

Original Article

Comparison of Diagnostic Yield of Endoscopic Ultrasound-guided Fine-needle Aspiration Cytology and Cell Block in Solid Lesions

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ABSTRACT

Background and Aim: Endoscopic ultrasound-guided fine-needle aspiration (EUS-FNA) is a procedure of choice for the diagnostic evaluation of submucosal and periluminal lesions. Tissue sample can be obtained by EUS-FNA cytology (FNAC) or cell block (CB). The aim of the present study is to compare diagnostic yield of EUS-FNA CB and cytology in the absence of onsite pathologist following a protocol-based EUS-FNA approach in solid lesions. **Patients and Methods:** Participants who underwent EUS-FNA at our center for solid submucosal or periluminal lesions (pancreas, lymph node, and liver) between 2014 and 2016 were included, retrospectively. The indication for the procedure along with the clinical and other investigation details and the final etiological diagnosis were recorded on uniform structured data forms. The diagnostic yield of cytology and CB were compared using McNemar's test. The $P < 0.05$ was considered statistically significant. **Results:** EUS-FNA for solid lesion was performed in 130 lesions in 101 patients during the study period. Their mean age was 52.5 ± 12 years and 42.5% were female. Pancreatic masses were the most common lesions (37.7%) followed by lymph nodes (36.9%). Submucosal lesions (17.7%) and liver lesions (7.7%) accounted for rest of the cases. The overall diagnostic yield for EUS-FNAC (70%) and CB (74.6%) was not significantly different ($P = 0.3$) and their combined yield was 85.3%. For the 23 patients with submucosal lesion, diagnostic yield of CB (82.6%) was significantly better than cytology (47.8%, $P = 0.04$). **Conclusions:** EUS-guided CB has better yield compared to cytology in gastrointestinal submucosal lesions. The combination of CB with cytology improves the overall yield of the procedure; and hence, they should be considered complimentary rather than alternatives.

KEYWORDS: Cell block, cytology, endoscopic ultrasound, fine-needle aspiration

INTRODUCTION

Endoscopic ultrasonography (EUS) has evolved as an important tool for the diagnosis and therapy of lesions located in the gastrointestinal (GI) tract as well as around the lumen. This has especially become possible by introduction of linear scanning instruments that place needles into the ultrasound plane of view, permitting biopsies/interventions to be performed.^[1,2] EUS-guided fine-needle aspiration (EUS-FNA) is a very sensitive technique for establishing tissue diagnosis in patients with suspected GI wall lesions and periluminal lesions. Diagnostic accuracy of FNA is up to 90%

depending on site and lesion (higher for nodes and lower for pancreatic malignancies) with a complication rate of 1%–2.5%.^[3-6] The European Society of GI Endoscopy published the guidelines for EUS-FNA sampling, with technical prerequisites for maximizing the diagnostic yield of this procedure.^[7] Factors that can influence

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EUS-FNA yield are experience of endosonographer and cytopathologist, technical difficulty (FNA by transesophageal/transgastric with straight scope position is easier than transduodenal with angulated scope tip position), needles (22G/25G needles better for cytology and 19G for histology, 22G for cell block [CB]), and on-site pathology.^[8]

Lesion characteristics such as the necrosis or desmoplastic reaction can also affect the yield. Repeated sampling along same trajectory can increase bloodiness of aspirate. Fanning with aspiration across lesion in multiple trajectories (4 × 4) from one margin to the other can increase the yield.^[9] CB procedure allows better preservation of cell architecture. The sections obtained from CB can be stained with hematoxylin and eosin (H and E) and subjected to immunohistochemistry (IHC) or molecular analyses.^[10,11] Due to limited availability of on-site pathologist, multiple passes are made to increase the chance of collecting adequate sample. Making a CB from the material obtained may improve the yield, and in this study, we aim to compare individual and combined diagnostic yields of CB and cytology in solid lesions in the absence of on-site pathologist while following a protocol-based approach.

PATIENTS AND METHODS

We screened the records of all the patients who underwent EUS-FNA at our center between January 2014 and December 2016. Patients who underwent EUS-FNA for solid lesions from the following sites were included– (i) pancreas, (ii) lymph node, (iii) upper GI submucosal lesion, and (iv) liver. Those with FNA for cystic lesions or lesions at other sites were excluded. The etiological, clinical, and investigation details were recorded on uniform structured data forms. The general prerequisites for FNA were similar to those for any therapeutic endoscopic procedure.^[12]

EUS was performed using a Pentax curvilinear array echoendoscope (EUS) EG-3870 UTK connected to a Hitachi Avius estiva ultrasound machine (Pentax, Tokyo, Japan, 2012). We used 22G or 25G EUS-FNA needles (Wilson-Cook Corporation, Winston-Salem, North Carolina, USA) with removable stylet.

