

Endoscopic Ultrasound Guided Core
Biopsy Needle Versus Fine Needle
Aspiration (FNA) In Tissue Sampling Of
Pancreas Cancer For Whole Exome
Sequencing And Genomic Profiling: A
Prospective Randomized Controlled
Trial

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Endoscopic ultrasound guided core biopsy needle versus fine needle aspiration (FNA) needle in tissue sampling of pancreas cancer for whole exome sequencing and genomic profiling: a prospective randomized controlled trial.

CLINICAL STUDY PROTOCOL

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Protocol Synopsis

Title of Study	<i>Endoscopic ultrasound guided core biopsy needle versus fine needle aspiration (FNA) needle in tissue sampling of pancreas cancer for whole exome sequencing and genomic profiling: a prospective randomized controlled trial.</i>
Hypothesis	Flexible core biopsy needles are more capable than fine needle aspiration in acquisition of tumor tissue that is important for pathological evaluation and genomic analysis. This would specifically allow us to perform whole exome sequencing of a tumor tissue and could provide path for future individualized pancreas cancer treatment. Thus, we hypothesized that FNB is superior to FNA in acquiring tumor tissue from pancreas mass that is key for genomic profiling.
Specific Aims	<p>To compare FNB versus conventional FNA for whole exome sequencing and genomic profiling in tissue sampling of pancreas cancer.</p> <ol style="list-style-type: none">1. Per needle adequacy for un-amplified whole exome sequencing2. Per needle quantity of DNA.3. Per needle adequacy for cytology4. Per needle adequacy for histology5. Adverse events
Study design	Prospective, single blinded, randomized controlled trial of FNB versus FNA for whole exome sequencing and genomic profiling.

Sample size	50cases
Inclusion criteria	<ul style="list-style-type: none"> ▪ Male and female patients who are 18 years old or older and are referred for the evaluation of pancreatic mass lesion ▪ International Normalized Ratio (INR) less than 1.5 and platelet count of more than 50,000. ▪ Medically stable to undergo sedation for EUS. ▪ Signed informed consent
Exclusion criteria	<ul style="list-style-type: none"> ▪ Medical condition that preclude the patient from having a therapeutic procedure regardless of the EUS finding ▪ Pregnant patients
Study design	<p>This is a prospective, single blinded randomized controlled trial with a paired evaluation of FNB vs FNA for whole exome sequencing and genomic profiling. A minimum 2 passes (1 with each needle) will be performed from form pancreas stratified by the lesion location (pancreas head tumor vs pancreas body/tail). Based on the location and type of the abnormal lesion, if further investigation via FNA is deemed necessary by the participating endosonographer, conventional FNA will be alternated with FNB in the usual fashion for obtaining histological material. The procedure will be performed with rapid onsite evaluation. Onsite cytopathologist will evaluate the adequacy and the degree of pathological changes. Based on the information provided by the cytopathologist, the endosonographer will repeat the FNA until enough histological material is obtained to confirm a diagnosis. Adverse effect of procedure will be assessed immediately after procedure and during the first 30 days with a follow-up phone call.</p>
Statistical analysis	For descriptive analyses, continuous variables will be reported as a mean \pm SD or median (interquartile range) and comparison

	<p>between two groups will be done by Paired <i>t</i>- test. Categorical variables will be reported as frequencies with percentages and compared using chi-square test or Fisher's exact test. Two sided P values less than 0.05 will be considered statistical significance. Descriptive analysis will be performed in terms of DNA yield per sample, ability to complete WES, histology and cytology yield per sample.</p>
Trial duration	Approximately 1 year

List of Abbreviations

This list includes all abbreviations used in the document in alphabetic order. Abbreviated terms are spelled out and the abbreviation is indicated in parentheses at its first appearance in the text section.

