

B.L.O.C.S
(BLOCS -Benign Liver Optimal Core Study)
Comparison of 2 techniques using EUS guided Liver biopsies via 19g CORE biopsy needle to
Obtain Optimal Core Liver Biopsies in Benign Disease

NCT 03245580

Protocol Version: 4.0

Date: 05Feb2020

Previous Version	Original 11DEC2017
Previous Version	Version 1.1 08Jan2018
Previous Version	Version 2.0 05Apr2018
Previous Version	Version 3.0 25Nov2018

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Confidentiality Statement

BLOCS Benign Liver Optimal Core Study

Version 4.0 05Feb2020

The information provided in this document is provided in confidence to Investigators, potential investigators, and consultants for review by said individuals, their study staff, and applicable Independent Ethics Committee/Institutional Review Board. It is understood that this information will not be disclosed to others without written authorization, except to the extent necessary to obtain informed consent from potential study participants.

Comparison of 2 techniques using EUS guided Liver biopsies via 19g CORE biopsy needle to Obtain Optimal Core Liver Biopsies in Benign Disease

The clinical study is sponsored by Parkview Research Center.

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1.0 Purpose

The primary purpose of this prospective, randomized, multicenter study is to evaluate and compare the amount and quality of tissue samples yielded in a liver biopsy comparing 2 different techniques of EUS guided CORE liver biopsy for benign disease. The two techniques: “Modified Wet suction” and “Slow pull” technique of collecting tissue from a liver biopsy via Endoscopic Ultrasound (EUS).

2.0 Background

Endoscopic Ultrasound (EUS) for tissue acquisition has undergone significant advances over the past 10 years. EUS guided fine needle biopsy (FNB) and CORE biopsy techniques have rapidly come to the forefront. With the advent of changes to needle design and the study of techniques for tissue acquisition, FNB and Core biopsies will allow for a revolution in tissue acquisition.

The safety and efficacy of EUS guided liver biopsy for acquisition of histologic samples (FNB, “biopsy” or “core”) has been demonstrated in initial studies¹⁻⁷. These initial studies demonstrated the ability to safely obtain adequate liver specimens based on size of specimen and number of portal tracts¹⁻⁷. Diehl et al also noted that EUS guided liver biopsy offers significant advantages including increased patient satisfaction, ability to avoid a second procedure for biopsy, and EUS guidance can be used to avoid large vessels and perform diagnostic evaluation of adjacent structures³. In addition to patient satisfaction, a reduction in cost may be seen as well in the appropriate settings where a patient already requires EUS for evaluation of dilated biliary system or other indications – as opposed to having the liver biopsy done as a separate additional procedure. While there has been a high level of adequacy, diagnostic yields have varied from 86-100%^{1,3,5,6} indicating the technique must be refined and studied further.

The efficacy and safety of EUS guided biopsy is dependent on technique, needle and specimen processing, which provides opportunity for improvement of clinical outcomes.

The use of suction has not offered a clear benefit in the quality of specimen. The cellularity, yield and bloodiness of the specimens, account for the lack of clarity. The use of a wet suction technique has been introduced to provide a larger tissue volume and decrease bloodiness^{8,9}. Endoscopic Ultrasound (EUS) with Fine Needle Aspiration (FNA) has traditionally been performed using a dry suction technique. As opposed to the wet suction technique, which is performed using a needle flushed with saline, the dry suction technique utilizes an empty needle, essentially filled with air. The proposed advantage of the wet suction technique and the basis for the study relies on the fundamentals of fluid mechanics, as saline water is substantially less compressible than air. This difference in compressibility allows a greater “pulling force” to be generated from drawing back the plunger upon aspiration and in turn, more tissue is suctioned for biopsy.

Software simulation demonstrated the wet suction technique to allow faster aspiration and to aspirate 70% more tissue for a simulation time of 0.1 seconds, as compared to the dry technique ⁴.

Recently two companies have released novel designs to obtain histologic specimens for core biopsy. The technique for obtaining optimal tissue samples has not been well defined, but there has been tendency towards slow pull or “wicking” to obtain larger histology ¹².

We aim to further refine EUS guided liver biopsy with use of novel needle technology in conjunction with Fine needle biopsy – “FNB” – for Core histology acquisition by comparing two widely accepted techniques in a randomized, multi-center prospective trial.

3.0 Study Objectives

3.1 Objective

- a. To determine if there is a significant difference in pathological yield as determined by fragmentation, length and number of portal tracts of the biopsy sample between a “modified wet suction,” and a “slow pull” techniques in obtaining CORE of histologic tissue from nonmalignant liver per EUS.

