Assessing Effects of Heparin Priming and Pass Number on Tissue Quality of Fine Needle Biopsies

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Principal Investigator: Jorge D. Machicado, MD, MPH

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SIGNATURE PAGE

The signature below constitutes the approval of this protocol and the attachments, and provides the necessary assurances that this study will be conducted according to all stipulations of the protocol, including all statements regarding confidentiality, and according to local legal and regulatory requirements and applicable US federal regulations.

Site Investigator:*

Signed: _____ Date: _____

Name
Title

** The protocol should be signed by the clinical site investigator who is responsible for the day to day study implementation at his/her specific clinical site.*

LIST OF ABBREVIATIONS

EUS	Endoscopic Ultrasound
FNB	Fine Needle Biopsy

1 BACKGROUND/SCIENTIFIC RATIONALE

Previous studies evaluating the effect of needle priming with heparin for liver biopsies have shown that heparinized needles improved histologic yield and tissue adequacy.[1,2] With regards to pancreatic lesions, two retrospective studies showed that needle priming with heparin during EUS fine needle aspiration (FNA) of pancreatic solid lesions was not superior to conventional methods on tissue yield, sample adequacy, bloodiness, and number of passes.[3,4] In contrast, a recent crossover RCT from Taiwan, found that among 50 patients with solid pancreatic masses, EUS-guided biopsy with a heparinized needle obtained longer white tissues with less blood contamination than with conventional EUS-FNB.[5] However, the study measured tissue length with macroscopic on-site evaluation (MOSE), which is prompt to investigator and measurement bias. In addition, the investigators used the same needle for each patient at the time of the crossover to the 2nd intervention, which introduces the possibility of needle contamination in those who were randomized to undergo sampling with the heparinized needle first. Finally, EUS sampling was performed using an old needle design not commercially available in the United States (20-gauge EchoTip ProCore needle) and by a single endoscopist, which limits the external validity of the results. Although these results are important, they cannot be generalizable and need to be confirmed in high-quality RCTs.

When sampling pancreatic masses with EUS-FNN, multiple passes are often performed to improve diagnostic accuracy and reduce false negative results. However, the impact of successive EUS-FNB passes on tissue quality is unknown. This may have a direct impact on specimens collected for purposes other than diagnostic histopathology, since the endoscopist typically reserves the first FNB passes for clinical histopathology and may collect the last passes for personalized medicine applications (e.g. next generation DNA or RNA sequencing).[6] However, it is unclear whether successive passes will have the same tissue quality, as needles may progressively become contaminated with blood.

In this proposal, we aim to provide guidance on how tissue quality is affected by successive EUS-FNB passes, and whether this may be mitigated by heparinizing the FNB needle. The results of this study will provide guidance to endoscopists regarding whether heparin can improve tissue quality when sampling solid pancreatic masses, the number of passes that is needed to obtain adequate tissue, and how to divide up the number of FNB passes for multiple applications. For pancreatic cancer patients, this is highly relevant, as precision platforms that may impact their care such as Know Your Tumor[7], and clinical trials such as Precision Promise[8], require adequate tumor tissue quality for eligibility.This will be a pilot study, as there have been no studies specifically looking at the effects of heparin priming and fine needle biopsy on tissue yield and blood contamination of pancreatic masses.

2 OBJECTIVES

Primary objective:

- *Determine whether heparin priming of FNB needle improves tissue quality as compared to standard FNB for EUS-tissue acquisition of pancreatic masses.*

Secondary objectives:

- *Determine whether heparin priming of FNB needle improves cellularity.*
- *Determine whether heparin priming of FNB needle reduces blood contamination.*
- *Determine whether heparin priming of FNB needle improves histologic yield.*
- *Determine whether increasing number of FNB passes affects tissue quality, block contamination, and histologic yield.*
- *Determine the incremental diagnostic yield with a second or third FNB pass as compared to a single FNB pass.*

Hypothesis:

We hypothesize that the tissue quality of EUS-FNB specimens of pancreatic masses decreases with increasing pass number due to blood contamination. This blood contamination can be ameliorated with priming of the needle with an anticoagulant such as heparin, resulting in improved tissue quality of EUS-FNB of pancreatic masses.

