—Original Article—

Canalization technique to obtain deep tissue biopsy of gastrointestinal subepithelial tumors as an alternative to conventional known techniques

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ABSTRACT

Background and Objectives: The most accurate technology to detect and diagnose subepithelial tumors (SETs) is the endoscopic ultrasonography (EUS) combined with puncture techniques, such as the endoscopic ultrasonography-guided fine-needle aspiration (EUS-FNA) or the endoscopic ultrasonography-guided fine-needle biopsy. Going further in the improvement of the results of tumor samples obtained endoscopically to diagnose the SETs, the canalization technique guided by miniprobes (MPs) to obtain biopsies of SET could be an alternative to EUS-FNA. The objective of this study is to analyze the results of samples obtained by this procedure. **Materials and Methods:** A multicenter, retrospective study of a review of a database of 32 consecutive patients with a SET in the digestive tract, from 2000 to 2015 was conducted. All patients underwent EUS-performed by MP, to define the size, internal echostructure, and layer of origin of tumor. Once the echostructure was defined, it proceeded to the canalization technique to arrive to the tumor tissue. **Results:** The average diameter of SETs in this series (32 patients) was about 21.6±11 mm (range: 5–41 mm). The diagnostic accuracy was 28/32, 87.50% (Confidence interval 95%: 76.04%–98.99%), and there were no major complications. All procedures were performed on outpatients, none of which required additional hospitalization. The 50% of patients were operated or endoscopically resected and in all cases, the previous pathological diagnosis was confirmed. **Conclusions:** This is a feasible, safe, and effective procedure that allows to access to inside of SET to obtain deep biopsies. Tumor samples obtained by deep biopsy, with prior performing of the canalization technique guided by MP, were sufficient for histopathological and immunohistochemical diagnosis and similar to those obtained with other known methods (FNA Trucut, ProCore®, etc.). However, more prospective comparative studies with a larger number of patients and different specialists carrying out the procedure to reach a higher statistical significance are necessary.

Key words: Canalization technique, deep biopsy, endoscopic ultrasonography-guided fine-needle aspiration, endoscopic ultrasonography-guided fine-needle biopsy, miniprobe, subepithelial tumor, submucosal tumor

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INTRODUCTION

There is a wide consensus regarding the endoscopic ultrasonography (EUS) as the gold standard method for the evaluation of subepithelial tumors (SETs) with a very high sensitivity, over 90%.[1,2] SETs may arise from deep mucosa to deeper serosa, depending on the histological type. However, the endoscopic biopsy which tends to be superficial, does not usually confirm the etiologic diagnosis of SET. That is why new EUS-guided methods have been devised such as fine-needle aspiration (EUS-FNA) (average diagnostic yield 87%), EUS-trucut biopsy, and ProCore® (average diagnostic yield 85%) for acquisition of tissue samples.

The EUS-guided by miniprobe (MP) is important to define the tumor size and EUS characteristics (internal echostructure, edge and layer of origin) since these data can help in the tumor management. The most relevant predictor of malignancy is the tumor size with cut offs of 4.05 and 6.40 cm, according to various authors, especially concerning to the gastrointestinal stromal tumor (GIST)[3] [Figure 1]. When the tumors are about 2 cm in size, an annual revision is recommended to control the growth and possible degeneration comparing with the initial histological material obtained by techniques guided by EUS.[4]

The purpose of our original work is to evaluate the canalization technique to perform deep biopsies of SETs as an alternative to known techniques, such as EUS-FNA and ProCore®, through a multicenter, retrospective, and open study of an endoscopic database from 2000 to 2015.

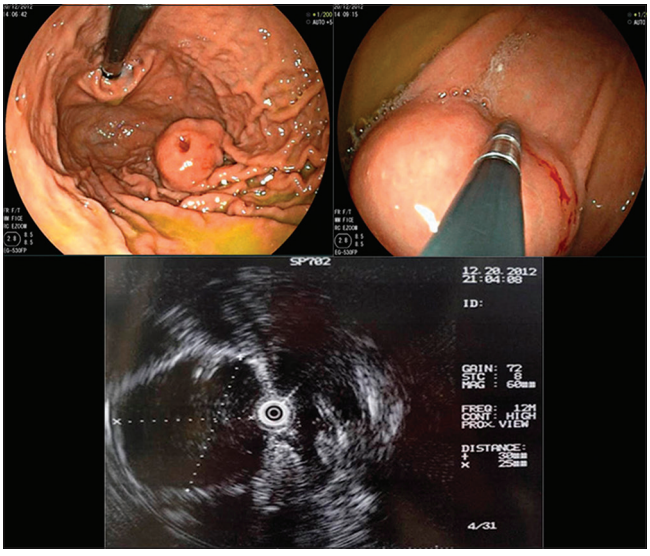

Figure 1. Subepithelial tumor (gastrointestinal stromal tumor) and miniprobe EUS (12 MHz)