3.2 Secondary Objective

- a. To identify complications within one week that are associated with each technique.
- b. To identify the time required for each step of the individual procedures.
- c. To identify additional findings of the procedure not related to liver disease
- d. To identify any diagnostic differences in pathological findings between left and right liver lobe specimens

4.0 Study Design

This is a randomized prospective multicenter trial. At least 160 subjects will be enrolled. Enrollment is projected to be completed within approximately 12 months. Subjects will undergo a liver biopsy consisting of two passes using one of 2 methods: “modified wet suction” (**Arm 1**) and “slow pull” (**Arm 2**).

Up to twenty (20) participating U.S. sites will consist of tertiary referral centers (either academic or community). The participating physicians are required to have experience as advanced interventional expert endoscopists as demonstrated by prior experience of performing a minimum of twenty EUS guided liver biopsies, and performing greater than 350 EUS procedures per year.

Adult (> 18 years old) subjects who will undergo EUS guided liver biopsy may be included in this study. EUS liver biopsy will be performed if clinically indicated as part of standard of care for clinical workup. This includes patients who need an Esophagogastroduodenoscopy

(EGD) and have any indication for liver biopsy. The initial indication for EUS may not be solely for EUS liver biopsy but rather may be for other indications as well (such as chronic pancreatitis, ductal dilation, right upper quadrant abdominal pain, etc.).

The study duration will commence at the time of Informed Consent for the EUS guided liver biopsy procedure and conclude one-week post procedure.

Subjects will undergo a liver biopsy consisting of two passes using one of 2 methods: “modified wet suction” (**Arm 1**) or “slow pull” (**Arm 2**). A randomization process will determine which Arm will be utilized. Each subject shall undergo two passes (two cores of tissue will be obtained – ideally from the right and left lobe of the liver. However, if this is not technically possible – per discretion of the endoscopists – it will be noted and two biopsies will be obtained from the same lobe of the liver.). Standard hospital protocol will be followed for patient care management following procedure.

Quality of tissue obtained via the two different techniques will be evaluated by number of fragments, length of specimens and number of tracts observed by the local pathologist and compared between the tissues obtained from the two techniques. We will also note technical success rate (ability to insert the needle twice into the liver for obtaining two specimens, incidence of a dry pass and ability to pass into the right and left lobe of the liver). Complications will be observed and reported up to one-week post procedure. (Complications may include additional visit to clinic or ER visit, bleeding requiring medical attention, infection requiring interventions, liver laceration, admission post procedure, or need for surgery).

The EUS guided biopsy will follow the procedural description in Section 8.

Follow up evaluations and treatment will be performed in accordance with standard of care procedures and procedures deemed necessary by the attending physician.

The participating site will obtain appropriate Institutional Review Board (IRB) approval prior to participating in any research activities. A Central IRB review will be conducted for those sites that are able to accept a Central IRB determination. For other sites that require local IRB approval, the participating site will provide PRC with a copy of their IRB approval prior to any study procedures. PRC will also need to collect the local IRB’s IRB registration and/or FWA to confirm compliance.

Once IRB approval has been confirmed by the coordinating site and the Site Initiation Visit (SIV) is completed, the participating site will receive enrollment instructions, randomization envelopes and case report forms.

All processing of specimens will be performed according to The College of American Pathologists. The designated pathologist(s) at each site will be responsible for reading all study specimens as described in Appendix A.

5.0 Selection of Patients

Candidates for this study are patients who will be undergoing liver biopsy under EUS guidance and are deemed to have clinical need for liver biopsy for non-malignant processes.

All patients determined by the physician to be clinically in need of a liver biopsy via EUS may be approached for possible enrollment. Usually these are patients requiring an upper endoscopy and a liver biopsy as part of workup for liver disease.

6.0 Eligibility

6.1 Inclusion Criteria

- 6.1.1 Subjects that plan to undergo a liver biopsy via EUS to confirm possible underlying liver disease or to determine stage, grade and presence of fibrosis for suspected benign etiology.
- 6.1.2 Subjects with a history of abnormal LFTs (as designated by attending gastroenterologist), documented history of chronic liver disease, history of fatty liver disease or have a question of underlying liver disease as cause of abnormal imaging, labs or symptoms which may be attributed to liver disorder and may benefit from liver biopsy or other clinical indication for liver biopsy
- 6.1.3 Subjects 18 years of age or older
- 6.1.4 Subject must be able to hold anticoagulants as per institutional standard of care
- 6.1.5 Subjects must be deemed physically able to undergo anesthesia. This includes either Monitored Anesthesia Care (MAC) or general anesthesia.
- 6.1.6 Subjects that have agreed to participate in the study and have signed Informed Consent
- 6.1.7 Women of child bearing potential who are not pregnant as proven by a negative pregnancy test.