Anticipated Results

- *We anticipate that histologic yield will decrease and blood contamination will increase with increasing number of FNB passes*
- *We anticipate that the intervention of heparin priming of the biopsy needle will decrease blood contamination and increase histologic yield.*

Trial design

- Single center, double-blind, superiority, parallel-group, 1:1 ratio, randomized controlled trial

3 EXPECTED RISKS/BENEFITS

There are no immediate benefits to participating in this study. Of note, IF a diagnosis of malignancy is made on the tissue obtained for research by a blinded clinical pathologist AND the diagnosis was not made on the passes obtained for clinical purposes, the patient will be notified. In this event, either the PI or one of the co-investigators (all of which are practicing physicians) will reach out to the patient personally to inform them of the discrepancy and make arrangements for the research block to be submitted to pathology for further evaluation. Furthermore, the patient's physician will be notified, and the patient will be referred to the appropriate medical specialist to treat the incidentally found condition.

The known or expected risks are:

1) Bleeding - EUS fine needle aspiration and EUS FNB rarely (<0.01%) cause bleeding but this is not expected to be increased measurably by the one additional pass made for research purposes. The risk of bleeding will be minimized by the use of experienced gastroenterologists who will perform the EUS FNBs with Doppler assistance to identify and avoid intervening vasculature during needle puncture.

2) Pancreatitis - A risk of EUS-FNB is pancreatitis, which is inflammation of the pancreas that is uncommonly severe. The reported risk ranges from 0-2% in the literature. It is unknown whether increased numbers of FNB passes increases the risk of pancreatitis. The risk of pancreatitis as a complication will be minimized by the use of experienced gastroenterologists who will perform the EUS procedures.

3) Infection - EUS fine needle aspiration and EUS FNB rarely (<0.01%) cause infection cases but this is not expected to be increased by the one additional pass made for research purposes.

4) Prolongation of procedure time - Acquisition of additional specimens during EUS will extend procedure duration by no more than 5 to 10 minutes. The only risk associated with this would be additional discomfort. This risk will be minimized through the continued use of sedative and pain medications given during the procedure. As per the standard of care, vital signs including oxygen saturation, heart rate, blood pressure, and cardiac rhythm will be continuously monitored.

5) Confidentiality breach - Data produced and collected might be revealed to persons outside the study. This risk will be minimized through the use of a code number for the patient's name. The samples will be shared with other institutions and companies to conduct the research tests, but no information that identifies the patient will be shared.

Of note, we do not anticipate any extra risks from heparin priming of the needle, as the amount of heparin present in the needle would be a trace amount.

To further minimize research-related risk, only patient undergoing endoscopic FNB for clinical purposes will be consented for the research study.

4 ELIGIBILITY

Inclusion criteria:

- Patient must be at least 18 years old.
- Patient identified as having a possible solid pancreatic lesion on computed tomography or magnetic resonance
- Patient scheduled for EUS for sampling of pancreatic mass.

Exclusion criteria:

1. Patients with known history of coagulopathy
2. Patients with history of heparin allergy
3. Patients with evidence of vascular tumors on imaging
4. Patients with history of chronic pancreatitis
5. Pregnant patients
6. Medically unstable patients
7. Unwillingness or inability to consent for the study

Patient must be willing and able to provide written informed consent.
We anticipate that 98 patients will be seen within the University of Michigan Health System and the surrounding community for this study.

5 SUBJECT ENROLLMENT

Patients or their medical records from the Michigan Medicine health system will be used to screen for recruitment.

Patients who present for care and/or consultation to Michigan Medicine, as an inpatient or an outpatient, with known or suspected solid mass lesion will be approached for recruitment. Patients will be approached by a study team member after their initial consultation with the medical staff in a private consultation room. Patient privacy will be protected by discussing the study in the private consultation rooms available adjacent to the medical procedure unit waiting area before they are brought back to the pre-operative area.

If individuals that can be consented for this study are identified ahead of procedure date, we may attempt to call them by telephone once up to 7 days in advance to discuss the research study in an effort to save time and avoid delays on the day of procedure.

