



Optimal number of needle punctures in endoscopic ultrasound-guided fine-needle biopsy for gastric subepithelial lesions without rapid on-site evaluation

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Abstract

Purpose The utility of endoscopic ultrasound-guided fine-needle biopsy (EUS-FNB) for gastric subepithelial lesions (SELs) has been reported. In this study, we examined the optimal number of needle punctures during EUS-FNB for gastric SELs without rapid on-site evaluation (ROSE). The factors that allowed for a single needle puncture to arrive at the correct diagnosis were also analyzed.

Methods We conducted a retrospective study of all patients who underwent EUS-FNB to evaluate gastric SELs between April 2015 and September 2020; 51 patients with 57 gastric SELs were enrolled. The optimal number of needle punctures was determined when additional needle passes did not increase diagnostic sensitivity by more than 10%. Factors allowing for only a single needle puncture to arrive at the correct diagnosis were identified by univariate and multivariate logistic regression analyses.

Results EUS-FNB resulted in a definitive final diagnosis in 48 of 57 lesions (84%). Lesions in the gastric body (odds ratio [OR] 6.15, 95% confidence interval [CI] 1.75–21.6; $P < 0.01$) and lesions punctured using a 22G Franseen needle (OR 3.61, 95% CI 1.07–12.3; $P = 0.04$) were independent factors that allowed for only a single needle puncture to arrive at the correct diagnosis. The optimal number of needle punctures for lesions using a 22G Franseen needle in the gastric body and other lesions was two and three, respectively.

Conclusion The optimal number of needle punctures in EUS-FNB for gastric SELs without ROSE was two or three, depending on the location and type of needle used.

Keywords Endoscopic ultrasound-guided fine-needle aspiration · Endoscopic ultrasound-guided fine-needle biopsy · Subepithelial lesion · Number of needle punctures · Stomach

Introduction

The utility of endoscopic ultrasound-guided fine-needle aspiration (EUS-FNA) was first demonstrated in 1992 [1]. With the development of endoscopic technology, the utility of EUS-FNA for lesions such as lymph nodes and subepithelial

lesions (SELs) has been reported [2–5]. Recently, there has been an increasing need to collect an adequate volume of tissue for genetic diagnosis and anticancer drug-sensitivity assays. In addition, the choice of needles for fine-needle biopsy (FNB) has expanded. Although rapid on-site evaluation (ROSE) has been shown to be useful during EUS-FNA for SELs, the facilities where it is routinely available are limited [6]. For pancreatic lesions, Uehara et al. recommended the optimal number of needle punctures without ROSE to be between one and three, but the optimal number for SELs was unclear [7]. At our hospital, EUS-FNB is performed without ROSE, and three or four needle punctures are performed for SELs. The incidence of EUS-FNB complications for SELs is low, but unnecessary punctures should be avoided, because the risk of complications increases as the number of

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punctures increases [8]. In this study, we examined the optimal number of needle punctures required for SELs. We also examined the factors that allowed for only a single needle puncture during EUS-FNB to arrive at the correct diagnosis.

Materials and methods

Study design and setting

We conducted a retrospective study of all patients who underwent EUS-FNB at Yokohama Rosai Hospital between April 2015 and September 2020 to evaluate gastric SELs. During this period, we enrolled 51 consecutive patients with 57 gastric SELs.

EUS-FNB procedure

Before EUS-FNB, pharyngeal anesthesia with 8% xylocaine was administered via pump spray (Aspen Japan Corp., Tokyo, Japan), and midazolam and pentazocine were administered intravenously. The heart rate, blood pressure, and peripheral oxygen saturation were monitored during the procedure. EUS-FNB was performed using a curved linear-array echoendoscope (GF-UCT260; Olympus Medical Systems Corp., Tokyo, Japan) paired with an ultrasound system (EU-ME1 or EU-ME2 Premium; Olympus Medical Systems Corp.). We used Doppler imaging when puncturing the lesion to avoid the vascular structures. After the lesion was punctured, we removed the stylet, and aspiration was performed with a 20-ml syringe. Approximately 20 rapid strokes were created within the lesion, suction was released, and the needle was removed. The aspirated samples were placed in 10% formalin and sent to the Department of Pathology for histopathological assessment. Since ROSE was not performed, the puncture procedure was repeated until visual confirmation of a macroscopic white specimen.

