—Original Article—

Role of endoscopic ultrasound and endoscopic-ultrasound-guided fine-needle aspiration in endoscopic biopsy negative gastrointestinal lesions

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ABSTRACT

Background and Objectives: Many cases of gastrointestinal (GI) tumors as lymphoma, adenocarcinoma, and most of submucosal tumors (SMT) such as gastrointestinal stromal tumor (GIST) and leiomyoma are difficult to diagnose as they frequently yield negative endoscopic biopsies. We evaluated the accuracy of endoscopic ultrasound (EUS) and EUS-guided fine-needle aspiration (EUS-FNA) in the diagnosis of endoscopic biopsy negative GI tumors. **Patients and Methods:** One hundred and nine patients with biopsy negative GI tumors were included in this prospective study. EUS and EUS-FNA were performed to all patients with cytopathologic examination. **Results:** There were 109 patients with endoscopic biopsy negative GI lesions, including 61 males (56%) and 48 females (44%), with the mean age of 54 years. Sixty-three cases (57.8%) were proved to have malignant lesions, among them there were 15 cases with high-risk GIST as proved by FNA and excision biopsy. Forty-six cases (42.2%) were proved to be benign; among them there were 21 cases presented with non-high-risk GIST. Endoscopic ultrasound had a sensitivity of 96.8%, specificity of 89.1%, positive predictive value (PPV) of 92.4%, negative predictive value (NPV) of 95.3%, and accuracy of 93.6%. EUS-FNA had a sensitivity of 87.3%, specificity of 100%, PPV of 100%, NPV of 85.2%, and accuracy of 92.7%. **Conclusion:** EUS with EUS-FNA is an accurate procedure in the diagnosis of GI tumors with negative endoscopic biopsies.

Key words: Biopsy negative, endoscopic-ultrasound-guided fine-needle aspiration (EUS-FNA), submucosal lesions

INTRODUCTION

Submucosal Tumor (SMT) is a term used by endoscopists to describe any bulge covered with intact mucosa. The commonest SMT are mesenchymal cell tumors as gastrointestinal (GI) stromal cell tumors (GIST), leiomyomas and schwannomas.[1] Other SMT are lipomas, carcinoid tumors and duplications cysts. The etiology of most SMTs cannot easily be determined by endoscopy. Although experienced

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endoscopists may often make a speculation on the etiology of SMTs on the basis of size, shape, firmness, surface color, and overall appearance, histological diagnosis is limited.[2] Evaluation of SMT is one of the classical indications of endoscopic ultrasound (EUS). It is the most important tool to assess its layer of origin, differential diagnosis, classification and follow up of these lesions.[3]

Some cases of diffuse circumferential GI malignancy as diffuse signet ring adenocarcinoma and lymphoma involve mainly the submucosal layer. Endoscopic mucosal forceps biopsy is the standard procedures for establishing diagnoses in patients with GI tumors. However, the false negative rate of endoscopic mucosal forceps biopsy can be as high as 50%. Possible reasons for this false negative rate include infiltrative and stenotic diseases as well as lesions in submucosal locations, such as lymphoma.[4] In such cases, alternative techniques for obtaining tissue diagnoses are warranted. We conducted this study to evaluate the efficacy and accuracy of EUS and EUS-guided fine-needle aspiration (FNA) biopsy in the diagnosis of conventional endoscopic biopsy negative GI tumors.

PATIENTS AND METHODS

This prospective study was conducted from January 2011 to April 2015 on 109 patients with GI lesions diagnosed by endoscopy. The study design was approved by the ethical committee and all the patients were informed about the protocol and clarified written consents were obtained from them. Endoscopic biopsies taken from the lesions were proved to be negative for benign or malignant neoplasms. Biopsies were taken by a biopsy-over-biopsy technique up to 5 biopsy from same site to involve a deeper layer from the mucosa using a biopsy forceps (Wilson-Cook, Winston Salem, NC).