6 STUDY DESIGN AND PROCEDURES

Study setting: University of Michigan- Medical Procedure Unit

Study type: Clinical Trial

Assignment of Interventions:

Enrollment: 98 patients

Randomization: Subjects will be randomized in a 1:1 allocation ratio to the heparin group or the non-heparin control group at the time of intra-procedural decision to sample the mass. We will use restrictive block randomization to ensure balanced number of participants per group. The randomization sequence will be generated centrally from a computerized binary number generator at the University of Michigan. The treatment assignments will be in opaque sealed envelopes for allocation concealment. The study coordinator will enroll participants and will assign participants to the intervention. The randomization key will be kept under lock by the study coordinator until completion of the study period.

Blinding: The participants and the pathologist will be blinded to the group allocation. The proceduralist will not be blinded to allocation given the design of the study and for patient safety.

Interventions: all subjects will undergo EUS-FNB using linear echoendoscopes (GF-UCT180 Olympus America, Center Valley, Pa, USA). All EUS-FNB procedures will be performed by 1 of 5 experienced endosonographers (EW, AS, RW, GP, JM). Once the site is identified, the lesion will be punctured using a 22-gauge Fork-tip needle (Sharkcore needle). The sampling technique will be at the discretion of the endoscopist (e.g. stylet vs. not stylet, suction vs. not suction, fanning technique vs. to-and-fro technique, number of strokes). A total of 3 FNB passes will be performed on every procedure. The use of rapid on-site cytopathology (ROSE) will be used at the discretion of the endoscopist and will not be mandatory for this study. The tissue specimens from each the 3 passes will be collected in 3 separate jars of 10% formalin for tissue analysis. The use of heparin flushing vs. not heparin flushing, will be based on their randomized group assignments:

- Intervention group: The FNB needle will be flushed with 1 mL of heparin (100 USP/mL) and then flushed with air. Pass 1, 2, and 3 will be collected in separate jars and sent to pathology, as per standard clinical procedures. The study will pay for the cost of utilizing and submitting extra jars to pathology so that no extra cost is incurred to the patient for participating in the study. Between passes, after tissue is extracted from the needle, the needle will be flushed with 1 mL of heparin (100 USP/mL) and flushed with air before next pass is made.

- Control group: FNB will per performed as current standard methods in the medical procedure unit without the use of heparin priming. Pass 1, 2, and 3 will be collected in separate jars and sent to pathology, as per standard clinical procedures. The study will pay for the cost of utilizing and submitting extra jars to pathology so that no extra cost is incurred to the patient for participating in the study. Between passes, after tissue is extracted from the needle, the needle will be flushed saline and or air as per current standards of care.

If proceduralist feels that it is unsafe to continue with the research protocol during the procedure (examples would include inadequate tissue acquisition in the clinical jar, or clinical instability of patient during procedure), he or she reserves the right to withdraw the patient from the protocol. For patient safety, the proceduralist will not be blinded to whether the patient was randomized to the heparin or non-heparin arm. If the proceduralist deems more than 3 passes are needed to for clinical purpuses, the extra passes will go into a fourth jar.

Tissue Analysis:
H&E slides from the passes 1, 2, and 3 (which will be requested from pathology) will be compared in the following manner:

- A blinded clinical pathologist will examine each slide and determine
    - Is a tissue diagnosis able to be made?
    - Is the tissue adequate quality? The pathologist will create a reference scoring system and rate specimens accordingly.
- In parallel, using the HALO® image analysis platform (Indica Labs), each slide will be digitally scanned. The number of cells present on each H&E slide (cell-based analysis), the amount of blood present on each H&E slide (area-based analysis), and the size of each biopsy will be quantified (area-based analysis).

Tracking and labelling system
Each research specimen will be given a specimen ID. A spreadsheet key matching the specimen ID to the patient name and medical record number will be kept in a password and network protected database. Only study team members will have access to the spreadsheet.