CBC	Complete Blood Cell count
EUS	Endoscopic Ultrasound
FNA	Fine Needle Aspiration
FNB	Fine Needle Biopsy
ROSE	Rapid onsite evaluation
GCP	Good Clinical Practice
GI	Gastrointestinal
INV	Investigator
INR	International Normalized Ratio
IRB	Internal Revenue Board
LN	Lymph Node
SNI	Standardized Numerical Identifier

1.0 INTRODUCTION

1.1 Background

Pancreas cancer is a highly fatal disease with a 5 year overall survival is of only 8%¹. The shorter survival and the poor outcome may be due to late stage presentation, tendency toward early metastases and aggressive biologic behavior of pancreas cancer with diverse genetic alterations that drive the tumor to early dissemination. Endoscopic ultrasound (EUS) has been established as a one of the valuable tools for evaluation of pancreatobiliary disease. EUS-guided FNA is important for cytological and pathological evaluation of pancreatic cancer. It is minimally invasive, rapid and accurate for staging and diagnosis of pancreas cancer. The overall sensitivity of EUS-FNA is 85% (95% CI, 0.84-.86) and specificity of 98% (95% CI, 0.97-0.99)² for pancreatic tumor diagnosis. Over the past 20 years there have been significant developments done in the field of needle technology and sampling techniques to obtain the good quality of cytologic materials. However, over time it has been obvious that size of needle, use of stylet and suction techniques has small value in increasing the diagnostic yield of pancreas cancer³⁻⁵.

There has been recent advancement in the field of FNB design including needle tip and use of more flexible shaft. However, multiple randomized trials and metaanalysis have failed to show the superiority of FNB vs FNA for pancreatic lesions^{6,7}. Therefore difficulties still persist to diagnose pancreas cancer at early stage. The development of pancreas cancer is a multistep process that involves wide range of genetic alteration. However, the paradigm shift for early diagnosis of pancreas cancer has changed over recent years. Now a day multiple gene mutation and expression profiling of pancreas cancer has become a research priority. A number of efforts have been made for molecular diagnosis of pancreas cancer at early stage that would facilitate to design a specific chemotherapy preoperatively.

EUS- guided FNA is not only important for histopathologic characterization of pancreas lesion but has also opened the door for genomic analysis of pancreas cancer tissue. Several techniques

have been investigated for improving the pancreatic cancer diagnosis, mainly in on the genetic analyses⁸⁻¹⁰. Studies have demonstrated that the feasibility of genomic profiling, next generation sequencing and performing the multiple genetic tests from a small sample of pancreas tumor tissue obtained by endoscopic-ultrasound guided fine needle aspiration¹¹.

With the advances in the needle technology to increase the yield of core tissue in addition to cytology, a new needle has been developed termed “Shark Core needle”. The needle has a sharpened point in the shape of a fork-tip that promotes the collection of core sample by shearing a material from the target lesion during to-and fro-movement of the needle. Recently published studies have demonstrated its benefit in acquiring adequate core tissue compared to conventional FNA needle^{12,13} for pathological evaluation. With this advancement, it will be possible to obtain more DNA from the tumor tissue and carry out comprehensive whole exome sequencing of pancreas cancer. The contribution will be significant because a large amount of DNA will facilitate to carry out genomic profiling and chemo sensitivity test of pancreas cancer preoperatively. This will be a significant breakthrough in the field of precision medicine. Though several studies have been conducted to characterize the expression profiles of pancreas cancer¹⁴ using FNA specimens, the ability to characterize the global genetic mutational status from FNB specimens has not been proven yet. It has been understood that the low quantity of cells, obtained from the FNA specimens preclude the precise determination of a tumors genetic status¹⁵. None of the studies have reported the comparison between FNB needle “Shark Core” and Standard FNA needle for genomic profiling of pancreas cancer. Thus, to facilitate a development of personalized cancer treatment we sought to determine whether the new FNB needle “Shark Core” would be capable of performing widespread whole exome sequencing of pancreas tissue from FNB specimens. Therefore the main of this study is to compare the DNA yield and genomic profiling of pancreas cancer between FNB needle and standard FNA needle.

1.2 Rationale for performing the study

Based on the above facts, there is still a need of prospective randomized trial to evaluate the efficacy of FNB in conducting the comprehensive whole exome sequencing of pancreas cancer. . We designed the trial to compare of FNB versus FNA for whole exome sequencing and genomic profiling of pancreas cancer. The rationale for choosing the 25g as the standard comparator is based on the recent systematic review of needle devices by Wani et al. which concluded “*The use of a 25-gauge needle is associated with a higher diagnostic yield compared with a 22-gauge needle in patients undergoing EUS-FNA of pancreatic masses.*”¹⁶.

1.3 Hypothesis

Hypothesis: FNB is superior compared to FNA for whole exome sequencing and genomic profiling of tissue sampling of pancreas cancer.

