A Comparative Study of the Diagnostic Utility of Endoscopic Ultrasound-Guided Fine Needle Aspiration Cytology (EUS-FNA) versus Endoscopic Ultrasound-Guided Fine Needle Biopsy (EUS-FNB) in Pancreatic and Non-Pancreatic Lesions

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Abstract

Objectives: Endoscopic ultrasound-guided fine-needle aspiration (EUS-FNA) has become the procedure of choice to obtain samples from pancreatic lesions. However, it still has limitations affecting its diagnostic yield. The endoscopic ultrasound-guided fine-needle biopsy (EUS-FNB) needle was developed to allow acquisition of histological core. We conducted this study to compare the diagnostic yield of the Echotip 22Gauge FNA needle with the 22Gauge acquire FNB needle in pancreatic and non-pancreatic lesions. Materials and Methods: This prospective study was carried out on 100 cases of pancreatic and non-pancreatic lesions referred to El-Ebrashi unit of Gastroenterology and Hepatology, internal medicine department, Kasr Al-Aini hospital. The patients included were then randomized for sampling using either the standard Echotip 22Gauge FNA needle or 22Gauge acquire FNB needle. Results: Patients were 57 males and 43 females with a mean age of 58±15 years. Seventy-eight patients had pancreatic lesions, while twenty-two patients had non-pancreatic lesions. Half of the patients (50 cases) underwent EUS-FNA, and the other half (50 cases) underwent EUS-FNB. The presence of adequate tissue core was significantly higher in the FNB group. In contrast, smear cellularity was not significantly different between both groups. FNB had more sensitivity and accuracy depending on cell block/tissue core examination only for diagnosing pancreatic lesions. Blood contamination was higher in cell blocks of the FNA group. The sensitivity, specificity, and accuracy in the combined cytologic and histologic evaluation were 100%. Based on smear only or tissue only, the specificity was 100%, but the sensitivity and accuracy were decreased in both techniques. No complications were reported in both techniques. Conclusion: EUS-guided FNA and FNB are safe with comparable diagnostic accuracy in pancreatic and non-pancreatic lesions. FNB improved the histopathological quality of specimens with little blood contamination. Depending on tissue examination only in diagnosing pancreatic lesions, FNB had more sensitivity and diagnostic accuracy.

Keywords: EUS- FNA- FNB- diagnostic yield- accuracy- pancreatic- non-pancreatic- lesions

Introduction

Endoscopic ultrasound-guided fine-needle aspiration (EUS-FNA) has emerged as the procedure of choice to obtain samples to reach a definitive diagnosis and proper staging of lesions of the gastrointestinal tract and adjacent organs (Fuccio and Larghi, 2014). The diagnostic accuracy of EUS-FNA for pancreatic cancer ranges from 78% to 95% and has a sensitivity and specificity of 85% to 95% and 95% to 98%, respectively (Kandel et al., 2016).

This variation in the diagnostic utility is dependent on many factors, including lesion location, the availability of rapid on-site evaluation (ROSE), the skill and experience of the endo-sonographer, and the size and type of needle selected for tissue acquisition. One of the limitations of EUS-FNA is that it does not provide core tissue with preserved architecture, which is required for immunohistochemical staining needed for the diagnosis of some lesions such as lymphoma, gastrointestinal stromal tumor (GIST), and autoimmune pancreatitis (Na et al., 2015).

Core biopsy needles have been developed to procure histology samples to overcome these limitations. Although randomized trials have shown that fine needle biopsy (FNB) needles reliably procure histology-grade specimens, their impact on routine clinical practice is unclear (Bang et al., 2019).

Until now, limited data have been published on the...
impact of needle type (FNA or FNB) on the diagnostic yield and the technical success among Egyptian patients. Therefore, our study aims to compare the diagnostic accuracy and technical success of the standard 22-G FNA needle to the acquire FNB needle with different gauges in both pancreatic and non-pancreatic lesions.

