Review Article

When to puncture, when not to puncture: Submucosal tumors

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ABSTRACT

Subepithelial masses of the gastrointestinal (GI) tract are a frequent source of referral for endosonographic evaluation. Subepithelial tumors most often appear as protuberances in the GI tract with normal overlying mucosa. When there is a need to obtain a sample of the mass for diagnosis, endoscopic ultrasound (EUS) - guided fine-needle aspiration (FNA) is superior to other studies and should be the first choice to investigate any subepithelial lesion. When the decision is made to perform EUS-guided FNA several technical factors must be considered. The type and size of the needle chosen can affect diagnostic accuracy, adequacy of sample size and number of passes needed. The use of a stylet or suction and a fanning or standard technique during EUS-guided FNA are other factors that must be considered. Another method proposed to improve the efficacy of EUS-guided FNA is having an on-site cytopathologist or cytotechnician. Large or well-differentiated tumors may be more difficult to diagnose by standard EUS-FNA and the use of a biopsy needle can be used to acquire a histopathology sample. This can allow preservation of tissue architecture and cellularity of the lesion and may lead to a more definitive diagnosis. Alternatives to FNA such as taking bite-on-bite samples and endoscopic submucosal resection (ESMR) have been studied. Comparison of these two techniques found that ESMR has a significantly higher diagnostic yield. Most complications associated with EUS-FNA such as perforation, infection and pancreatitis are rare and the severity and incidence of these adverse events is not known. Controversy exists as to the optimal method in which to perform EUS-FNA and larger prospective trials are needed.

Key words: Endoscopic ultrasound, fine needle aspiration, gastrointestinal tract, submucosal tumor

INTRODUCTION

While the prevalence of subepithelial masses in the gastrointestinal (GI) tract is unknown, these lesions are a frequent source of referrals for endosonographic evaluation. These lesions are most often found incidentally during endoscopic or radiologic examinations being done for other reasons. The majority are asymptomatic, although they may sometimes present with obstruction, dysphagia or hemorrhage. Rarely, they can cause jaundice or pancreatitis if the lesion is in close proximity to the ampulla. Subepithelial tumors most often appear as protuberances in the GI tract with normal overlying mucosa (although the overlying mucosa can be ulcerated with certain lesions). These protuberances can be very subtle and the origin of the lesion (i.e., extramural *vs.* intramural) can be difficult to determine. Subepithelial masses are classified as intramural, when the lesion originates from within the layers of the GI wall. They are classified as extramural, when the lesion originates from outside the GI wall. Some of these lesions can be benign and require no additional evaluation or

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intervention, whereas others can be premalignant and may need close follow-up. Still others are malignant, thus requiring medical or surgical interventions. Endoscopic ultrasound (EUS) has become the primary modality in the evaluation of subepithelial lesions. When there is a need to obtain a sample of the mass for diagnosis, EUS-guided fine-needle aspiration (FNA) is an indispensable tool for tissue acquisition. The objective of this article is to review the types and endosonographic features of the subepithelial lesions that may be encountered and to some of the key aspects of EUS-guided FNA that can impact the efficacy of the technique.

EUS AND LAYERS OF THE GI WALL

Familiarly with the histologic layers of the GI wall and their sonographic counterparts is essential in obtaining an accurate diagnosis of subepithelial lesions. The GI wall consists of 5 layers: The epithelium, lamina propria, submucosa, muscularis propria and serosa (adventitia). At usual EUS frequencies (5-12 MHz) these are displayed in a 5-layer pattern numbered from the lumen out: First and second (mucosa including the muscularis mucosa), third (submucosa), fourth (muscularis propria) and fifth (serosa or adventitia).

EUS is superior to other studies (computed tomography, barium studies and endoscopic studies with biopsies) in delineating the origin of the mass, hence EUS should be the first choice to investigate any subepithelial lesion.[1] Studies have shown that around 14% to 42% of the lesions suspected to be subepithelial lesions during a routine endoscopic examination turned out to be extramural lesions or compressions during EUS examinations.[2,3] Structures that are commonly found to be compressing the GI wall during EUS are usually benign. In one study that included 238 patients who underwent EUS to investigate subepithelial lesions, 55 lesions were found to represent extramural structures. More than half of these cases (58%) were impressed upon by neighboring organs such as the spleen, splenic vessels, gallbladder, liver and pancreas [Figure 1 and Video 1]. Totally 12 cases were related to benign lesions (hepatic cysts, hepatic hemangiomas, splenic cyst and pancreatic cyst). Nearly 10% of extramural cases were thought to represent transient impression. About 9% of the cases represented malignant lesions (pancreas, liver and spleen).[3] Hence extramural lesions may be malignant, though as such are encountered infrequently.