1.4 Benefit/Risk Aspects

Procedures used in this study are standard medical practice for the evaluation of lesions within or adjacent to the GI tract. The shark core FNB is an FDA approved device for sampling of submucosal lesions, mediastinal masses, lymph nodes and intraperitoneal masses. FNB was introduced as a less invasive alternative to open surgical biopsy. FNB has been proposed to increase the sampling yield and proposed to potentially require fewer needle passes compared to conventional FNA. This could potentially improve the tissue yield which is measured by the tumor DNA and will facilitate to perform whole exome sequencing of pancreas cancer. This will provide significant development for “personalized cancer therapy”.

2. STUDY OBJECTIVES

2.1 Primary Objective:

To compare EUS guide fine needle biopsy versus fine needle aspiration for whole exome sequencing and genomic profiling in tissue sampling of pancreatic solid tumors.

2.2 Secondary Objectives:

- To compare the DNA yield of FNB versus FNA in tissue sampling of pancreas cancer including sufficient DNA to complete un-amplified whole exome sequencing.
- To compare the number of needle passes necessary to obtain adequate tissue sample to make a cytological/histological diagnosis when FNB is used as compared to the conventional FNA for pancreas cancer
- To compare the histology quality of the specimens obtained via FNB as compared to conventional FNA.
- To compare the yield of tissue sufficient for targeted (Foundation Medicine Assay) genomic profiling of pancreas tumors.
- To estimate the safety profile of FNB as compared to conventional FNA. (by historical comparison)
- Per needle adequacy for cytology

3. STUDY POPULATION

3.1 Number of Patients:

50

3.2 Inclusion criteria

- Male and female patients who are 18 years old or older and are referred for the evaluation of pancreatic solid mass requiring FNA or FNB.
- International normalized ratio (INR) less than 1.5 and platelet count of more than 50,000.
- Medically stable to undergo sedation for EUS.
- Signed informed consent.

3.3 Exclusion criteria:

- Any medical condition that preclude the patient from having a therapeutic procedure regardless of the EUS finding.
- Pregnant patients.

3.4 Patient Informed Consent

The Institutional Review Board (IRB) of Mayo Clinic will approve a consent document with information about the nature and possible adverse events of the procedure. Patients that might be

included in the trial will be given sufficient time to study the written consent form and ask questions before signing the consent document.

3.5 Replacement Policy

Any patient who does not complete the study will be replaced. In the case of any withdrawal or dropouts, existing data will be retained for evaluation at the end of trial.

3.6 Compensation and Financial Responsibility

Since this procedure is “standard of care”, the cost of procedure will be charged to the patient or their insurer. The cost of the FNB will be covered by a grant from Medtronic Corporation (grant application in process).

3.7 Patient Remuneration

No patient remuneration will be provided for this study.

4. PRE-PROCEDURE EVALUATION

4.1 History and Physical Examination (all standard of care)

- 1) Inclusion/Exclusion Criteria
- 2) Medical History
- 3) Medication Review
- 4) Brief Physical Examination (heart, lungs, abdomen) with vital signs (BP, pulse), and weight

4.2 Laboratory Studies

- 1) CBC and INR (within 30 days of enrollment which is standard of care)

5. INTRA AND POST- PROCEDURE EVALUATION

5.1 Evaluations during Procedure

Standard monitoring, including continuous cardiopulmonary evaluations, will be performed during the EUS exam. In case of immediate complications, including but not limited to, visceral perforation or extensive bleeding, the procedure will be terminated and appropriate standard care will be provided. The precise time for each FNA procedure as defined by the first puncture attempt to the completion of expressing the cytologic material on the glass slide and formalin.

5.2 Post-Procedure Evaluations

All of the participants of the study will receive the standard post-EUS care until discharge. Any adverse effects of the procedure will be assessed immediately after procedure and during the first 30 days with a follow-up phone call.

6. SUBJECT WITHDRAWAL AND STUDY TERMINATION CRITERIA

6.1 Subject Withdrawal

Subjects will be withdrawn from the study for any of the following reasons:

- 1) Voluntary withdrawal - Any patient may remove himself from the study at any time without prejudice to his medical care.
- 2) Complication related Withdrawal - Any patient with a serious immediate complication due to the EUS-FNA will be withdrawn.

6.2 Confidentiality

Only the investigators and their office staff will have access to the data that identifies the patient by name. Patients will be identified only by Standardized Numerical Identifier (SNI) and patient identity will remain confidential if material from the record is used for publication or for educational purposes.