6.2 Exclusion Criteria

- 6.2.1 Subjects that are undergoing the EUS procedure for malignant process.
- 6.2.2 Subjects unable to tolerate anesthesia for the procedure
- 6.2.3 Subjects who cannot undergo or refuse EUS guided procedure
- 6.2.4 INR >1.5 (if tested per individual institutional protocol)
- 6.2.5 Subjects that have taken aspirin in the 5-7 days prior to procedure (or as required by individual institutional protocol)
- 6.2.6 Platelets 50,000 uL or less

- 6.2.7 Subjects with evidence of significant upper gastrointestinal bleeding prior to endoscopy
- 6.2.8 Subjects requiring endoscopic mucosal resection
- 6.2.9 Subjects with large volume ascites as determined by subject's clinician
- 6.2.10 Subjects requiring pancreatic biopsies
- 6.2.11 Subjects that have not held antiplatelet or other anticoagulant medications for adequate time prior to procedure as per institutional policy

7.0 Schedule of Events

	Screening	Biopsy	1 Week post-Biopsy
	+/- 7 days	Day of Procedure	
Informed Consent	X		
Eligibility Checklist	X		
Demographics	X		
Medical History	X		
Concomitant Medications	X		
Laboratory Assessments *	X		
Randomization		X	
EUS Guided Liver Biopsy		X	
Pathology Assessment		X	
Adverse Event Evaluation		X	X
Post Biopsy Clinical Summary			X
* Laboratory assessments may include Albumin, Alkaline Phosphatase, ALT/SGPT, AST/SGOT, Total Bilirubin, Direct Bilirubin, Indirect Bilirubin, Lipase, Total Protein, platelets, INR, serum pregnancy if applicable, Fibroscan, Fibrosure and Hepatitis Panel results (these laboratory assessments will be performed per institution Standard of Care)			

8.0 Conduct of Study Assessments and Study Procedures

8.1 Informed Consent

Informed consent must be obtained before performing any study-specific procedures. Subjects will receive a consent form to read in person. They will be instructed to read the consent and write down any questions they may have. They will be allowed as much time as they need to read the consent form.

It will be stressed to the subjects that this is voluntary and in no way will it impact their medical care. Study Staff will then review the consent document with the subject to ensure that they understand the study.

The subject must have all questions pertaining to the study answered satisfactorily. The consent form that is used must be approved by both the reviewing IRB and by PRC. One copy of the

completed informed consent form must be given to the subject and the original must be placed in the subject's medical record as a source document.

8.2 Eligibility Checklist

Prior to any study procedures, the eligibility checklist will be completed and signed.

8.3 Subject ID # Assigned

Upon enrollment into the clinical study, a unique subject study ID will be assigned. The subject number will be assigned consecutively in ascending order per site. The subject study ID number should be recorded on all source documents.

8.4 Demographics

The following demographic information will be collected:

Age, Gender, Race, Ethnicity

8.5 Medical History

Record all past and/or concomitant medical conditions, diagnoses and surgeries.

8.6 Concomitant Medications

All concomitant medications (including start/stop dates and indication) must be recorded from the start of treatment through the study treatment follow-up period. Blood-thinning agents as well as any herbal supplements will be recorded.

8.7 Laboratory Assessments

Lab studies may be performed per local Standard of Care. Labs may include Albumin, Alkaline Phosphatase, ALT/SGPT, AST/SGOT, Total Bilirubin, Direct Bilirubin, Indirect Bilirubin, Lipase, Total Protein, Platelets, INR, Hepatitis Panel, Fibroscan and Fibrosure. No labs will be required for the sole purpose of this research study. The most recent lab results will be reported to the sponsor for study purposes.

For female patients of child bearing potential, pregnancy tests will be performed per local Standard of Care.

8.8 Randomization

Subjects will undergo a liver biopsy consisting of two passes using one of 2 methods: "modified wet suction" (Arm 1) and "slow pull" (Arm 2). Subjects in each of the two specimen processing groups will be randomized into each arm in a balanced manner to either the "modified wet suction" technique (ARM 1), or the "slow pull" technique (ARM2) when performing liver biopsy according to a predetermined randomization. Randomization will be stratified by site, with a block size of 4.

A numbered, sealed envelope will be utilized to identify randomization of each subject; these will be provided by study sponsor. A sheet of paper inside the envelope will indicate either ARM 1 –

Modified Wet Suction technique or ARM 2– Slow Pull technique. The envelope may be opened after the Informed Consent and Eligibility Checklists are completed. Often the Randomization envelope will be opened once the patient is in the procedure room.

8.9 EUS Guided Liver Biopsy

Upper endoscopy is performed with necessary biopsies or upper dilation completed if needed. Patients with evidence of significant upper gastrointestinal bleeding prior to endoscopy are exempt.

Once upper endoscopy is completed, the upper endoscope will be removed, and the Linear Array EUS scope is introduced per os. Diagnostic EUS examination is completed. EUS requiring pancreatic biopsies or interventions are exempt (See section 6). The patient is randomized to one of 2 techniques. The study will use block randomization stratified per site. The Randomization envelope is opened and the endoscopist is told which EUS guided liver biopsy technique is to be used.