The EUS operator should try to identify the layer of origin for any subepithelial lesions because this can help significantly to narrow the diagnosis. Lesions originating from the submucosal layer are usually lipomas, fibromas, carcinoid tumors, granular cell tumors, pancreatic rests and duplication cysts. Lesions arising from the muscularis propria usually represent GI stromal tumors (GIST), leiomyomas and schwannomas.[2,4] Metastatic disease to the GI track will generally involve the 4th and 5th layers and can be confused with a GIST [Table 1].

Echogenicity is an important feature to describe when investigating subepithelial lesions. Anechoic lesions may represent cysts, varices, lymphangiomas, or cavernous hemangiomas. A hypoechoic lesion can represent GI mesenchymal tumor (GIST, leiomyoma, schwannoma),

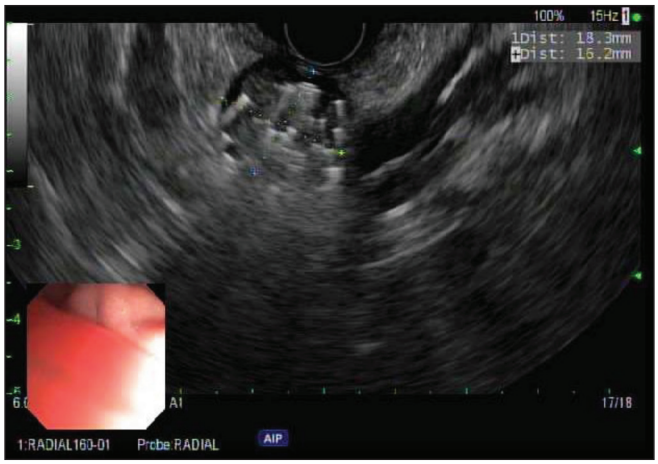

Figure 1. A splenic artery aneurysm causing extrinsic compression of the stomach (post-coil embolization)

Table 1. Differential diagnosis of subepithelial lesions by echogenicity and wall layer of origin

Subepithelial lesion	Echogenicity	Wall layer of origin
Duplication cysts	Anechoic	2-4 or extraluminal
Varices	Anechoic	3rd
Lymphangiomas	Anechoic	3rd
GIST	Hypoechoic	4th layer (rarely 2nd or 3rd)
Leiomyoma	Hypoechoic	2nd, 3rd or 4th
Schwannoma	Hypoechoic	3rd or 4th
Granular cell tumor	Hypoechoic	2nd or 3rd
Carcinoid	Hypoechoic	2nd or 3rd
Inflammatory fibroid polyp	Hypoechoic	2nd or 3rd
Metastasis	Hypoechoic	Any or all
Lymphoma	Hypoechoic	2nd, 3rd or 4th
Pancreatic rest	Hypoechoic	2nd, 3rd or 4th
Lipomas	Hyperechoic	3rd

GIST: Gastrointestinal stromal tumors

granular cell tumor, neuroendocrine tumor, inflammatory fibroid polyp, metastasis, subepithelial cancer, lymphoma, amyloid, focal inflammation and endometriosis. Hyperechoic lesions usually represent lipomas or fibrolipomas. Mixed echogenicity (bright and dark areas) can be seen and may represent heterotopic pancreas, malignant mesenchymal tumor, fibrovascular polyp, spontaneous esophageal hematoma, or wall abscess.[2,5]

Measuring the size of the lesion is important as it may help to narrow the diagnosis and may provide prognostic value in certain situations. For example, in a patient with a GIST, size of less than 1 cm is less likely than the size of 5 cm to be malignant.[5,6] The extent of the mass has implications for treatment and prognosis. If the subepithelial lesion is seen to extend to the 4th layer or beyond, it makes the chance of removing the lesion endoscopically risky due to the risk of perforation.[7] In addition, if the lesion is seen to extend to or invade surrounding organs, this raises the concern of an invasive malignancy.

Determining vascularity and presence of surrounding vessels is also important, especially before attempting to obtain a biopsy, perform FNA or remove a lesion. For example, gastric varices can be easily misdiagnosed as a gastric mass [Figure 2]. EUS can safely and reliably identify gastric varices and vessels with tumors, improving safety.[8,9] Identifying the presence of lymphadenopathy and performing EUS-guided FNA of suspicious lymph nodes can be helpful in the locoregional staging of malignant lesions, with implications for the management of these lesions.[10,11]

