practice. Third, the possibility that EUS-FNB procedure may have affected the unroofing biopsy procedure could not be ruled out. Fourth, there was a relative high drop out rate because only one procedure was performed for various reasons, including failure of needle penetration, failure of identification of the lesion with white light endoscopy, minorbleeding, and severe belching.

In conclusion, there was no significant difference in the histological diagnostic yield for SETs between EUS-FNB and unroofing biopsy, if technically successful. Therefore, it is necessary to decide the biopsy technique considering various factors, and further study is needed to confirm the efficacy of EUS-FNB and unroofing biopsy in a larger, prospective study population (CinicalTrials.gov identifier NCT02646241).

Conflict of interest

None declared.

Acknowledgment

None.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.dld.2019.01.028.

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