Endoscopic ultrasound-guided liver biopsy using a 22-G fine needle biopsy needle: a prospective study

Authors
Muhammad K. Hasan1, Kambiz Kadkhodayan1, Evgeny Idrisov2, Saeed Ali2, Ehsan Rafiq3, Dana Ben-Ami Shor1,4, Ala Abdel-Jalil1, Uday Navaneethan1, Ji Bang1, Shyam Varadarajulu1, Robert Hawes1, Peter Pernicone5

Institutions
1 Center for Interventional Endoscopy, AdventHealth Orlando, Florida, USA
2 Department of Internal Medicine, AdventHealth Orlando, Florida, USA
3 Department of Gastroenterology and Hepatology, AdventHealth Orlando, Florida, USA
4 Sackler Faculty of Medicine, Tel-Aviv University, Tel-Aviv, Israel
5 Department of Pathology, AdventHealth Orlando, Florida, USA

submitted 24.2.2019
accepted after revision 16.5.2019

Bibliography
DOI https://doi.org/10.1055/a-0967-3640
Published online: 31.7.2019 | Endoscopy 2019; 51: 818–824
© Georg Thieme Verlag KG Stuttgart · New York
ISSN 0013-726X

Corresponding author
Muhammad Khalid Hasan, MD, Center for Interventional Endoscopy, AdventHealth Orlando, 601 East Rollins Street, Orlando, Florida 32803, USA
Fax: +1-407-303-2585
muhammad.hasan.md@adventhealth.com

ABSTRACT

Background Endoscopic ultrasound-guided liver biopsy (EUS-LB) using a 19-gauge (19-G) EUS needle is becoming increasingly popular. We evaluated the efficacy and safety of a 22-G EUS fine needle biopsy (FNB) needle for performing EUS-LB.

Methods Patients referred for evaluation of elevated liver enzymes and without obstructive disease requiring endoscopic retrograde cholangiopancreatography (ERCP) were included. Using a 22-G FNB needle, two passes were made from the left lobe and one from the right. The main outcome measure was adequacy of the specimen for histology interpretation, and the secondary outcome was the safety of EUS-guided liver biopsy with a 22-G FNB needle. Patients were followed for post-procedure complications for 30 days.

Results 40 patients (median age 61 years; 26 women) underwent EUS-LB. Analyzing by needle passes, the median longest core fragment was 12 mm (1st quartile – 3rd quartile 10 mm–15.75 mm, interquartile range [IQR] 6.25 mm) from the left lobe and 11 mm (10 mm–15.75 mm, IQR 5.75 mm) from the right lobe. The median cumulative core length per patient was 55 mm (44.5 mm–68 mm, IQR 23.5 mm). The median cumulative number of complete portal triads (CPTs) per patient was 42 (28.5–53, IQR 24.5). The specimen was considered adequate in all 40 patients (100%). Self-limiting abdominal pain was reported in 6 patients (15%).

Conclusions EUS-LB using a 22-G FNB needle is a safe and viable alternative to the use of larger gauge needles, yielding adequate tissue for evaluation of parenchymal disease in 100% of the patients.

Introduction
Tissue acquisition and histopathological evaluation continue to play an essential role in the management of patients with liver disease. Liver biopsy can reveal a diagnosis in up to 96% of patients and may alter management in up to 18% of patients [1]. With the increasing incidence of liver disease, particularly non-alcoholic fatty liver disease, liver biopsy is expected to play an increasingly important role in its diagnosis, staging, and management [2]. Traditional methods of liver biopsy include percutaneous (“blind,” image-guided), transvenous (transjugular, transfemoral), and surgical (open, laparoscopic). Endoscopic ultrasound (EUS) evaluation of cholestatic and mixed-type liver chemistry abnormalities is frequently sought, to exclude non-parenchymal etiology. If EUS evaluation is unrevealing, such patients have traditionally been referred to a separate specialist for a liver biopsy, frequently causing a delay in the diagnostic workup in addition to the extra time and cost of a separate procedure.
In recent years, “same session” EUS-guided liver biopsy (EUS-LB) has gained traction amongst endosonographers and may offer several potential advantages over existing techniques for liver tissue acquisition. With a histologic diagnosis rate of 93.9% and adverse events rate of 2.3%, EUS-LB appears to be a safe and viable alternative [3–10]. However, despite the advantages it may offer over traditional methods of liver biopsy, concerns regarding tissue fragmentation, tissue adequacy, and bleeding risk continue to hamper the widespread adoption of EUS-LB in clinical practice.

