EUS-guided single-incision needle-knife biopsy: description and results of a new method for tissue sampling of subepithelial GI tumors (with video)

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Background: The diagnostic efficacy of current tissue sampling techniques for upper GI subepithelial tumors (SETs) appears to be limited. Better tissue acquisition techniques are needed to improve the diagnostic yield in this setting.

Objective: Our purpose was to determine the safety and diagnostic yield of EUS-guided needle-knife incision and forceps biopsy (SINK biopsy) of upper GI SETs.

Design: Retrospective database review.

Setting: Academic tertiary-care referral center.

Patients: This study involved 14 consecutive patients referred for EUS evaluation of upper GI SETs with previous unsuccessful attempts at tissue diagnosis by conventional forceps biopsy.

Intervention: EUS-guided needle-knife incision and forceps biopsy.

Main Outcome Measurements: The safety and diagnostic yield of this method, compared with EUS-guided fine-needle aspiration (EUS-FNA), when possible.

Results: SINK biopsy provided tissue samples that were sufficient for definite histologic diagnosis in 13 of 14 cases (diagnostic yield 92.8%). There were 8 gastric GI stromal tumors. In 7 of 8, the size of SINK specimens allowed immunohistochemical analysis, and the evaluation of malignant potential was carried out by means of mitotic index determination in 5 cases (71.42%). SINK biopsies determined the pathological diagnosis of all (4 of 4) nonmesenchymal lesions. Eight patients underwent both EUS-FNA and SINK, with final histologic diagnosis determined in 6 of 8 cases (75%) by SINK versus 1 of 8 cases (12.5%) by EUS-FNA (Fisher exact test, $P = .023$). There were no procedure-related complications.

Limitations: A single-center, retrospective analysis with small sample size.

Conclusion: SINK biopsy appears to be an easy, safe, and effective technique for determining the definitive pathological diagnosis, evaluation of the malignant potential, and planning management of SETs. It could be a reliable alternative to conventional FNA, providing larger samples that improve the histologic yield.

EUS is the imaging technique of choice for evaluation of upper GI subepithelial tumors (SETs) because of its capability to typify size, layer of origin, and echo pattern of the lesions. However, EUS imaging alone frequently is not sufficient to provide an accurate diagnosis or evaluation of malignant potential, essentially in hypoechoic intramural masses. Therefore, a tissue diagnosis of this type of lesion is advisable. Endoscopic forceps biopsies usually fail to provide specimens adequate for diagnosis, so EUS-guided needle biopsies performed either with fine-needle aspiration (FNA) or trucut biopsy are needed. EUS-guided FNA (EUS-FNA) is currently considered the standard method for samples of GI SETs; however, the diagnostic yield in previous case series varies from 38% to 82%.[1-4] Moreover, most SETs are of mesenchymal origin, and FNA samples have only limited value in this setting by the lack of sufficient material for immunohistochemical analysis. It has been hypothesized that this problem might be overcome by using larger-bore needles or trucut biopsy (TCB), but recent studies report similar diagnostic yields because

Abbreviations: SET, subepithelial tumor; SINK, single-incision needle-knife; TCB, trucut biopsy.

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of the high rate of technical failures of TCB.[5] Safety concerns and difficulties in obtaining large enough samples to determine the mitotic index reliably, also have arisen around TCB.[5,6]

We present a novel method for histologic diagnosis of SETs, consisting of EUS-guided single-incision with needle-knife (SINK) and deep forceps biopsies. Results were compared when possible with those from EUS-FNA samples.

Take-home Message

- Histologic diagnosis of upper GI subepithelial tumors is challenging: EUS-guided single incision with needle-knife and forceps biopsy sampling (SINK biopsy) may represent a safe and effective technique for tissue diagnosis, treatment management, and evaluation of malignant potential.

METHODS

Data were collected retrospectively between April 2010 and February 2011 from consecutive patients with upper GI SETs and unsuccessful attempts at tissue diagnosis by conventional forceps biopsies. Procedure and pathology reports were reviewed and entered into an Excel database. The investigation was approved by the Institutional Review Board of Río-Hortega Hospital. A specific informed consent was obtained from all patients because permission to analyze their data in a retrospective study was required.