MATERIALS AND METHODS

In this study, three private centers participated with a total of 32 patients, 19 men and 13 women included (average age of 58 years). All explorations were carried out after the patients signed the corresponding informed consent.

All centers used the same protocol and all procedures were performed by the same specialist, who worked in the three hospitals at the same time and used the canalization as the unique procedure for obtaining deep biopsy of SET, with the assistance of an anesthetist for the sedation.

As this is not a generalized method, all procedures were performed by the same specialist since this technique or procedure was a Dr. Abad's invention that along this time has been developing to reach an optimized method. The rest of specialists of these hospitals used the EUS-FNA procedure to acquisition of tissue samples of SET.

Canalization was applied to all patients that were attended by Dr. Abad in three hospitals. Patients were not chosen for this procedure, they underwent canalization because the day that they requested the doctor's appointment, coincided with Dr. Abad's visit days.

After observing the good results of canalization procedure, it would be interesting to carry out a study with multiple investigators, previously trained by Dr. Abad, to strengthen the validity of this method.

Inclusion criteria

Consecutive patients with a SET in the digestive tract that required the acquisition of tissue for the anatomopathological diagnosis and therapeutic management of tumor were included in the study.

Exclusion criteria

Patients with coagulopathies that could hinder the biopsy, patients with polyps, cystic SETs, or extrinsic compression of the digestive tract were excluded from the study.

Procedure

An endoscopy with sedation (propofol) was performed to all patients (EG 530 FP-XL 4450 with processor VP4450 HD, Fujinon, Tokyo, Japan).

When the endoscope arrived in the tumor zone, the EUS was conducted by MP of 12 MHz (Olympus and Fuji, Tokyo, Japan) [Figure 1], to measure the maximum size, to define the internal echostructure (hypoechoic, hyperechoic or mixed), the edge and layer of origin of SET.

Endoscopic intervention

Once the tumor data were obtained by EUS-guided by MP, the SET was typified and an extrinsic compression was discarded, the endoscopist proceeded with the steps of the canalization technique:

a. The mucosa covering the subepithelial was pressed perpendicularly with the tip of a disposable polypectomy snare (Boston Scientific and Interplex Industries, Inc., Andrei. Hangzhou, China) [Figure 2]
   At the same time, some millimeters were electrofulgurated to reach the inside of SET (the fulguration was of one-third of the tumor size, previously measured by EUS MP)
   This procedure allowed us to build a suitable canal for the passage of the biopsy forceps toward the tumor and decrease the risk of bleeding due to the electrofulguration of the tissue vessels that the technique offered as well [Figure 3]
b. When the canal was performed, the conventional disposable biopsy forceps (Boston Scientific and Fuji) were introduced through the channel, to blindly obtain an average of five random samples of tumoral tissue for histopathological and histochemical study [Figure 4].
   After each biopsy, the endoscope was removed with the biopsy forceps at the tip and the sample was not passed along the operator's channel. This procedure was made for each sample to prevent loss tissue (average amount of tissue obtained ≃4 mm).
   Further, if it was required due an arterial bleeding, we proceeded to fulgurate (electrode scalpel ERBE ICC 300 HINT) using the same polypectomy snare with ionized argon gas (emed. Spectrum-line trolley) and/or with a metallic clip (Boston Scientific, EEUU) placed at the insertion point.

Anatomopathological studies

Formalin-fixed and paraffin-embedded tissue samples were cut in 4-μm-thick sections on a microtome using standard procedures. Hematoxylin and eosin was the stain of choice for routine examination [Figure 5]. When immunohistochemical differential diagnosis was needed, different diagnostic panels were used, depending on the morphological pattern. For the distinction between GIST and gastric smooth muscle or neural tumors, the panel of markers was smooth muscle actin, DOG1, c-kit (CD117), CD34, and PS100. To establish the diagnosis and classification of lymphomas, we used a pankeratin antibodies cocktail (AE1, AE3), leukocyte common antigen, and different B- and T-cell markers to identify the immunophenotype (CD20, CD79a, CD3, CD5).