Endosonographic examination was performed in all the patients using a linear Echoendoscope Pentax EG3830UT (HOYA Corporation, PENTAX Lifecare Division, Showanomori Technology Center, Tokyo, Japan) connected to an ultrasound unit Hitachi EUB-7000 HV (Hitachi Medical Systems, Tokyo, Japan). All examinations were performed by one endosonographer. For the assessment of SMTs, it is a routine practice to record the tumor location, layer of origin, maximal diameter, regularity of extraluminal border, echopattern, and presence of cystic spaces or echogenic foci in order to provide a presumptive diagnosis and predict their malignant risk. Endosonographic features suggestive of a risk of malignancy including large size (>5 cm), irregular extra-luminal border, heterogeneous echopattern, presence of cystic spaces have been well described in previous studies.[5]

EUS-FNA was done for all patients. All FNA procedures were performed using a 19 and 22-gauge needle (Echotip®; Wilson-Cook, Winston Salem, NC). Color flow and Doppler sonography were performed to exclude intervening vascular structures and to select a vessel-free needle track. Once the tip of the catheter was visualized, the needle was advanced from the catheter sheath through the wall of the GI tract into the target lesion under ultrasound guidance [Figure 1]. The stylet was removed, and the initial passes were performed by moving the needle back and forth within the target lesion for 15-30 s. No suction was applied during biopsy unless the biopsy did not yield any material. On-site cytopathological examination was available in nine of our patients (8.3% of all cases), if not available at least three passes were done. Slides were dried and then fixed with with 95% alcohol and formalin block were used in all cases. Immunostaining of c-kit 117 was done to all cases with GIST. Also staining for CD-3 and CD-20 was done for cases with lymphoma. Cytokeratin was done in 12 out of 28 cases with adenocarcinoma.

To determine the role of EUS-FNA in diagnosing GI tract neoplasms, sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and diagnostic accuracy were calculated.

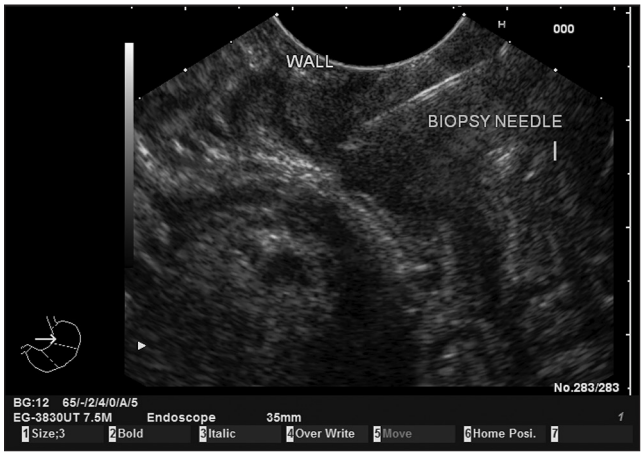

Figure 1. EUS-FNA of diffuse gastric antral lesion

RESULTS

There were 109 patients with biopsy negative submucosal lesions, including 61 males (56%) and 48 females (44%), with the mean age of 54 years. The age was ranging from 25 years (youngest) to 77 years (oldest). Table 1 shows the clinical presentation of submucosal lesions.

Table 2 shows the site of the SMTs. Table 3 shows the layer of origin and the nature of the lesions. Table 4 shows the final diagnosis of the lesions reached after histopathological examination. Table 5 shows the efficacy of the EUS-FNA for reaching the final diagnosis. Table 6 shows the statistical parameters of EUS and EUS-FNA diagnosis (sensitivity and specificity).

Sixty-three cases (57.8%) were proved to have malignant lesions, among them there were 15 cases with high-risk GIST as proved by FNA and excision biopsy. Forty six cases (42.2%) were proved to be benign; among them there were 21 cases were proved to be non-high-risk GIST as confirmed by FNA [Table 4]. All benign lesions were followed up for at least 12 months with no progression of the size of the lesion [Figures 2-4].

Endoscopic ultrasound had a sensitivity of 96.8%, specificity of 89.1%, PPV of 92.4%, NPV of 95.3%, and accuracy of 93.6% [Table 6].

Eight out of 46 patients were diagnosed with benign lesions by FNA that were finally proved to be malignant [Table 5]. The pathological report of two of those eight patients was indefinite for the diagnosis.

Those 8 patients that were not properly classified, were finally diagnosed by surgical laparoscopic full thickness biopsy that was requested upon high suspicion of malignancy based on EUS findings as marked wall thickening and focally localized lesions. EUS-FNA had a sensitivity of 87.3%, specificity of 100%, PPV of 100%, NPV of 85.2%, and accuracy of 92.7% [Table 6].

