# Utility of a 20G needle with a core trap in EUS-guided fine-needle biopsy for gastric submucosal tumors: A multicentric prospective trial

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#### ABSTRACT

Background and Objectives: Differential diagnosis to estimate the malignant potential of gastric submucosal tumor (g-SMT) is important for decision-making. This study evaluated the use of a 20G needle with a core trap for EUS-guided fine-needle biopsy (EUS-FNB) for g-SMT. Methods: This multicentric prospective trial was registered in the University Hospital Medical Information Network (UMIN000021410). Consecutive patients with g-SMT who presented at one of the nine Japanese Referral Centers between June 2017 and November 2018 were enrolled. All patients underwent EUS-FNB using a 20G needle with a core trap. Samples obtained with the first-needle pass were used for central pathological review. EUS-FNB was evaluated in terms of (i) technical success rate, (ii) adequacy for histological evaluation, (iii) rate of complications, (iv) accuracy for histological diagnosis of gastrointestinal stromal tumor (GIST), and (v) concordance between GIST mitotic index determined by EUS-FNB and after tumor resection. Results: The study included 52 patients. The technical success rate of EUS-FNB

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was 100%. The adequacy rate for histological evaluation was 90.4% (P < 0.001). There were no complications related to EUS-FNB. Of the 38/52 patients who underwent surgical resection, 36 were finally diagnosed with GIST. The sensitivity, specificity, and accuracy of EUS-FNB for the histological diagnosis of g-SMT were 80.6%, 100%, and 81.6%, respectively. The concordance rate between the mitotic index on EUS-FNB and that after analysis of the resected tumor was 89.7%. **Conclusions:** EUS-FNB using a 20G needle with a core trap is feasible, providing histological samples of sufficient quality for diagnosing g-SMT.

Key words: 20G needle, core trap, EUS-FNA, EUS-guided fine-needle biopsy, submucosal tumor

#### **INTRODUCTION**

Gastric submucosal tumors (g-SMTs) include gastrointestinal stromal tumors (GISTs), which are an indication for surgery, as well as benign lesions such as leiomyoma. The differential diagnosis of g-SMT and evaluation of malignancy are important for selection of the most effective therapy. A pathological diagnosis of g-SMT requires sufficient tissue sample to determine the histological type. EUS-guided fine-needle biopsy (EUS-FNB) is widely used for preoperative histological evaluation of lesions inside or outside the digestive tract detected by EUS.<sup>[1-5]</sup> In a multicentric study from our group, we reported that a 25G EUS-FNB needle with a core trap is more effective for pancreatic tumor tissue sampling than a standard 25G needle.<sup>[6]</sup> In addition, a 25G needle with a core trap is useful for grading pancreatic endocrine tumors.<sup>[7]</sup> Although the 25G needle with a core trap is useful for EUS-FNB sampling of pancreatic disease, a 25- or a 22-G needle may be too thin to obtain sufficient tissue for diagnosing g-SMT.<sup>[8,9]</sup> A recent multicentric randomized trial suggested that a 20G needle with a core trap outperforms a standard 25G needle with respect to histological yield and diagnostic accuracy in patients with g-SMT; however, the study included mainly pancreatic or lymph node lesions.<sup>[10]</sup> The aim of the present prospective multicentric trial was to evaluate the use of a 20G needle with a core trap for histological evaluation by EUS-FNB in patients with g-SMT.

#### **METHODS**

#### Study design and patient enrollment

This multicentric prospective study was approved by the institutional review board of each participating hospital. All patients provided written informed consent. The trial was registered with the University Hospital Medical Information Network (number UMIN000021410). Consecutive patients with g-SMT on imaging studies who presented at one of the nine Japanese referral centers for EUS-FNB between June 2017 and November 2018 were enrolled prospectively via a designated website. Patients were included if they were >20 years of age, had a g-SMT of  $\geq 10$  mm, had no severe comorbidities, required a definitive pathological diagnosis of g-SMT to determine treatment, and had provided written informed consent. Patients were excluded if they had a high risk of bleeding (a platelet count <50,000/mm<sup>3</sup> or prothrombin time-international normalized ratio  $\geq 1.5$ ), interposing vessels were present, the tumor could not be visualized clearly on EUS, or if they did not/could not provide informed consent.

