19 G aspiration needle versus 19 G core biopsy needle for endoscopic ultrasound-guided liver biopsy: a prospective randomized trial

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ABSTRACT
Background Endoscopic ultrasound-guided liver biopsy (EUS-LB) is a safe and effective method for accomplishing parenchymal liver biopsy. The aim of this study was to compare a 19 G aspiration needle (FNA) with a 19 G Franseen-tip core biopsy needle (FNB) for EUS-LB.

Methods This was a prospective, parallel group, randomized trial comparing the tissue yields and adequacy of a 19 G FNA needle vs. a 19 G FNB needle for EUS-LB. The primary outcome was length of the longest piece of liver core specimen. Secondary outcomes were aggregate specimen length, number of complete portal triads (CPTs), and adverse events. One transgastric pass and one transduodenal pass were performed with the same needle in each patient. Specimen lengths were measured before and after histological processing.

Results 40 patients referred for EUS-LB were randomized to either the FNA group (n = 20) or the FNB group (n = 20). Both groups had similar patient characteristics. FNB biopsies yielded longer mean (standard deviation) specimen lengths (pre-processing mean 2.09 cm [0.41] vs. mean 1.47 cm [0.46], and post-processing mean 1.78 cm [0.66] vs. mean 1.05 cm [0.42]; both \( P < 0.001 \)), a longer aggregate specimen length (pre-processing mean 15.78 cm [5.19] vs. 10.89 cm [4.38]; \( P = 0.003 \)), and more CPTs (mean 42.6 [25] vs 18.1 [9.3]; \( P < 0.001 \)) compared with the FNA needle. There were no severe adverse events or difference in adverse event rate between the two needles. Post-biopsy pain was noted in 37.5 %.

Conclusion EUS-LB using the FNB needle delivered longer liver biopsy specimens with more CPTs than the regular

Introduction
Chronic liver disease has several causes and is an important cause of morbidity and mortality. Although blood testing and noninvasive diagnostic imaging modalities are useful, these may not be able to determine the etiology of a patient’s chronic liver disease [1–4], and in other cases may be unable to accurately estimate the degree of hepatic fibrosis. Liver biopsy remains an important tool in diagnosis and treatment of liver disease.

Percutaneous liver biopsy remains the predominant means of parenchymal liver biopsy, but endoscopic ultrasound-guided liver biopsy (EUS-LB) can obtain robust cores of parenchymal liver tissue and is being used with increasing frequency [5–8]. A benefit of EUS-LB is the ability to quickly and safely perform bilobar sampling under real-time sonographic imaging during the entire procedure, minimizing the risk of inadvertent puncture of a large vessel or other organ [8]. Other potential benefits include patient comfort during the procedure, and increased efficiency and convenience for patients who require both liver biopsy and esophagastroduodenoscopy or EUS.
Several studies using the 19 G EUS fine-needle aspiration (FNA) needle have shown the feasibility and accuracy of the EUS-LB technique [9–13], with tissue yields exceeding 90%. A small retrospective study comparing liver biopsy by EUS-LB with either percutaneous or transjugular routes showed that the different methods for biopsy were comparable in terms of amounts of tissue obtained and complete portal triad (CPT) counts [14].

Ongoing research has focused on how to improve the samples obtained with this technique. Initial studies on EUS-LB utilized either a 19 G FNA needle [6, 10] or the 19 G TruCut needle (Merit Medical, South Jordan, Utah, USA) [9, 13]. Since the development of core needles for EUS-guided biopsy, these devices have been tried for EUS-LB, with encouraging results [8, 15]. However, no head-to-head in vivo prospective comparison of these needles has been conducted. In this study, we aimed to compare tissue yield of a 19 G core biopsy EUS needle (FNB) with that of a 19 G regular EUS-FNA needle in a prospective randomized trial. The hypothesis of our study was that the 19 G FNB core needle would provide better tissue yields than the currently used FNA needle.

