19-Gauge Fine Needle Aspirate (FNA) Versus 19-Gauge Fine Needle Biopsy (FNB) Needles for Endoscopic Ultrasound Guided Liver Biopsy (EUS-LB): A Randomized Prospective Trial

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1 ABBREVIATIONS USED IN THE PROTOCOL

Abbreviation	Term
AE	Adverse event
ASGE	American Society of Gastrointestinal Endoscopy
ALS	Aggregate specimen length
BMI	Body mass index
CA	California
CLD	Chronic liver disease
Cm	Centimeter
Co-I	Co-Investigator
CPT	Complete portal tracts
CRNA	Certified Registered Nurse Anesthetist
EUS	Endoscopic ultrasound
EUS-LB	Endoscopic ultrasound guided-liver biopsy
EGD	Esophagogastroduodenoscopy
FNA	Fine needle aspirate
FNB	Fine needle biopsy
g	Gauge
GIRB	Geisinger IRB
HRPO	Human Research Protection Office
In	Inches
Inc	Incorporated
IRB	Institutional Review Board
Lb	Pounds
LB	Liver biopsy
mm	Millimeters
PA	Pennsylvania
PI	Principle investigator
PLB	Percutaneous liver biopsy
PPT	Partial portal tracts
QASM	Quality and Safety Monitoring
SA	Specimen adequacy
SAE	Serious adverse event
SLB	Surgical liver biopsy
TLB	Transjugular liver biopsy
US	United States

2 ABSTRACT

Chronic liver disorders (CLD) are a major cause of morbidity and mortality for individuals in the US. Though serologic analysis will often lead to a conclusive diagnosis, liver biopsy remains an important method for helping to determine the etiology and stage of LD. Percutaneous liver biopsy (PLB), transjugular liver biopsy (TLB) and surgical liver biopsy (SLB) are alternative methods for obtaining hepatic tissue. In recent years endoscopic ultrasound guided-liver biopsy (EUS-LB) has come to the forefront as a safe and effective method for obtaining tissue in CLD. There are several studies of the safety of EUS-LB as well as the adequacy of specimens obtained in this fashion. Most studies involve a 19-g needle, therefore in this study we hope to compare the tissue yields of a 19-g FNB needle, in comparison to conventional 19-g FNA needle. We predict that 19-g FNA and 19-g FNB needle will demonstrate similar diagnostic accuracy, with less visible blood artifact. Similarly, we predict the safety to be equal.

3 BACKGROUND AND SIGNIFICANCE

Chronic liver disease has a number of causes, and leads to significant mortality and morbidity in the United States. It has been estimated that roughly 36,000 individuals die annually from the burden of chronic liver disease, thus early diagnosis and intervention are paramount to preventing such complications [1]. Though serologic markers and non-invasive diagnostic imaging modalities are used as a method for determining the underlying disease process, these methods lack the specificity of determining etiology of a patient's chronic liver disease [2-5]. Therefore, liver biopsy remains the "gold standard" for obtaining valuable diagnostic and prognostic information.

At present there exist several methods for liver tissue acquisition. The most widely accepted method remains percutaneous route (PLB), which utilizes percussion or imaging to localization the biopsy site [6-8]. The issue with this approach is its potential complication of post-procedural pain in up to 84%, bleeding in 1/2500-10,000 procedures, with under 1/10,000 of these cases being fatal [7-17]. Another means for obtaining tissue samples is the transjugular route (TLB), which also allows for portal pressure measurement, and is usually reserved for patients with coagulopathy[18,19].

More recently, endoscopic ultrasound guided liver biopsy (EUS-LB) has been developed as a newer LB technique [23, 24]. The feasibility of EUS-LB for liver lesions has been validated yielding excellent diagnostic results in several studies [25-27]. This technique has also been evaluated for hepatic parenchymal disease with up to 90% diagnostic yield. Subsequently, EUS-LB using a 19-g needle was compared to percutaneous/transjugular routes showing at least comparative, and in some instances improved sample acquisition, versus other methods [28]. Different 19-g needles have been utilized in this setting yielding variable diagnostic specimens [29-33]. However, there has yet to be comparison of 19-g FNA versus a 19-g core biopsy needle for EUS-LB.

Primary End Points

1. Proportion of cases for which a histologic diagnosis could be made based upon the amount of tissue obtained with the needle.
2. Number of portal tracts (PT) in the specimen. [34-36]
3. Aggregate specimen length (ASL), length of the longest piece (LLP), and degree of fragmentation.

Secondary End Points

1. Presence of a visible core specimen.
2. Presence of visible clots in specimen.
3. Adverse events (AE) and serious adverse events (SAE).