EUS-LB is a technically reproducible approach regardless of body habitus, because the needle needs only to traverse the gastric or duodenal wall to reach the hepatic parenchyma. The procedure is performed in an outpatient setting and provides the comfort of sedation and analgesia. The EUS provides high resolution images of the left lobe of the liver along with a sizable portion of the right lobe of the liver. This, coupled with the capability of Doppler imaging, allows the biopsy needle to be safely directed into the liver for sampling under real-time image guidance.

It has been recognized that sampling error can lead to diagnostic inaccuracy of a biopsy from a single site [11]. In comparison with ultrasound or computed tomography (CT) scans, EUS allows an easy and safe biopsy of both left and right lobes of the liver in the same setting, while potentially addressing concerns about sampling error. Also the main expense of the EUS-LB is the endoscopic procedure, along with the cost of the fine-needle biopsy (FNB) needle which is not different in expense to the cost of the needle for either percutaneous or transjugular approaches. Thus, this approach may be best utilized for patients requiring EUS for evaluation of elevated liver function test findings. If no obstructive lesion that would require endoscopic retrograde cholangiopancreatography (ERCP) is identified, then EUS-LB can be performed in the same session.

To our knowledge, the EUS needles that have been most studied for EUS-LB for evaluation of parenchymal liver disease are a 19-gauge (19-G) fine needle aspiration (FNA) needle and, more recently, an FNB needle [12]. While smaller in size than most non-EUS needles used for liver biopsy, the 19-G needle may be difficult to maneuver, especially in the duodenum while accessing the right lobe of the liver. There is scant literature on the use of potentially safer and presumably easier-to-maneuver smaller gauge EUS needles that are commercially available. Despite the potential advantages, such needles have not been used for EUS-LB largely because of concerns surrounding tissue adequacy for evaluating parenchymal liver disease. We sought to address this issue by evaluating a 22-G FNB needle while using a technique in which the needle is kept straight during slow expulsion of the specimen from the needle. In our clinical practice, this technique has consistently yielded adequate core samples. We hypothesized that a small gauge FNB needle may yield adequate histology grade tissue with minimal adverse events.

### Methods

#### Overview

The study was a single-center, prospective, open-label, nonrandomized, trial that was conducted from August 2017 to June 2018 at the Center for Interventional Endoscopy (CIE), AdventHealth Hospital, Orlando, USA. Informed consent was obtained for each patient and the hospital’s Institutional Review Board (IRB) approved the study. A data and safety monitoring board composed of nonparticipating members of our hospital provided oversight during this trial. This trial was registered online at clinicaltrials.gov (NCT03307811) prior to enrolment of any participants.

#### Patients

Patients recruited to the study were adults (age ≥19 years) with abnormal liver chemistries and otherwise nondiagnostic serological and/or imaging studies, who were referred for EUS to rule out extrahepatic causes of abnormal liver chemistries (►Table 1). Patients on anticoagulation or antiplatelet therapy at the time of the procedure, with coexisting coagulopathy (international normalized ratio [INR] >1.6 or platelet count <50 000/µL), or unable to safely undergo the EUS procedure for any reason were excluded. Patients also were excluded if they were unable to provide informed consent, were pregnant, or were less than 19 years of age.

At EUS, the liver, gallbladder (if present), extrahepatic bile duct, and pancreas were thoroughly examined. If biliary obstruction was not evident, EUS-guided LB was undertaken. All patients were observed for 4 hours post-procedure, and adverse events were recorded. Patients were discharged home.

