Gastrointestinal subepithelial lesions (SELs) include several neoplastic and non-neoplastic lesions that can be difficult to diagnose. The term “subepithelial” seems more appropriate than “submucosal” because these lesions can originate from each layer of the gastrointestinal wall or even can be caused by extramural compression [1]. Most of the gastrointestinal SELs are asymptomatic, therefore their real incidence is unknown. The highest incident of SELs throughout the gastrointestinal tract has been documented in the stomach [2]. When a SEL is found, establishing the exact nature is mandatory for subsequent management. Conventional endoscopic biopsies are frequently inconclusive, because mucosa overlying SELs is usually normal. Similarly, radiological investigations such as barium contrast radiography, computed tomography (CT) or abdominal ultrasound are of limited value in defining the exact nature of the lesion [1, 3].

Endoscopic ultrasound (EUS) is currently recommended as first-choice investigation to assess SELs because of its accuracy.

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**ABSTRACT**

**Background and study aims** This study was designed to evaluate the impact of additional tissue obtained with endoscopic ultrasound (EUS)-guided 25-gauge core biopsy needle (25G-PC) following an unsuccessful fine-needle biopsy (FNB) performed with larger-bore needles for the characterization of gastrointestinal subepithelial lesions (GI-SELs).

**Patients and methods** We prospectively collected and retrospectively analyzed information in our database from January 2013 to June 2017 for all patients with GI-SELS who received an EUS-guided FNB (EUS-FNB) with 25G-PC during the same procedure after failure of biopsy performed with larger-bore needle. Diagnostic yield, diagnostic accuracy and procedural complications were evaluated.

**Results** Sixteen patients were included in this study, 10 men and 6 women, median age 67.8 (range 43 to 76 years). Five patients were found to have a SEL localized in the distal duodenum, five in the gastric antrum, two in the gastric fundus and four in the gastric body. The mean size of the lesions was 20.5 mm (range 18 – 24 mm). EUS-FNB with 25G-PC enabled final diagnosis in nine patients (56.2 %). Regarding the subgroup of duodenal lesions, the procedure was successful in four of five (80%). Final diagnoses with EUS-guided sampling were GIST (n = 6), leiomyoma (n = 2) and metastatic ovarian carcinoma (n = 1). No procedure-related complications were recorded.

**Conclusion** In patients with small GI-SELS, additional tissue obtained with 25G-PC could represents a “rescue” strategy after an unsuccessful procedure with larger-bore needles, especially when lesions are localized in the distal duodenum.
in differentiating them from extrinsic compression and providing information about morphology and layer of origin [1]. EUS can sometimes provide information in case of lesions with typical morphological features, such as lipomas or duplication cysts. However, tissue diagnosis is often required, especially in neoplasms for which immunohistochemistry (IHC) is mandatory, such as gastrointestinal stromal tumors (GISTs). EUS needles of different size and shape have been used, with variable success/complication rates [4–7]. Recently, a new needle with reverse bevel technology has been developed to simultaneously obtain cytological aspirates and histological core samples, thereby leading to an ideal EUS-guided fine needle biopsy (EUS-FNB) [8–11]. The majority of reports on EUS-FNB needles have focused on pancreatic masses. Data on the diagnostic performance of 25-gauge (G) core needle to assess GI-SELS are lacking.

The aim of this study was to evaluate the impact of additional tissue obtained with EUS-guided 25G-ProCore needle biopsy following an inconclusive EUS-FNB performed with larger-bore needles for characterization of GI-SELS.

**Patients and methods**

**Patients**

All consecutive patients who received, during the same procedure, an EUS-guided FNB with 25G-ProCore (25G-PC) needle (EchoTip ProCore; Cook Endoscopy) as a “rescue strategy” after an initial unsuccessful biopsy performed with larger ProCore needles (22G-PC and/or 19G-PC) to diagnose upper GI-SELS were prospectively enrolled and retrospectively analyzed. EUS-FNB with 25G-PC was considered as a rescue strategy after a prior attempt with EUS-FNB with a larger-bore needle when: (1) puncture of the lesion was not feasible for technical reasons (i.e., difficulty to advance the needle through the scope in angulated position); or (2) specimens obtained were considered macroscopically suboptimal (i.e., not suitable to put in a formalin bottle for histological examination).

Inclusion criteria for EUS-FNB were: (1) presence of upper GI-SELS revealed by endoscopy, (2) need for pathological assessment to make a diagnosis and/or to guide management decision, (3) age older than 18 years, and (4) ability to provide informed consent. Exclusion criteria were: (1) inability to provide informed consent; (2) evidence of coagulation disorder.