All the procedures were performed by the same dedicated endosonographer (C.S.H.), with the patients under conscious sedation. SETs initially were characterized by using a radial scanning echoendoscope (GF UM160; Olympus America Inc, Melville, NY), and then they were re-examined by linear EUS (GFUCT 140; Olympus America) for color and pulsed Doppler to scan the area for vessels. The accessory channel was used to pass through devices for tissue sampling.

A conventional needle-knife sphincterotome (Microknife XL; Boston Scientific Inc, Natick, Mass) connected to an electrosurgical unit (ICC 200; ERBE Electromedizin, Tübingen, Germany) was used. Blended current cautery at a setting of 30 W and 60 W output was selected. Under direct endoscopic vision, a 6 to 12–mm linear incision was made over the highest convexity zone of the lesion (Fig. 1; Video 1, with the demonstration of the entire procedure, available online at www.giejournal.org). A conventional biopsy forceps (Radial Jaw 4; Boston Scientific) was then deeply introduced, and 3 to 5 samples were obtained (Figs. 2-5). EUS-FNA (Fig. 2) was performed by using a linear echoendoscope with a 19 or 22 gauge (EUSN3 EchoTip; Wilson-Cook Medical Inc, Winston-Salem, NC) according to standard techniques, under real-time US guidance and color/pulsed Doppler control. Three to 5 (mean 3.5) passes were performed for each lesion. All patients were closely monitored and discharged 1 or 2 hours after the procedure was finished. Adverse events were evaluated at discharge from the endoscopy unit, by telephone call 24 to 48 hours after the procedure, and finally, by reviewing retrospectively the charts of each patient. Incisions were prophylactically closed with 2 to 3 endoclips (Resolution Clip; Boston Scientific).

Forceps biopsy specimens were fixed in formalin, embedded in paraffin, and stained with hematoxylin-eosin

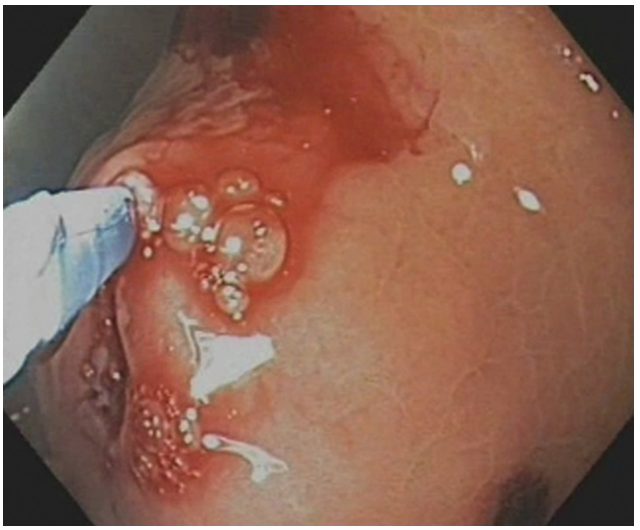

Figure 1. Needle-knife sphincterotome performing a linear incision over the center of a subepithelial mass in the second portion of the duodenum.

for histopathological examination. Examination included identification of cell type, cellularity, cytoplasmic features, nuclear atypia, immunohistochemical findings, and mitotic index. FNA material was processed for liquid-based cytology and placed in Cytorich red (Thermofisher Scientific, Waltham Mass), and a cell block was prepared. The mitotic index was determined on 50 consecutive high-power fields (HPF)[7] and only from SINK biopsy samples, because 19 to 22–gauge FNA specimens contain a maximum of 20 HPF.[5]

When conventional cytologic analysis revealed features of mesenchymal origin, further differentiation into GI stromal tumor and non-GI stromal tumor was performed by immunohistochemistry. The most specific immunohistochemical markers for GI stromal tumors (c-KIT-CD117, CD34, smooth muscle actin, Ki-67, S-100) were analyzed with commercially available monoclonal/polyclonal primary antibodies. There was no cytotechnologist or cytopathologist on site.