Endoscopic ultrasound guided fine-needle aspiration technique and protocol

EUS-FNA was done under conscious sedation with the assistance of an anesthesiologist by a single echoendoscopist. FNA was done by targeting of the lesion at the center of EUS image, closest to the transducer and avoiding intervening vessels by color doppler imaging. Needle size and technique of FNA were chosen according to the following protocol.

Before the procedure, patients were informed about the indication for EUS-FNA and possible complications associated with it and written informed consent was obtained. Institutional ethical committee approved this retrospective study.

All FNA were done using 22G needles except for transduodenal FNA where 25G needle was used.

1. Approach for acquiring tissue was transesophageal, transgastric, or transduodenal depending on the site of the lesion
2. An attempt to fan 4X4 times was made as shown in the Figure 1 in all the lesions to improve the yield
3. Lymph node FNA was done without suction but with gradual withdrawal of stylet (wicking technique). In rest of the lesions, 10 mL of suction force was applied with syringe
4. Stylet was used for expressing the aspirate onto 2 slides for cytology and remaining aspirate into formalin bottle for CB
5. If excessive bloodiness on specimen evaluation by endosonographer or senior nurse (SEEN) was seen as in Figures 2 and 3, suction was switched to wicking. Visual inspection for straw-colored, pink, red, chocolate-colored, and whitish-yellow-tinted material was done as in Figures 4-6 which represent tissue. Slide was seen for granularity. In formalin bottle, aspirate should sink and not disperse or float as it may indicate inadequate tissue sample
6. There was no on-site pathologist.

The yield of EUS-FNA CB and cytology was compared in solid lesions.

Sample/specimen processing

One drop of material was expressed at the corner of a clean properly labeled slide. Direct smears were made by gently spreading the material on the slide utilizing a spreader slide to create a monolayer of cells with minimal to no distortion to avoid tissue loss, artifacts, and interpretation difficulties

The slides were air-dried, and staining was done using Romanowsky-type staining kits consisting of a fixative (methanol), an acidophilic (eosin) dye for cytoplasmic staining, and a basophilic (methylene blue) dye for nuclear staining. Microscopic examination was performed by two-experienced pathologists. Cytological specimens obtained consist mainly of “loose” cells or cluster of cohesive cells rather than “cores” of tissue.

CBs were done with excess material after smear preparation. Additional pass dedicated for CB was made if SEEN evaluation does not reveal adequate specimen. The specimen was placed in formalin preservative. The

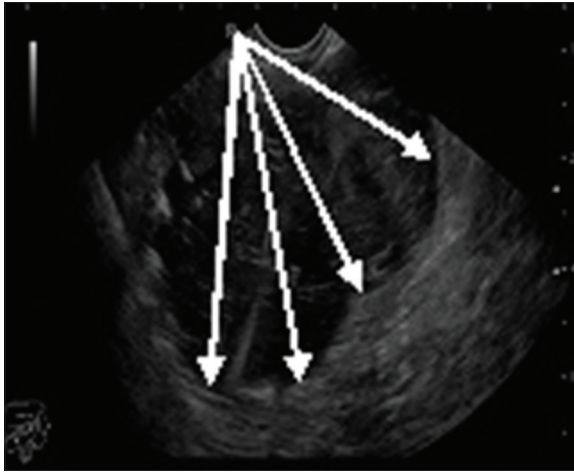


Figure 1: Endoscopic ultrasound image with demonstration of possible fanning trajectories - 4 × 4 rule



Figure 2: Thread biopsy using standard fine-needle aspiration – NEEDLE specimen for cell block - less bloody sample with wicking

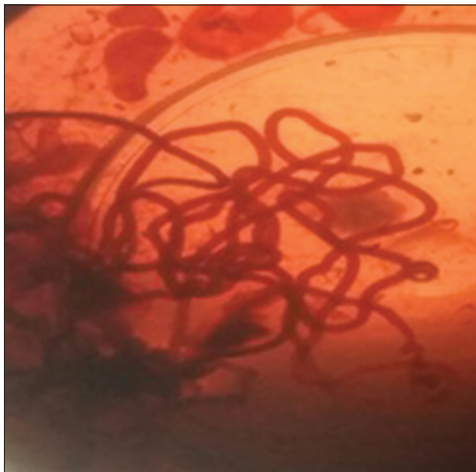


Figure 3: Thread biopsy showing a larger sample but more bloody with suction



Figure 4: Specimen evaluation by endosonographer or senior nurse for straw-colored or whitish-yellow-tinted material in between red clot of blood



Figure 5: Specimen evaluation by endosonographer or senior nurse for straw-colored material against light



Figure 6: Specimen evaluation by endosonographer or senior nurse showing black pigmented sample suggestive of hemorrhage

solution would be centrifuged to provide a pellet, fixed in 10% Formalin, and embedded in paraffin. The tissue was

then sectioned into thin slices, and stained with H and E and reviewed by the pathologists.