Baseline variables are presented as numbers (percentage) and mean values (range).

**EUS-FNB procedure**

EUS-FNBs were performed by using convex array echoendoscopes (UCT-140, Olympus America, Inc. Melville, New York, United States) with the patient in the left lateral position under conscious sedation (intravenous fentanyl and midazolam) or deep sedation (propofol). After targeting the optimal puncture site, each puncture was done using a core biopsy needle (EchoTip ProCore; Cook Endoscopy) guided by real-time EUS imaging. Two different suction techniques (slow-pull and “wet”) were used at the operator’s discretion. In the slow-pull technique, the stylet was left inside the needle and, after puncturing the lesion, it was slowly and continuously removed as the needle was moved to-and-fro for 10 to 15 times inside the lesion. In the “wet” technique, the stylet was removed and the needle was filled with saline to replace the column of air with water, then the needle was passed into the lesion and the suction applied with a 10-cc pre-vacuum syringe. Thereafter, the needle was moved to-and-fro 10 to 15 times inside the lesion, suction-suction was then turned off before withdrawing the needle from the lesion [12, 13]. The ProCore (PC) needle size for the first attempt (19G or 22G) and the number of needle passes were at discretion of the endosonographer. The procedure was stopped when biopsy specimens were considered sufficient by the operator at gross examination. A maximum number of three biopsy attempts were allowed for each needle.

All EUS-FNBs procedures were performed by a single experienced endoscopist (FA) who has performed more than 1000 EUS procedures and at least 100 EUS-FNAs per year. This study was approved by the institutional review board.

**Specimen evaluation and histological process**

During the procedure there was no on-site cytopathologist. After EUS-FNB, the sample obtained was expelled onto slides. All macroscopically visible cores specimens (defined as whitish or yellowish piece of tissue with an apparent bulk) considered adequate by the endosonographer were put into formalin for histological process. Specimens considered inadequate were submitted for cytology assessment.

Histologic specimen was categorized “diagnostic” when considered adequate to reach a definitive diagnosis by the pathologist (including cases where IHC was mandatory), and “non-diagnostic” when the sample did not meet this requirement. IHC staining was performed using commercially available antibodies against c-kit (CD117), CD34, S-100, DOG-1, and smooth-muscle actin.

**Endpoints**

The primary endpoint was adequacy, defined as the rate of cases in which an adequate tissue specimen for histological examination was obtained.

Secondary endpoints were accuracy, defined as proportion of correct diagnoses, and adverse event rate. Standard references for the diagnosis were the surgical specimen when available or other diagnostic investigations and a follow-up of at least 6 months. Early (within 48 hours) and late (>48 hours) adverse events (AEs) were recorded.

All patients were evaluated for procedural AEs with a phone call or clinic visit at 24 to 48 hours and at 7 to 10 days following the procedure.

**Results**

Between January 2013 and June 2017, a total of 108 patients were referred to our department for tissue sampling of upper GI-SELS. Among them, 16 (14.8%) patients (10 male; median age, 67.8 years; range, 43 to 76 years) underwent EUS-FNB with 25G-PC as a rescue strategy after an initial inconclusive biopsy performed with larger-bore needles during the same EUS procedure (Table 1). Five patients had a SEL localized in...
the distal duodenum, five in the antrum, two in the gastric fundus and four in the gastric body. All SELs originated from the fourth sonographic layer of the gastrointestinal wall (i.e., muscularis propria) and showed a homogeneous hypoechoic echo pattern on EUS. Mean size of the lesions was 20.5 mm (range 18–24 mm). Previous EUS-FNB with larger size-needle (11 cases with 22G-PC needle and 5 cases with 19G-PC needle) failed in 10 cases because macroscopically suboptimal specimens were retrieved and in the other six cases because of technical issues (Table 2). Technical failure was mainly due to difficulty in advancing a large needle through the scope in an angulated position (such as the distal duodenum) and for the tendency of the needle to push the scope away from the gastrointestinal wall (as it happens in the greater curvature of the stomach).