#### Outcome measures

Samples obtained with the first-needle pass were used for the central pathological review. EUS-FNB using a 20G needle with a core trap was evaluated in terms of (i) technical success rate, (ii) adequacy for histological evaluation (primary outcome), (iii) complication rate, (iv) accuracy for the histological diagnosis of GIST, and (v) concordance between the mitotic index of GIST determined by EUS-FNB and that obtained after analysis of the resected tumor. Technical success was defined as successful puncture of the g-SMT on the first pass. The complication rate was defined as the percentage of enrolled patients with an adverse event requiring treatment during EUS-FNB or within 3 months after EUS-FNB. The accuracy of histological diagnosis of GIST was estimated from patients who underwent surgical resection. Final diagnoses were obtained by surgical resection or EUS-FNB including in-house diagnoses. Cases for which the pathological diagnosis could not obtained by surgical resection or EUS-FNB were denoted "no definitive diagnosis."

#### EUS-guided fine-needle biopsy technique

EUS-FNB was performed by an expert endosonographer with experience of more than 100 EUS-FNB procedures using a linear-array echoendoscope (GF-UCT 240 or GF-UCT 260, Olympus Optical, Tokyo, Japan; or EG-530UT2, FUJIFILM, Tokyo, Japan) with the patient under conscious sedation. All patients underwent EUS-FNB using a 20G needle with a core trap (EchoTip ProCore, Cook Medical, Bloomington, IN, USA). The slow-pull technique was used while fanning the needle throughout the lesion twenty times after the mass had been punctured.<sup>[5]</sup> Tissue samples were expelled into formalin bottles with a stylet or air and then processed for histological evaluation; no rapid on-site evaluation was performed. The sample was not divided for cytologic examination including cell blocks. Samples obtained from additional needle passes were used to ensure an accurate diagnosis. The number of additional passes was determined by the physician.

#### Tissue processing and histological assessment

Formalin-fixed samples obtained by EUS-FNB were sent to a single designated facility at 1 day postharvest and processed for histological evaluation prior to the central review. The technique, which was the same for each sample, was described previously.<sup>[6]</sup> The entire formalin-fixed sample was spread onto a mesh sheet, dehydrated, and embedded in paraffin. For the diagnosis of g-SMT, ten slides bearing serial sections were prepared: two were stained with hematoxylin and eosin and eight were stained immunohistochemically to detect c-kit, CD34, S-100, chromogranin A, synaptophysin, and CD56. Spindle cell tumors positive for c-kit or CD34 were diagnosed as GIST. The mitotic index was calculated by including the maximum number of high-power fields (HPFs) obtained from the EUS-FNB specimens. A sample was defined as "adequate" for histological evaluation if the tissue architecture was preserved. Histological evaluations were performed by two expert pathologists (SY and AY). If their conclusions differed, the samples were re-evaluated and the results were discussed until a consensus was reached.

#### Statistical analysis

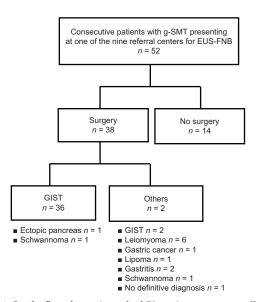
A previous study that used a 22G EUS-guided fine-needle aspiration (EUS-FNA) needle or a 19G EUS-FNB needle for g-SMT reported that the rate of adequacy for histological evaluation was 61.8% overall and 77.8% in the group using only the 19G needle.<sup>[9]</sup>

The 20G needle with a core trap used in the present study is thinner than a 19G needle but has a similar diameter. The expected adequacy rate for histological evaluation in the present study was 80%, and the expected threshold adequacy rate for histological evaluation was 60%. Under this assumption, a type I error of 0.05 (one sided), a power of 80%, and a sample of 39 patients would be required. Assuming a certain dropout rate, a target sample size of fifty patients was established. If the lower limit of the 90% confidence interval (CI) was  $\geq 60\%$ , EUS-FNB using a 20G needle with a core trap would be considered valid. For reference, the 95% CI was also calculated. P values were calculated by performing Fisher's exact test based on a binominal distribution, with a null hypothesis of 60%: a one-sided test with a 5% significance level was used. Continuous and categorical variables were analyzed using *t*-tests and Chi-square tests, respectively. Statistical analysis was performed using SAS software version 9.4 (SAS Institute, Cary, NC, USA).