Methods
Study design and population
This was a prospective, parallel-group, randomized trial of consecutive adult patients referred for EUS-LB at the Geisinger Medical Center between October 2017 and December 2017 for suspected hepatic parenchymal disease. Patients included in the study were randomly assigned to undergo EUS-LB with either the EUS-FNA or EUS-FNB 19 G needle. The trial was registered at ClinicalTrials.gov (NCT03408171) and approved by the institutional review board at Geisinger Medical Center (IRB 2017–0391, approved 10/9/2017). Informed consent for the study was obtained from all patients. Four endosonographers with extensive previous experience with EUS-LB performed the biopsies.

Inclusion criteria were patients over 18 years of age with abnormal liver enzyme tests of uncertain etiology, or the need to grade and stage autoimmune, viral, or metabolic liver disease. Exclusion criteria were pregnancy, platelet count <50 000/mL of blood, international normalized ratio >1.5, inability to provide informed consent, inability to discontinue anticoagulation or antiplatelet agents, treatment with low molecular weight heparin, hemophilia, known liver cirrhosis or presence of ascites.

Data were collected on patient demographics, alcohol use, pertinent laboratory tests, pre- and post-processing specimen lengths, CPTs, liver fragment size, pathology results, and adverse events.

EUS-LB protocol
Patients undergoing EUS-LB received propofol-based anesthesia during the procedure, with appropriate cardiorespiratory monitoring by a certified registered nurse anesthetist, as per routine practice. A linear array echoendoscope was used (GF-UC140-ALS; Olympus America, Center Valley, Pennsylvania, USA). Doppler imaging was used to ensure that no vascular structures were present along the expected trajectory of the needle. The EUS-LB was performed in widely separated regions of the liver (right and left lobes) using a 19 G EUS-FNA needle (Expect Flexible 19 G; Boston Scientific, Marlborough, Massachusetts, USA) or a 19 G FNB Franseen-tip needle (Acquire 19 G; Boston Scientific) (Fig. 1s in the online-only supplementary material). A computer-generated randomized table determined needle type selection; the table was concealed, and the endosonographer was told the group immediately before the biopsy.

Prior to biopsy, the stylet was removed, 2–3 mL of heparin (100 units/mL) was flushed through the needle, and the suction syringe was set and attached to the needle hub [16]. The needle was then introduced into the echoendoscope channel. A transgastric approach was used to obtain samples from the left lobe of the liver, a few centimeters below the gastroesophageal junction. A transduodenal approach, with the linear echoendoscope placed in the duodenal bulb, was utilized to acquire biopsies from the right liver lobe. Once liver parenchymal penetration was achieved with the needle (1–2 cm), full suction was applied with the 20 mL vacuum syringe. One pass consisted of a total of 7–10 to-and-fro needle motions using the fanning technique applied under direct and continuous endosonographic visualization of the tip of the needle. After the biopsy, and before the needle was removed from the liver parenchyma, the suction was turned off by using the stopcock on the vacuum syringe. Visualization of the puncture site with Doppler post-biopsy was not routinely done.

The needle was then removed from the echoendoscope. The sample was pushed from the needle with the stylet directly onto a small nylon mesh sieve (made from part of a histopathology cassette), and the sample was rinsed gently with saline, which removed all or most of the admixed blood (Fig. 1). Small pieces of light brown tissue – the cores of liver tissue – remained on the sieve; these were, then “floated” off the sieve into formalin solution. Heparin was again flushed through the needle lumen. A single pass per liver lobe was performed in...
each patient. Only one type of needle was used per patient as determined by the randomization table.

Pathology processing and data analysis
Liver samples were left in formalin for at least 1 hour before processing. The contents of the formalin jar were poured into a petri dish, and liver tissue cores were picked out with small forceps. These fragments were arranged in a linear fashion on lens paper, and the specimens were photographed alongside a ruler to measure pre-processing tissue lengths (Fig. 2). The lengths of the pieces of liver tissue were measured from the photograph after calibrating with a millimeter-graded ruler using ImageJ software (imagej.nih.gov, version 1.51. [17]). These measurements were made by a collaborator who was blinded to needle type. Samples from each lobe were submitted for evaluation separately. Tissue cores were processed in the usual fashion and, after paraffin embedding, slide blanks were made (5 μm tissue thickness). These blanks were stained with hematoxylin and eosin, trichrome, and reticulin, and additional immunohistochemistry as required.