EUS-FNB with 25G-PC was technically feasible in all subjects and enabled final diagnosis in nine out of 16 cases (56.2 %). IHC was feasible in all these adequate specimens. Regarding the subgroup of duodenal lesions, the procedure was successful in four of five (80 %) (Fig. 1). Final diagnoses with EUS-guided sampling were GIST (n = 6), leiomyoma (n = 2) and metastatic ovarian carcinoma (n = 1). All six patients with EUS-proven GIST were treated by surgery and confirmed at final pathology. Patients with leiomyoma were planned for follow-up. The patient with metastasis from ovarian cancer started palliative chemotherapy. Regarding the seven patients with non-diagnostic results with 25G-PC, two underwent wedge resection and GIST was confirmed on surgical specimens in both cases, five had endoscopic follow-up (no change was seen in a mean follow-up period of 23 months, ranging from 7 to 38 months).

No major procedure-related AEs were recorded irrespective of needle size.

| Table 2 | Technical results of EUS-guided fine-needle biopsy. |
|---------|-----------------------------------------------|
| Tumor location | Tumor size on EUS | Needle size at the first attempt (Gauge) | EUS-FNB results of first attempt | Histological results with 25-Gauge needle | Follow-up |
| A | 19 | 22 | Failure | Nondiagnostic | Follow-up |
| A | 20 | 22 | Suboptimal | GIST | Surgery |
| A | 20 | 22 | Suboptimal | GIST | Surgery |
| A | 21 | 22 | Failure | Nondiagnostic | Follow-up |
| A | 23 | 19 | Failure | Metastatic cancer | Chemotherapy |
| B | 18 | 22 | Suboptimal | Nondiagnostic | Follow-up |
| B | 20 | 22 | Suboptimal | Nondiagnostic | Follow-up |
| B | 22 | 19 | Suboptimal | Nondiagnostic | Surgery |
| B | 22 | 20 | Suboptimal | Nondiagnostic | Follow-up |
| B | 22 | 22 | Suboptimal | Nondiagnostic | Follow-up |
| B | 23 | 22 | Failure | Metastatic cancer | Chemotherapy |
| B | 24 | 22 | Suboptimal | Nondiagnostic | Surgery |

*EUS-FNB: endoscopic ultrasound fine-needle biopsy; GIST: gastrointestinal stromal tumor. Tumor location: A, antrum; B, body; D, distal duodenum; F, fundus; suboptimal, specimen macroscopically suboptimal for histology; failure, technical failure.*
Discussion

Endoscopic ultrasound (EUS) is considered the primary modality for evaluation of SELs. Furthermore, EUS-FNA enables tissue acquisition when needed. EUS-FNA has an overall diagnostic accuracy ranging from 60% to 80% in SELs [14, 15]. Several factors have been associated with inadequate tissue yield but the main ones are size and location of the lesion [16]. In fact, sampling adequacy increases proportionate with tumor size and poorer diagnostic yield has been generally associated with lesions smaller than 30 to 40 mm. Evidence from the literature supports this statement. In a retrospective study by Hoda et al on 112 upper GI-SELs, the diagnostic yield was 44.4% for lesions less than 10 mm and increased up to 58.3% for lesions ranging from 11 to 30 mm, and to 69.7% for lesions > 30 mm [14]. In another study on 53 subepithelial gastric lesions, EUS-FNA had an
overall diagnostic yield of 71% for lesions measuring up to 20 mm, 86% for lesions ranging 20 to 40 mm and 100% for lesions larger than 40 mm [17]. More recently Akahoshi’s group obtained a diagnostic rate of 73% from EUS-FNA of 90 gastric SELs smaller than 20 mm [18]. However, Sekine et al demonstrated that GIST can be correctly identified by EUS-FNA even in small lesions, with an overall sensitivity of 82.5% for GIST of any size, and 81.3% for GIST smaller than 20 mm [19].