#### Table 1 Characteristics of 40 patients who underwent endoscopic ultrasound (EUS)-guided liver biopsy using a 22-G fine-needle biopsy (FNB) needle.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>n</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sex</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>26</td>
<td>65</td>
</tr>
<tr>
<td>Male</td>
<td>14</td>
<td>35</td>
</tr>
<tr>
<td><strong>Race</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>29</td>
<td>72.5</td>
</tr>
<tr>
<td>Hispanic</td>
<td>8</td>
<td>20</td>
</tr>
<tr>
<td>Black</td>
<td>1</td>
<td>2.5</td>
</tr>
<tr>
<td>Asian</td>
<td>1</td>
<td>2.5</td>
</tr>
<tr>
<td>Other</td>
<td>1</td>
<td>2.5</td>
</tr>
<tr>
<td><strong>Presentation</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Elevated LFT results</td>
<td>40</td>
<td>100</td>
</tr>
<tr>
<td>Abdominal pain</td>
<td>11</td>
<td>27.5</td>
</tr>
<tr>
<td>Other</td>
<td>6</td>
<td>15</td>
</tr>
</tbody>
</table>

LFT, liver function test
afterward, if clinically stable. A research nurse coordinator conducted post-procedure follow-up calls at 24–72 hours and at day 30.

**Technique of tissue acquisition and tissue handling**

The EUS procedure was performed by one of five experienced endosonographers, using a curved linear-array echoendoscope (GF-UCT180; Olympus America, Center Valley, Pennsylvania, United States). At EUS, the left lobe of the liver was identified in the proximal stomach. Doppler imaging was utilized to identify a suitable position, and the liver capsule was punctured using a 22-G FNB needle (Acquire; Boston Scientific, Marlborough, Massachusetts, United States) with the stylet slightly retracted. The stylet was then withdrawn and 3–4 slow back and forth movements were performed in a “fanning” motion while avoiding major vessels or intrahepatic bile ducts. The needle was then withdrawn from the echoendoscope. An effort was made to avoid excessive use of the elevator.

After retraction of the needle from the echoendoscope, the needle shaft was maintained straight. The stylet was re-inserted slowly while keeping the assembly straight, and core tissue was expelled from the needle tip into 10 % formalin. Once tissue expulsion had begun, the stylet was advanced without interruption to prevent tissue fragmentation. A nonserrated forceps was used to gently separate any tissue that remained attached to the needle. The stylet was then withdrawn completely, and any residual material was air-flushed using a standard 20-mL syringe. The presence of macroscopic core tissue was assessed and recorded.

The needle was then re-inserted into the echoendoscope, and an additional pass was made in the left lobe of the liver. After completion of the second pass, the echoendoscope was advanced to the duodenal bulb and, using a similar technique, the right lobe (segment 5) was sampled.

According to the protocol, the first two passes were performed without application of any suction. Suction was applied in the third pass only if there was inadequate visible core tissue formed without application of any suction. Suction was applied using a 22-G FNB needle (Acquire; Boston Scientific, Marlborough, Massachusetts, United States) with the stylet slightly retracted. The stylet was then withdrawn and 3–4 slow back and forth movements were performed in a “fanning” motion while avoiding major vessels or intrahepatic bile ducts. The needle was then withdrawn from the echoendoscope. An effort was made to avoid excessive use of the elevator.

After completion of the second pass, the echoendoscope was retracted post-procedure follow-up calls at 24–72 hours and by a telephone follow-up call at 72 hours afterward, if clinically stable. A research nurse coordinator conducted post-procedure follow-up calls at 24–72 hours and at day 30.