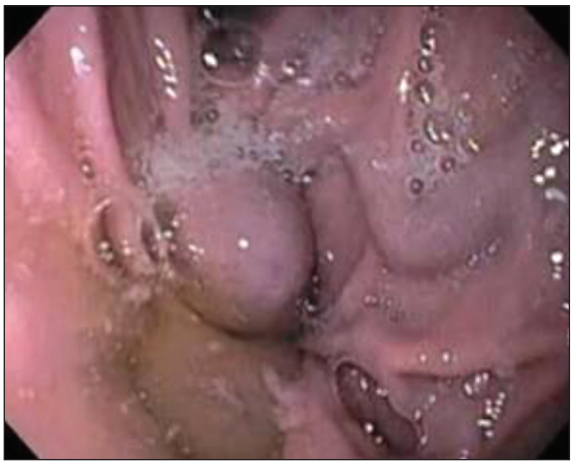

Figure 2. A gastric varix misdiagnosed as a gastric mass

GIST

GISTs are considered a subset of mesenchymal tumors and are the most common mesenchymal neoplasm of the GI tract.[12] They usually appear as firm and protruding lesions and may be discovered by endoscopy done for unrelated reasons. Ulceration or an umbilicated appearance is common. They can present with abdominal pain, bleeding, obstruction or intussusception, or be found on cross sectional imaging done for unrelated reasons. They are most common in the stomach (60%) and small bowel (35%) and are rarely found in the esophagus or rectum (<5%). They generally arise from the 4th layer (muscularis propria) and appear as a round, hypoechoic lesion with a homogeneous to ground-glass echotexture [Figure 3 and Video 2]. Histologically, they consist of spindle-shaped, epithelial or mixed type cells. More than 95% are cKIT positive (CD117 positive). 60-70% are CD34 positive. And 95% are positive for DOG1 (discovered on GIST), but this recently described test is mostly used in cases that are cKIT negative. These tests can be performed on FNA material to distinguish GIST from other similar-appearing spindle cell tumors.

LEIOMYOMA

Leiomyoma is a benign tumor of the smooth muscle. Most leiomyomas are intraluminal or intramural tumors and are often asymptomatic until they have reached a large size. They are most commonly asymptomatic but their clinical presentation depends on the size, location and direction of tumor growth. They are the most common tumor of the esophagus but can occur anywhere in the GI tract. Endoscopic

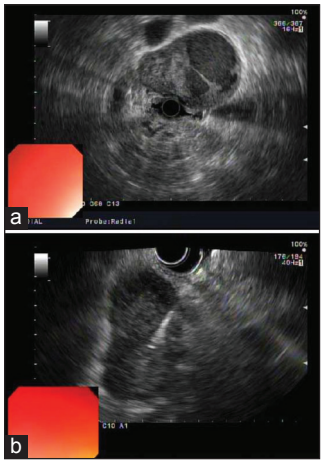

Figure 3. Image of a large gastrointestinal stromal tumor. (a) Endoscopic ultrasound (EUS); (b) EUS-guided fine-needle aspiration

ultrasonography (EUS) typically shows a hypoechoic lesion arising from the second (muscularis mucosa) or 4th layer (muscularis propria) [Figure 4 and Video 3]. Asymptomatic cases do not require FNA or further intervention. Clinically, endosonographically and histologically leiomyomas are indistinguishable from other spindle cell neoplasms such as GIST and Schwannomas. They can be defined using immunohistochemical stains as being positive for actin and desmin and negative for cKIT and S100.

CARCINOID TUMORS

Carcinoid tumors originate from neuroendocrine cells. Although they commonly occur in the ileum, at endoscopy they are usually discovered in the rectum, stomach, or duodenum.[13] EUS examination of GI carcinoids shows a hypoechoic and homogeneous oval to round tumor with a clear margin and smooth contour, arising from the 3rd layer (submucosa).

LIPOMA

Lipomas are frequently asymptomatic and can occur anywhere in the GI tract (although they are most commonly encountered in the right side of the colon).[14] They rarely can cause GI hemorrhage, intussusception and bowel obstruction.[15] On EUS, a lipoma appears as a homogeneous hyperechoic (bright) mass localized in the submucosal (3rd) layer [Figure 5].

SCHWANNOMA

Schwannomas are tumors of the nerve sheath. Schwannomas can present with vague abdominal pain, vomiting, weight loss, dysphagia, obstruction and GI hemorrhage.[16] In a series of GI schwannoma, most of the cases arose in the stomach (around 70%), around 15% in the colon and rectum and the rest in the esophagus. On EUS, a schwannoma appears as a hypoechoic lesion that originates from the 3rd or 4th layer and has an appearance similar to a GIST or leiomyoma. On immunohistochemical stains, they are positive for S100 but negative for GIST or leiomyoma markers.

PANCREATIC REST

Pancreatic rest refers to pancreatic tissue that is found outside the pancreas without anatomic or vascular connection with the pancreas itself. Most of the cases are asymptomatic. When symptomatic, patients may present with mucosal ulcer and hemorrhage, intussusception, intestinal obstruction and bile duct obstruction.[17] On EUS, most of the lesions originate from the 2nd, 3rd and/or 4th layers. Nearly all of these lesions have a heterogeneous echotexture, are mainly hypoechoic or of mixed echogenicity and the majority have indistinct borders [Figure 6].