#### RESULTS

The study included 52 patients [Table 1]. All the enrolled patients were eligible for EUS-FNB, and all g-SMT samples were obtained by puncture from the stomach. Figure 1 shows the study flowchart. Of the 52 patients enrolled, 38 underwent surgical resection, and 36 of these were diagnosed with GIST after examination of a resected specimen. The remaining two were diagnosed as ectopic pancreas and schwannoma. In these two cases, GIST could not be completely ruled out by EUS-FNB, which resulted in surgery. Of 14 patients who did not undergo surgery, 12 were not diagnosed with GIST, including one patient with no definitive diagnosis, and two patients refused surgery after being diagnosed with GIST by EUS-FNB. The technical characteristics and outcomes of EUS-FNB are shown in Table 2. The technical success rate of EUS-FNB (i.e., successful puncture of the g-SMT) was 100%. The rate of adequacy for histological evaluation was 90.4% (90% CI; 80.8–96.1, 95% CI; 79.0–96.8, P < 0.001). The incidence rate of complications was 0%. Five inadequate cases (i.e., patients without an adequate sample obtained by EUS-FNB) were older patients and those with smaller tumors compared to the adequate cases (i.e., patients with an adequate sample obtained by EUS-FNB); however, the difference was not significant [Table 1]. The sensitivity, specificity, positive predictive value, negative predictive value, and accuracy with 95% CI of EUS-FNB for the

histological diagnosis of g-SMT in the 38 patients who underwent resection were 80.6% (77.0-80.6), 100% (35.8-100), 100% (95.6-100), 22.2% (8.0-22.2), and 81.6% (74.8-81.6), respectively [Table 3]. Seven cases that were diagnosed with GIST by surgery received a different diagnosis by first-pass EUS-FNB: three were diagnosed with gastritis and four were inadequate. These cases underwent surgery because additional EUS-FNB specimens revealed GIST.



**Figure 1.** Study flowchart. A total of 52 patients were enrolled and analyzed for the primary outcome. g-SMT: Gastric submucosal tumor; GIST: Gastrointestinal stromal tumor

The number of HPFs used to evaluate the mitotic rate in EUS-FNB specimens was 1–5 in 19 cases, 6–10 in eight cases, and >10 in four cases (the maximum was 40 HPFs). Table 4 shows the concordance of the mitotic index between EUS-FNB and surgical specimens in the 29 patients diagnosed with GIST by both EUS-FNB and surgical specimen. The concordance rate was 89.7%, although the mitotic index from EUS-FNB samples was 0 in all cases. Figure 2 shows representative adequate EUS-FNB samples from a case with a low-risk GIST measuring 34 mm (c-kit positive, CD34-positive, S-100-negative, and desmin-negative; mitotic index: 0/10 HPF).

#### DISCUSSION

EUS-FNB is performed using needles of different diameters (19-, 20-, 22-, and 25-G in order of increasing thickness). A thicker needle has limited puncture ability but better tissue acquisition, whereas a thinner needle has better puncture ability but inferior tissue acquisition.<sup>[11]</sup> A meta-analysis evaluating EUS-guided needle sampling for SMT reported a pooled diagnostic rate of only 59.9%.<sup>[12]</sup> However, needles of different gauges (19-, 22-, or 25-G) and types (EUS-FNA, trucut needle biopsy, or FNB) were used. One reason for the poor diagnostic rate is that core biopsy or immunostaining was difficult to perform