Slides were digitized using a whole slide scanner at ×40 allowing digital zoom to ×8 (Ventana iScan Coreo; Roche Diagnostics USA, Indianapolis, Indiana, USA). The digitized images were used for post-processing quantitative analysis with accompanying software (Ventana Virtuoso; Roche Diagnostics). This software has been validated by CE and FDA (in vitro diagnostic validation). All samples were measured pre- and post-processing for maximum intact core tissue length and aggregate specimen length, and the total number of CPTs was determined by examination of the whole-slide scanning images. The investigator who performed the measurements and CPT counts was blinded to needle type used for the biopsy.

Follow-up after biopsy sampling
All patients were closely observed in the recovery area for 1 hour after the procedure and were followed up by a phone call the day after the procedure, as per our standard policy. Subsequent in-person follow-up in the hepatology or general gastroenterology clinic was done in most patients.

Study definitions
The primary outcome measure was the pre-processing length of the longest piece of liver biopsy specimen; this was used because it is the most objective and meaningful measurement of a good liver core biopsy. Aggregate specimen length does not adequately account for multiple small fragments, which are less useful for histological interpretation. Secondary outcomes were post-processing length of the longest piece of liver biopsy, pre- and post-processing aggregate specimen lengths, number of CPTs in the specimen, and adverse events. We also looked at the percentage of samples that were ≥2 cm compared with those that were <2 cm, technical success rate, and ability of pathologist to provide a diagnosis on the specimen.

Technical success with biopsy attempts with either needle was described as effective conclusion of all phases from needle insertion into the scope to successfully obtaining a sample. Any unexpected incident occurring for any patient enrolled in the study was considered an adverse event of EUS-LB. Adverse events were graded according to the American Society for Gastrointestinal Endoscopy lexicon [18]. In these recommendations, an adverse event is an event that prevents completion of the planned procedure and/or results in admission to the hospital, prolongation of existing hospital stay, another procedure (needing sedation/anesthesia), or subsequent medical consultation. Abdominal pain was categorized using a 1–10 pain scale, as mild (1–3), moderate (4–6), or severe (7–10). Patients were assessed by post-procedure phone calls and office visits. Adverse events that were screened for included abdominal pain, nausea, vomiting, sore throat, hemorrhage, infection, perforation, pneumothorax, bile leak, and anesthesia-related side-effects.

Statistical analysis
Based on our prior study [16,19], the pre-processing length of the longest piece of liver tissue from an FNA needle is 1.5 cm with a standard deviation (SD) of 0.5 cm. In our initial experience with 10 patients using the FNB needle, we observed a pre-processing longest length of 1.95 cm. Using these values, for 80% power with an alpha (significance level) of 0.05, the sample size for a two-tailed test would be 39 patients. For this reason, 40 patients were enrolled (20 patients in the FNA group and 20 patients in the FNB group).

For analysis, continuous variables were described as mean and SD or median and range. Categorical variables were expressed as simple proportions. Statistical analyses were performed using SPSS version 21 (IBM Corp., Armonk, New York, USA). One-way analysis of variance and Tukey correction were used. A P value of <0.05 was considered to be significant for continuous data and categorical data using chi-squared test or Fisher’s exact test.
Results

A total of 40 patients were referred for EUS-LB from October 2017 to December 2017, typically for evaluation of abnormal liver enzymes, and none declined participation in this study (Fig. 2s). Study population demographics and indications for liver biopsy are presented in Table 1s and Table 2s. Patients who underwent EUS-LB were most commonly white (Non-Hispanic) (n = 34; 85%) and obese (n = 24; 60%), with the indication of abnormal liver biochemical tests (n = 32; 80%). A total of 20 patients underwent EUS-LB with the FNA needle; these patients had a median age of 51 years (range 28–67 years) and a median body mass index (BMI) of 32.3 kg/m² (range 21.6–49.54 kg/m²). The remaining 20 patients underwent biopsy with the FNB needle. The median age of this group was 49 years (range 27–69 years), and median BMI was 30.2 kg/m² (range 21.6–50.0 kg/m²).