DUPLICATION CYST

Foregut duplication cysts are uncommon congenital anomalies. Duplication cysts are usually asymptomatic in adults. When symptomatic, they can present with abdominal pain, dyspnea, dysphagia, or coughing. On EUS they appear as anechoic (although they can sometimes be hypoechoic), homogeneous lesions with regular margins arising from the 3rd layer or extrinsic to the gut wall. It is recommended that FNA be avoided

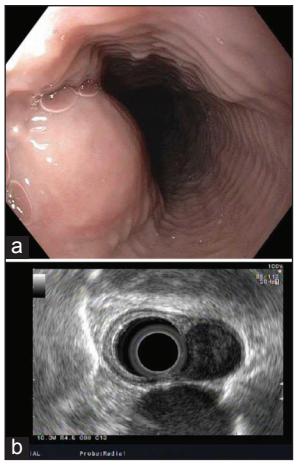

Figure 4. Esophageal leiomyoma. (a) Endoscopic image; (b) Endoscopic ultrasound image

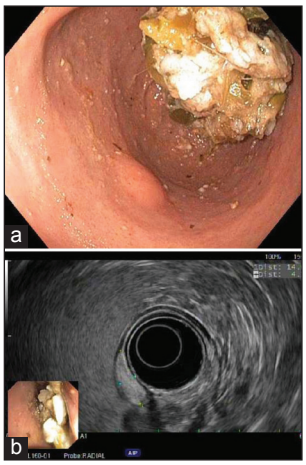

Figure 5. Gastric lipoma. (a) Endoscopic image; (b) Endoscopic ultrasound images

for cysts with typical EUS appearance of duplication cyst in the mediastinum. This is due to several reported case of infection from the FNA of duplication cysts in the mediastinum despite the use of prophylactic antibiotics [Figure 7].

METASTASIS

More than 50% of metastases to the stomach may present as subepithelial lesions. Metastases to the stomach tend to involve the upper and middle-third of the stomach. Metastasis to the stomach most commonly originates from the lung, esophagus, breast and malignant melanoma.[18] The EUS appearance of the tumors can show a hypoechoic or heterogeneous mass[4] and endoscopic biopsy may be diagnostic in up to 90% of cases. Endometriosis can have the appearance of a hypoechoic mass of the 4th and 5th layers and is most typically in the colon of affected women.

MANAGEMENT OF SUBEPITHELIAL LESIONS

Management of subepithelial lesions depends on the etiology, location, size, symptoms and patients' characteristics such as age, comorbidities and need and frequency of follow-up examinations. Asymptomatic benign lesions do not require follow-up or intervention. Such lesions include most pancreas rests, leiomyomas, schwannomas, lipomas, duplication cysts, hemangiomas and inflammatory fibroid polyps. Lesions with malignant or invasive risk should be resected or undergo endoscopic or EUS surveillance. These lesions include carcinoids, granular cell tumors and GISTs. Endoscopic resection is indicated for all carcinoids of less than 1 cm in size as well as most type 1 and type 2 gastric carcinoids. Most granular cell tumors may be resected endoscopically, as can small GISTs arising from the 3rd layer (submucosa or muscularis mucosa).[19]

Controversy exists as to the management of small incidentally found GISTs, especially gastric lesions less than 2 cm in size. These tumors appear to have a low risk of malignant behavior and may be considered for EUS surveillance without resection.[20] Factors to be considered in selecting patients for surveillance include patient's age, comorbidities and life expectancy. Although the optimal timing and number of surveillance examinations and duration are unknown, a survey reported 70% would survey annually.[21] Any change in size should prompt surgical resection.[22,23]

EUS-GUIDED FNA

In many cases, EUS is not capable of providing a definitive diagnosis of the subepithelial lesion based on its ultrasound characteristics alone. In these cases, tissue sampling is needed in order to provide a diagnosis. If the lesion is within or in close proximity to the GI tract a cytologic specimen may be obtained through EUS-guided FNA. EUS-guided FNA has proven itself to be a safe and effective technique for sampling subepithelial lesions of the GI tract. Indeed, FNA via EUS guidance is now considered to be the procedure of choice to acquire tissue from subepithelial lesions in the GI tract, particularly those arising from the 4th layer (muscularis propria).[24-27]

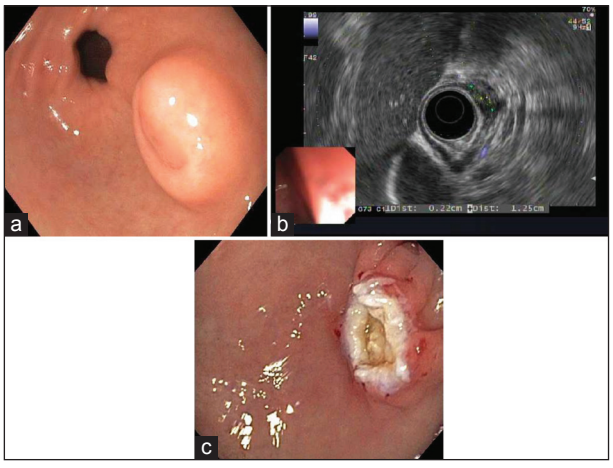

Figure 6. Pancreatic rest. (a) Endoscopic view; (b) Endoscopic ultrasound view; (c) Lesion after endoscopic submucosal resection