Heparin priming

This study will use heparin for needle priming. The heparin product used will be BD PosiFlush™ Pre-Filled Heparin Lock Flush Syringe, 5 mL (100 USP/mL) in a 10 mL syringe. The product will be stored in a secure location within the endoscopy unit at room temperature, as per manufacturer instructions. The product has a shelf life of 18 months and will be discarded after the expiration date. We anticipate actual exposure of patient to heparin to be minimal, as after heparin flushing, needles will be air-flushed before proceeding with the next pass. We do not anticipate any adverse events from heparin priming of the needle.

Saline Flush
While there is a saline-only PosiFlush, the hospital changes its supplier of saline flushes depending on cost. Therefore, because use of saline for flushing is the routine standard of care, we will not be using specialized research PosiFlush saline syringes and instead will be using the available supply our unit keeps on stock

7 DATA COLLECTION AND MANAGEMENT PROCEDURES

Collection of medical record data for this study will include the following:
Initial collection:

- Patient demographic data:
    - Age
    - Sex
    - BMI
    - Pack Years
    - Presence of Diabetes, Type 1 or 2
    - Alcohol use
    - History of Pancreatitis
    - History of IPMN (intraductal papillary mucinous neoplasms)
    - Prior Cancer History

Follow up collection (approximately 4 weeks post-procedure):

- Was patient reached for follow-up?
- Did patient develop post-procedural complications
- Did patient need interventions for post-procedural complications?
- Was hospital admission required for post-procedure complication?
- Did patient require repeated EUS for sampling?
- Age Patient demographic data:

Chart review at end of study (after 98 patients collected)

- Development of new pancreatic disease other than cancer
- Overall survival (date of death)
- Cancer Stage
    - If resectable, did patient receive surgery?
        - If yes
            - What surgery (distal pancreatectomy vs Whipple?)

        - Final surgical staging
        - Tumor grade
- Chemotherapy treatment
    - Palliative, neoadjuvant, or adjuvant chemo
    - Regimen (FOLFIRINOX, Gemcitabine/abraxane, Gemcitabine/cisplatin)
- ECOG score
- Final Diagnosis

. Data will be pulled from patient's medical charts via both DataDirect and chart review. Regarding data management we will seek assistance with the MICHR database development service. The study team may collect data from each research participant for up to 5 years from when they were consented. This data will be stripped of patient name and stored using REDCap; only the study team members have access to this database. On the database, patient identity will not be discernable to the casual observer. As indicated in section 6, separate from this, a spreadsheet key matching the specimen ID to the patient name and medical record number will be kept in a password and network protected database. Only study team members will have access to this spreadsheet.

SCHEDULE OF EVENTS8 DATA ANALYSIS

Primary Outcomes

- Heparin priming increases cellularity captured in fine needle biopsies
- Heparin priming decrease blood contamination in fine needle biopsies

Secondary Outcomes

- Successive fine needle biopsy passes result in more blood contamination
- Successive fine needle biopsy passes have lower cellular yield
- Successive fine needle biopsy passes have more blood contamination
- Need for repeated procedure (at U of M)

Exploratory Outcomes

- Identify correlations in patient metadata (i.e. tumor grade, cancer stage) and tissue quality, cellular yield, and blood contamination

Tissue analysis:

H&E slides from the pass 1, 2, and 3 (which will be requested from pathology) will be compared in the following manner:

- A blinded clinical pathologist will examine each slide and determine
    - Is a tissue diagnosis able to be made?
    - Is the tissue adequate quality (pathologist will make a reference scoring system and rank specimens accordingly)

  In parallel, using image processing software, the number of cells present on each H&E slide will be quantified, the amount of blood present on each H&E slide will be quantified by area quantification, and the tissue yield on each H&E slide will be quantified by area quantification.