The mean of wall thickness of benign lesions was 11 mm with a SD of 2 mm while the mean for malignant lesions was 18 mm with a SD of 11 mm. There was no statistical significance between both groups in term of wall thickness. The mean gastric wall thickness for diffuse type adenocarcinoma was 2.8 cm with a SD of 2.1 cm [Figure 5]. As for lesions diagnosed as lymphoma, the mean gastric wall thickness was 2.4 with a SD of 0.9 cm [Figure 6]. There was no statistically significant difference between these groups of patients as regards gastric wall thickness.

Table 1. Presentation of submucosal lesions

Presentation	No
Dyspepsia	32
Dysphagia	29
Weight loss	20
Bleeding	14
Pyloric obstruction	5
Accidental	3
Pain	3
Constipation	1
Fever	1
Jaundice	1

Table 2. Site of submucosal lesions

Site	No	Percentage (%)
Gastric	72	66.1
Esophageal	14	12.8
Gastroesophageal	9	8.3
Duodenal	7	6.4
Rectal	6	5.5
Gastrojejunal stoma	1	0.9

Table 3. Layers of origin

Layer of origin	Number	diagnosis
Mucosa	0	
Muscularis mucosa	10	8 GIST, 2 leiomyoma
Submucosa	22	12 inflammatory 3 Lipoma 2 adenocarcinoma 2 benign polyps 1 squamous cell carcinoma 1 lymphoma 1 pancreatic rest
Muscularis propria	28	24 GIST 2 hypertrophic pyloric stenosis 1 leiomyoma 1 achalasia
All layers	49	26 adenocarcinoma 12 lymphoma 4 GIST 2 carcinoma *in situ* 2 high grade dysplasia 1 neuroendocrine 1 squamous cell carcinoma 1 TB

DISCUSSION

SMTs are not uncommon, their exact incidence is difficult to assess because most of them are asymptomatic, thus it is estimated to be around 0.3%.[1] For GI tract lesions, EUS is particularly helpful in

identifying the layer of origin of the lesion and whether it arises in the wall or is caused by an extrinsic lesion compressing the GI lumen.[5] However, using EUS only as an imaging technique to identify the nature of the lesion is not enough and tissue examination is required to reach a definite diagnosis especially for malignant lesions. FNA under the guide of endoscopic ultrasound has been used to solve this issue.[6,7]

The use of EUS-FNA has proven to be successful in the evaluation of pancreatic masses and lymphadenopathy.[8] However, only a few studies published have focused specifically on evaluating the use of EUS-FNA in GI tract lesions.[9-11] Those studies as well as others that included pancreatic lesions and lymphadenopathies found that EUS-FNA was less useful in the diagnosis of GI tract lesions, and particularly submucosal tumors. A multicenter study that included a series of 115 GI tract lesions reported that the sensitivity, specificity, and accuracy of EUS-FNA in diagnosing neoplastic GI tract lesions were 61%, 79%, and 67%, respectively.[12] Our study on EUS-FNA gave sensitivity of 87.3%, specificity of 100%, PPV of 100%, NPV of 85.2%, and accuracy of 92.7% Our results are higher than those cited in the literature. This may be due to the availability of on-site cytopathological examination in 50 of our patients (46% of all cases), if not available at least 3 passes were done. Immunostaining of c-kit 117 was done to all cases with GIST (36 cases). Also, staining for CD-3 and CD-20 was done for all 13 cases with lymphoma. Cytokeratin was done in 12 out of 28 cases with adenocarcinoma.

Table 4. Diagnosis of submucosal lesions

Diagnosis	No	Percentage (%)
Diffuse malignant Lesions:	48	44.1
Adenocarcinoma	28	25.7
Lymphoma	13	11.9
Squamous cell carcinoma	2	1.8
Carcinoma in situ	2	1.8
High grade dysplasia	2	1.8
Neuroendocrine	1	0.9
Submucosal tumors (SMT):	43	39.5
GIST	36	33.2
High risk: 15 cases		
Non-high risk: 21 cases		
Lipoma	3	2.7
Leiomyoma	3	2.7
Pancreatic rest	1	0.9
Benign lesions:	18	16.4
Nonspecific inflammation	12	11
Hypertrophied pyloric stenosis	2	1.8
Benign polyp	2	1.8
Esophageal TB	1	0.9
Achalasia	1	0.9

Table 5. EUS-FNA efficacy in reaching the final diagnosis

EUS-FNA diagnosis	Final diagnosis	
	Malignant (63 patients)	Benign (46 patients)
Malignant	55	0
Benign	8	46

On-site cytopathology is considered by some studies a good way to overcome over- or underestimation of specimens. Klapman *et al.* observed that an EUS center with on-site cytologic interpretation had a significantly lower rate of unsatisfactory specimens and a higher rate of positive or negative cytologic diagnoses for malignancy compared with an EUS center without on-site cytologic interpretation.[13] They estimated an improvement of diagnostic sensitivity by approximately 10-20%.