Each technique will be used to perform 2 passes. A “pass” is defined as entry into the liver and then removal of tissue. An “actuation” is defined as a to and fro movement once the needle is within the liver.

An attempt to biopsy both left and right lobes of the liver will be made. If this cannot be completed for technical reasons, then the endoscopist will make a note and perform 2 passes to the lobe which is able to be biopsied.

8.10 Arm 1- “Modified Wet suction”

The right or left lobe of the liver is initially targeted.

Doppler and EUS imaging are used to avoid significant vessels and large ducts when identifying appropriate biopsy tract.

The stylet is removed from the onset of the procedure. It will only be used to push the specimen out of the needle. A syringe is used to flush the needle with 5 cc of saline. Then a syringe that contains 3cc of saline with preloaded 20cc of suction with the valve in the off position is attached to the back of a 19g Acquire Boston Scientific needle. The needle is advanced into the liver just beyond the capsule. An initial pass of at least 4-5 cm is made into the liver with a quick forceful forward movement, the needle is then slowly pulled back to the edge of the capsule. The valve is switched to the open position. 2-4 actuations are made driving the needle at least 4-5 cm in length every time while the angle is changes slightly (1-3mm) with each actuation. No fanning. The needle is then pulled out of the liver with the valve switched to the closed position just prior to removing the needle from the liver. Often a flash of blood may be seen. The needle is removed and the specimen is taken for processing (see processing below).

The endoscopist then finds a second site to biopsy within the other lobe. If it is deemed by the endoscopist to not be able to biopsy the other lobe for technical reasons, this will be noted and tracked and an additional tract will be biopsied within the same lobe. Again, taking care to avoid vessels and large bile ducts, a second pass is made with the same technique.

In the event that a pass returns with no or inadequate tissue, the endoscopist will switch to the opposite technique and proceed as per protocol.

8.11 Arm 2- “Slow Pull”

The right or left lobe of the liver is initially targeted.

Doppler and EUS imaging are used to avoid significant vessels and large ducts when identifying appropriate biopsy tract.

The stylet is maintained from the onset of the procedure. It will later be used to push specimen through the needle. Using the 19g Acquire Boston Scientific needle, the needle is advanced into the liver just beyond the capsule. No fanning. The stylet is pulled back about 2cm. The needle is passed at least 4-5 cm into the liver with a quick forceful forward movement, as it is slowly withdrawn the nurse pulls back the stylet part with each withdrawal after actuation. This is repeated 2-4 times while the angle is changed slightly (1-3mm) with each actuation. The end the stylet is almost but not completely withdrawn from the needle. The needle is then pulled out of the liver. The needle is removed. The specimen is then taken for processing (see processing below).

The endoscopist then finds a second site to biopsy within the other lobe. If it is deemed by the endoscopist to not be able to biopsy the other lobe for technical reasons, this will be noted and tracked and an additional tract will be biopsied within the same lobe. Again, taking care to avoid vessels and large bile ducts, a second pass is made with the same technique.

In the event that a pass returns with no or inadequate tissue, the endoscopist will switch to the opposite technique and proceed as per protocol.

8.12 Total EUS time

Beginning of EUS examination which begins when the EUS scope is placed into the patient’s mouth to when the EUS scope is withdrawn. EGD time prior to EUS is not counted. Room set up and anesthesia time is variable and not counted.

8.13 Total Liver Biopsy time

After completing the EUS examination including non-liver biopsies, the operator pays attention to the liver. The time starts for EUS liver biopsy. This should be the start time for the liver biopsy. The end time is then when the EUS scope is removed.

8.14 Specimen Processing within Surgical Suite

After completion of each pass, the specimen is processed by the technician or nurse:

- Process as surgical specimen – histologic sample in 10% formalin
- Caution should be used to avoid fragmentation of specimens
- **ARM 1:** A specimen (in the modified wet suction technique) should not come into the chamber of the suction syringe. If this should happen, remove the plunger from the syringe to limit fragmentation and pour contents into the specimen jar. Assuming that the specimen does not go into the syringe, use the stylet to slowly push the specimen out. As the specimen touches the formalin the capillary pull of the formalin will help to withdraw the tissue. Once the specimen makes contact with the formalin slowly swirl the specimen in an outward circle around the jar. The swirling and gentle expression avoids fragmentation
- After expression of specimen, the needle is flushed with 10 – 20 cc of saline. Any additional tissue from this flush should also be captured in the specimen jar.
- The needle is taken back to the endoscopist for second pass or discarded if both passes have been completed and the procedure is over.
- Repeat the above specimen prep for the second pass.
- Specimens should be labeled according to the lobe of acquisition.
- **ARM 2:** For slow pull technique, the stylet can be used to express the specimen slowly ensuring that it remains intact. Use the same technique of capillary pull and swirling to avoid fragmentation.
- After expression of specimen, the needle is flushed with 10 – 20 cc of saline. Any additional tissue from this flush should also be captured in the specimen jar.
- The needle is taken back to the endoscopist for second pass or discarded if both passes have been completed and the procedure is over.
- Repeat the above specimen prep for the second pass
- Specimens should be labeled according to the lobe of acquisition.