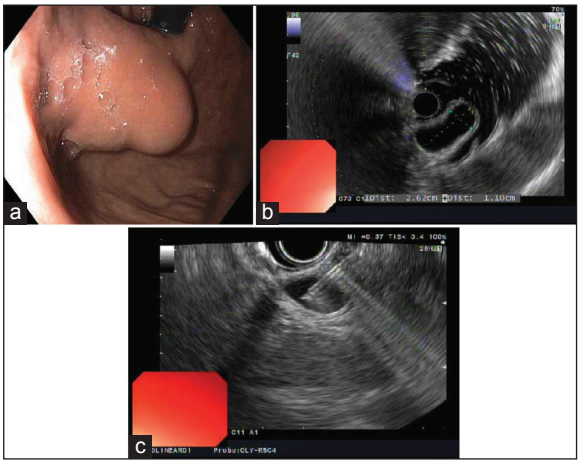

Figure 7. Gastric duplication cyst. (a) Endoscopic view; (b) Endoscopic ultrasound (EUS) view; (c) EUS-guided fine-needle aspiration of the same lesion

Whereas earlier studies reported relatively poor accuracy[28] more recent studies have reported high diagnostic yields reaching 75-100% respectively.[24,25,29-32] The sensitivity of EUS FNA for GISTs has been estimated at 84-89%.[26,31] Higher yield of EUS FNA of subepithelial lesions may be obtained from larger lesions, from a gastric location and in the presence of on-site cytopathology.[27] The overall rate of EUS FNA-specific morbidity is low, estimated to be 0-2%.[33] Complications after FNA of subepithelial masses are very rare and mostly consist of post-procedural abdominal pain.[33] Infection following FNA is also rare and seen mainly when FNA is used to aspirate fluid from a cystic lesion. Antibiotic prophylaxis is now recommended as part of routine practice in cases when FNA of cystic lesions are performed.[19]

Several factors can affect the efficacy of EUS-guided FNA of sub-epithelial lesions. These factors include the type and size of the needle used for FNA, whether or not a stylet is used during the tissue acquisition process as well as what maneuvers are used to acquire the sample once the needle is passed into the target lesion. More recently, the impact of having an on-site cytopathologist or cytotechnician present in the endoscopy suite has been studied.

SIZE OF FNA NEEDLE

After the decision is made to perform EUS-guided FNA, the first decision that must be made is to select the type of needle to be used. There are many types of commercially available needle systems that can be used for tissue acquisition during EUS-guided FNA. The currently available needle sizes for FNA are 19, 22 and 25 gauge needles. There are many factors to be considered when deciding which size needle to choose for FNA. Factors such as the type of lesion, the location of the lesion within the GI tract and the degree of angulation en route to the target lesion need to be taken into account when choosing a needle. A 19 gauge needle with its larger bore has the ability to obtain a larger sample size. Whether this leads to a higher diagnostic yield and better cellularity is controversial as the specimen may also be more blood dilute. In addition, the mechanical factors of a larger needle such as its stiffness may make it more difficult to maneuver into an area of the GI tract that is sharply angulated. This may in turn lead to a technical failure of the needle and an inability to acquire a sample in lesions that are in locations in which a higher degree of torque may be needed. Large series involving the 19 gauge needle are limited. The standard 22 gauge needle is the one most commonly employed in published series.[24-26,29-32] The 25 gauge needle has been advocated as causing less trauma and having as good if not slightly better yield due to less blood dilution of the specimen. A recent meta-analysis and systematic review[34] attempted to evaluate the utility and effect of needle size on diagnostic accuracy, adequacy of sample size, number of needle passes and complications. This review found that although there was a paucity of randomized controlled trials comparing needle sizes, there was a slight trend of the available data favoring the smaller 25 gauge needle. In comparison to other needle sizes, the 25 gauge needle showed a slightly better rate of obtaining an adequate sample. However, there was no significant difference in accuracy, complication rates, or number of needle passes. In order to allow for a more detailed comparison between needle sizes, larger series prospective randomized trials are needed.