Liver biopsy specimen results

Bilobar liver biopsies (transgastric and transduodenal) were obtained from all patients. The quantitative data are presented in ▶ Table 1, ▶ Fig. 3, and ▶ Fig. 4 (see also Fig. 3s) and represent per-patient data (with left and right lobar biopsy results combined). The mean pre-processing length of the longest specimen piece was 2.09 cm (SD 0.41) in the FNB group vs. 1.47 (SD 0.46) in the FNA group (P < 0.001). The length of the longest piece was further subclassified as < 2.0 cm or ≥ 2.0 cm; 10 (50%) were ≥ 2.0 cm in the FNB group vs. 3 (15%) in the FNA group (P = 0.04). The mean pre-processing aggregate specimen length was 15.78 cm (SD 5.19) with the FNB needle compared with 10.89 cm (SD 4.38) with the FNA needle (P = 0.003). The median number of CPTs was 38.0 (range 0–81) with the FNB needle vs. 16.5 (range 6–38) with the FNA needle (P = 0.004) (Table 1).

Pathological diagnosis was possible in 100% of liver biopsies and included alcoholic and nonalcoholic liver disease, viral hepatitis, drug-induced liver injury, primary biliary cholangitis, autoimmune cholangiopathy, and metabolic and genetic diseases including hemochromatosis. Both needles produced aggregate specimen ≥ 2 cm in length.

Adverse events

Clinical follow-up was available in all patients to check for delayed adverse events. A summary of adverse events is given in ▶ Table 2. Of the 40 patients who underwent EUS-LB, 13 complained of abdominal pain, 7 (35%) in the FNA group vs. 6 (30%) in the FNB group (P = 0.74). The pain was severe in 5 patients (25%) in the FNB group compared with 1 (5%) in the FNA group (P = 0.18). Five patients (25%) in the FNB group required analgesics for abdominal pain compared with 2 (10%) in the FNA group (P = 0.41). All patients were discharged from the endoscopy unit. One patient was evaluated after discharge in the emergency department for severe abdominal pain; this patient underwent colonoscopy on the same day. An abdominal computed tomography was obtained, which was unremarkable. The pain subsided without analgesics and the patient was discharged.

| Table 1 Quantitative outcomes comparing fine-needle aspiration versus core biopsy needles. |
|-----------------|-----------------|-----------------|-----------------|
|                  | FNA (n = 20)    | FNB (n = 20)    | P value        |
| Aggregate specimen length, mean (SD), cm |
| Pre-processing  | 10.89 (4.38)    | 15.78 (5.19)    | 0.003*         |
| Post-processing | 11.4 (5.55)     | 15.32 (5.24)    | 0.028*         |
| Length of longest piece, mean (SD), cm |
| Pre-processing  | 1.47 (0.46)     | 2.09 (0.41)     | < 0.001*       |
| Post-processing | 1.05 (0.42)     | 1.78 (0.66)     | < 0.001*       |
| Total specimen complete portal triads |
| Mean (SD)       | 18.1 (9.3)      | 42.6 (25.0)     | < 0.001*       |
| Median (range)  | 16.5 (6–38)     | 38.0 (0–81)     | 0.004*         |
| Portal triads groups, n (%) |
| < 11            | 6 (30)          | 2 (10)          | 0.24           |
| ≥ 11            | 14 (70)         | 18 (90)         | 0.24           |
| No. of fragments ≥ 9 mm, mean (SD) |
| Pre-processing  | 3.5 (2.4)       | 7.7 (3.7)       | < 0.001*       |
| Post-processing | 1.1 (1)         | 4.8 (3.6)       | < 0.001*       |

FNA, fine-needle aspiration; FNB, fine-needle biopsy; SD, standard deviation.
* Significant P value of 0.05.
There were no severe adverse events observed with either biopsy needle. There were no cases of anesthesia complications in either group.

**Discussion**

Despite advances in noninvasive assessment of liver diseases, the need for liver biopsy has not gone away, particularly with increased awareness of the spectrum of nonalcoholic fatty liver disease. Among the various means of performing parenchymal liver biopsy, EUS-LB has been shown to be a safe and effective means of obtaining good cores of liver tissue [6,7,9,10]. A retrospective comparison study suggested that tissue yields by EUS-LB are comparable to those obtained by the percutaneous or transjugular approaches [14]. More recent research has focused on changes in technique and devices to optimize sample acquisition. The advent of core needle technology for EUS has presented new possibilities for improving liver biopsies.