8.15 Pathologist Assessment

The specimen processing in pathology lab will use standard practice techniques in accordance with the College of American Pathologists standards as described in Appendix A.

Designated pathologist(s) at each site should be identified, and is/are responsible for reading all specimens from the study.

The pathologist(s) will remain blinded as to the specimen acquisition technique used.

8.16 Post Biopsy CRF

A summary of lobes biopsied, pathology findings, EUS findings and EGD findings.

8.17 Adverse Event Evaluation

Adverse events will be collected starting after randomization occurs and for one (1) week post-Biopsy. No additional visits are required for the study. Record review may suffice for obtaining follow-up information, See Section 9, Adverse Event Reporting, for more information.

8.18 Recovery:

From time out of room until patient is ready to be released. This time will be noted by recovery room personnel. The patient does not need to actually leave; but ready for discharge.

9.0 Adverse Event Reporting

An adverse event is defined as the appearance of (or worsening of any pre-existing) undesirable sign(s), symptom(s), or medical condition(s) that occur after patient's signed informed consent has been obtained.

Adverse events that begin or worsen after informed consent should be recorded in the Adverse Events CRF. Conditions that were already present at the time of informed consent should be recorded in the Medical History page of the patient's CRF.

Adverse events (including lab abnormalities that constitute AEs) should be described using a diagnosis whenever possible, rather than individual underlying signs and symptoms. When a clear diagnosis cannot be identified, each sign or symptom should be reported as a separate Adverse Event.

All adverse events should be treated appropriately. If a concomitant medication or non-drug therapy is given, this action should be recorded on the Adverse Event CRF.

9.1 Serious Adverse Event Reporting

All serious adverse events will be reported to the PRC Medical Monitor within 24 hours.

Definition of an SAE: Any adverse event that results in any of the following outcomes:

- Death;
- A life-threatening adverse experience;
- Inpatient hospitalization or prolongation of existing hospitalization;
- A persistent or significant disability/incapacity;
- A congenital anomaly/birth defect;
- Important medical events that may not result in death, be life threatening, or require hospitalization may be considered an SAE, when, based upon medical judgment, they may jeopardize the patient and may require medical or surgical intervention to prevent one of the outcomes listed in the definition

Serious adverse event (SAE) is defined as one of the following:

- Is fatal or life-threatening
- Results in persistent or significant disability/incapacity
- Constitutes a congenital anomaly/birth defect
- Is medically significant, i.e., defined as an event that jeopardizes the patient or may require medical or surgical intervention to prevent one of the outcomes listed above
- Requires inpatient hospitalization or prolongation of existing hospitalization, note that hospitalizations for the following reasons should not be reported as serious adverse events:
 - Routine treatment or monitoring of the studied indication, not associated with any deterioration in condition
 - Elective or pre-planned treatment for a pre-existing condition that is unrelated to the indication under study and has not worsened since signing the informed consent
 - Social reasons and respite care in the absence of any deterioration in the patient's general condition
- Note that treatment on an emergency outpatient basis that does not result in hospital admission and involves an event not fulfilling any of the definitions of a SAE given above is not a serious adverse event

For patients who sign the study ICF, SAE collection starts at time of study informed consent.

Any additional information for the SAE including complications, progression of the initial SAE, and recurrent episodes must be reported as follow-up to the original episode within 24 hours of the investigator receiving the follow-up information. An SAE occurring at a different time interval or otherwise considered completely unrelated to a previously reported one should be reported separately as a new event.

Information about all SAEs is collected and recorded on the Serious Adverse Event Report Form; all applicable sections of the form must be completed in order to provide a clinically thorough report. The investigator must assess and record the relationship of the SAE to the study

treatment, complete the SAE Report Form, and submit the completed form within 24 hours to PRC Medical Monitor.

Follow-up information is submitted in the same way as the original SAE Report. Each re-occurrence, complication, or progression of the original event should be reported as a follow-up to that event regardless of when it occurs. The follow-up information should describe whether the event has resolved or continues, if and how it was treated, and whether the patient continued or withdrew from study participation.

If an investigator is in doubt about the applicable reporting obligations, he/she should consult with the PRC.

When required, and according to local law and regulations, serious adverse events must be reported to the Ethics Committee and Regulatory Authorities.

PRC will report all adverse events that are serious, unexpected, and considered at least possibly related.