The needles used for EUS-FNB were either a 22G Franseen needle (Acquire; Boston Scientific Corp., Natick, MA, USA) or one of these two conventional needles: 22G EZ Shot 3 Plus (Olympus Medical Systems Corp.) or 22G Echo Tip Ultra (Cook Medical Inc., Winston-Salem, NC, USA). Conventional needles were used between April 2015 and March 2017; from April 2017 onward, we primarily used 22G Franseen needles, but occasionally used 22G EZ Shot 3 Plus needles, depending on the lesion. We used EZ Shot 3 Plus needles for lesions that were difficult to puncture, i.e., those difficult to hold with a scope.

The EUS-FNB procedures were performed by eight endosonographers. Seven endosonographers were trainees (< 3 years of EUS-FNA/FNB experience), and one was an expert (> 3 years of EUS-FNA/FNB experience). All

EUS-FNB procedures were conducted under the guidance of the expert.

Histological assessment

The pathological specimens were placed in 10% formalin and sent to the Department of Pathology for histopathological assessment. In this study, pathological diagnosis was based only on histopathological diagnosis. The tissue sections were stained with hematoxylin and eosin for evaluation by a pathologist. Immunohistochemistry staining for c-kit, CD34, DOG-1, smooth muscle actin, and S-100 was performed in the presence of spindle cell lesions. We examined whether the diagnosis was confirmed for every puncture.

Definitions

EUS-FNB was indicated for the following SELs: (1) smaller than 20 mm with signs of malignancy such as ulceration, irregular borders, and increasing size; (2) 20–50 mm without signs of malignancy; and (3) requested by a surgeon for a preoperative pathological diagnosis.

The final diagnosis was determined by the histological diagnosis from surgery in patients who underwent surgery. In patients who did not undergo surgery, the final diagnosis was determined by their clinical course, imaging follow-up after ≥ 6 months, and subsequent histological assessment of the EUS-FNB specimens [9].

Aberrant pancreas, schwannomas, and lipomas were diagnosed by histological assessment of hematoxylin and eosin staining.

GIST, schwannomas, and leiomyomas were diagnosed by immunohistochemistry staining. The details were as follows:

- GIST: positive c-kit staining, or negative c-kit staining but positive CD34 and DOG-1 staining
- Schwannoma: positive S-100 staining
- Leiomyoma: positive smooth muscle actin staining

The optimal number of needle punctures was determined when the additional punctures did not increase diagnostic sensitivity by more than 10%. We cited this value from a report from Uehara et al. who examined the optimal number of needle punctures for pancreatic lesions without ROSE [7].

The number of needle punctures recorded was the number of attempted punctures. For example, if four punctures were attempted but the lesion was punctured only twice under ultrasound guidance, we recorded the number of needle punctures as four.

Adverse events in this study were described using the lexicon recommended by the American Society for Gastrointestinal Endoscopy [10].

Statistical analysis

Factors that allowed for only a single needle puncture to arrive at the correct diagnosis were identified by univariate and multivariate logistic regression analyses. Statistical analyses were carried out using EZR 64-bit (Jichi Medical University Saitama Medical Center, Saitama, Japan). We considered a *P* value of <0.05 as statistically significant.

Results

The demographic characteristics of the patients, gastric SELs, and technical characteristics of EUS-FNB are shown in Table 1. We performed EUS-FNB for 57 gastric SELs in 51 patients. Confirmation was not achieved during the first EUS-FNA/FNB procedure for six patients, and the procedure was repeated.

Twenty-five patients underwent surgery. GIST and schwannoma were diagnosed in 23 and two patients, respectively. Except for one case of schwannoma, the diagnosis of the surgical and FNB specimens concurred.

Twenty-six patients did not undergo surgery, and the final diagnosis was determined by their clinical courses and imaging follow-up after ≥ 6 months. The final diagnosis in these

patients was as follows: eight had aberrant pancreas, six had GIST, five had schwannomas, four had leiomyomas, one had a lipoma, and two had a diagnosis of “other.” The two lesions that were classified as “other” were suspected cysts, because only epithelial components were acquired. The two lesions were regarded as non-definitive diagnostic cases.

All malignant diseases were confirmed based on the progression of the lesion or the presence of metastases on follow-up imaging. All benign lesions were stable with no metastasis.