Unfortunately, cytology is often not sufficient to reach a definitive diagnosis of GI-SELs and usually a proper histological sample is required, especially in view of IHC analysis. EUS-FNB PC needles have been conceived to obtain more tissue and ideally to provide histological specimen (core biopsy). Studies on core biopsy needles were mainly conducted on patients with pancreatic masses, while only a few studies are available looking at characterization of SELs [7,20–22]. In the first experience of Iglesias-Garcia et al on heterogeneous study population with intestinal and extra-intestinal lesions, EUS-FNB with 19G-PC was technically feasible in 98.2% of cases (112/114). In this study, 11 patients presented with upper GI-SELs and correct diagnoses were achieved in nine of them (81.8%) [8]. Kim et al have evaluated 12 patients with upper SELs, including esophageal, gastric and duodenal lesions, and EUS-FNB with a 22G-PC needle reached a diagnostic yield of 75% [20]. Similarly, Lee et al evaluated the efficacy of EUS-FNB with 22G-PC needle in gastric SEL, obtaining an overall diagnostic yield of 86% [21]. According to tumor location, the highest diagnostic yield was in the fundus (100%), followed by the body (89.5%), cardia (83.3%), and antrum (50%). In this study there were only two cases of antral lesions and only one had final diagnosis with EUS-FNB [21]. More recently, a larger study of 77 upper GI-SELs with EUS biopsy needle has been conducted to evaluate performance of EUS-FNB using a 22G-PC where diagnosis was achieved in 96.8% of cases [22]. Core biopsy tissue was obtained in 96.8% of the cases. Only a single case of post-procedural bleeding was recorded [22]. Recently, a new 20G-PC needle has been developed, which is expected to be a balanced compromise between flexibility, facility of use proper of the smallest needles, and quality of the tissue sampling, typical of the larger needle, providing echo endoscopists a new tool to accurately target lesions, regardless of their size or location [7]. Antonini et al published the first experience with this needle in a multicenter retrospective study for the diagnosis of SELs. A total of 50 SELs were included and after a mean number of passes of 2.2 (range 1–4), definitive diagnosis with full histological assessment including IHC was obtained in 88% of patients (44/50) without any major complications [7].

The external validity of these studies was strongly limited by the fact that most of the punctured lesions were >20 mm in diameter and a 22G-PC needle was used. Notably, in the current study, all the lesions were sampled with a 25G-PC needle and all of them were less than 25 mm. Indeed, our study showed than even in lesions ≤20 mm, the 25G-PC was able to achieve a diagnosis in 70% of cases (7/10).

Up to now, management algorithms for small GIST have been a matter for debate [23,24]. Natural history of small GISTs has not been well defined but even these lesions may present with malignant behavior and evolve into clinically relevant lesions [25,26]. Therefore, the European Society for Medical Oncology (ESMO) recommends EUS assessment for esophagogastric or duodenal SELs <20 mm and surgical excision of histologically proven small GISTs, unless that entails major morbidity [27].

EUS-guided tissue acquisition with 25G-PC needles in patients with pancreatic lesions resulted in high diagnostic yield, similar to standard 25-gauge FNA needles, able also to provide sufficient tissue specimen for histological assessment [28,29]. In the study by Iwashita et al, despite the low yield (32%) of a real “core,” histological analysis was possible in 63% of patients on the first pass and in 80% of cases on subsequent passes [28]. This indicated that a definitive diagnosis could be obtained based also on tissue fragments that do not meet the criteria for architecturally intact histology but can still yield a diagnosis based on cell morphology. In our study both histological core and tissue fragments were considered by the pathologist for the final diagnosis, including full IHC when required. The results show that EUS-FNB with 25G-PC enables definitive diagnosis in most of the assessed small upper GI-SELs, otherwise not fully characterized by other larger-bore needles. Indeed, correct diagnosis rates were 56.2% overall but 80% in duodenal lesions.

Other authors have highlighted the better performance of the 25G needle for SELs located in certain positions, such as the greater curvature of the stomach, where the needle tip may rebound, making it difficult to puncture the lesion [19]. The major advantage of the 25G needle is its thin caliber which makes EUS-guided sampling easier even in difficult sites. Transduodenal EUS-guided tissue acquisition can be technically challenging due to the angulated position that may hamper advancement of the needle through the scope and into the targeted lesion. Moreover, to avoid instrumental damage with larger-bore needles, often the scope has to be withdrawn into the stomach so the tip can be straightened.

Our study presents some limitations that should be acknowledged. The number of patients was relatively small and there were recruited in a single center (reducing the external validity of our findings). Follow-up was relatively short, ranging from 7 to 38 months after EUS-FNB, and not all patients underwent surgical resection as the gold standard for diagnosis. Follow-up of small GI-SELs is controversial. Koizumi et al. have showed that doubling time differs according to the type of SELs, and GISTs were confirmed to have a significantly shorter doubling time (17.2 months) than the other types of tumors, thus suggesting that even small SELs should initially be followed up within at least 6 months after detection [30].

To the best of our knowledge, this study represents the first investigation of the role of EUS-FNB with 25G-PC biopsy following a failed FNB performed with another size needle for characterization of small subepithelial lesions of the upper gastrointestinal tract. Therefore, larger prospective studies are warranted to confirm our results.
Conclusion
In conclusion, our study shows that in patients with small GI-SELS, additional tissue obtained with 25G-PC may represents a “rescue” strategy after an unsuccessful procedure with larger-bore needles, especially when lesions are localized in the distal duodenum.

Competing interests
None

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