USE OF A STYLET

The use of a stylet during the acquisition of the tissue sample of solid subepithelial lesions has been proposed as a way to optimize the diagnostic yield. The commonly used and commercially available EUS-guided FNA needles have an internal stylet included with the needle. The use of a stylet during needle insertion is thought to prevent contamination and clogging of the needle lumen as it passes through the walls of the GI tissue en route to the target lesion. This may, in turn, increase the ability of needle to aspirate tissue from the target lesion and improve the quality of the specimens obtained for analysis. One method for using the stylet involves slightly withdrawing the stylet from the needle to sharpen the tip in order to enable passage through the GI tract and into the lesion.[35] It is then pushed in so as to clear the needle tip of any debris when the needle is fully in the target lesion. The stylet then needs to be withdrawn in order to obtain the tissue sample and must be reinserted between subsequent passes which increases the total time of the procedure. The use of the stylet has not been shown to increase the diagnostic yield or improve the quality of the cytology sample that is obtained. A recent prospective randomized, controlled trial was performed by Wani *et al*.[36] to evaluate the samples obtained by EUS-FNA with and without a stylet for solid subepithelial lesion. The samples were evaluated for

diagnostic yield as well as cytopathology characteristics such as cellularity, degree of contamination and specimen adequacy. The study found no difference in the diagnostic yield or the proportion of inadequate samples obtained by FNA with the use of a stylet and without the use of a stylet. Whether to use a stylet or not during routine EUS-guided FNA remains controversial. Other studies comparing the diagnostic yield and quality of the FNA samples obtained with and without a stylet[37,38] have also failed to show a difference between the two techniques. The use of suction during aspiration is also controversial. While this theoretically may increase the size of the sample, blood dilution may decrease the diagnostic yield. One common technique is to slowly withdraw the stylet during the FNA providing a capillary aspiration suction or microsuction.[39] We favor this technique as it appears to provide a good tissue specimen while minimizing blood dilution.

FANNING TECHNIQUE VERSUS STANDARD TECHNIQUE

The best way in which to sample a solid subepithelial mass by FNA so as to obtain the greatest diagnostic yield remains an area of debate. The "standard" technique for obtaining an FNA sample involves positioning the needle tip at a single location within the mass and moving it in and out in line with the direction of trajectory of the needle as it passes into the mass. This is considered to be one individual pass. For each subsequent pass, the needle is inserted into a different point of the mass but remains confined to the same area within the mass. By contrast, the "fanning" technique involves sampling multiple areas within the mass during each individual pass. This can be accomplished by inserting the needle into the mass and moving the needle in a fan-like motion within the mass by using a combination of the elevator and the up/down dial control as the needle is moved back and forth. The rationale behind using a fanning technique is that when the center of a mass is more necrotic than the periphery (as can be the case with cancerous lesions), sampling multiple areas within the mass may increase the diagnostic yield during each individual pass which may in turn lead to fewer overall passes in order to obtain a diagnosis.[40] The ability to obtain a diagnosis with fewer passes has multiple advantages and may lead to shorter procedure duration and improved patient safety.

BIOPSY NEEDLES

Although the standard cytologic sample obtained by EUS-guided FNA of subepithelial lesions has a high sensitivity and specificity, there are certain lesions in which obtaining a histologic rather than cytologic is desirable.[31,32] Large or well-differentiated tumors may be more difficult to diagnose by standard FNA techniques. In cases when conventional FNA needles fail to obtain a diagnosis, a core histopathology sample may be useful in establishing a definitive diagnosis. The Trucut biopsy (TCB) (Quick-Core; Wilson-Cook Medical Inc., Winston-Salem, NC, USA) has been used to obtain core biopsy samples. The Tru-cut device uses a 19 gauge needle with a spring-loaded biopsy in order to obtain a larger tissue sample.[4] Larger core biopsy samples can allow the tissue architecture and cellularity of the lesion to be preserved, which can allow for a more accurate diagnosis. However, the use of this needle is cumbersome in areas of the GI tract such as the antrum, fundus and duodenum. In areas where there is a high degree of endoscope angulation there is a higher rate of technical failure and the use of this needle beyond the duodenal apex is not recommended.[41] In practice, the diagnostic yield of EUS-TCB is modest relative to other techniques.[35,42] In their prospective study of 49 patients with hypoechoic gastric submucosal tumors, Dewitt *et al.* found that EUS-TCB was most successful in tumors located on the lesser curvature of the stomach (odds ratio, 7.4; 95% confidence interval, 1.9-28) and the immunohistochemical diagnostic yield (63%) was not superior to EUS-FNA.[42] Furthermore, septic complications with EUS-TCB in this study were particularly high.[42] In a prospective crossover study in 40 patients where all patients received both EUS-TCB and EUS-FNA in random order, per protocol analysis in the 27 patients with adequate specimens appeared to show an advantage for EUS-TCB (91%) over EUS-FNA (74%). In a separate study did not show any advantage to using EUS-TCB.[27] In this prospective crossover study, 40 patients with GISTs underwent EUS-FNA and EUS-TCB in random sequence. However, the high rate of technical failure associated with EUS-TCB negated this qualified advantage of EUS-TCB, which in intention-to treat analysis had a yield of 55%.[43] The current role of EUS-TCB is in those with initially non-diagnostic FNA.[42]

A new coring needle (EchoTip® ProCore™, Cook Medical, Bloomington, IN) is now available in 22 and 19 gauge, but there are no published data using this

approach to establish a diagnosis of GIST. Although it had been hoped that EUS-TCB of GIST would allow determination of the mitotic index, in practice, the specimen is rarely large enough to supply 50 high-powered fields.