Thirty-seven lesions were located in the gastric body, 14 in the cardia or fundus, and six in the antrum. The median size of the SELs was 20 (9–80) mm, and 26 of the 57 lesions were smaller than 20 mm. Thirty-four lesions were punctured with a 22G Franseen needle; five were initially punctured with a 22G Franseen needle that was changed to a conventional needle from the second puncture onward, because although these five lesions could be punctured, only a small amount of sample could be acquired.

EUS-FNB resulted in a definitive final diagnosis in 48 of 57 lesions (84%). The diagnosis in six patients could not be confirmed during the first procedure, and a definitive diagnosis was made at the second procedure in five of them. The needles were changed during the procedures in four patients, and the endosonographer in one procedure was replaced by an expert. None of the patients reported complications from EUS-FNB in this study.

The cumulative diagnostic sensitivities of EUS-FNB with repeated needle punctures in all patients are shown in Table 2. Thirty-three lesions (58%) were confirmed during the first needle puncture. The first needle puncture was unsuccessful in only three lesions; a Franseen needle was used in these cases. In these patients, a trainee performed the first needle puncture, whereas an expert performed all succeeding punctures. All of the other needle punctures were successful.

Analysis of factors that allowed for only a single needle puncture to arrive at the correct diagnosis is shown in Table 3. Univariate analysis using a logistic regression model identified lesions located in the gastric body and use of a 22G Franseen needle as factors allowing for only a single needle puncture to arrive at the correct diagnosis. In the multivariate analysis using a logistic regression model,

Table 1 Patient demographics and clinical characteristics, gastric SELs, and technical characteristics of EUS-FNB

Patients	
Age (years)	
Median (range)	57 (36–82)
Sex	
Male/female	19/32
Gastric SELs	
Locations	
Cardia or fundus/body/antrum	14:37:6
Size of tumors on EUS (mm)	
Median (range)	20 (9–80)
< 20 mm/ \geq 20 mm	26/31
Final diagnosis	
GIST	32
Aberrant pancreas	10
Schwannoma	8
Leiomyoma	4
Lipoma	1
Others	2
EUS-FNB	
Diagnostic sensitivities	48/57 (84%)
Needle	
22G Franseen needle/conventional needle	34/23

SELs subepithelial lesions, EUS-FNB endoscopic ultrasound-guided fine needle biopsy, GIST gastrointestinal stromal tumor

Table 2 Cumulative diagnostic sensitivities of EUS-FNB by repeated needle punctures

Puncture number	Diagnostic sensitivities by repeated needle punctures			
	1	2	3	4
<i>n</i> = 57	33/57 (58%)	43/57 (75%)	48/57 (84%)	48/57 (84%)

EUS-FNB, endoscopic ultrasound-guided fine needle biopsy; n, number of lesions

Table 3 Univariate and multivariate analyses of factors that allowed for only single needle puncture to arrive at a correct diagnosis during EUS-FNB for gastric SELs

Factors	Univariate			Multivariate		
	OR	95% CI	<i>P</i> value	OR	95% CI	<i>P</i> value
Age (< 65 years)	0.61	0.17–2.02	0.42			
Sex (female)	1.30	0.39–4.29	0.79			
Size (≥ 20 mm)	0.74	0.23–2.41	0.60			
Location (body)	6.07	1.65–25.3	< 0.01	6.15	1.75–21.6	< 0.01
GIST	2.75	0.83–9.56	0.10			
Needle (22G Franseen needle)	3.64	1.07–13.2	0.03	3.61	1.07–12.3	0.04

EUS-FNB endoscopic ultrasound-guided fine needle biopsy, *SELs* subepithelial lesions, *OR* odds ratio, *CI* confidence interval, *GIST* gastrointestinal stromal tumor

lesions in the gastric body (odds ratio [OR] 6.15, 95% confidence interval [CI] 1.75–21.6; $P < 0.01$) and puncture using a 22G Franseen needle (OR 3.61, 95% CI 1.07–12.3; $P = 0.04$) were identified as independent factors that allowed for only a single needle puncture to arrive at the correct diagnosis.