Materials and Methods

Patients

This prospective study included 100 patients who presented with pancreatic and non-pancreatic lesions at Kasr Al-Aini hospital, internal medicine department, from May 2018 to March 2021. Patients were divided into two groups: Group (A) includes 50 cases; who underwent EUS-FNA using an Echotip 22G needle (Cook Medical, Bloomington, IN). Group (B) includes the remaining cases, and EUS-FNB was done using 22G acquire needles (Boston Scientific, Marlborough, MA).

Inclusion criteria: Patients who require endoscopic ultrasound and tissue sampling after imaging examination (MRI, CT, and ultrasonography) that shows either pancreatic, intra-abdominal or mediastinal solid lesions (size > 1cm).

Exclusion criteria:
1) Hemoglobin ≤ 8.0 g/dL
2) Patient has any coagulation disorder
3) History of taking oral anticoagulation agents in the past week
4) Experienced acute pancreatitis in the past 2 weeks
5) Has cardiorespiratory dysfunction that cannot tolerate the procedure
6) Unable to provide informed consent

Technique of EUS-FNA and FNB

On the procedure day, eligible patients were appointed to the endoscopy room for EUS examination under intravenous propofol sedation. EUS examination was performed in all patients with a linear Echoendoscope Pentax EG3870UTK attached to Hitachi Avius Ultrasound machine. The same technique was used for tissue sampling with both Echotip FNA (Cook medical) and acquire FNB (Boston scientific) needles to avoid technical biases. The endoscopist used color Doppler to identify the optimal position for puncture without intervening vessels between the needle and target lesion. The needle was then inserted into the target tissue under EUS guidance. After the needle penetrated the lesion, back and forth movements were done with simultaneous minimal negative pressure by pulling the needle stylet slowly and continuously. Then continuous suction was applied with a 10-ml syringe, and the needle was moved back and forth 20 times within the lesion. Suction was released, and then the needle was withdrawn from the lesion. For each lesion, one to three passes were carried out.

Cytopathologic analysis

Smears were prepared from the material obtained by either FNA or FNB and immediately fixed in 95% ethyl alcohol for 20 minutes, then stained with Papanicolaou stain. Moreover, abundant material obtained from FNA and FNB (blood containing cellular aggregates and/or tissue fragments) was directly fixed in 10% buffered formaldehyde and then routinely processed. Tissue blocks were cut into 4-μm slides. Sections were stained with hematoxylin and eosin.

Assessment standard

All samples were analyzed to assess cytological material quality (including smears cellularity and blood contamination) and histopathological quality (including tissue integrity and blood contamination) of each needle following a three-class grading system for each item as described by (Alatawi et al., 2015; Fabbri et al., 2015). For tissue integrity; Grade A: an architecturally intact piece of tissue measuring at least 550 microns in the greatest axis, as the diameter of a high-power microscopic field clearly characterizes the lesion sufficient for diagnosis; Grade B: tissue fragments present, the tissue does not meet the criteria for architecturally intact histology but can still yield a diagnosis based on cell morphology; Grade C: no lesion tissue found and cannot yield a diagnosis. For smear cellularity; Grade A: satisfactory, more than four clusters, with a minimum of ten cells in each cluster; Grade B: adequate, approximately two to four clusters, with a minimum of ten cells in each cluster; Grade C: unsatisfactory, fewer than two clusters, or no cellular smear). Assessment of blood cell contamination was graded into: Grade A: little blood contamination, minimal surface area (SA) < 25 % of the slide, Grade B: medium blood contamination, SA 25–50 % of the slide, Grade C: much blood contamination, SA > 50 % of the slide).

Immunocytochemical staining

Immunostaining was performed on cell block/core material when needed according to the differential diagnosis, following the standard immunoperoxidase methods using Ventana BenchMark. The antibodies used included CK, CK7, CK20, P63, Ki67, Synaptophysin, Chromogranin, CD10, CD68, LCA, TTF1, CEA, ER, PR, HER2, GATA-3, SMA, CD117, DOG-1, Beta-catenin, CD34, S100, Glypican-3 and HepPar-1.