**Tissue processing, quantification, and pathologic interpretation**

After on-site visual assessment of the core tissue (Fig. 1a) the specimens obtained from each pass were submitted in separate, labeled containers to the surgical pathology laboratory for routine processing followed by embedding in paraffin and sectioning. The length of intact core tissue in terms of longest fragment per pass, aggregate length per pass, and aggregate length per patient were measured. Using an Olympus BX41 compound light microscope fitted with an eyepiece micrometer, the average core thickness also was measured. Three separate levels were evaluated on each specimen. The levels were obtained at approximately 40-micron intervals using a microtome and slides were prepared. Three hematoxylin and eosin (H&E)-stained slides were examined for each location. Routine stains, including trichrome, reticulin, iron, and periodic acid–Schiff (PAS) with diastase, were evaluated. Also, a variety of ancillary studies were used as indicated, including immunohistochemical stains, e.g. CK7, and other histochemical stains including copper (Fig. 1b–d).

The number of portal triads represented in each specimen was counted using H&E-stained sections and trichrome-stained sections. A portal triad was counted if at least an interlobular bile duct was identified.

A single, experienced surgical pathologist with expertise in liver pathology evaluated all the biopsies and provided a pathologic diagnosis.

**Study definitions**

**Adequate gross tissue specimen** Intraprocedural tissue adequacy was visually assessed after each pass. A specimen was considered adequate, if at least one core fragment was present and the aggregate core length per pass measured at least 15 mm by visual estimation using a ruler as a reference.

**Adequate diagnostic yield** A specimen was considered adequate if there was sufficient material for the pathologist to successfully render a diagnosis. Congruently with several published EUS-LB studies [7, 9, 13, 14], an aggregate core length of 15 mm and presence of six complete portal triads (CPTs) was used as a benchmark for comparison.

**Adverse events** These were defined as any deviation from an uneventful post-procedure recovery that was significant enough to warrant medical attention. Patients were evaluated for pain, bleeding, signs of peritonitis or perforation, and otherwise unexplained cardiopulmonary or hemodynamic changes, for 4 hours post-procedure and by a telephone follow-up call at 24–72 hours post-procedure.
Outcome measures

The primary outcome measure of the study was to determine the diagnostic adequacy of liver biopsy specimens procured using a 22-G FNB needle. Secondary outcomes included adverse events and the need for suction in EUS-LB.

Results

From August 2017 to June 2018, 48 patients with elevated liver function test findings and who met inclusion criteria were recruited. Amongst these, eight patients (20%) were found to have a biliary obstruction that required endoscopic retrograde cholangiopancreatography (ERCP) for further management and were therefore excluded. Median age was 61 years (1st quartile – 3rd quartile 46.7 – 68.2, interquartile range [IQR] 21.5). Other patient characteristics are presented in ▶Table 1.

Three passes were made in each of the 40 patients (total 120). No patients required more than three passes. An adequate tissue specimen, as judged by on-site visual estimation, was obtained in 119 passes (99.2%); for one patient there was an on-site evaluation of inadequate core tissue on the first pass, but there was adequate core tissue on the second pass and the use of suction was not required. All 40 patients (100%) had adequate core tissue samples by visual estimation within the first two passes and no patient required the use of suction in the third pass.

A single 22-G FNB needle was used in 39 patients for EUS-LB. One patient required the use of an additional, similar 22-G FNB needle, because of dysfunction of the original needle after the first pass. Both lobes were sampled in 38 patients (95%).
left lobe only was sampled in two patients because of surgically altered anatomy. None of the patients required the use of a larger bore needle to obtain an adequate sample.

The median length of longest core fragment from the left lobe was 12 mm (10–16.25 mm, IQR 6.25 mm) and from the right lobe it was 11 mm (10–15.75 mm, IQR 5.75 mm). The median core aggregate lengths for left lobe and right lobe were 20 mm (14–25 mm, IQR 11 mm) and 20 mm (13.3–25, IQR 11.7 mm), respectively (Table 2). In the per-patient analysis, the median cumulative core length was 55 mm (44.5–68 mm, IQR 23.5 mm). The median core thickness was 0.1 mm. In the per-patient analysis, the median cumulative number of portal triads (CPTs) was 42 (28.5–53, IQR 24.5) (Table 3). A moderate amount of post-processing tissue distortion, characterized by crush and compression of portal venules, arterioles, and bile ducts was noted in most patients. Despite this limitation, the core samples from all patients contained adequate portal tracts for histopathological evaluation, and the specimen was considered adequate in all 40 patients (100%). The most common diagnosis was fatty liver disease with fibrosis in nine (22.5%) patients (Table 4).