Gastric SELs were divided into four groups according to their location and needle used during the puncture, which were factors that allowed for only a single needle puncture: Group 1, lesions in the gastric body punctured with a 22G Franseen needle; Group 2, lesions in other parts of the stomach punctured with a 22G Franseen needle; Group 3, lesions in the gastric body punctured with a conventional needle; and Group 4, lesions in other parts of the stomach punctured with a conventional needle. The cumulative diagnostic sensitivities of EUS-FNB with repeated needle punctures in these four groups are shown in Table 4. The optimal number of needle punctures in groups 1, 2, 3, and 4 was two, three, and three, respectively. The diagnostic sensitivities by the optimal number of needle punctures in groups 1, 2, 3, and 4 were 96%, 80%, 85%, and 60%, respectively.

The European Society of Gastrointestinal Endoscopy (ESGE) guidelines recommend EUS-FNA/FNB be performed for SELs sized ≥ 20 mm [11]. The cumulative diagnostic sensitivity of EUS-FNB with repeated needle punctures for SELs sized ≥ 20 mm in these four groups is shown in Table 5. The optimal number of needle punctures for SELs sized ≥ 20 mm in groups 1, 2, 3, and 4 was one, two, three, and three, respectively. The diagnostic sensitivities by optimal number of needle punctures for SELs sized ≥ 20 mm in groups 1, 2, 3, and 4 were 91%, 86%, 78, and 75%, respectively.

Discussion

To the best of our knowledge, this is the first study to examine the optimal number of needle punctures required for SELs without ROSE and factors that allow for only a single

Table 4 Cumulative diagnostic sensitivities of EUS-FNB by repeated needle punctures in four groups of SELs

Group	Diagnostic sensitivities by repeated needle punctures			
	1	2	3	4
Group 1 (<i>n</i> = 24)	20/24 (83%)	23/24 (96%)	23/24 (96%)	23/24 (96%)
Group 2 (<i>n</i> = 10)	4/10 (40%)	7/10 (70%)	8/10 (80%)	8/10 (80%)
Group 3 (<i>n</i> = 13)	7/13 (54%)	9/13 (69%)	11/13 (85%)	11/13 (85%)
Group 4 (<i>n</i> = 10)	2/10 (20%)	4/10 (40%)	6/10 (60%)	6/10 (60%)

Group 1: Lesions were punctured with a 22G Franseen needle in the gastric body

Group 2: Lesions were punctured with a 22G Franseen needle in other parts of the stomach

Group 3: Lesions were punctured with a conventional needle in the gastric body

Group 4: Lesions were punctured with a conventional needle in other parts of the stomach

EUS-FNB endoscopic ultrasound-guided fine needle biopsy, *SELs* subepithelial lesions, *n* number of lesions

needle puncture during EUS-FNB to arrive at the correct diagnosis.

EUS-FNA/FNB has a high diagnostic sensitivity and specificity and carries a low risk of complications. The diagnostic yield of EUS-FNA for SELs is reported to be 48.9–83.9% [5, 12–16], whereas that of EUS-FNB is 81.8–86.7%. El Chafic et al. reported that the diagnostic yield of EUS-FNB was significantly higher than that of EUS-FNA [13, 17, 18].

The ESGE reported a complication rate of approximately 1% for EUS-FNA [19]. However, increasing the number of needle punctures increases the risk of infection and bleeding. ROSE reduces the number of needle punctures required, but it is not available in many facilities due to its cost or personnel issues. Older studies have reported that the diagnostic

Table 5 Cumulative diagnostic sensitivities of EUS-FNB by repeated needle punctures in 4 groups of SELs sized ≥ 20 mm

Group	Diagnostic sensitivities by repeated needle punctures			
	Puncture number 1	2	3	4
Group 1 ($n=11$)	10/11 (91%)	11/11 (100%)	11/11 (100%)	11/11 (100%)
Group 2 ($n=7$)	3/7 (43%)	6/7 (86%)	6/7 (86%)	6/7 (86%)
Group 3 ($n=9$)	5/9 (56%)	6/9 (67%)	7/9 (78%)	7/9 (78%)
Group 4 ($n=4$)	1/4 (25%)	2/4 (50%)	3/4 (75%)	3/4 (75%)

Group 1: Lesions were punctured with a 22G Franseen needle in the gastric body

Group 2: Lesions were punctured with a 22G Franseen needle in other parts of the stomach

Group 3: Lesions were punctured with a conventional needle in the gastric body

Group 4: Lesions were punctured with a conventional needle in other parts of the stomach

EUS-FNB endoscopic ultrasound-guided fine needle biopsy, SELs subepithelial lesions, n number of lesions

accuracy of EUS-FNA for SELs reaches a plateau after four punctures [19, 20]. However, it is expected that the number of needle punctures required will decrease due to the recent development in FNA/FNB needles.