Final diagnosis

Pancreatic cases were diagnosed and categorized according to “The Papanicolaou Society of Cytopathology System for Reporting Pancreaticobiliary Cytology: standardized terminology and nomenclature for pancreaticobiliary cytology” (Pitman and Layfield, 2014). These categories include; (I) Non-diagnostic, (II) Negative for malignancy, (III) Atypical, (IV) Neoplastic (Benign or others), (V) Suspicious for malignancy and (VI) Positive for malignancy. The non-pancreatic lesions were diagnosed following five categories, “unsatisfactory,” “positive for malignancy,” “suspicious for malignancy,” “atypia,” and “negative for malignancy.” (Fabbri et al., 2015).

Statistical methods

The collected data was entered on the computer using Microsoft Office Excel Software Program 2019. Pre-coded data was then transferred and entered into the
Statistical Package of Social Science Software program, version 26 (SPSS), to be statistically analyzed. For quantitative variables, they were described as mean, standard deviation, median, minimum and maximum. For qualitative variables, they were described as frequency and percentage and compared using the Chi-square test, where the p-value is significant if less than 0.05.

### Results

The study included 100 patients fulfilling the inclusion criteria, equally divided between FNA and FNB groups. The majority of the included patients had pancreatic lesions (78%), and most of them were located in the pancreatic head. The non-pancreatic lesions included lymph nodes, gall bladder, esophagus, duodenum, stomach, rectum, and mediastinum. The main patient characteristics are displayed in Table 1.

Among pancreatic lesions, forty cases (40) underwent EUS-FNA, and thirty-eight cases (38) underwent EUS-FNB. On the other hand, ten (10) non-pancreatic lesions underwent EUS-FNA, and the remaining twelve cases (12) underwent EUS-FNB. In both procedures, the median number of needle passes was two. Sampling outcomes for FNA and FNB are presented in Table 1.

For both pancreatic and non-pancreatic lesions, the presence of adequate tissue core was significantly higher in the FNB group (P-value =0.001). Examples of grade A and B tissue integrity are shown in Figures 1 and 2, respectively. In contrast, smear cellularity was not significantly different between the two types of needles. Cell blocks of FNA were significantly higher in blood content than FNB (P-value =0.002). However, no complications or technical difficulties were recorded.

### Table 1. Comparison between EUS-FNA and FNB in Clinical Variables, Diagnostic Categories and Sampling Outcomes

| Characteristic          | FNA (N=50) | FNB (N=50) | p-value |
|-------------------------|------------|------------|---------|
| Age (mean±SD)           | 58±16.7    | 57.5±12.5  | 0.626   |
| Sex (male:female)       | 29:21:00   | 28:22:00   | 0.84    |
| Site of lesion          |            |            | 0.629   |
| Pancreatic              | 40         | 38         |         |
| Non-pancreatic          | 10         | 12         |         |
| Diagnostic categories   |            |            | 0.251   |
| Negative                | 10         | 4          |         |
| Positive                | 40         | 46         |         |
| Tissue integrity        |            |            | 0.001   |
| A                       | 13         | 43         |         |
| B                       | 31         | 6          |         |
| C                       | 6          | 1          |         |
| Tissue blood contamination |          |            | 0.002   |
| A                       | 12         | 23         |         |
| B                       | 15         | 20         |         |
| C                       | 23         | 7          |         |
| Smear Cellularity       |            |            | 0.576   |
| A                       | 45         | 42         |         |
| B                       | 3          | 6          |         |
| C                       | 2          | 2          |         |
| Smear blood contamination |          |            | 0.071   |
| A                       | 16         | 8          |         |
| B                       | 15         | 25         |         |
| C                       | 19         | 17         |         |