10.0 Data and Safety Monitoring

It is the responsibility of the Principal Investigator to oversee the safety of the study at his/her site. This safety monitoring will include careful assessment and appropriate reporting of adverse events as noted above. Medical monitoring will include a regular assessment of the number and type of serious adverse events.

11.0 Sample Size

12.0 With a sample size of 72 subjects per group completing the study, 144 total subjects, the study will have 80% power to detect a 20% difference in means between the groups for the length of tracts and number of tracts, assuming two-sided tests of means using ratios, each conducted at a 5% significance level, and a coefficient of variation of 0.4. To account for up to 10% dropouts and lost samples, the study will enroll 80 subjects per group, 160 total subjects. Sample size calculations were made using PASS 13 (NCSS, Kaysville, UT). Statistical Analysis Plan

12.1 Primary Endpoint

- The primary endpoint for the study is determination of the optimal EUS guided core liver biopsy technique based on the fragmentation, total specimen length, number portal tracts of the tissue as evaluated by pathologist.

12.2 Secondary Endpoints

- To identify complications within one week that are associated with each technique.
- To identify the time required for each step of the individual procedures.
- To identify additional findings of the procedure not related to liver disease.
- To identify any diagnostic differences in pathological findings between left and right liver lobe specimens

12.3 Statistical Analysis

The two techniques will be compared for differences in fragmentation, total specimen length, and number portal tracts of the tissue using generalized linear mixed effects models (GLMM). The GLMMs will compare the two techniques while incorporating random effects for study site and endoscopist using the appropriate distribution and link function for each outcome (e.g. negative binomial distribution with log link for count data, binomial distribution with logit link for binary data). The GLMMs will also include a random effect to allow correlation between the two biopsy samples from each subject and a fixed effect to allow comparison between the two sides; an interaction between side and technique will be examined but removed from the model if not significant. Similar GLMM analyses will be performed for the comparisons of complications, technical success rate, total EUS time, total liver biopsy time, and recovery time. Analyses will be performed with sample processing technique included as a covariate in the analyses. Because of the small number of subjects with the mesh prep technique, the interaction between study group and sample processing technique will not be examined. Additionally, the analyses will be repeated after removing the subjects with the mesh prep technique. A 5% significance level will be used for all tests.

13.0 Subject Withdrawal

A subject has the right to withdraw from the study at any time for any reason without prejudice to their future medical care by the physician or the institution. The investigator and sponsor also have the right to withdraw subjects from the study in the event of comorbidities, adverse events, treatment failure, protocol violation, or other reasons. Should a subject decide to withdraw; all efforts will be made to complete and report the observations as thoroughly as possible.

14.0 Data Collection and Management

For Each Subject who has given informed consent, an eligibility CRF must be completed and signed by the physician. Every effort should be made to respond to all questions on each CRF page. Each CRF should be completed to identify the physician, subject number, and subject initials. At no time should the subject name appear in the CRFs. Complete data is required in order to provide statistical analysis for each subject.

Completed source documentation CRFs should be faxed upon request to 260-266-5656
Attention: Angela Hamman

14.1 Data Handling

The Investigator will maintain a file for each subject that includes the signed informed consent and copies of all CRFs completed for that subject. The following study-related records should also be retained and stored:

- Study Protocol and all amendments
- IRB approved Informed Consent Forms
- Signed Statement of Investigator form
- Approval letter(s) from and all other correspondence to and from the IRB
- The certification of the clinical laboratory used for this study and a listing of control ranges used by that laboratory
- Curriculum vitae for the Investigator(s) and Sub-Investigators
- A list of ancillary study personnel including delegation of responsibilities
- Copies of all laboratory test results and other original data from which CRF information was obtained
- Correspondence to and from the Sponsor

14.2 Retention of Data

An investigator shall retain records required to be maintained under this part for a period of 7 years after the investigation is discontinued.

14.3 Case Report Forms (CRFs)

This study will utilize an electronic data capture format. The case report forms (CRFs) contain confidential information.

Specific instructions to complete the CRFs shall be provided to the Investigator and other site personnel as appropriate. The Investigator is responsible for reporting appropriately and in a timely manner.

14.4 Audit and Supervision

Investigator sites and study documentation may be subject to Quality Assurance (QA) audits during the course of the study. In addition, inspections may be conducted by regulatory bodies at their discretion, during and after study completion.

The Investigator agrees to allow inspectors from regulatory agencies to have access to all study records, including subject source documents. By participating in this study, the Investigators agree to these requirements and will assist the inspectors in their duties. The Investigator should immediately notify PRC of an upcoming inspection.

14.5 Data and Quality Management

The clinical study will be monitored according to the current PRC standard operating procedures.

Monitoring activities at the site or virtually include, but are not limited to:

- Source document verification,
- Review of investigator site files.