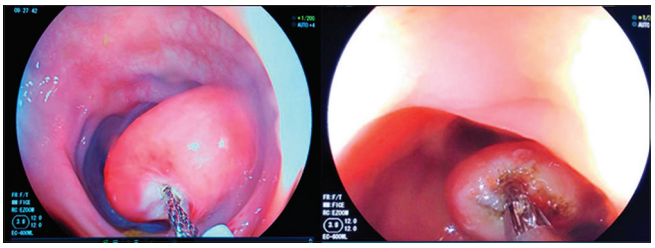

Figure 2. Performing of canal by pressing with the tip of the polypectomy snare (subepithelial tumor of the colon)

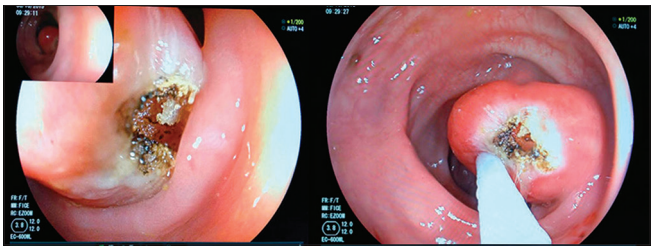

Figure 3. View of the canal after the canalization technique (subepithelial tumor of the Colon)

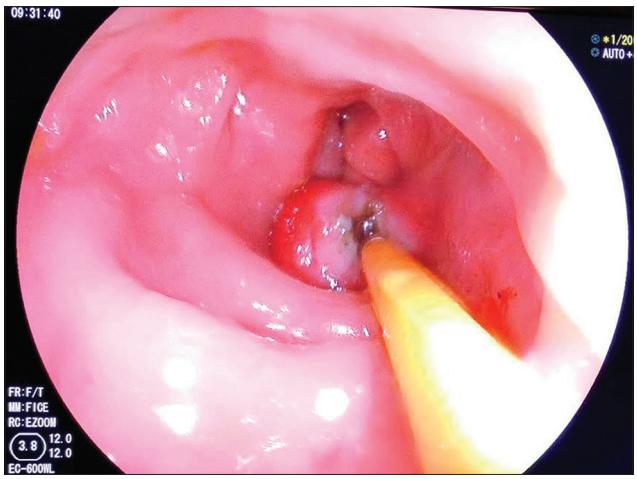

Figure 4. Tumor deep biopsy through the canal with the biopsy forceps to obtain samples for pathological diagnosis (subepithelial tumor of the colon)

Statistical analysis

Statistical analyses and modeling were carried out with the statistical software package version 3.0.2 (R Core Team 2015). Several functions including confidence intervals (CIs) of diagnostic sensitivity were done using the R-package binom (Dorai-Raj S, 2015).

The continuous variables were expressed as the arithmetic mean, median, standard deviation interval, and percentages. The diagnostic sensitivity, regarding to the biopsy result, was obtained by the formula: TP/(TP + FN), where TP and FN mean true positive and false negative, respectively. The CI of 95% of sensitivity was calculated using the exact Pearson-Klopper method.

RESULTS

The sample size was of 32 patients (19 men and 13 women) with a mean age of 58 ± 16 years (range: 16–83 years) and all of them with a SET in the digestive tract that was biopsied (an average of 5 samples per patient). The average diameter of SETs was 21.6 ± 11 mm (range: 5–41). The distribution of tumors was 5 esophageal (15.63%), 22 gastric (68.76%), 1 duodenal (3.12%), 1 ileal (3.12%), and 3 located at colon (9.37%) [Tables 1 and 2].

Diagnostic sensitivity, after anatomopathological confirmation or follow-up, was 28/32 (87.5%: [71.01%–96.49%]) and 26/28 (92.85%: [76.50%–99.12%]) for SETs ≥10 mm. When the SETs were ≥12 mm, the diagnostic yield was (22/22) (100%: [84.56%–100%]) (exact Pearson-Klopper method 95% CI).