### Table 2. Different Diagnoses of Pancreatic and Non-Pancreatic Lesions

| Diagnosis                        | FNA (N=50) | FNB (N=50) | Total |
|----------------------------------|------------|------------|-------|
| Pancreatic lesions (N=78)        |            |            |       |
| Adenocarcinoma, ductal type      | 30         | 33         | 63    |
| Acinar cell carcinoma            | 1          | 0          | 1     |
| IPMN                              | 0          | 1          | 1     |
| Metastatic breast duct carcinoma | 1          | 0          | 1     |
| Neuroendocrine tumor             | 1          | 2          | 3     |
| Pancreatitis                     | 6          | 1          | 7     |
| solid pseudopapillary neoplasm   | 1          | 0          | 1     |
| Undifferentiated pancreatic carcinoma with osteoclast-like giant cells | 0 | 1 | 1 |
| Non-pancreatic lesions (N=22)    |            |            |       |
| Adenocarcinoma                   | 2          | 5          | 7     |
| Signet ring carcinoma            | 2          | 1          | 3     |
| GIST                              | 1          | 2          | 3     |
| Granuloma (Sarcoidosis)          | 1          | 0          | 1     |
| Granuloma, TB                    | 0          | 3          | 3     |
| Hepatocellular carcinoma         | 0          | 1          | 1     |
| Cholecystitis                    | 1          | 0          | 1     |
| Reactive lymphoid hyperplasia    | 2          | 0          | 2     |
| Squamous cell carcinoma          | 1          | 0          | 1     |
during the EUS procedure for both types of needles. Based on both histologic and cytologic assessment criteria, eighty-six (86%) out of all cases (71 pancreatic cases and 15 non-pancreatic cases) were positive for malignancy and fourteen cases (14%) were negative for malignancy (7 cases for each group).

Among pancreatic lesions, adenocarcinoma, ductal type was the dominant diagnosis (diagnosed in sixty-three cases). Different diagnoses for both pancreatic and non-pancreatic lesions were demonstrated in Table 2.

Immunohistochemistry was indicated for twelve cases. The different antibodies used with the final diagnosis are shown in Table 3. Examples of cases that underwent immunohistochemistry are shown in Figures 3 and 4.

There is no significant difference in establishing diagnosis using smear only between FNA and FNB groups. Cell block/core biopsy confirmed the cytologic diagnosis in all adequate cases. However, in pancreatic cases, FNB had more diagnostic outcome than FNA when depending on cell block/tissue core only (p-value=0.002), as six FNA cases had inadequate cell blocks for diagnosis. In non-pancreatic cases, there was no significant difference between smears only and cell block/tissue core only (p-value=0.746)

When combining cytological evaluation of smears and histological evaluation of cell blocks/core biopsies,
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Table 3. Different Markers Done for Final Diagnosis

| Site                        | Number of cases | Markers done                                      | Diagnosis                                      |
|-----------------------------|-----------------|---------------------------------------------------|-----------------------------------------------|
| Pancreas, two at the head   | 3               | Chromogranin, Synaptophysin, and Ki67             | Neuroendocrine tumor, grade 2                 |
| and one at body             |                 |                                                   |                                               |
| Pancreas, body              | 1               | Beta-Catenin, CD10, PR, Chromogranin, and Synaptophysin | Solid pseudopapillary neoplasm                |
| Pancreatic tail             | 1               | GATA-3, ER, PR & HER2                             | Metastatic breast carcinoma to pancreas       |
| Stomach                     | 2               | CK, LCA, S100, and CD68                           | Signet ring adenocarcinoma                    |
| Stomach                     | 2               | CD117, DOG-1, SMA and CD34                        | GIST                                           |
| Duodenum                    | 1               | CD117, DOG-1, SMA and CD34                        | GIST                                           |
| Esophagus                   | 1               | CEA, CD15, P63, LCA, ER, PR and HER2             | Signet ring adenocarcinoma                    |
| Para-aortic lymph node      | 1               | CK7, CK20, Glypican-3, AFP, Chromogranin, CDX-2, TTF-1 and HepPar-1 | Hepatocellular carcinoma                     |
| Total                       | 12              |                                                   |                                               |

Table 4. Comparison of Sensitivity, Specificity, PPV, NPV and Accuracy between EUS-FNA & EUS-FNB when Using Smears Only, Tissue Only, and Both Smears and Tissue in Diagnosis of All Cases

| Site                        | Smears only     | Tissue only | Combined smears and tissue |
|-----------------------------|-----------------|-------------|----------------------------|
|                            | EUS-FNA | EUS-FNB | EUS-FNA | EUS-FNB | EUS-FNA | EUS-FNB |
| Sensitivity                 | 98%     | 98%     | 88%     | 98%     | 100%    | 100%    |
| Specificity                 | 100%    | 100%    | 100%    | 100%    | 100%    | 100%    |
| PPV                         | 100%    | 100%    | 100%    | 100%    | 100%    | 100%    |
| NPV                         | 91%     | 80%     | 67%     | 80%     | 100%    | 100%    |
| Accuracy                    | 98%     | 98%     | 90%     | 98%     | 100%    | 100%    |