The ProCore (EchoTip® ProCore™, Cook Medical, Bloomington, IN) is a newer type of needle available to obtain a core histologic sample in order to improve diagnostic accuracy. The ProCore needle is now available in 19, 22 and 25 gauge sizes. The smaller caliber core biopsy needle may provide an advantage in terms of maneuverability and allow for a higher success when obtaining a sample from areas of the more angulated portions of the GI tract. It consists of an aspiration needle with a second bevel on the needle shaft. It is used similar to standard FNA needles, with or without suction. Studies in pancreatic lesions and lymphadenopathy have found similar diagnostic yields to aspiration needles, but may require fewer passes.[39,44] There are no published studies specifically evaluating ProCore needles to establish a diagnosis of subepithelial lesions or GIST, although one series included 13 patients with gastric subepithelial lesions established the feasibility of the approach.[45]

ON-SITE CYTOPATHOLOGY EVALUATION

Another method that has been proposed as a way to improve the diagnostic and safety performance of EUS-guided FNA is to have a direct smear of the FNA sample processed and analyzed at the point of acquisition in the endoscopy suite. This process involves taking the newly acquired FNA sample and preparing a direct smear for rapid evaluation. The smear is then processed and examined by light microscopy in the endoscopy suite by a trained cytopathologist or cytotechnician. The goal is to perform this analysis in a rapid fashion at the point of care while the patient remains sedated so as to be able to acquire additional FNA samples as needed to obtain a diagnosis. The rationale for having the cytopathology evaluation occur in the endoscopy suite during the procedure is that this method can provide direct feedback to the endosonographer as to whether the samples obtained are diagnostic or not. This immediate analysis can then allow the endosonographer to perform additional FNA passes or adjust their FNA technique in order to increase the likelihood of obtaining a diagnostic sample.[35,46] This in turn may allow for fewer FNA passes or decrease the need for a second endoscopic procedure in order to obtain a diagnosis. A recent study done by Collins *et al*.[47] looked at the impact of having a rapid on-site cytopathology evaluation on the diagnostic yield and the incidence of repeat EUS-FNA biopsy procedures for solid lesions of the pancreas. The study was performed retrospectively by searching a database for cases undergoing EUS-FNA both before and after the implementation of a rapid on-site cytopathology evaluation. This study found that the use of on-site cytopathology evaluation decreased the number of patients requiring repeat procedures EUS-FNA procedures by approximately 50% ($P = 0.024$) and also provided a higher rate of definitive diagnosis in patients who did require a repeat procedure. However, large randomized studies evaluating the efficacy of on-site cytopathology are limited and further studies are needed before its use in the standard practice of EUS-guided FNA can be recommended. Another factor that may play a role in the efficacy of having an on-site cytopathology evaluation is whether the evaluation is performed by a trained cytopathologist or a cytotechnician. Having a trained cytopathologist available for every EUS-guided FNA procedure may not be feasible in smaller centers with limited resources or larger centers that perform a high number of FNA procedures. On-site cytotechnicians may be one solution but prospective studies comparing the efficacy of on-site cytopathologists and cytotechnicians are needed.

When clinically indicated, FNA samples should be submitted for analysis by immunohistochemical stains. Immunohistochemistry has become a vital part in the evaluation of subepithelial tumors. A variety of immunohistochemical stains are currently available which can greatly assist in the diagnosis of subepithelial lesions. Leiomyomas usually stain positive for actin and desmin while schwannomas stain positive for S100 and GISTs are usually positive for CD117 or c-kit and variably positive for CD-34.[30,33] Ki67 (MIB-1) is a marker of proliferation and can be assessed in resected GISTs and EUS-guided FNA specimens but its ability to predict GIST behavior remains unclear and in need of further study.[48,49] The PDGFRA gene mutation is another useful tool in the evaluation of cKIT negative GISTs. Up to 14% of GISTs are cKIT negative, but are positive for PDGFRA gene mutation. These lesions may be diagnosed through mutational analyses of c-kit and PDGFRA genes or staining for DOG-1.[50-52]