The CRA ensures that original, signed source documents (or certified copies) are available for verification against the CRFs at the site during each monitoring visit. As part of the source document verification process, the monitor confirms the following:

- All relevant adverse events, concomitant medications, medical history and concurrent illnesses have been entered into the appropriate sections and reconciled for logical relationships.
- Missed subject visits, tests or examinations are adequately documented in the CRF.
- All data queries are source verified and/ or required and resolved.

Source data verification (SDV) is completed as described the in the Monitoring Plan. CRAs use the following criteria when monitoring source data:

- Only original source notes are reviewed.
- Verification (by verification of source data) that all subjects exist.
- The Investigator keeps a written or electronic patient file for every subject participating in the clinical study. In this patient file, the available demographic and medical information of a subject has to be documented, in particular the following: name, date of birth, gender, height, weight, subject medical history, concomitant diseases and concomitant medications (including changes during the study), statement of entry into the study, study identification, randomization number, the date of informed consent, all study visit dates, protocol- defined examinations and clinical findings, observed Adverse Events, and reason for withdrawal from the study, if applicable.
- It must be possible to verify the inclusion and exclusion criteria for the study from the available data in this file.
- It must be possible to identify each subject by using this patient file.
- Any other documents with source data, especially original printouts of data that were generated by technical equipment have to be filed. This includes laboratory value listings.
- All of these documents have to bear, at a minimum, the subject identification and the printing date printed by the recording product to indicate to which subject and to which study procedure the document belongs.
- The Investigator should document the medical evaluation of such records as necessary and sign and date.
- Computerized subject files will be printed whenever the monitor performs source data verification. Printouts must be signed and dated by the Investigator, countersigned by the monitor and kept in a secure place.

The following can be used as source documents:

- Hospital records, clinical and office charts, memoranda, subject diaries, patient questionnaires, evaluation checklists, laboratory reports, computer printouts, and any other

documentation regarding the subject. Verification that all safety and efficacy information is clearly documented.

- The Investigator/institution shall allow PRC to carry out audits, as an integral part of the Quality Assurance system. Audits are controls, independent and separate from monitoring, of study activities and documents, with the aim of verifying whether study-related activities have been performed and data have been recorded, analyzed, and forwarded in compliance with the protocol, GCP, standard operating procedure (SOPs), and applicable regulatory requirements.
- All clinical laboratory determinations will be performed in local certified laboratories throughout the study. For laboratory assessments performed at a local facility, the Investigator will provide the Sponsor with a completed, signed, and dated Normal Laboratory Values Form and appropriate lab certifications from the laboratory performing the tests.

15.0 Responsibility of Lead Principal Investigator

- Analysis of Complications and Adverse Events with respect to their clinical relevance and cause relation.
- Evaluation of necessary protocol amendments.
- Provisional evaluation of the final results in the form of a written report which will be the basis for an investigator reviewed publication.
- Assure compliance with the ICMJE's Uniform Requirements for Manuscripts submitted to biomedical journals

16.0 Sponsor Responsibilities

Required Sponsor's records include the following:

- All correspondence (Monitor, Investigator)
- Shipment and disposition of supplies (CRFs, etc.)
- Signed Investigator Agreement
- Maintenance of monitoring documentation.

PRC or designee will monitor the study following standard procedures and applicable regulations.

17.0 Investigator Responsibilities

The Investigator is responsible for complying with all local, state, and federal regulations relating to performing clinical research with an investigational product.

The Investigator is responsible for ensuring that the investigation is conducted according to the signed Investigator Agreement, the approved protocol, and applicable regulations for protecting the rights, safety, and welfare of study subjects under the Investigator's care. The Investigator is

additionally responsible for the control of investigational product and for providing accurate and verifiable data to PRC.

The Investigator must obtain the Informed Consent or LAR of each subject before participation in the study. The Investigator should make every effort to participate in all PRC teleconferences. The Investigator must assure initial and continuing review of the study by an IRB that complies with applicable national and local regulations.

Other Investigator responsibilities relative to the IRB include the following:

- Submit to the IRB for review any advertisements that will be used to recruit subjects.
- Submit all protocol amendments to the IRB for review.
- Report to the IRB any information received from PRC about serious adverse events. Provide the IRB with any other information it requests before or during the conduct of the study.
- Report to the IRB all adverse events that are serious, unexpected, and possibly or probably related to the investigational material.
- Maintain a file of IRB/study-related information.
- Update the IRB on a minimum of a yearly basis.

18.0 Conduct of Study

18.1 Ethical Conduct of the Study

This study will be conducted in accordance with applicable local, national laws and requirements.

18.2 Subject Confidentiality and HIPAA Compliance

To ensure that the confidentiality of subjects' identification is maintained and medical records are protected, subject names will not be used in the study. A unique subject identifier will be assigned to each subject enrolled in the study. Each participating site will maintain a list that cross-references subjects' identification. The participating site will control their subject's identity. To ensure that subjects' identification and medical records are protected, the list will be kept confidential and only accessible to the PI, his/her staff, and the appropriate research representative responsible for ensuring the quality of the reported data. Subject's records may be reviewed by research representative to verify the quality of the reported data; however, confidentiality will be maintained.