Patient demographics and SET characteristics were analyzed. Continuous variables were expressed as mean and range. The comparison between the diagnostic yields of EUS-FNA and SINK was assessed by using the Fisher exact

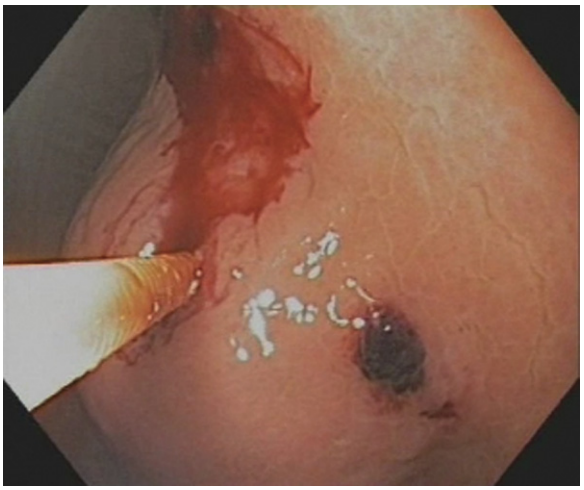

Figure 2. Biopsy forceps introduced through the hole, taking multiple tissue samples inside.

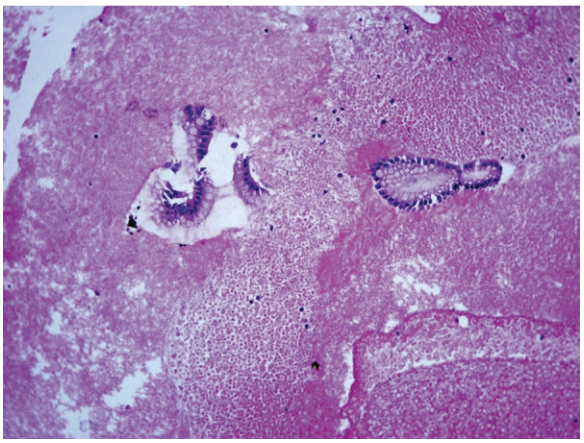

Figure 3. Cell block specimen from the lesion obtained with 22-gauge FNA: fibrin-hematic background with scattered glandular structures without signs of atypia. There are no mesenchymal or spindle-shaped cells. (H&E orig. mag. × 10)

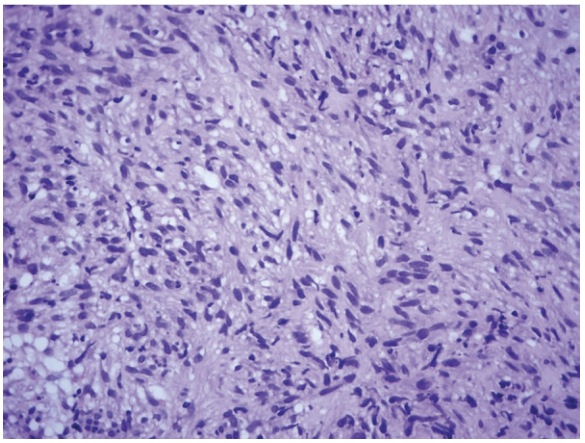

Figure 4. Forceps biopsy specimen from the same lesion obtained by the single-incision needle-knife biopsy procedure: cluster of spindle cells with oval-shaped prominent nuclei, anysochariosis, and irregular cytoplasmic margins (H&E, orig. mag. × 40).

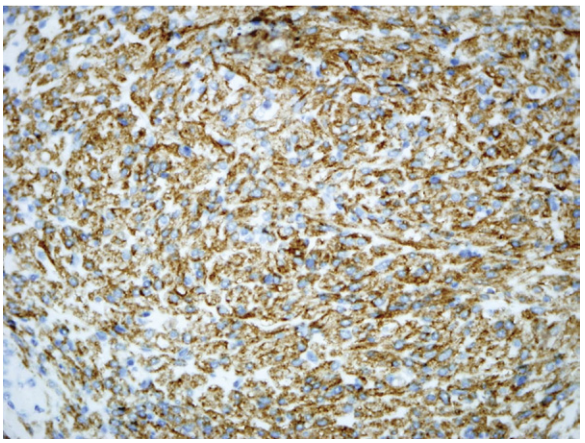

Figure 5. Biopsy sample revealing brown immunostaining, positive for c-KIT (CD117) (Orig. mag. × 100).

test performed by a statistical software package Epidat 3.0 (Dirección Xeral de Saùde Publica, Xunta de Galicia, Panamerican Health Organization, 2003).