ALTERNATIVES TO FNA

When EUS-guided FNA is non-diagnostic, several alternative tissue sampling methods can be utilized in order to obtain a diagnosis. One method involves obtaining deep biopsies by taking bite-on-bite samples using jumbo forceps. This technique uses large-capacity forceps to take successive biopsies with each bite directly on top of the previous bite. This is an attempt to tunnel into the lesion and obtain a deeper tissue sample. However, the diagnostic yield of taking bite-on-bite biopsies can be low and is estimated to be between 14% and 42% respectively.[53] Another technique used to obtain a larger tissue sample is endoscopic submucosal resection (ESMR). ESMR has adapted the techniques used for endoscopic mucosal resection to the removal of submucosal tumors (3rd layer). This technique involves either injecting saline or suctioning the lesion into a cap fitted endoscope to raise the lesion away from the muscularis propria. The lesion is then resected using an electrosurgical snare. This technique has the advantage of simultaneously providing a definitive diagnosis and therapy of smaller lesions (up to 20 mm in size) with the main complications being bleeding (4-13%) and perforation (up to 5%).[54-57] A study by Cantor *et al.* compared the diagnostic yield between forceps biopsies using the bite-on-bite technique and ESMR for subepithelial lesions.[58] This prospective study looked at the diagnostic yield for these two techniques when sampling subepithelial lesions arising from the 3rd layer. The authors found that the diagnostic yield of the jumbo forceps biopsies was 17% and for the ESMR was 87%. They concluded that ESMR has a significantly higher diagnostic yield than the bite-on-bite technique.

Forceps biopsies of 4th layer lesions such as GIST are usually non-diagnostic. However, in GISTs with mucosal ulceration, biopsies taken from within the ulcerated portion of the suspected GIST can have a high diagnostic yield, but can also increase the risk of bleeding. Another alternative to FNA that has been described to achieve a diagnostic tissue sample is the use of endoscopic submucosal dissection (ESD). This technique utilizes an insulated tipped knife to first cut the mucosa surrounding the lesion, then dissecting the submucosa beneath it and finally closing the defect with clips.[57,59-61] ESD is an advanced therapeutic technique and can be technically challenging. The main risk of ESD is the risk of perforation, which has been reported to be as high as 28%. The endoscopic partial technique uses a conventional snare with electrical current to remove the mucosa to obtain sufficient tissue from the exposed underlying tumor.[62] A non-comparative study of 16 patients with hypoechoic subepithelial tumors <3 cm originating in the muscularis propria found a 93.7% (95% confidence interval, 80.4-100%) diagnostic yield.

COMPLICATIONS OF EUS-GUIDED FNA

Although EUS-guided FNA is an established and safe procedure, such as any technique that involves tissue sampling and the advancement of a needle through the mucosa of the GI tract, there are risks associated with the procedure. Fortunately, most complications associated with EUS-FNA are rare. These complications may include bleeding, perforation, infection and pancreatitis (when a lesion is being sampled in the pancreas). Due to the relative rarity of the complications associated with EUS-guided FNA the severity and incidence of these adverse events is not known. Several retrospective studies have attempted to evaluate the incidence and mortality of EUS-guided FNA for subepithelial lesions. A recent study by Hamada *et al.*[63] retrospectively examined the records of 1135 consecutive patients (via a national database) who underwent EUS-FNA of submucosal tumors at 219 hospitals in Japan. Of the patient records reviewed, only five patients (0.44%) experienced severe bleeding requiring RBC transfusion (one patient) or endoscopic treatment (four patients). No deaths as the result of bleeding due to EUS-FNA were found. Furthermore, no GI tract perforation was observed in any of the patients.

SUMMARY

Subepithelial masses in the GI tract are frequently encountered endoscopic findings and their evaluation by EUS is becoming more common. These masses encompass a heterogeneous group of lesions that range from benign to malignant. EUS is highly useful in their evaluation and tissue sampling when needed via EUS-guidance has become the standard first-line sampling modality for many subepithelial lesions. Management of these lesions depends on many factors. A working knowledge of the EUS characteristics of these lesions can help when deciding whether or not tissue sampling is needed. While 3rd layer lesions may be biopsied or removed, most 4th layer lesions will require FNA to establish a histological diagnosis and differentiate benign (e.g., leiomyomas, Schwannomas) from malignant/premalignant tumors (e.g., GIST, lymphoma, metastasis). A variety of factors such as the

size and type of needle, whether or not to use a stylet or suction, use of the fanning technique or biopsy needles may affect the efficiency of sample acquisition as well as the diagnostic yield. Other factors such as having an on-site cytopathologist or cytotechnician have the potential to impact the efficacy of EUS-guided FNA. Cytological evaluation of the specimen should include immunohistochemical stains to differentiate GIST (cKIT, CD117, DOG1) from leiomyomas (actin, desmin), Schwannomas (S100) or other lesions. The optimal way in which to perform a EUS-guided FNA has not been established and larger prospective studies are needed. The role of endoscopic resection of 4th layer lesions is currently investigational.

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