In the present study, we concluded that the optimal number of needle punctures was three for many SELs, whereas it was two when using a 22G Franseen needle in the body of the stomach. Lesions located in the body of the stomach were found to be independent factors that allowed for only a single needle puncture to arrive at the correct diagnosis. These lesions are often easy to hold with a scope; therefore, they can be easily punctured. However, they are more difficult to puncture than pancreatic tumors, because SELs are generally hard and movable [21]. In contrast, it is often difficult to puncture lesions in the fundus and antrum, because they are difficult to hold with a scope. In such cases, a cap-assisted forward-viewing endoscope is extremely helpful [22]. Using this scope, the lesion can be caught in the cap and punctured. Since only an oblique-viewing scope was used in this study, it was concluded that the optimal number of needle punctures would depend on the location of the lesions. However, it may be possible to diagnose lesions in the fundus and antrum using a smaller number of needle punctures when the new cap-assisted forward-viewing endoscopes are used.

The use of a 22G Franseen needle was also an independent factor that allowed for only a single needle puncture to arrive at the correct diagnosis. In 2016, the Franseen needle was developed to improve the diagnostic performance of needles [23]. Franseen needles allow for the acquisition of more tissue for a more accurate histological diagnosis with fewer needle passes than conventional needles [24–26]. These factors are believed to also apply to SELs and are reflected in the results of the present study [13, 17, 18]. Fujita et al. reported that Franseen needles tended to have a higher adequate sample rate than conventional needles, especially in lesions smaller than 20 mm [27]. However, owing to the unique tip design of the Franseen

needle, it is harder to correctly puncture lesions with a Franseen needle than with a conventional needle. Therefore, if the endosonographer is not highly skilled, a proper puncture cannot be performed using a Franseen needle.

Lesion size ≥ 20 mm and gastrointestinal stromal tumor were not identified as independent factors that allowed for a only single needle puncture to arrive at the correct diagnosis. Based on the results, it is considered that the improvement of the EUS-FNA/FNB needle and the location of the lesion contributed to the diagnostic yield of small lesions, but the type of lesion did not.

This study has several limitations. First, eight endosonographers with different skill levels performed the puncture procedures. However, all procedures were performed under the guidance of a single expert. Therefore, we believe that the quality of EUS-FNB was maintained. Second, different types of needles were used, because the study was conducted over the course of 5 years, and the choice of puncture needle was left to the discretion of each endosonographer. Puncture is more difficult to achieve with a Franseen needle than a conventional needle, so it may have only been used for uncomplicated lesions. However, even if the use of a Franseen needle was limited to SELs sized ≥ 20 mm, these SELs were punctured less than those wherein a conventional needle was used. Third, we listed the independent factors in Table 3, including GIST. The diagnosis of GIST can only be determined after EUS-FNB has been performed. However, the results suggest that additional needle punctures should not be indicated when a white specimen has been acquired by the initial needle puncture for a lesion with a strong suspicion of GIST (hypoechoic lesion originating from the fourth layer with a hypervascular pattern on contrast echography) [28]. Finally, as this was a retrospective and single-center study, there are inherent limitations. Therefore, a prospective study is needed to determine the optimal number of needle punctures for SELs and the factors that may allow for only a single needle puncture to arrive at the correct diagnosis.

Conclusion

The optimal number of needle punctures in EUS-FNB for SELs without ROSE was two or three, depending on the location of the lesion and the FNA/FNB needle used.

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Author contributions MS and YS conceived the study. All EUS-FNA/FNB procedures were performed under the guidance of YS. MS performed the statistical analysis. AN supervised the study and gave final approval of the version to be published. All authors read and approved the final manuscript.

Declarations

Conflict of interest Masato Suzuki, Yusuke Sekino, Kunihiro Hosono, Kenichi Kawana, Hajime Nagase, Kensuke Kubota, and Atsushi Nakajima declare that they have no conflicts of interest.

Ethical approval All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1964 and later versions. This study was approved by the ethics committee of Yokohama Rosai Hospital. We obtained written informed consent for EUS-FNB from all participating patients.

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