RESULTS

During the study period, a total of 14 patients were included: mean age 63.5 years (range 35-85 years), 4 men, 10 women. Table 1 summarizes data of procedures, location and size of the lesions, histopathological results, and patient characteristics. All patients with SETs diagnosed during the study period were primarily included. The first 9 procedures were performed by using the radial scanning echoendoscope first and then the linear echoendoscope, but finally only the linear echoendoscope was used to simplify the technique to a 1-step procedure (patients 10-14).

The global diagnostic accuracy of SINK biopsy tissue sampling was 92.8% (13 of 14 cases), with immunohistochemical analysis in 9 patients. There were 8 gastric GI stromal tumors, with immunohistochemical diagnosis in 7 and samples amenable for mitotic index evaluation in 5 of 7 (71.42%). Four cases were classified as low risk for malignant potential (mitotic index <5/50 HPF).

Tissue sampling was carried out only by SINK biopsy in 6 of 14 patients (42.85%). In the remaining 8 patients (57.14%), both EUS-FNA and SINK were performed to obtain more material and compare both techniques. In this group of patients, SINK provided a final histological diagnosis in 6 of 8 cases (75%) versus 1 of 8 cases (12.5%). FNA: ($P = .023$, 1-tailed Fisher exact test) only in this case (number 4) was immunohistochemical study possible from FNA specimens. Another two patients had FNA cytology specimens suspicious for GI stromal tumor on account of isolated spindle cells, with the final diagnosis settled by SINK.

In 1 case (number 5) both techniques were nondiagnostic. Final surgical confirmation was obtained in 3 patients (5, 6,

TABLE 1. Endoscopic, demographic, and clinicopathologic characteristics of the patients and lesions

Patient no., tumor location	Tumor size (cm)	Age/sex	Layer	Endoscopes	FNA(Ø)	FNA cytology	SINK biopsy
1. Esophagus (distal)	3.54	59/M	4th	EUSr + EUSl	22 G	Nondiagnostic	Leiomyoma
2. Stomach (fundus)	3.93	85/F	4th	EUSr + EUSl	22 G	Suspicious (spindle cells)	GIST, IH+, MI ↓
3. Stomach (body)	2.82	66/F	4th	EUSr + EUSl	No	No	GIST, IH+
4. Stomach (body)	3.50	74/F	4th	EUSr + I + GS	22 G	GIST, IH+	GIST, IH+
5. Stomach (body)	6.43	51/M	2nd	EUSr + EUSl	19 G	Nondiagnostic	Nondiagnostic
6. Stomach (body)	3.37	82/F	4th	EUSl	No	No	GIST, IH+, MI ↓
7. Stomach (antrum)	1.56	43/F	3rd	EUSr + EUSl	No	No	Heterotopic pancreas
8. Stomach (antrum)	2.81	35/F	3rd	EUSr + EUSl	22 G	Nondiagnostic	Inflammatory fibroid polyp
9. Duodenum (2nd portion)	3.83	69/M	3rd	EUSr + EUSl	No	No	Lipoma
10. Stomach (antrum)	4.95	66/M	4th	EUSl	22 G	Suspicious (spindle cells)	GIST, IH+, MI ↑
11. Stomach (antrum)	1.23	69/F	3rd	EUSl	19 G	Nondiagnostic	Inflammatory fibroid polyp, IH+
12. Duodenum (2nd portion)	1.95	58/F	3rd	EUSl	No	No	Gangliocytic paraganglioma, IH+
13. Stomach (body)	2.46	53/F	4th	EUSl	22 G	Nondiagnostic	GIST, IH+, MI ↓
14. Stomach (fundus)	1.34	79/F	4th	EUSl	No	No	GIST, IH+, MI ↓

SINK, Single-incision needle-knife biopsy; *M,* male; *EUSr,* radial echoendoscope; *EUSl,* linear echoendoscope; *G,* gauge; *F,* female; *GIST,* GI stromal tumor; *IH,* immunohistochemical; *MI,* mitotic index; *GS,* gastroscope.

10). There were 10 mesenchymal tumors (8 gastric GI stromal tumors) and 4 of nonmesenchymal origin (inflammatory fibroid polyp, 2; heterotopic pancreas, 1; atypical EUS lipoma, 1), with final histologic diagnosis achieved through SINK in all of them. No cautery artifacts were described in any sample. There were no procedure-related complications.