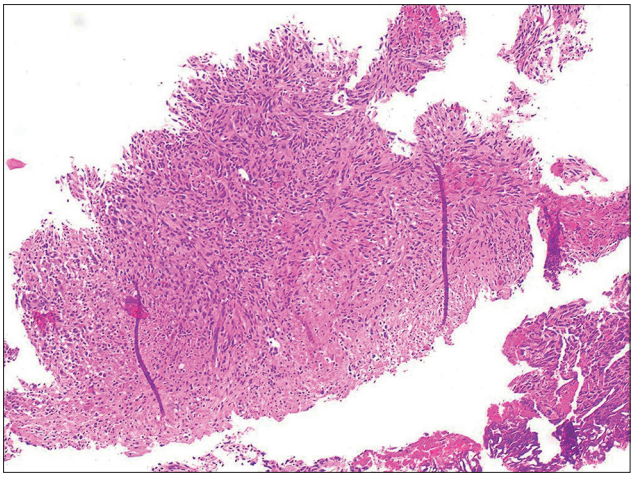

Figure 5. Gist with spindle cells. The sample was obtained by deep biopsy after canalization technique

The anatomopathological diagnosis of the samples obtained was 5 lipomas, 2 fibroid polyps, 7 leiomyomas, 1 schwannoma, 5 ectopic pancreases, 2 lymphomas, 1 epithelial stromal tumor, 3 GISTs, and 2 leiomyosarcomas. There were no major complications such as perforation or arterial bleeding, only two small bleedings (6%) with drooling that were solved with conventional hemostatic methods (fulguration using the same polypectomy snare). All procedures were performed on outpatients and none of them required further hospitalization. 50% of patients were operated or endoscopically resected accordingly to the anatomopathological results and in all cases, the previous pathological diagnosis was confirmed.

Despite leiomyoma is a benign smooth muscle neoplasm and very rarely becomes cancer (0.1%), has the ability to ulcerate and bleed. This was the reason why the small tumors such as cases 1 and 3 [Table 1], after anatomopathological diagnosis, were resected. Endoscopic resection was the procedure chosen, due to the small size of lesions and the risk-benefit of the procedure.

In other cases with larger lesions, the decision to proceed with surgery was taken based on the location of tumor and the risk-benefit ratio for patients.

DISCUSSION

In this study, we have shown that the canalization technique is feasible and safe to take deep biopsies from SETs and has allowed us to make 87% of

Table 1. Demographic and clinicopathological characteristics of the study patients with tumor size ≤10 mm (*n*=8)

Age/gender	Size (mm)	Echostructure/layer of origin/localization	Biopsy result	Complications	Diagnosis	Surgery/follow-up
32 female	5	Hypoechoic/4th/esophageal	B+	No	Leiomyoma	Endoscopic resection
16 female	7	Hypoechoic/4th/gastric	B−	No	Leiomyoma	Follow-up
27 male	8	Hypoechoic/4th/gastric	B+	No	Leiomyoma	Endoscopic resection
56 female	9	Hypoechoic/-/gastric	B−	UGB: 3 clips	-	Follow-up
72 female	10	Hypoechoic/2nd/gastric	B+	No	Leiomyoma	Follow-up
55 male	10	Hypoechoic/3rd/gastric	B+	No	Lipoma	Follow-up
55 male	10	Hyperechoic/3rd/gastric	B+	No	Lipoma	Follow-up
69 male	10	Hypoechoic/3rd/gastric	B−	No	Ectopic pancreas	Follow-up

UGB: Upper gastrointestinal bleeding, B+: Positive biopsy correct diagnosis, B−: Negative biopsy, without diagnosis

Table 2. Demographic and clinicopathological characteristics of the study patients with tumor size >10 mm (*n*=24)