4 HYPOTHESIS AND SPECIFIC AIMS

4.1 Hypothesis

We predict that the 19-g FNA needle and 19-g FNB needle will have similar ability to obtain adequate EUS-LB specimens

4.2 Specific Aim 1

To determine the adequacy of EUS-LB using a 19-g FNB needle as compared with 19-g FNA needle.

4.3 Specific Aim 2

To determine if the 19-g FNB needle will demonstrate less blood artifact during the time of EUS-LB as compared with 19-g FNA needle.

5 PRELIMINARY DATA

Our EUS group has used the 19-g EUS FNB needle in several patients undergoing EUS-guided liver biopsy, and cores of liver tissue can be obtained. We have found that special tissue handling after biopsy is required to prevent fragmenting the tissue. We have improved the technique of tissue handling, and can minimize post-biopsy fragmentation. This can allow a better comparison of different needle gauges.

6 STUDY DESIGN

6.1 Description

This is a prospective randomized trial comparing the biopsy specimen adequacy (SA) of 19-g FNA versus 19-g FNB needle for EUS-LB.

6.2 Study Population

6.2.1 Approximate Number of Subjects

Approximately 32 subjects will participate in this study.

6.2.2 Inclusion Criteria

1. Patients undergoing EUS-LB
2. Platelet count $\geq$ 50,000
3. International normalized ratio (INR) < 1.5
4. Age > 18 years

5. Non-pregnant patients

6.2.3 Exclusion Criteria

1. Age < 18 years
2. Pregnant patients
3. Inability to obtain consent
4. Anticoagulants or anti-platelet agents use (excluding aspirin) within the last 7-10 days
5. Platelet count < 50,000
6. INR ≥ 1.5
7. Presence of ascites
8. Known liver cirrhosis
9. Hemophilia

6.3 Recruitment

Patients shall be recruited in the pre-procedural endoscopy area. After identifying subjects, a study investigator shall discuss the study in detail in person. The patient will be given time to read the consent form and ask questions.

6.4 Study Duration

6.4.1 Approximate Duration of Subject Participation

Participation is just for the duration of the EUS-LB.

6.4.2 Approximate Duration of Study

The duration of the study shall last until 6 months from enrollment of the last study participant. This shall allow for analysis of final data points and construction of a manuscript.

6.5 Procedures

EPIC electronic health records database will allow for availability of demographic data and office-based follow-up records. ProVation MD software information will provide details regarding endoscopic parameters and intervention performed.

Electronic records gathered for study purposes will only be available to study investigators and will be stored on an encrypted hard drive on a computer. Data will initially be entered with PHI attached so that all information can be obtained. Once all data collection is complete identifiers will be removed and random number assigned to the patients.

Upon initial encounter, the study shall be described to the patient in detail by one of the study investigators and informed consent obtained.

Once the patient has agreed to participate, demographic data will be obtained from the medical record including; age, gender, height [inches (in)], weight [pounds (lb)], body mass index (BMI) (lb/in^2), past medical history [in particular diagnosis of liver disease, biliary or pancreatic disease, ascites, encephalopathy, portal hypertension, portal hypertension-related bleeding (i.e. varices), liver cancer or masses]. Past surgical history shall be obtained regarding prior cholecystectomy, hepatobiliary or pancreatic surgery (i.e. pancreatojejunostomy) or bariatric surgery (i.e. Roux-en-Y gastric bypass). Medication and social history shall be performed regarding alcohol intake per week and hepatotoxic medications (i.e. acetaminophen). A baseline INR and platelet count shall be performed on all individuals prior to EUS-LB, as is the standard of care.

EUS-LB Protocol

Patients undergoing EUS-LB receive anesthesia during the procedure, as per normal practice. This is provided by a certified registered nurse anesthetist (CRNA). The endosonographic study will be conducted with a linear array echoendoscope (GF-UC140-AL5; Olympus America, Center Valley, PA). Before needle puncture of the desired lobe, color Doppler imaging will be used to ensure the lack of vascular structures in the trajectory of the needle. The EUS-LB will be performed in widely separated regions of the liver using a 19-g EUS-FNA needle (Expect Flexible 19g, Boston Scientific, Marlborough, MA) or a 19-g FNB needle (Acquire 19g, Boston Scientific, Marlborough, MA). A computer-generated randomized schema shall determine needle type selection.