All patients were discharged within 4 hours after the procedure. Mild abdominal pain was reported in six patients (15%) prior to discharge. At the 24–72-hour follow-up post-procedural abdominal pain had completely resolved in all six patients. One patient developed chest pain that required re-hospitalization and cardiac workup, and one patient developed self-resolving fever. One patient died; this was reported to have occurred within 24 hours of the procedure. The patient was a 72-year-old woman with a history of factor V Leiden mutation (activated protein C resistance) and remote history of a right-sided pulmonary embolism. She was on therapeutic anticoagulation (apixaban) and had been referred for evaluation of elevated liver function test findings. The therapeutic anticoagulant had been stopped 2 days prior to the procedure. The procedure was uneventful and the patient was observed for 4 hours and had no complaints or noted adverse events during this time. The patient was discharged with recommendations to restart the anticoagulant after 2 days. The patient reportedly died the next morning; as no autopsy was performed the cause of death was unclear.

No early post-procedural or delayed bleeding reported. No statistically significant difference in tissue yield when the two lobes were compared or between the first and second pass in the left lobe. No patients were lost to follow-up.

Discussion

Recently, EUS-guided liver biopsy has been reported as an alternative to traditional methods (percutaneous, transvenous, and surgical techniques) of liver biopsy for the evaluation of parenchymal liver disease [7, 9, 13, 14]. In all previously reported EUS-LB studies, 19-G or larger bore needles have been used. Although EUS-LB has been reported to be safe, it has not gained wide acceptance by endosonographers, because of concerns regarding potential complications, i.e., bleeding with such larger bore EUS needles. The present study is a prospective one that describes the adequacy and safety of a smaller 22-G FNB needle for EUS-guided liver biopsy. To the best of our knowledge, this is the first prospective study to describe the utility of a 22-G FNB needle for EUS-LB to evaluate liver parenchymal disease.

A post-procedural death was reported in one patient within 24 hours of the procedure. Unfortunately, the family refused an autopsy and the exact cause of death remains unclear. Pre-procedural anticoagulation was managed in accordance with current guidelines [15, 16] and after prior consultation with the patient’s primary physician. The patient’s anticoagulation would have been managed similarly for a non-EUS liver biopsy. Overall, while it is possible that the death may have occurred from a procedure-related adverse event such as bleeding, we believe that this is less likely given the patient’s underlying hypercoagulable state and the relatively smaller gauge needle
used. It is more likely, that the patient’s death was caused by an event related to the withdrawal of anticoagulation, such as a pulmonary embolism or acute coronary syndrome. This highlights the importance of appropriate post-procedural anticoagulation management and risk stratification. Further studies are necessary to determine optimal timing for re-initiation of therapeutic anticoagulation after EUS-LB.

Current guidelines from the American Association for the Study of Liver Disease guidelines recommend obtaining core aggregate lengths of 20–30 mm, and more than 11 CPTs [2]. It is also recommended that in cases with fewer than 11 CPTs, it should be noted in the biopsy report that the diagnosis, grading, and staging may be incorrect because of incomplete sample size [2]. The core aggregate lengths and the aggregate numbers of CPTs in our study were greater than 20 mm and 11 CPTs, respectively, in all patients. This surpasses required guideline benchmarks. This may translate to mean that provided adequate core tissue can be seen at visual inspection, then one only pass from each lobe may suffice. While the core lengths and CPT numbers far exceeded current recommendations, there was a moderate amount of post-processing tissue distortion in the portal tracts. This was described by the reviewing pathologist as crush and compression of portal venules, arterioles, and bile ducts in some portal tracts. The cause of such distortion remains unclear and is perhaps a result of the smaller caliber needle and mechanical stress associated with routine handling of the relatively thinner core fragments. Despite some tissue distortion, the samples remained adequate, and there was intact tissue and more than sufficient CPTs that allowed for ancillary staining and histopathological interpretation in all patients (100%).