Age/gender	Size (mm)	Echostructure/layer of origin/localization	Biopsy result	Complications	Diagnosis	Surgery/follow-up
64 male	12	Hypoechoic/3rd/gastric	B+	No	Fibroid polyps	Follow-up
64 male	12	Hypoechoic/4th/gastric	B−	No	Leiomyoma	Follow-up
31 male	12	Hypoechoic/3rd/gastric	B+	No	Ectopic pancreas	Follow-up
57 female	15	Hyperechoic/3rd/gastric	B+	UGB: Argon	Fibroid polyps	Follow-up
66 male	15	Hypoechoic/3rd/gastric	B+	No	Ectopic pancreas	Follow-up
56 male	15	Hypoechoic/3rd/gastric	B+	1 metallic clip	Ectopic pancreas	Follow-up
57 female	20	Hypoechoic/3rd/gastric	B+	No	Ectopic pancreas	Follow up
45 female	20	Hyperechoic/3rd/colon	B+	No	Blind lipoma	Follow-up
55 male	21	Hypoechoic/4th/esophageal	B+	No	Leiomyoma	Follow-up
50 male	30	Mixed/4th/duodenal	B+	No	Sarcoma	Surgery
51 female	30	Hyperechoic/3rd/gastric	B+	No	Lipoma	Endoscopic resection
63 male	30	Hypoechoic/4th/gastric	B+	No	GIST	Surgery
70 male	30	Hypoechoic/4th/gastric	B+	No	Sarcoma	Surgery
80 male	30	Hypoechoic	B+	No	Lymphoma	Surgery
64 male	30	Hypoechoic/4th/gastric	B+	No	GIST	Surgery
50 male	30	Mixed/3rd/gastric	B+	No	Ectopic pancreas	Follow-up
40 female	30	Hypoechoic/3rd/gastric	B+	No	Schwannoma	Endoscopic resection
65 male	31	Hypoechoic	B+	No	Lymphoma	Surgery
60 female	35	Hyperechoic/3rd/ileal	B+	No	Lipoma	Surgery
83 male	35	Hypoechoic/4th/gastric	B+	No	GIST	Surgery
79 female	35	Hypoechoic/4th/gastric	B+	No	Epithelioid	Surgery
59 female	40	Hypoechoic/4th/esophageal	B+	No	Leiomyoma	Surgery
57 male	40	Hypoechoic/4th/esophageal	B+	No	Leiomyoma	Surgery
58 male	41	Hypoechoic/4th/esophageal	B+	No	Leiomyoma	Follow-up

UGB: Upper gastrointestinal bleeding, B+: Positive biopsy correct diagnosis, B−: Negative biopsy, without diagnosis, GIST: Gastrointestinal stromal tumor

diagnostics; nevertheless, the number of patients is limited to 32 cases and is not enough to obtain a strong statistical result.

The main difficulty and limitation for diagnosis of SETs are the tumor size.[5] The efficiency is not optimal when the SET is smaller than 10 mm.[6] In a very recent review, it was stated that the acquisition of tissue of SETs depends on the localization, layer of origin, size, ulceration, vascularization, characteristics of patient, and the experience of the endoscopist; quoting only four studies as alternative to the FNA.[7,8-10] In Table 2, it can be checked that the studies conducted subsequently by Japanese and Korean authors, as well as ours, showed high rates of diagnostic sensitivity, the average value of which would be 89%.

Diagnostic sensitivity of our study was 87.5% (28/32). Excluding the data of those patients with SET smaller than 10 mm, the diagnostic sensitivity was 92.85% (26/28), similar to that of Kataoka *et al.* (18/18, 100%) and of Kobara *et al.* (8/8, 100%).[11,12] The same as us, they also used MP for the assessment of SETs before performing the tunneling technique and the deep biopsy. With our technique, we obtained a diagnostic yield of 100% (22/22) when the diameter of SET was greater to 12 mm.

One of the most recent reviews about the convenience of the puncture of SETs establishes that the EUS-FNA succeeds in making a diagnosis in the 75%–100% of the cases (mean 87%) with a very low mortality rate (0%–2%).[13] Using ProCore®, the diagnostic sensitivity seems to be between 80% and 90%, mean 85% although more exhaustive studies might be necessary since the sample size in these two studies was n = 11 and 13, respectively.[8,9] Our data are similar to their results with EUS-FNA and upper to ProCore®. Our group has obtained and published a yield of 80% with the EUS-FNA in the cytohistological diagnosis of SETs and an efficacy of 98% in the endoscopic resection with only 4% of upper gastrointestinal bleeding (UGB).[4,14]

The procedure of canalization described and applied in our work could be an alternative to EUS-FNA or be useful in those cases where EUS-FNA had

a previous negative result. The advantages would be that this technique can be performed during the endoscopic-echoendoscopic diagnosis, and otherwise, the samples obtained are usually better to evaluate the microanatomy of the tissue and the immunohistochemical profile than the FNA material.

Consequently, less time is required, a pathologist in the room is not required, it can be done using conventional materials and might be cost-effective, according to a recent study[15] [Table 3].

Alternatives to the EUS-FNA would also be the "biopsy-on-biopsy" with conventional forceps or using a macrobiopsy forceps, with percentages of UGB lower than 42%, according to the review of Lee *et al.*[16] However, some variations based on this technique with better results have been recently published. These studies are compared in Table 4.