The left lobe is described as liver parenchyma identified a few centimeters below the gastroesophageal junction with the echoendoscope torqued clockwise. The right lobe is considered the large area of liver tissue can be seen through the duodenal bulb, near the gallbladder [37]. The stylet is removed, heparin flushed through the needle lumen, and the suction device set and attached to the needle hub. The prepared needle is then inserted into the echoendoscope. A transgastric approach will be used to obtain samples from the left lobe of the liver; a transduodenal approach, with the linear echoendoscope positioned in the duodenal bulb, will be used to obtain samples from the large amount of liver parenchyma seen in that location. Once adequate liver parenchymal penetration will be achieved with the needle (~2-6 cm), full suction will be applied with a 20-mL vacuum syringe. One pass consists of a total of 7 to 10 to-and-fro needle motions with the fanning technique applied under direct and continuous endosonographic visualization of the tip of the needle.

The needle will then be removed from the echoendoscope. The specimen will be pushed from the needle with the stylet directly into a microseive, and blood washed from the specimen with a gentle saline rinse. The endosonographer looks for multiple pieces of light brown tissue approximately 5 to 15 mm in length. The tissue cores are then “floated” off the microseive into formalin solution. Heparin is flushed through the needle lumen prior to the next pass. The biopsy process is then repeated on the opposite liver lobe. A pass per liver lobe will be performed in each patient; using the standard 19-g EUS-FNA needle or the 19-g EUS FNB

needle. All patients are closely observed in the recovery area for 1 hour after the procedure, as per our standard policy. Patients will be followed-up with by a phone call the next day and at 1 week after the procedure, as per standard of care.

Sample Processing
The surgical pathology department, per a specific protocol for clinical practice, will process the EUS-LB samples. Tissue samples are left in formalin for at least 1 hour before processing. The contents of the formalin jar will be poured into a petri dish, and visible cores of liver tissue picked out with small forceps by the surgical pathology technician. These pieces are arranged in a linear fashion on lens paper, then the specimen photographed alongside a ruler to estimates pre-processing tissue lengths. Samples from both lobes and the different needles will be submitted for evaluation separately. The tissue will be processed in standard fashion, and slide blanks made (5-µm tissue thickness). These blanks are stained with hematoxylin and eosin, trichrome, and reticulin, with other special stains done as needed. The slides are digitized using a whole slide scanner (ScanScope CS; Aperio Technologies, Inc, Vista, CA), and the digitized images used for quantitative analysis (eSlide Manager; Aperio Technologies, Inc). Quantification of sample length (mm) and portal triads is performed by 2 of the investigators, annotating the digital images with the software. Fellowship-trained GI pathologists then perform histologic interpretation for clinical use.

Post-Procedural Follow-up
After undergoing the procedure, patients will receive a 1week follow-up phone call to monitor for adverse events (i.e. bleeding), as per standard of care.

6.5.1 Study Time and Events Table

Study Procedures			
Study Interval	Pre-Endoscopy Procedure	Day of Procedure	Follow-up
Informed consent	X		
Demographics	X		
Medical history	X		
Surgical history	X		
Medication History	X		X
INR	X		
Platelet Count	X		
Height (in)	X		
Weight (lb)	X		
BMI	X		
Urine Pregnancy test, when applicable	X		
Randomization regarding needle type		X	
EUS-LB		X	
Adverse events[a]	X----------X		

[a]From the signing of the informed consent form to 1 week post-EUS-LB
INR = international normalized ratio, BMI = Body Mass Index (lb/in^2), EUS-LB = Endoscopic Ultrasound Guided Liver Biopsy

All activities are standard of care, except for the selection of needle type.

Follow-up will be conducted via phone call.

6.6 Primary Endpoints

1. Proportion of cases for which a histologic diagnosis could be made based upon the amount of tissue obtained with the needle.
2. Number of portal tracts (PT) in the specimen [34-36]

3. Aggregate specimen length (ASL), length of the longest piece (LLP), and degree of fragmentation

6.7 Secondary Endpoints

1. Presence of a visible core specimen
2. Presence of visible clots in specimen
3. Adverse events (AE) and serious adverse events (SAE)

6.8 Statistics

A representative from the Biostatistics & Research Data Core will be doing the statistical analysis.

6.8.1 Statistical Analysis Plan

Assuming the 19-g FNB needle has a success percentage of 98% (ie,>= 5 portal triads), and margin over FNA needle of 5% (98% vs. 93%), the study needs to evaluate 32 patients to achieve 80% power for a 1-sided non-inferiority test. This also assumes each patient provides 2 specimens. If the margin is increased to 10% (98% vs. 88%), then the study needs to evaluate 18 patients to achieve 80% power.

Descriptive statistics will be utilized to represent continuous and categorical variables, with results expressed as medians with ranges. Multiple comparisons between the aggregate tissue length and CPT yield from bilobar, left lobe only, and right lobe only biopsies will be carried out using the Mann-Whitney-Wilcoxon test. A P-value of <0.05 will be considered statistically significant.