The pathology reports of the specimen sent were recorded. The yield of EUS-FNA and CB were recorded separately and their combined yield was also estimated. The yields of two techniques in different type of lesions were also noted.

Statistical analysis

The baseline characteristics were expressed as mean with standard deviation for continuous data and as percentage for categorical data. Comparison between the overall diagnostic yield of CB and cytology and the yield based on type of lesion was done by McNemar test. The $P \leq 0.05$ was considered statistically significant.

RESULTS

EUS-FNA/CB was performed in 114 patients during the study period. Of these, 101 met our inclusion criteria based on site of the lesion, and these patients had altogether 130 lesions, which were subjected to EUS-FNA/CB. Rest of the 13 patients had EUS-FNA from other sites (lung lesion – 6, omental deposit – 5, kidney lesion – 1, and parathyroid lesion – 1) and was excluded from this study. The demographic characteristics, lesion, and procedure details of the included participants were summarized in Table 1. Their mean age was 52.5 ± 12 years, and there were more males. Most FNA were transesophageal or transgastric, and hence, 22G needle was more frequently used.

The diagnostic yield of EUS-FNA cytology (FNAC) for the entire group was 70% and for CB was 74.6%, and this difference was not significant statistically ($P = 0.3$). When the yields of two techniques were combined together, a diagnosis could be achieved in 111 (85.3%) lesions. Table 2 shows the site-specific and combined diagnostic yield of EUS-FNA and CB. Pancreatic solid masses were the most common indication (37.7%) followed by the lymph nodes (36.9%), GI wall lesion (17.7%), and liver lesions 10 (7.7%). Suspicious cytology was also considered as positive for statistical analysis. The yield of CB was significantly better than cytology for GI wall lesions ($P = 0.04$). The yield of CB was more in liver and pancreatic lesions and less in lymph nodes compared to cytology, but these differences were not significant statistically as shown in Table 2.

Among 34 pancreatic solid lesions diagnosed by both EUS-FNAC and CB, adenocarcinoma, inflammatory, neuroendocrine tumor, neuroendocrine carcinoma, and intraductal mucinous tumor were noted in 14, 8, 6, 3, and 3 cases, respectively. Among 6 missed cases who underwent surgical exploration, the final diagnosis was adenocarcinoma in 4 and intraductal mucinous neoplasm in 2.

Table 1: Demographic, lesion, and procedure details of 101 patients (130 procedures) included in the study

1	Age in years- Mean \pm SD (Range)	52.5 \pm 12 (13-72 years)
2	Gender – Male <i>n</i> (%): Female <i>n</i> (%)	58 (57.5%): 43 (42.5%)
3	Location of lesion	
	a. Transesophageal- <i>n</i> (%)	a. 56 (43%)
	b. Transgastric- <i>n</i> (%)	b. 48 (37%)
	c. Transduodenal- <i>n</i> (%)	c. 26 (20%)
4	Size of lesion-in centimeter- mean \pm SD (range)	2.8 \pm 1.4 (1-8 cm)
5	Type of standard fine needle used- 22G: 25G- <i>n</i> (%)	104 (80%): 26 (20%)
6	Number of passes- mean \pm SD	3.6 \pm 0.5

The diagnosis in 28 cases with lymph node diagnosed on both FNAC and CB was tuberculosis (10), reactive (8), metastasis (6), sarcoidosis (3) and lymphoma (1). The other ten pathology in lymph nodes which were not diagnosed by EUS-FNAC/CB included metastasis (5), tuberculosis (3) and sarcoidosis (2) which was subsequently diagnosed after surgery or endobronchial biopsy.

In other GI wall lesions, 9 cases diagnosed by both EUS-FNAC and CB, 7 were GI stromal tumors (GIST) and 2 were leiomyoma. The two missed cases were GIST with large size (>5 cm) and with cystic spaces where EUS-FNAC and CB specimen revealed hemorrhage and necrosis. Two lesions in cytology were suspicious for GIST which showed only necrosis in CB evaluation. Five GISTs, 2 each of leiomyoma and Schwannoma and one carcinoid were diagnosed only in CB and were subcategorized with IHC.