In a series of 265 consecutive patients with GI tract malignancies, Zargar *et al.* reported that the diagnostic accuracy of EUS-FNA (94%) was significantly greater than the accuracy of endoscopic mucosal forceps biopsy (87%); this was particularly true in the case of submucosal lesions and infiltrative malignancies.[14] Furthermore, the same investigators pointed out that FNA was diagnostic in 21 of 24 lesions that were negative on both brush cytology and mucosal forceps biopsy. Therefore, EUS-FNA should be the diagnostic procedure of choice when standard methods, such as endoscopic mucosal forceps biopsy, fail to provide a definitive diagnosis.

GIST may be one of the most diagnostically challenging lesions encountered in EUS-FNA of GI tract lesions. Thirty-six cases were diagnosed with GIST in our study representing 33.2% of all endoscopic biopsy negative lesions and 84% of all submucosal lesions.

Table 6. Statistical parameters of EUS diagnosis and EUS-FNA

Procedure	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Accuracy (%)
EUS diagnosis	96.8	89.1	92.4	95.3	93.6
EUS-FNA	87.3,	100	100	85.2	92.7

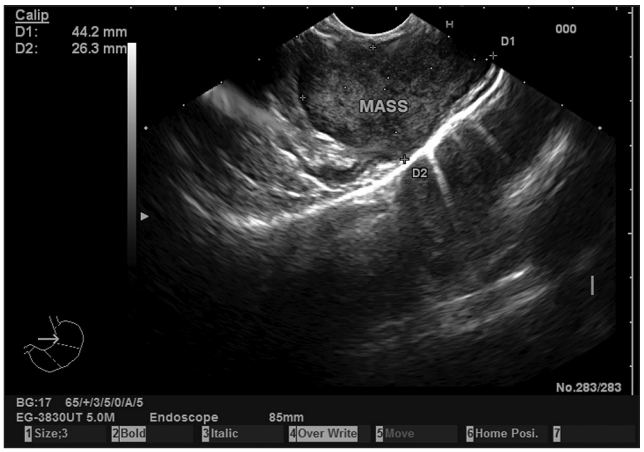

Figure 2. Gastric body GIST with high risk

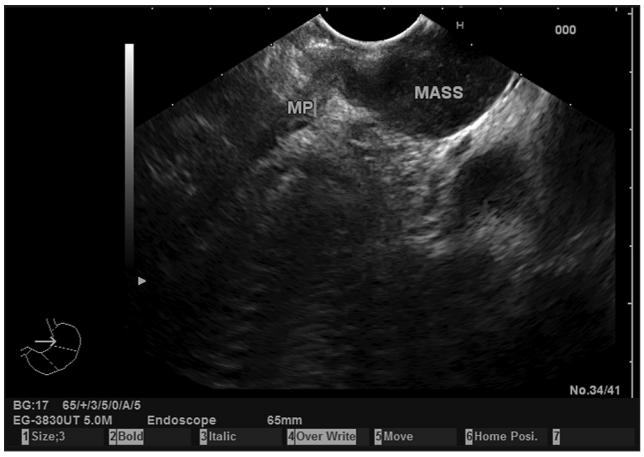

Figure 3. Gastric body GIST with low risk

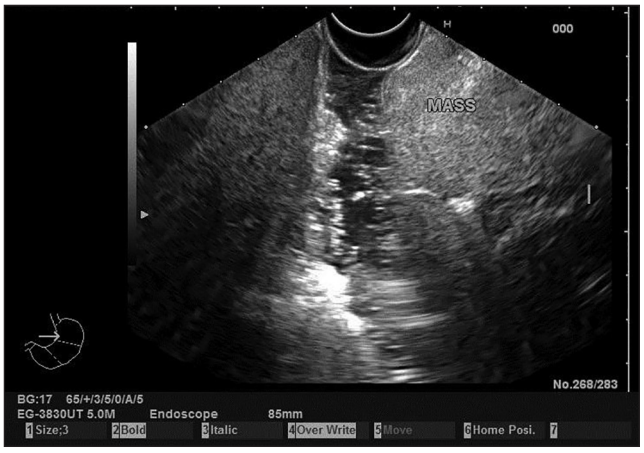

Figure 4. Large gastric antral lipoma

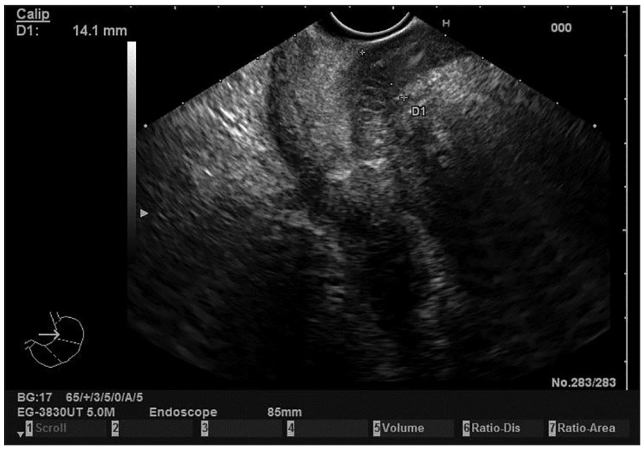

Figure 5. Adenocarcinoma of the gastric body

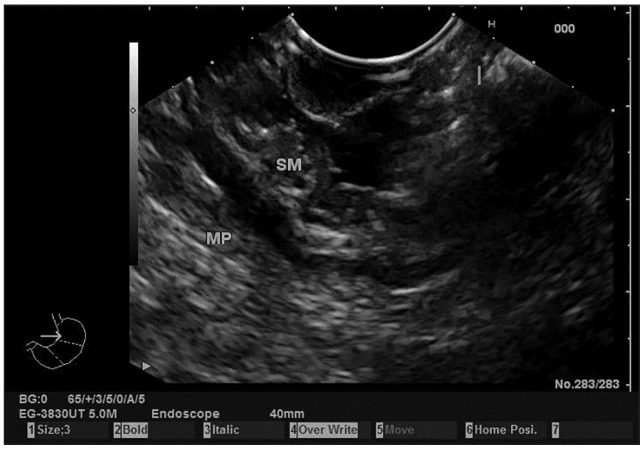

Figure 6. Lymphoma of gastric body and antrum

One author commented that because of the fibrosis and firmness of GIST, which requires substantial force for penetration, it may be difficult to obtain cytologic material via aspiration. Others have reported success in diagnosing GIST when combining cytologic and immunocytochemical methods.