6.8.2 Statistical Power and Sample Size Considerations

The purpose of this study to determine if the 19-g FNB EUS needle provides liver cores equal or superior to 19-g FNA needle for histologic interpretation. A few preliminary cases utilizing the 19g core needle have been found to provide adequate cores. It is felt that 32 cases collected prospectively should be adequate to learn how the 19g FNB needle compares to the 19g FNA needle in different patients with different liver conditions.

6.9 Data Management

6.9.1 Data Collection and Storage

EPIC electronic health records database will allow for availability of demographic data and office-based follow-up records. ProVation MD software information will provide details regarding endoscopic parameters and intervention performed.

Electronic records gathered for study purposes will only be available to study investigators and will be stored on an encrypted hard drive on a computer. Data will initially be entered with PHI attached so that all information can be obtained. Once all data collection is complete identifiers will be removed and random number assigned to the patients.

6.9.2 Records Retention

Records shall be retained for a total of 6 years as per Geisinger policy

7 SAFETY MONITORING

7.1 Adverse Event Reporting

Clinical adverse events (AEs) will be monitored throughout the study. All AEs will be reported to the institutional review board (IRB) regardless of whether they are considered study related. The date and time of onset and outcome, course, intensity, action taken, and causality to study treatment will be assessed by the study PI. In the event of a serious AE (SAE), this will be reported to the Geisinger IRB (GIRB) according to the GIRB guidelines. All other AEs will be summarized and submitted to GIRB during continuing review.

7.2 Definitions

An **adverse event** (AE) is any untoward, undesired, or unplanned event in the form of signs, symptoms, disease, or laboratory or physiologic observations occurring in a person given a test article or in a clinical study. The event does not need to be causally related to the test article or clinical study.

[Include as applicable to study]

An AE includes, but is not limited to, the following:

- Any clinically significant worsening of a preexisting condition.
- An AE occurring from overdose of a test article, whether accidental or intentional. Define overdose for each test article here or in the Overdose section. Overdose is a dose greater than

that specified in the protocol. OR Overdose is a dose greater than that specified in the investigator's brochure/label. OR define overdose

- An AE occurring from abuse (e.g., use for nonclinical reasons) of a test article.
- An AE that has been associated with the discontinuation of the use of a test article.
- For reports from post marketing studies, any failure of expected pharmacologic action of a test article. For over-the-counter products, the recommended daily dose must be administered before failure of expected pharmacologic action can be attributed.

A **serious adverse event** (SAE) is an AE that:

- Results in death.
- Is life-threatening (see below).
- Requires inpatient hospitalization or prolongation of an existing hospitalization (see below).
- Results in a persistent or significant disability or incapacity (see below).
- Results in cancer.
- Results in a congenital anomaly or birth defect.
- Additionally, important medical events that may not result in death, be life-threatening, or require hospitalization may be considered SAEs when, based on appropriate medical judgment, they may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in hospitalization, or development of drug dependency or drug abuse.

Life-threatening refers to immediate risk of death as the event occurred per the reporter. A life-threatening experience does not include an experience, had it occurred in a more severe form, might have caused death, but as it actually occurred, did not create an immediate risk of death. For example, hepatitis that resolved without evidence of hepatic failure would not be considered life-threatening, even though hepatitis of a more severe nature can be fatal. Similarly, an allergic reaction resulting in angioedema of the face would not be life-threatening, even though angioedema of the larynx, allergic bronchospasm, or anaphylaxis can be fatal.
Hospitalization is official admission to a hospital. Hospitalization or prolongation of a hospitalization constitutes criteria for an AE to be serious; however, it is not in itself considered an SAE. In absence of an AE, a hospitalization or prolongation of a hospitalization should not be reported as an SAE by the participating investigator.

In addition, a hospitalization for a preexisting condition that has not worsened does not constitute an SAE.

Disability is defined as a substantial disruption in a person's ability to conduct normal life functions.

If there is any doubt about whether the information constitutes an SAE, the information is treated as an SAE.

A **protocol-related adverse event** is an AE occurring during a clinical study that is not related to the test article, but is considered by the investigator or the medical monitor (or designee) to be related to the research conditions, i.e., related to the fact that a subject is participating in the study. For example, a protocol-related AE may be an untoward event occurring during a washout period or an event related to a medical procedure required by the protocol.