Statistical Comparisons using image analysis:
The student *t* test will be utilized to assess if there is a significant difference in the following:

- Blood contamination area between passes 1, 2, and 3in the heparin group
- Blood contamination area between passes 1, 2, and 3 in the non-heparin group
- Blood contamination area in pass 1 between the heparin group and non-heparin group
- Blood contamination area in pass 2 between the heparin group and non-heparin group
- Blood contamination area in pass 3 between the heparin group and non-heparin group
- Tissue area between passes 1, 2, and 3 in the heparin group
- Tissue area between passes 1, 2, and 3 in the non-heparin group
- Tissue area in pass 1 between the heparin group and non-heparin group
- Tissue area in pass 2 between the heparin group and non-heparin group
- Tissue area in pass 3 between the heparin group and non-heparin group
- Cell number between passes 1, 2, and 3 in the heparin group
- Cell number between passes 1, 2, and 3 in the non-heparin group
- Cell number in pass 1 between the heparin group and non-heparin group
- Cell number in pass 2 between the heparin group and non-heparin group
- Cell number in pass 3 between the heparin group and non-heparin group

Statistical Comparisons using pathologist scoring:
The Pearson's Chi-squared test will be utilized to determine if there is a significant difference in the following:

- Tissue score between passes 1, 2, and 3 in the heparin group
- Tissue score between passes 1, 2, and 3 in the non-heparin group
- Tissue score in pass 1 between the heparin and non-heparin group
- Tissue score in pass 2 between the heparin and non-heparin group
- Tissue score in pass 3 between the heparin and non-heparin group

In a previous study the standard deviation for tissue area with fine needle biopsy of pancreatic masses was 5 mm^2.[9] Based on our preliminary results and on results of

recent crossover RCT, we will assume a difference between randomized groups of 3 mm^2.[5] Therefore, if the true mean difference in total tissue area between the heparin and non-heparin groups is 3 mm^2, we will need to study 44 subjects in the heparin group and 44 subjects in the non-heparin group, to reject the null hypothesis with 80% power and with a type I error of 0.05. To account for 10% dropouts, we will need to recruit 98 subjects.

9 QUALITY CONTROL AND QUALITY ASSURANCE

Sample collection and processing will be conducted by highly trained proceduralists and staff.

Tissue scoring will be performed by blinded clinical pathologist Jiaqi Shi, MD, PhD.

10 STATISTICAL CONSIDERATIONS

Statistical tests for significance are outlined in section 8. To be able to achieve statistical power of *0.80* with identified differences between groups (with variables of pass number and heparin priming) with $\alpha = 0.05$, we anticipate a sample size of 50 patients in each arm will be required (98 patients total).

11 REGULATORY REQUIREMENTS

11.1 Informed Consent

The study team will be responsible for reviewing medical charts in MiChart or approved registries of patients that have agreed to be contacted for research for potential patients being seen at Michigan Medicine. Potential candidates will be seen in their designated patient rooms after consultation with healthcare staff.

In person, the research coordinator or study team member will be responsible for explaining the study in full, gauging patient interest, answering questions, and consenting patients, when applicable. Estimated time for the first patient visit is 5-15 minutes. Future interactions between the patient and the research coordinator or study team member will be minimal.

11.2 Subject Confidentiality

Research records will be maintained that will document patient name and medical record number. This data will be stripped of patient name and stored using REDCap; only the study team members have access to this database. On the database, patient identity will not be discernable to the casual observer. The Principal Investigator and study team will retain the ability to determine the identity of patients in the database by use of a master list. The master list will be kept in a password protected and network protected file that will only be accessible to the study team.

11.3 Unanticipated Problems[1]

Upon becoming aware of any other incident, experience, or outcome that may represent an unanticipated problem, the study team will immediately alert the PI. The PI will assess whether the incident, experience, or outcome represents an unanticipated problem. If the PI determines that the incident, experience, or outcome represents an unanticipated problem, he will report it promptly to the IRB.

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[1] The Office for Human Research Protections (OHRP) considers unanticipated problems involving risks to subjects or others to include, in general, any incident, experience, or outcome that meets **all** of the following criteria:

- unexpected (in terms of nature, severity, or frequency) given (a) the research procedures that are described in the protocol-related documents, such as the IRB-approved research protocol and informed consent document; and (b) the characteristics of the subject population being studied;
- related or possibly related to participation in the research (in the guidance document, possibly related means there is a reasonable possibility that the incident, experience, or outcome may have been caused by the procedures involved in the research); and
- suggests that the research places subjects or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized.

An incident, experience, or outcome that meets the three criteria above generally will warrant consideration of substantive changes in order to protect the safety, welfare, or rights of subjects or others.

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