Subjects will provide the appropriate authorization to allow the use and disclosure of their personal health records in accordance with the applicable laws and regulations including, but not limited to, HIPAA requirements.

18.3 Informed Consent

Informed Consent will be obtained from each participant and must be appropriately signed. It is the physician's responsibility to obtain written informed consent from the subject prior to any protocol specific screening procedures being done. All subjects in this study should be

completely informed about the purpose, risks, benefits, and other pertinent details of this study. The original signed copies of consent forms will be maintained by physician.

Subjects will provide the appropriate authorization to allow the use and disclosure of their PHI in accordance with applicable laws and regulations, including but not limited to, HIPAA requirements.

18.4 Institutional Review Board/Ethics Committee

Written approval of the protocol and ICD must be obtained prior to subject enrollment. A copy of the letter indicating IRB approval must be provided to the sponsor. Annual updates must be provided to the IRB by the physician for studies longer than one year. Serious or unanticipated adverse events occurring during the study must also be sent to the IRB. A final report will be provided to the IRB by the physician at study completion.

18.5 Publication

It is intended that the results from this study will be published jointly by the participating gastroenterologists.

Note: In signing this protocol, the endoscopic interventionist agrees to the release of the data from this study and acknowledges the above publication policy and per the agreement to participate.

18.6 Institutional Review Board (IRB)

This study will be conducted in accordance with the U.S. Code of Federal Regulations governing the protection of human subjects (21 CFR 50), Institutional Review Boards (IRB) (21 CFR 56), and the obligations of clinical Investigators (21 CFR 312.100).

The Protocol Informed Consent to be used must be approved by the Investigator's IRB before the study is initiated; documentation of this approval (i.e., a copy of the document showing IRB approval that should include the IRB chairperson's [or designee's] signature and the date of IRB's approval) is to be provided to the Sponsor.

PRC expects the Investigator to comply with local IRB regulations. The Investigator will also comply with current GCPs particularly in reference to the safety and rights of the subjects.

Investigators are encouraged to discuss any ethical issues that arise prior to or during the conduct of the study with PRC.

18.7 Declaration of Helsinki

The study will be conducted according to the guidelines established in the Declaration of Helsinki, U.S. GCPs and local ethical and legal requirements. Subjects will be free to withdraw from the study at any stage without prejudice to their subsequent treatment.

19.0 References

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14. DiMaio CJ, Et Al. Initial experience with a novel EUS-guided core biopsy needle (SharkCore): results of a large North American multicenter study. *Endoscopy International Open*. 2016. September. E974-E979

Appendix A: Pathologist Assessment

The specimen processing in pathology lab will use standard practice techniques in accordance with the College of American Pathologists standards.

A designated pathologist(s) at each site will be identified and is/are responsible for reading all specimens from the study.

The designated pathologist will complete all entries on the Case Report Form "Pathology Assessment".

The Pathologist(s) will remain blinded as to the specimen acquisition technique used.

Specimen Processing

After completion of each pass, the specimen is processed by the technician or nurse:

- Process as a surgical specimen- histologic sample in 10% formalin fixative
- Caution should be used to avoid fragmentation of specimens

Technique/Arm 1 (modified wet suction)

- A specimen (in the modified wet suction technique) should not come into the chamber of the suction syringe. If this should happen, remove the plunger from the syringe to limit fragmentation and pour contents into the specimen jar.
- Assuming that the specimen does not go into the syringe, use the stylet to slowly push the specimen out.
- As the specimen touches the formalin the capillary pull of the formalin will help to withdraw the tissue.
- Once the specimen makes contact with the formalin slowly swirl the specimen in an outward circle around the jar.
- The swirling and gentle expression avoids fragmentation.
- After expression of specimen the needle is flushed with 10-20cc of saline. Any additional tissue from this flush should also be captured in the specimen jar.
- Specimens should be labeled according to the lobe of acquisition.
- The specimen processing in pathology lab will use standard practice techniques.

Technique/Arm 2 (slow pull)

- For slow pull technique, the stylet can be used to express the specimen slowly ensuring that it remains intact. Use the same technique of capillary pull and swirling to avoid fragmentation.
- As the specimen touches the formalin the capillary pull of the formalin will help to withdraw the tissue.
- Once the specimen makes contact with the formalin slowly swirl the specimen in an outward circle around the jar.
- The swirling and gentle expression avoids fragmentation.

- After expression of specimen the needle is flushed with 10-20cc of saline. Any additional tissue from this flush should also be captured in the specimen jar.
- Specimens should be labeled according to the lobe of acquisition.
- The specimen processing in pathology lab will use standard practice techniques.