The results from the current study demonstrate that the 19G FNB needle outperformed the 19G FNA needle for EUS-LB on every metric that was assessed: length of the longest piece of tissue core, number of CPTs, and aggregate specimen length. These data indicate that the 19G core FNB needle can obtain longer intact cores of liver parenchyma than the FNA needle.

Previous studies of EUS-LB utilizing regular 19G FNA needles demonstrated adequate specimens, with reasonable tissue lengths [6,7,10,14–16,19]. However, for some liver diseases, such as advanced fibrosis or cirrhosis, tissue fragmentation remains an issue, even with the 19G needle. In a series of comparative studies, we have found that tissue core fragmentation is a function of the underlying liver disease as well as the gauge and type of needle used.

An ex vivo study tested six types of EUS needles for parenchymal liver biopsy in cadaveric liver [8]. These included 22G, 18G, and 19G needle sizes. Needles had standard tip and reverse bevel side fenestration. A fork-tip needle and two percutaneous needles were also included for testing. Two needle “excursion patterns” (single pass vs. 3 fanning passes) were examined for each needle. In addition, different amounts of suction (from slow-pull to 30 mL) were tested. The primary end point of this study was the number of CPTs (complete plus incomplete) in the specimen; secondary end points were degree of fragmentation and specimen adequacy (≥15mm and ≥5 portal triads).

The authors found that the 19G fork-tip needle gave the highest mean number of portal tracts compared with all of the other needles. In addition, both the 22G and 19G fork-tip needle showed less tissue fragmentation than the other needles. However, it should be noted that in this study, fragmentation assessment was made only before tissue processing.

We have previously investigated whether a 22G core needle could be used for EUS-LB in the in vivo setting [19]. In a prospective randomized study, EUS-LB was performed from left and right lobes with a 19G FNA needle (19 G Expect Flexible; Boston Scientific) and with a 22 G FNB needle (22 G SharkCore; Medtronic, Sunnyvale, California, USA). Pre-processing tissue cores of reasonable length were obtained with both needles. However, tissue fragmentation occurred during histological tissue processing, resulting in more fragmented cores in the 22G FNB group. This is likely to be due to the smaller core diameter of tissue obtained with the 22G needle, which is less than that of the 19G needle. We have not had problems with adverse events related to the larger needle gauge, and therefore do not favor the 22G needle over the 19G for EUS-LB.

We did a previous study demonstrating that “wet suction” was better than “dry suction” for EUS-LB [16], and therefore...

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**Figure 4** Complete portal triad counts by needle type.

**Table 2** Adverse events.

<table>
<thead>
<tr>
<th>Adverse events n (%)</th>
<th>FNA</th>
<th>FNB</th>
</tr>
</thead>
<tbody>
<tr>
<td>Severity according to ASGE lexicon</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mild</td>
<td>8 (40)</td>
<td>7 (35)</td>
</tr>
<tr>
<td>Moderate</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Severe</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Fatal</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Anesthesia events</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Pain</td>
<td>8 (40)</td>
<td>7 (35)</td>
</tr>
<tr>
<td>Headache</td>
<td>0</td>
<td>1 (5)</td>
</tr>
<tr>
<td>Abdominal pain</td>
<td>7 (35)</td>
<td>6 (30)</td>
</tr>
<tr>
<td>Back pain</td>
<td>1 (5)</td>
<td>0</td>
</tr>
<tr>
<td>Abdominal pain severity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mild</td>
<td>6 (30)</td>
<td>0</td>
</tr>
<tr>
<td>Moderate</td>
<td>0</td>
<td>1 (5)</td>
</tr>
<tr>
<td>Severe</td>
<td>1 (5)</td>
<td>5 (25)</td>
</tr>
<tr>
<td>Pain medication</td>
<td>2 (10)</td>
<td>6 (30)</td>
</tr>
<tr>
<td>Emergency department visit</td>
<td>0</td>
<td>1 (5)</td>
</tr>
</tbody>
</table>