[15] One potential diagnostic pitfall is the misinterpretation of aggregates of spindle-shaped neoplastic cells from a GIST as the muscularis propria of the bowel wall, especially when cellularity is low. The use of immunocytochemistry may be helpful in making this differential diagnosis. Tumor cells from GIST should be positive for c-kit, whereas smooth-muscle cells from the bowel wall and from the spindle cell carcinoma should be negative for this marker. Spindle cell carcinoma is positive for cytokeratin expression, whereas GIST is not.[16]

EUS is a useful tool for assessing large gastric folds. Gastric wall thickness (≥9.8 mm) and thickened muscularis propria are significant features predictive of malignant disease on EUS.[17]

Although the mean wall thickness of malignant diseases was higher than that of benign diseases (18 mm versus 11 mm), yet this difference was not statistically significant. The minimal wall thickness of our patients (6 mm) was finally proved to be due to gastric wall adenocarcinoma. So, any wall thickening with negative

endoscopic biopsies should be followed by EUS-FNA especially in the presence of suspicious clinical or endoscopic findings.

CONCLUSION

In conclusion, our observations suggest that EUS-FNA is a very accurate and less invasive procedure with favorable sensitivity and specificity in the diagnosis of endoscopic biopsy negative GI lesions. Routine practice with on-site cytologic interpretation yields more accurate results. Additional immunohistochemical examination of the obtained specimens will add more diagnostic value especially to challenging GI tumors such as GIST and lymphoma.

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Conflicts of interest
There are no conflicts of interest.

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