Figure 3. Case of Solid Pseudopapillary Tumor of the Pancreas; EUS-FNA (22-gauge) (A) Smear showing pseudo-papillae having hyaline fibrovascular core covered by round cells (Chinese letter appearance) and many dispersed cells in the background. (Papanicolaou X40). (B) High power view showing pseudo-papillae covered by multiple layers of uniform round to oval cells. (Papanicolaou X400). (C) Cell block showing pseudo-papillae with hyaline fibrovascular cores. (Hematoxylin & Eosin X400). (D) Tumor cells show a diffuse positive nuclear reaction to Beta-Catenin. (E) Tumor cells show a diffuse positive nuclear reaction to PR. (F) Tumor cells are negative to chromogranin.
Discussion

EUS–FNA has a pivotal role in the diagnosis and staging of pancreatic and gastrointestinal as well as mediastinal lesions (Fujita et al., 2020). The acquisition of samples by (EUS) has proved to be the most effective diagnostic instrument, using 2 techniques: The first implemented and older method is fine-needle aspiration (FNA), while fine needle biopsy (FNB) has emerged as an alternative procedure in more recent years (Yan et al., 2018). EUS-FNA is a useful, safe, and highly accurate diagnostic tool because needles are generally more flexible and easier to use, as confirmed in several studies in the last 2 decades (van Riet et al., 2020). The greatest strength of FNA is its excellent specificity (around 100% in nearly all studies) while there are still conflicting results regarding its sensitivity (which ranges from 85 to 93% in most trials) (Marta Nicola et al., 2020; Facciorusso et al., 2018). However, many factors influence the diagnostic yield of pancreatic lesions, including anatomical location, tumor nature (presence of fibrosis and areas of necrosis), technical factors, like the difficulty to reach the sampling site, the needle size, the availability of rapid on-site evaluation (ROSE) for sample collection, the number of needle passes, the use of tissue blocks versus cytological smears which depends on the availability of experienced cytopathologist (Marta Nicola et al., 2020). All these factors remain unstandardized, and the procedure is still operator-dependent (Marta Nicola et al., 2020). In addition, it is difficult to differentiate between well-differentiated tumors and regenerative inflammatory tissue based on cytological specimens only (Wang et al., 2017). Also, accurate diagnosis of some tumors such as lymphoma, GIST and neuroendocrine tumors requires histologic assessment of tissue architecture and immunohistochemical staining (Marta Nicola et al., 2020).

Many advances in EUS-guided tissue acquisition have been made, including several novel needle designs. Tissue core needles have been shown to procure more histology-grade tissue than the regular FNA needles and maybe a suitable choice in the case of solid pancreatic lesions, especially in the era of molecular profiling and precision medicine (Kovacevic et al., 2021).

Studies addressing the feasibility of providing histologic samples with these needles and the additive diagnostic value of histological versus cytological assessment are still limited (Polkowski et al., 2017).

The current prospective study and previous studies (Tian et al., 2018; Altonbary et al., 2019) found that both FNA and FNB are applicable and safe during operation with no procedure-related complications or technical difficulties among all patients. The safety of EUS tissue sampling is well established, and a few or no adverse events have been reported in the literature (El Hajj et al., 2018).

When we correlated both needles with clinical parameters, no significant statistical difference was detected between the EUS-FNA and EUS-FNB groups regarding age, sex, and lesion site. These findings are similar to those obtained from studies done by (Tian et al., 2018; Altonbary et al., 2019).

In our study, the median number of needle passes required per lesion was 2, with no significant difference between FNA and FNB; this is in line with a study done by (Bang et al., 2012). In studies done by (Hucl et al., 2013; Lee et al., 2014; Tian et al., 2018), the mean number of needle passes was significantly lower for FNB than FNA. The discrepancy between our study and these studies might be due to immediate cytologic evaluation (ROSE) availability in their studies.