Other Reportable Information. Certain information, although not considered an SAE, must be recorded, reported, and followed up as indicated for an SAE. This includes:

- Pregnancy exposure to a test article, except for exposure to prenatal vitamins. If a pregnancy is confirmed, use of the test article must be discontinued immediately. Information about pregnancy exposure includes the entire course of pregnancy and delivery, and perinatal and neonatal outcomes, even if there are no abnormal findings. Both maternal and paternal exposure are considered other reportable information. For exposure involving the female partner of a male subject, the necessary information must be collected from the subject, while respecting the confidentiality of the partner.
- Lactation exposure to a test article with or without an AE.
- Overdose of a test article as specified in this protocol with or without an AE. Baby formula overdoses without any AEs are excluded.
- Inadvertent or accidental exposure to a test article with or without an AE.

7.3 Recording and Reporting

A subject's AEs and SAEs will be recorded and reported from the signing of the informed consent form to 1 week from EUS-LB Procedure.

7.4 Serious Adverse Event Reporting

David L. Diehl will notify GIRB of all study SAEs in accordance with policy guidelines. If an SAE has not resolved at the time of the initial report, a follow-up report including all relevant new or reassessed information (e.g., concomitant medication, medical history) will be submitted to GIRB. An SAE will be followed until either resolved or stabilized.

8 PROTECTION OF HUMAN SUBJECTS

8.1 Informed Consent

The investigator will provide for the protection of the subjects by following all applicable regulations. The informed consent form will be submitted to the IRB for review and approval.

Before any procedures specified in this protocol are performed, a subject must:

- Be informed of all pertinent aspects of the study and all elements of informed consent.
- Be given time to ask questions and time to consider the decision to participate.
- Voluntarily agree to participate in the study.
- Sign and date an IRB-approved informed consent form.

8.2 Protection of Human Subjects Against Risks

Potential Risks due to Study participation -
A potential risk is loss of the patients' privacy and loss of the confidentiality of their data.

Upper endoscopy possesses a 0.0004-0.00009% risk of perforation, and less than 0.5% risk of bleeding [38]. Adverse events described with the EUS and fine needle aspirate possess a complication rate of 1.72% in prospective studies and 0.64% in retrospective studies. The risk of perforation for EUS is roughly 0.06% and bleeding 0.13%. There have not been established increased risks of complication in patients who undergo EUS with different biopsy needles. However, the 19g needle is the standard needle size in use for EUS-LB, and has been found to be very safe.

Potential Benefits
Included patients are already undergoing the procedure to make a clinical diagnosis of the liver abnormality, and also have the benefit of requiring only one endoscopic procedure to evaluate digestive system problems while at the same time obtaining liver biopsy. As the potential complications with EUS-LB seem to be lower than other means of liver biopsy, a potential benefit is sparing patients from the complications caused by other methods of liver biopsy. This study shall also benefit future patients by providing data regarding prediction of success for EUS-LB with 19-g FNA versus 19-g FNB needles.

Risk: Benefit Ratio
A potential risk in this study involves loss of confidentiality. The use of password protected data storage, removal of PHI and assignment of randomly generated patient number and limitation of data to the study investigators shall limit this risk. Additionally, there is a small risk of perforation or bleeding in diagnostic endoscopy and EUS-LB. These risks are outweighed by the benefit of procuring a diagnosis behind their liver-related abnormality.

Procedures to Maintain Privacy and Confidentiality
As exposure of confidential information is a potential risk, subject identifiers shall not be recorded and subjects shall be given a randomly generated number. The project investigators shall be the only ones with access to the study data, which shall be kept on a password protected/locked Geisinger computer. All data will be destroyed at the completion of this study's manuscript completion.

Vulnerable Subjects
It is possible that terminally ill patients may be involved in this study. This study will however not involve direct intervention and not impact/delay the procedure for which they are undergoing. The observational nature of the study and the subject's ability to exclude them from the study at any time shall be reinforced by the PI/Co-I.
No individuals who require substituted consent shall be involved in this study, nor any children.

Compensation to Subjects
No compensation shall be granted to subjects as this is an observational study and does not deviate from the standard of care.

Treatment of Research-Related Injuries

Potential procedure-related injuries would include perforation or bleeding, which would be managed in a standard fashion. There are no additional "research-related" injuries. Any injuries in the study cohort will be a result of risks inherent to the procedure. This is always explained during the consent process for the procedure and is the standard of care.

8.3 Data Monitoring Plan

Procedures to Maintain Privacy and Confidentiality
As exposure of confidential information is a potential risk, subject identifiers shall not be maintained and subjects shall be given a randomly generated number. The project investigators shall be the only ones with access to the study data, which shall be kept on a password-protected Geisinger computer. All physical forms will remain in a locked storage device. All data will be destroyed at the completion of this study's manuscript completion.

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