There was no perforation, bleeding, or any significant complication associated with the study.

DISCUSSION

While EUS-FNA has proved to be an important diagnostic tool, efforts are on to improve the yield further. Among these, rapid on-site evaluation by pathologists (ROSE), making adequate number of needle passes and obtaining a CB have been proposed to affect yield although results are variable. Using ROSE, specimen acquisition can be improved with reduction of number of passes and complications.^[13] The presence of ROSE, size of the solid lesion >2 cm, and learning curve of cytopathologist have been shown to be significantly associated with diagnostic accuracy of EUS-FNA.^[14] However, ROSE may not be a viable, cost-effective strategy for many centers or institutions.^[15] Obtaining adequate specimen during EUS-FNA is a key issue, and in the absence of pathologist, adequacy of sample is difficult to confirm.

Table 2: Site/lesion specific and combined diagnostic yield of endoscopic ultrasound-guided fine-needle aspiration and cell block

Site/lesion type	CB diagnosis	Cytology diagnosis	Diagnosed in both	Undiagnosed in both	P
Pancreatic 49 (37.7%)	39 (79.5%)	38 (77.5%)	34 (69.3%)	6 (12.2%)	0.73
Lymph Node 48 (36.9%)	30 (62.5%)	36 (75%)	28 (58.3%)	10 (20.8%)	0.057
Other GI wall lesions 23 (17.7%)	19 (82.6%)	11 (47.8%)	9 (39.1%)	2 (8.6%)	0.04
Liver lesions 10 (7.7%)	9 (90%)	6 (60%)	6 (60%)	1 (10%)	0.25
Total 130 (100%)	97 (74.6%)	91 (70%)	77 (59.2%)	19 (14.6%)	0.303

An inadequate sample often results in repeat procedure which adds to the cost and delays diagnosis. Obtaining a CB may be one of the methods of improving yield and in the current study, where we had no ROSE, we noted a good diagnostic yield when FNAC was combined with CB.

Many studies on EUS-FNA of periluminal lesions have reported variable sensitivity and specificity depending on ROSE. In one study on 103 lesions, EUS-FNA sensitivity varied with the type of lesion (e.g., 89% for non-Hodgkin's lymphoma compared with 67% for stromal tumors).^[16] Diagnosis of GIST, which is a common GI submucosal lesion, requires IHC for KIT mutations apart from adequate sample to differentiate low-grade from malignant lesions, and this is much easier done on a CB.^[17] In a study of 65 patients undergoing EUS-FNA for GIST, EUS-FNA with IHC was diagnostic in 53 (80%) patients.^[18] With tissue available for multiple serial sections in CB, neoplasm can be better differentiated from contaminating or reactive tissues, and mitotic figures can be delineated, which may be difficult on cytology, smears.^[19,20] Average diagnostic accuracy rate of EUS-FNA has been reported to be 60% to 80% in other GI wall lesions.^[21] Our study showed that for GI wall lesions, CB was significantly superior to cytology and perhaps in these lesions, obtaining CB should be recommended to improve yield.

The likelihood of obtaining adequate tissue has been reported to be similar between EUS-FNA and EUS-true cut biopsy (TCB) with accuracy for specific diagnosis being higher in EUS-TCB compared to EUS-FNA (68.4% vs. 5.3%, $P < 0.005$).^[22] The stiffness and endoscopic tip angulation are technical drawbacks for EUS-TCB.^[23] However, when EUS-fine-needle biopsy (FNB) was used with suction, the angulation was not much of a concern. In this study, the CB had similar diagnostic yield as cytology but the combined yield of cytology and CB resulted a diagnostic yield of about 85%. FNB gave higher specimen adequacy than that of FNA in stromal tumors but converse was true in the lymph nodes (though not significant statistically). Both the techniques could be considered as complimentary

rather than competitive. Limitations of our study include the retrospective design with potential for the selection bias, but we had a uniform protocol for procedure and adequate follow-up.

CONCLUSIONS

During EUS, samples should be obtained for both cytology and histology if ROSE is not available. In cytology, the presence of large amounts of blood, necrotic material, or inflammatory cells can mask tumor cells leading to nondiagnostic specimen. The presence of CB provides a definitive diagnosis where tissue architecture is important in submucosal lesions such as GI stromal tumors and allows for special staining (IHC). Our results are limited due to small numbers and abovementioned methodological factors. However, they definitely add to the existing body of available literature showing that use of both CB and cytology improved the diagnostic outcome of EUS-FNA.

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Conflicts of interest

There are no conflicts of interest.

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