The technique discussed in the majority of literature on EUS-LB describes the use of suction as an aid to FNA/FNB. The role of suction in EUS-LB has not yet been investigated or validated, particularly in smaller gauge needles. The application of suction can be associated with increased bloodiness of the sample, particularly when sampling a vascular organ such as the liver [17]. It was our hypothesis that the use of suction may be avoided altogether, hence reducing the bloodiness of the sample. We therefore only planned to use suction if the first two passes did not yield visible core tissue. A total of 99.5% of the passes in the study yielded visually adequate tissue without the use of suction. Based on the above, we concluded that suction is not necessary for EUS-LB using a 22-G FNB needle.

In patients with elevated liver function test findings but no obstruction on endosonography, cost–effectiveness is the major potential advantage of the EUS-LB approach, because it precludes the need for a second procedure, thus expediting patient care.

There are some potential limitations to this study. First, its single-center nature may limit its generalizability with regard to centers in which endosonographers are not as experienced with EUS-LB and pathologists not as experienced with interpreting these specimens. Therefore, these results must be validated in a prospective multicenter study. Second, we recognize that an assumption of 100% specimen adequacy may be too high and the data acquired from this study may potentially be limited by the sample size. Moreover, a study for comparison of this technique using a 19-G FNA needle is needed.

One of the major strengths of our study is that it was prospective, thus enabling us to capture all the potential complications associated with this procedure. The most commonly encountered complication was mild abdominal pain in 15% of patients that completely resolved within 24–72 hours. These

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>n</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benign hepatic tissue</td>
<td>8</td>
<td>20</td>
</tr>
<tr>
<td>Nonspecific acute hepatitis</td>
<td>1</td>
<td>2.5</td>
</tr>
<tr>
<td>Nonspecific chronic hepatitis</td>
<td>7</td>
<td>17.5</td>
</tr>
<tr>
<td>Primary biliary cholangitis</td>
<td>2</td>
<td>5</td>
</tr>
<tr>
<td>Primary sclerosing cholangitis</td>
<td>2</td>
<td>5</td>
</tr>
<tr>
<td>Fatty liver disease</td>
<td>5</td>
<td>12.5</td>
</tr>
<tr>
<td>Fatty liver disease with fibrosis</td>
<td>9</td>
<td>22.5</td>
</tr>
<tr>
<td>Steatohepatitis</td>
<td>1</td>
<td>2.5</td>
</tr>
<tr>
<td>Steatohepatitis with liver cirrhosis</td>
<td>1</td>
<td>2.5</td>
</tr>
<tr>
<td>Non-Hodgkin’s lymphoma</td>
<td>1</td>
<td>2.5</td>
</tr>
<tr>
<td>Nonspecific ductular reaction with benign hepatic tissue</td>
<td>3</td>
<td>7.5</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>40</td>
<td></td>
</tr>
</tbody>
</table>

Hasan Muhammad K. et al. EUS-guided liver biopsy using a 22-G FNB needle... Endoscopy 2019; 51: 818–824
Complications are not unlike those encountered in previously reported studies; however, those studies were mostly retrospective and unlikely to capture any minor complications [7, 9, 12–14].

In conclusion, by using the techniques described above, of acquisition of liver tissue with EUS guidance and of expulsion, FNB even with a smaller caliber 22-G needle can provide adequate tissue for accurate histological evaluation and is likely very safe. In addition, the smaller caliber needle for EUS-LB may be an appealing alternative to a larger caliber needle for endosonographers at large.

Competing interests

The following authors disclosed financial relationships relevant to this publication. Muhammad K. Hasan is a consultant for Boston Scientific and Olympus America. Robert Hawes is a consultant for Boston Scientific, Olympus America, and Medtronic. Shyam Varadarajulu is a consultant for Boston Scientific and Olympus America. All other authors disclosed no financial relationships relevant to this publication.

References