	Total ( <i>n</i> =52)	Adequate cases (n=47)	Inadequate cases (n=5)	<b>P*</b>
Age, median (range), years	66.1 (36-84)	65.0 (36-84)	76.0 (68-79)	0.068
Sex, male:female, <i>n</i>	27:25	25:22	2:3	0.662
Tumor size, mean±SD, mm	27.4±17.4	28.0±18.1	21.4±5.4	0.422
Tumor location, <i>n</i>				
Cardia	11	11	0	0.145 <sup>†</sup>
Fundus	8	8	0	
Body	27	24	3	
Antrum	6	4	2	
Puncture site, stomach: duodenum, <i>n</i>	52:0	47:0	5:0	1.000
Final diagnosis, <i>n</i> (resected case)				
GIST	38 (36)	34 (2)	4 (4)	1.000‡
Leiomyoma	6 (0)	6 (0)		
Gastric cancer	1 (0)	1 (0)		
Lipoma	1 (0)	1 (0)		
Gastritis	2 (0)	2 (0)		
Ectopic pancreas	1 (1)	1 (1)		
Schwannoma	2 (1)	2 (1)		
No definitive diagnosis	1 (0)		1 (0)	

\*Comparison between adequate and inadequate cases; \*Comparison of percentage body/antrum; \*Comparison of percentage GIST. GIST: Gastrointestinal stromal tumor; SD: Standard deviation

on samples obtained by EUS-FNA with thin needles. The 19G needle with a core trap, which uses reverse bevel technology, was developed for EUS-FNB before development of the 20G needle with a core trap. The 19G needle with a core trap had a histological adequacy of 89.5% and a diagnostic accuracy of 86.0% in 114 intra-intestinal or extra-intestinal mass lesions and/or peri-intestinal lymph nodes.<sup>[1]</sup> Thus, the diagnostic yield and accuracy of the 19G needle with a core trap are high; however, it is limited by its stiffness and poor maneuverability. The 20G needle with a core trap, which has an antegrade-cutting side bevel, has a diameter equivalent to that of the 19G needle, and should be able to collect abundant tissue from SMT

# Table 2. Technical characteristics and outcomesof EUS-FNB

	20G EUS-FNB (n=52)
Technical success rate of EUS-FNB, %	100 (52/52)
Adequacy for histological evaluation, $\%$	90.4 (47/52)
Complication rate, %	0 (0/52)
FLIS-ENB: FLIS-guided fine-needle bionsy	

EUS-FNB: EUS-guided fine-needle biopsy

lesions. In addition, it is less likely than the 19G needle to be limited by puncture resistance or the angle of operation of the scope. In the present study, the use of a 20G needle with a core trap was technically feasible in all cases, including six cases with g-SMT located in the gastric antrum, which were relatively difficult to puncture. Moreover, the histological adequacy of this needle was 90.4%, which was better than that of the Trucut 19G needle and comparable with that of the 19G needle with a core trap.<sup>[1,12]</sup> However, three of seven incorrectly diagnosed cases were diagnosed as gastritis by EUS-FNB despite showing histological adequacy in EUS-FNB specimens [Table 3]. In these cases, only the surface mucosa of the stomach might have been taken. This suggests that a sufficient amount of EUS-FNB sample is needed to make an accurate diagnosis of GIST.

In many studies on EUS-FNB for gastrointestinal SMT, EUS-FNB is indicated for lesions >20 mm.<sup>[9,13-15]</sup> The Japanese guidelines or the National Comprehensive

# Table 3. Accuracy of EUS-FNB for histological diagnosis of gastrointestinal stromal tumor\* (accuracy,81.6%)

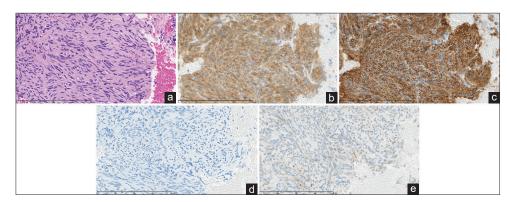
	GIST by surgical specimen, n	Others by surgical specimen, n	Total
GIST by EUS-FNB, n	29	0	29
Others by EUS-FNB, n	7	2	9
Total	36	2	38

\*The four (out of 38) cases with an inadequate EUS-FNB sample were diagnosed as GIST from surgical specimens; these are included in this analysis as "others by EUS-FNB." GIST: Gastrointestinal stromal tumor; EUS-FNB: EUS-guided fine-needle biopsy

# Table 4. Concordance of the mitotic index of gastrointestinal stromal tumor between EUS-FNB and surgical specimens

Mitotic index	Five or less in surgical specimens, n	More than five in surgical specimens, n	Total
Five or less in EUS-FNB, n	26	3	29
More than five in EUS-FNB, n	0	0	0
Total	26	3	29