The partial resection of SETs, as a method to obtain sufficient tissue samples for pathological diagnosis, has a yield of 93.7%.[16]

Komanduri *et al.*, de la Serna-Higuera *et al.*, and Buscaglia *et al.* compared the biopsy of SET with the EUS-FNA, with unfavorable results for this technique.[10,17,18]

Table 3. Features by technique

	EUS-FNA	ProCore®	Deep biopsy
Average sensitivity (%)	87	85	89
Pathologist at room	Yes	No	No
Complications (%)	0-2	ND	5
Cost-effectiveness	++	+	+++

EUS-FNA: Endoscopic ultrasonography-guided fine-needle aspiration

Ihara *et al.* and Kim *et al.* used EUS guided by miniprobe or radial EUS and subsequently, they performed the biopsy. They obtained excellent results, similar to our series, and only Kim *et al.* referred one perforation as complication (9%).[19,20]

Tae *et al.* analyzed a more sophisticated technique. after the EUS, they performed a submucosal dissection and deep biopsy, with whose results they obtained a change in the SETs management. Procedure-related bleeding and perforation rates were both 4%.[21]

The Japanese group of Kobara *et al.* using the submucosal dissection they have reached diagnostic results in 100% of biopsies, without complications such as perforation or UGB,[12,22] whereas Buscaglia *et al.* presented a far from negligible number of UGB of 35%.[18] Other studies as well as ours did not observe an evaluable UGB. In our series, the incidence of bleeding was 6%.

Nakai *et al.* have carried out a pilot study to evaluate safety and efficacy of EUS-guided through-the-needle forceps biopsy (EUS-TTNFB). Along the study, they performed eighteen sessions of EUS-TTNFB to 17 patients with solid lesions, using a combined technique of 0.75 mm biopsy forceps through a 19-gauge FNA needle, with a median of three passes per session, achieving very good results, providing additional tissue acquisition than a single method, with no adverse events, and good accuracy to diagnose malignancy.

This is the first work that combines both techniques. In our study with 32 cases of SET, only deep biopsy was

Table 4. Bibliographic review by authors

Author	Cases	Average diameter (mm)	Deep biopsy sensitivity, %	EUS-FNA sensitivity, %	Complications, %
Komanduri *et al.*[10]	72 SET	ND	92	58	NC
de la Serna-Higuera *et al.*[17]	14 SET	31	93-75	12.5	NC
Buscaglia *et al.*[18] (multicenter)	129 SET	15	59	45	35 UGB
Ihara *et al.*[19]	27 GIST	21.2	85 (23/27)	ND	NC
Kim *et al.*[20]	11 GSET	21	91 (10/11)	ND	9 PER
Kataoka *et al.*[11]	18 GSET	20.3	100	ND	NC
Tae *et al.*[21]	40 SET	20.3	90 (36/40)	ND	4
Kobara *et al.*[12]	8 GSET	ND	100	ND	NC
Kobara *et al.*[22]	26 GSET	20.25	100	ND	NC
Okuzono *et al.*[15]	27 SET	>15	85	90	NC
Abad 2015 (multicenter)	32 SET	21.6	87.5	ND	6 UGB

ND: No data, NC: No complication, UGB: Upper gastrointestinal bleeding, PER: Perforation, EUS-FNA: Endoscopic ultrasonography-guided fine-needle aspiration, SET: Subepithelial tumor, GIST: Gastrointestinal stromal tumor, GSET: Gastric subepithelial tumor

performed. It seems that only 2 of the 17 cases were SETs, so more studies with more patients with SET are needed to test the effectiveness of EUS-TTNFB for this type of tumors that probably will obtain good results.[23]

CONCLUSIONS

The canalization technique to perform a deep biopsy of SETs appears to be a feasible, safe, and effective procedure for determining the definitive pathological diagnosis since the samples obtained were sufficient to establish the diagnosis of SETs in the digestive tract in 87% of all cases, in 93% of SETs equal or larger than 10 mm, and in 100% when were larger than 12 mm.

It could be a reliable alternative to conventional known techniques, such as EUS-FNA and ProCore®. However, more prospective comparative studies with a larger number of patients and with different specialists carrying out the procedure to reach a higher statistical significance are necessary.

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Conflicts of interest

There are no conflicts of interest.

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