The acquire needle was developed as a histology needle. A histologic core is essential for diagnosing most...
non-pancreatic lesions such as GIST and lymphoma. Moreover, even in the case of pancreatic malignancies, immunohistochemical staining requires histological cores, which may become more important in the era of individualized therapy (Ang et al., 2019).

For both pancreatic and non-pancreatic lesions, we found that the integrity of tissue core obtained by FNB needles is better than those obtained by FNA needles with similar overall cytologic yield. Our data support what was previously reported by (Kandel et al., 2016; Cheng et al., 2017; van Riet et al., 2019).

Despite the higher blood cell content of the cell blocks of FNA samples in our cases, no clinical complication was recorded, as we mentioned before.

The performance of both needles in establishing diagnosis based on smear only or cell block only in both pancreatic and non-pancreatic lesions had been assessed. Our study, like the study of Asokkumar et al., (2019) did not observe any difference in the smear diagnostic adequacy between both needles for pancreatic and non-pancreatic lesions. Unlike the study of Aadam et al., (2014) who concluded that FNB was superior to FNA in diagnostic yield when examining the non-pancreatic lesions. Like the study of Cheng et al., (2017), our study has recorded that FNB achieved better histological diagnostic yield when assessing pancreatic masses with no significant difference between both needles in assessing non-pancreatic lesions.

Previous comparative studies on the diagnostic accuracy of EUS-FNA and EUS-FNB had yielded conflicting results (Levine et al., 2021). Our study and a previous meta-analysis, including six studies comparing the diagnostic accuracy of acquire needles and standard FNA needles for sampling various lesions (pancreatic, lymph nodes, gastric, pelvic lesions) did not record any significant difference in diagnostic accuracy between both needles (Hucl et al., 2013; Lee et al., 2014; Berzosa et al., 2015; Mavrogenis et al., 2015). In contrast, another two studies found that FNB was inferior to FNA in diagnosing pancreatic masses. However, both studies evaluated a small number of cases, and one of them used a different number of passes for FNA and FNB (Strand et al., 2014; Vanbiervliet et al., 2014). This conflict could be explained by the usage of different biopsy needles, the variable number of passes for FNA and FNB (Strand et al., 2014; Altonbary et al., 2019). Unlike the study of Aadam et al., (2014) who concluded that FNB was superior to FNA in diagnostic yield when examining the non-pancreatic lesions. The studies of Cheng et al., (2017) and our study found that the integrity of tissue core obtained by FNB needles is better than those obtained by FNA needles with similar overall cytologic yield.

In conclusion, EUS-guided FNA and FNB are safe, appropriate procedures with comparable diagnostic accuracy when assessing pancreatic and non-pancreatic lesions. EUS-FNB has more sensitivity and diagnostic accuracy in diagnosing pancreatic lesions depending on tissue examination only. EUS-FNB improved the histopathological quality of specimens with little blood contamination compared to EUS-FNA, thus can help in establishing diagnosis, especially in the absence of an experienced cytopathologist. This improvement in histopathological quality is also expected to facilitate the application of immunohistochemistry and molecular studies needed for precision genomics like next-generation sequencing and tailored therapy in favor of innovative therapeutic strategies.

Author Contribution Statement

All authors contributed to this study. All Samples were obtained by Dr Hussein Hassan Okasha. Preparation, data collection, review of the slides and analysis were performed by Dr Mohamed Yousri Ahmed. Monitoring of data collection, interpretation of results, revision and guidance were done by Dr Ahmed Hussein El-Habashi and Dr Eman Mahmoud Samy Abu-Sinna. The preliminary draft of the manuscript was written by Dr Mohamed Yousri Ahmed. All authors revised and commented on primary version of the manuscript and approved the final one.

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Ethical approval

The study was approved by the Institutional Review Board (IRB) no. IRB201920023.3 of National Cancer Institute (NCI), Cairo University. Oral and written informed consents were obtained from all patients or from their eligible relatives.

Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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Availability of data

The datasets are available from the corresponding author on reasonable request.

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