GIST: Gastrointestinal stromal tumor; EUS-FNB: EUS-guided fine-needle biopsy



**Figure 2.** EUS-guided fine-needle biopsy specimen showing a low-risk gastrointestinal stromal tumor. (a) Hematoxylin and eosin stain; (b) c-kit immunohistochemistry; (c) CD34 immunohistochemistry; (d) S-100 immunohistochemistry; and (e) desmin immunohistochemistry (all high-power views)

Cancer Network guidelines recommend follow-up for SMTs <2 cm without malignant findings or symptoms.<sup>[16,17]</sup> However, patients with GISTs <2 cm are common clinically, including a report of a case with GIST <2 cm that developed liver metastasis.<sup>[18-20]</sup> Diagnosis by EUS-FNB is important even for small lesions in patients with g-SMT. Yamabe et al. reported that EUS-guided sampling for SMT <10 mm was possible using a forward-viewing EUS.<sup>[21]</sup> In the present study, we did not use forward-viewing EUS. However, in cases with SMT <10 mm, it may be difficult to achieve effective operation of the core trap (antegrade-cutting side bevel) on the EUS-FNB needle. Therefore, EUS-FNB using a 20G needle with a core trap was indicated for lesions  $\geq 10$  mm. Inadequate cases tended to be smaller, as shown in Table 1, suggesting that the 20G needle with a core trap was unsuitable for small g-SMTs.

The adequacy rate for histological evaluation, which was the primary outcome of the present study, was better than expected. Furthermore, it was obtained using only the first-pass EUS-FNB sample. There are few studies on EUS-FNB using 20G needle with a core trap for gastrointestinal SMT.<sup>[14,15]</sup> One retrospective study reported that the mean number of passes required to obtain a histological diagnosis was 2.2, although the median size (range) of lesions on EUS was 43.1 mm (20-90 mm).<sup>[14]</sup> Another prospective study that performed at least three needle passes reported a rate of histological adequacy of 75.0% for the first pass, which increased to 88.9% after combining passes 1-3.<sup>[15]</sup> The median tumor size (range) on EUS in this prospective study was 25 mm (20-150 mm), which is comparable to that of the present study. One advantage of the present study is that all first-pass samples were obtained using the same procedure, namely, slow-pull and fanning techniques. In previous studies, these techniques were not used for all patients.<sup>[14,15]</sup> In addition, all samples were histologically analyzed in a single facility staffed by experienced pathologists who were blinded to the clinical information. The mitotic index on EUS-FNB was evaluated in all resected GIST cases and the results showed good concordance between the mitotic index determined by EUS-FNB and that obtained after analysis of the resected tumor; concordance was not achieved in three cases [Table 4]. The mitotic index in GISTs is usually examined by selecting fifty HPFs from surgically resected specimens.<sup>[22]</sup> In the present study, assessment of the mitotic index by EUS-FNB was limited because it was difficult to obtain a sufficient amount of tissue sample to evaluate fifty HPFs; this issue needs to be addressed in future. In addition, in most cases, the mitotic index from surgical specimens was  $\leq 5$ , and it was difficult to puncture the hotspot during EUS-FNB. The present study also has several limitations. First, only the first-pass EUS-FNB samples were analyzed. Second, all samples were obtained using a 20G needle with a core trap, and the efficacy of different needle types was not compared. In recent years, new needles, such as Franseen or Fork-tip needles with a 25- or 22-G, have been developed for tissue acquisition.<sup>[23,24]</sup> These needles may have the advantage of enabling EUS-FNB for small g-SMT; therefore, they could replace the 20G needle with a core trap. Further large-scale comparative trials are needed to evaluate the utility of a 20G needle with a core trap in patients with SMT. Third, a final diagnosis based on postsurgical assessment was not obtained for all cases. Two unresected cases with a final diagnosis of gastritis might indicate sampling error. In conclusion, EUS-FNB using a 20G needle with a core trap is technically feasible and provides histological samples of sufficient quality for the diagnosis of g-SMT.

#### CONCLUSION

EUS-FNB using a 20G needle with a core trap is technically feasible and provides histological samples of sufficient quality for the diagnosis of g-SMT.

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