FNA, fine-needle aspiration; FNB, fine-needle biopsy; SD, standard deviation; ASGE, American Society for Gastrointestinal Endoscopy. No significant differences between groups.
utilized this modification for the current study. A previous study of wet suction for non-liver EUS-FNA demonstrated improved yields [20]. For EUS-LB, where long intact cores are of critical importance for the sample, it is likely that the steady constant hydraulic “pull” on the tissue maintains the integrity of the core sample. In addition, we prime the needle with dilute heparin instead of saline. This decreases the formation of blood clots in the needle, improving tissue handling both in the procedure room and the surgical pathology laboratory. We have previously demonstrated that this heparin priming does not adversely affect histology of the sample, nor does it interfere with immunohistochemical staining [21]. Importantly, heparin priming does not lead to bloodier specimens, nor does it increase adverse events of FNA.

Minimizing sample handling appears to be important for EUS-LB specimens so that long tissue pieces do not undergo undue “iatrogenic” fragmentation. We have developed several steps to improve handling. First, we have implemented a “tissue sieve” process to allow efficient initial assessment of the sample adequacy. Use of the heparin priming of the needle then allows for blood to be washed off the specimen. The specimen is then “floated” into the formalin without further handling. When the specimen is received in the surgical pathology laboratory, the histology technician does not need to manually separate liver tissue from blood clot, further decreasing the chance of tissue fragmentation.

The core needle does seem to have a different interaction with liver tissue than the regular FNA needle. Besides longer lengths of tissue, we also noted increased portal structure yields, which is not completely accounted for by specimen length. We have noted that the margins of EUS-LB obtained with a 19 G FNA needle often produce specimens that have a slightly “wavy” appearance compared with the 19 G FNB needle in which the samples have more straight borders. Various measurements of the specimens obtained in this study showed decreased tissue fragmentation with the FNB needle (Table 1). Minimal specimen fragmentation is the ultimate goal of any liver biopsy device, and the core needle utilized here did appear to perform well in this regard. Longer cores are more likely to contain multiple CPTs and allow the pathologist to analyze “portal tract relationships,” meaning histological findings in parenchyma between two or more portal tracts. Fibrosis and inflammatory events are easier to diagnose in this way.

EUS-LB has a very low rate of adverse events [12, 13, 18, 22–25], and we did not see any patients with serious adverse events. No patients were lost to follow-up. Abdominal pain after the procedure is not uncommon, occurring in approximately 32.5% of patients, which is similar to pain experienced after percutaneous liver biopsy [1, 26, 27]. In the current study, the incidence of abdominal pain was similar between the two needles (30% for FNB and 35% for FNA). However, FNB biopsies had a higher rate of severe pain (25%) compared with FNA (5%). This may be due to peritoneal irritation from blood at the puncture site in the liver capsule, which is slightly larger with a core needle than with a standard beveled needle. The core biopsy needle also interacts differently with the gastric and duodenal wall compared with the regular beveled needle, in that tiny “core” specimens of stomach or duodenum can be readily identified in the core biopsy specimens. It is not clear whether pain arising from the duodenum or stomach, or bleeding at the puncture site in those organs, could be a factor to explain the increased rate of severe pain following biopsy. Increased analgesia requirements following the procedure might be anticipated when using the FNB needle.

A potential limitation of the study is that this research was completed at a high-volume center that performs over 350 EUS-LBs each year; therefore, our outcomes may not be fully applicable to practices with less familiarity with EUS-LB. There is a learning curve to achieving best possible tissue samples with EUS-LB, which appears to be between 10 and 20 procedures.

In conclusion, EUS-LB using a novel 19 G FNB needle delivered longer and less fragmented biopsy specimens than the usual 19 G FNA needle. In addition, there was less post-processing specimen fragmentation, and the CPT yield was also higher. These results indicate that use of a 19 G FNB needle is an improvement over the traditional 19 G FNA needle for EUS-LB.

Competing interests

Dr. Diehl is a consultant for Boston Scientific, Olympus America, Medtronic, and Cook (all manufacturers of needles used for endoscopic ultrasound).

References