DISCUSSION

Upper GI SETs or bulges are usually found incidentally during routine endoscopy. The differentiation between the different types is important because these lesions may have different management, prognoses, or therapeutic options. EUS morphologic features alone have limited specificity for the diverse subtypes of SETs, and EUS fails to provide data about benign or malignant origin: overt malignancy is rare, but potential malignancy is a usual scenario. The most common SETs encountered during gastroscopy are mesenchymal neoplasms, most often GI stromal tumors.

There is currently no consensus regarding the optimal management strategy for incidentally detected, small SETs,[8] and practice patterns of endosonographers in surveillance and management are highly variable.[9] It was previously said that lesions labeled GI stromal tumors based on EUS image features and size <3 cm had a low malignant potential, which would justify just EUS observation rather than cytohistologic diagnosis or removal.[10] However, the current thinking is that all GI stromal tumors have the potential for malignant behavior, even from a size of 1 cm.[11,12] Therefore, accurate diagnosis and evaluation of potential malignancy is mandatory, especially for those lesions that are hypoechoic, located in the stomach, and >2 cm.[13,14] So, appropriate techniques are required to obtain adequate specimens for cytological and immunohistochemical analysis.[4,13]

EUS-FNA has only limited value for conventional cytologic diagnosis of SETs, especially in nonmesenchymal lesions (nondiagnostic samples up to 100%[4]) but also in GI stromal tumors, with a high rate of failure in immunostaining of EUS-FNA samples, which drops the diagnostic yield from 70% to 81%[4,5,15,16] to 53%[5] after immunohistochemical analysis or even 34%.[4] Reasons for the low performance of EUS-FNA in this setting are probably secondary to the stiffness and rubbery consistency of mesenchymal tumors, which prevents deep insertion of the needle and limits aspiration of the amount of cells needed, even more with small-gauge needles. Large-caliber cutting needles were designed to overcome many of these problems, allowing us to acquire larger tissue specimens, preserving architecture, and providing histologic rather than only

cytologic diagnosis. However, only the further development of the trucut needle seemed to solve these limitations. EUS-guided TCB theoretically provides core tissue specimens that could increase histologic diagnostic performance by allowing taking of thicker samples, even though recent studies[5,6,16] show that the diagnostic yield of TCB in GI stromal tumors is moderate (47%-63%) and not superior to that of EUS-FNA. The high rate of technical failure secondary to the stiffness of the device hinders the needle from obtaining tissue when the needle is used in SETs of the gastric fundus and duodenum.[17,18] Moreover, TCB specimens are too small to determine the malignant potential of GI stromal tumors[6,13] because they cannot provide enough material to get 50 HPF. There are also concerns about safety, with two cases of sepsis among 52 procedures performed for gastric SETs,[6] and there is a risk of peritoneal spillage of malignant cells after puncture of the lesions.[19]

Endoscopic partial resection—the "unroofing" technique—has been advocated as a choice in this setting,[20] but preliminary series report a high rate (56%) of bleeding secondary to snaring. There are other technical limitations like difficulties in grasping SETs when they have extraluminal growth or slippage when the lesions are rubbery. Therefore, other EUS-based tissue acquisition techniques such as SINK biopsy are required, which ensure harvesting of adequate samples for histopathologic and immunohistochemical analysis and mitotic index evaluation that can be done in an easier and safer way. The results of our preliminary study show some advantages over the procedures used previously, providing sufficient tissue samples for definitive diagnosis, both in mesenchymal and nonmesenchymal tumors in a cost-effective manner, reducing the number of repeated explorations secondary to unsatisfactory samples or avoiding unnecessary follow-up. In addition, the technique is simple to perform, even more so after development of a single-step procedure (cases 10-14), which avoids the change of echoendoscope. Furthermore, SINK biopsy seems a safe procedure. There were no procedure-related complications such as perforation, pain, or bleeding, probably because of prophylactic insertion of clips. However, the study has several weaknesses including its retrospective nature, small sample size, and short follow-up of patients after procedures, which may not allow the detection of potential delayed complications.

In conclusion, SINK biopsy may represent an easy, safe, and effective technique for accurate diagnosis, evaluation of malignant potential, and treatment management of SETs. It could be a reliable alternative to conventional EUS-FNA and TCB, although larger, prospective, and comparative studies are required to confirm its superiority in this setting and to assess the potential impact in the diagnostic and therapeutic management of upper GI SETs.

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