6.3 Interim Analysis

No interim analysis is planned. Biannually the available data will be reviewed retrospectively and the rate of anticipated complications will be calculated. Serious adverse events including any life threatening events, inpatient hospitalization or prolongation of existing hospitalization or any unanticipated complication including any death as a result of the procedure will be reported to the Mayo Clinic IRB.

7. ETHICS AND REGULATORY

7.1 Institutional Review Board (IRB)

Mayo Clinic Institutional Review Board Committee (IRB) will review the study protocol and any amendments. The IRB will review the subject informed consent form, their updates (if any) and any written materials given to the subjects.

7.2 Regulatory Authority Authorization-Approval-Notification

The regulatory permission to perform the study will be obtained in accordance with applicable regulatory requirements. All applicable ethical and regulatory approvals will be available before a subject is exposed to any study-related procedures, including screening tests for eligibility.

7.3 Ethical Conduct of the Study

This study will be conducted in accordance with the ethical principles that have their origins in the Declaration of Helsinki, in compliance with the approved protocol, Good Clinical Practice (GCP) and applicable regulatory requirements.

7.4 Subject Information and Consent

The Investigator (or designee) will obtain a freely given written consent from each subject after appropriate explanation of the aims, methods, potential risks, and any other aspects of the study, which are relevant to the subject's decision to participate. The consent form must be signed and dated by the subject before conducting any study-related procedures, including screening tests for eligibility.

The Investigator will inform the subjects that they are completely free to refuse to enter the study or to withdraw from it at any time, without any consequences to their further care and without a need to justify. The subject will receive a copy of his/her signed informed consent.

Each subject will be informed that portions of his/her source records and source data related to the study may be reviewed and utilized in the study analysis. Data protection will be handled in compliance with Mayo Clinic bylaw.

7.5 Confidentiality of Subject Data

The Investigator will ensure that the confidentiality of the patients' data will be preserved in a safe database. The patients will not be identified by their names, but by SNI, which consists of their initials and number in the study.

7.6 Standardized Numerical Identifier (SNI)

Each subject will be assigned an identifier that will be used for identification purposes. SNI consists of the patient's initials, a ten digit number that correlates with the date and time of the procedure and the initials of the participating investigator in the following format:

I	I	I	M	M	D	D	Y	Y	H	H	M	M	I	I	I
---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---

Patient initial

Date

Time

Inv. Initial

8. STUDY DESIGN:

This is a single-center, prospective randomized controlled trial. All male and female patients 18 years old or older that are referred for evaluation of pancreatic solid mass can be considered for this trial.

8.1 Patient Registration and Documentation

Recruitment of patients will be initiated at the time of pre-procedure evaluation by one of the investigators. Any patient that has been referred to undergo an EUS exam for one of the above mentioned indications will be consulted regarding the ASPEN trial and his/her questions will be answered. If he/she is interested, the consent form will be provided to the patient and he/she will be asked to sign the consent form to participate in the study.

Patient registration section of the “Registration and Data Collection” form will be completed prior to performing FNA by the investigator. At this point, each patient will be assigned with a Standardized Numerical Identifier (SNI) that will be used for identification and data entry purposes in the study database. This form is designed to collect the following information (See Appendix A):

- Participants name and date of birth and medical record number
- Name of the investigator performing the EUS exam
- SNI
- Type of the cross-sectional study or endoscopic exam that warranted the EUS evaluation
- Size, location and characteristic of the abnormal lesion

After the EUS exam, the investigator will complete the post procedure information section of the “Registration and Data Collection” form that collects the following information (Appendix A):

- Size, location and characteristic of the lesion based on the EUS exam
- Size and type of the needle that was used for conventional FNA (25-gauge needle). 25g needle (brand of needle will be physicians choice) will be used as the standard needle for this study. If an adequate sample is not obtained after a total of 2 passes, the endoscopist may change to a different needle of their choice.
- Immediate complications that were noticed during the procedure or during the recovery period

8.2 Study Protocol

- 1) Filling the “Pre-Procedure Patient Registration” section of the “Registration and Data Collection” form
- 2) Review of the signed informed consent before the start of procedure.
- 3) All patients will undergo conscious sedation or Monitored Anesthesia Care (MAC) for the duration of the procedure. Conscious section and/or MAC are the standard method of sedation for all patients that have EUS at our institution, unless contraindicated.
- 4) Morphologic features of the lesion will be examined and documented in the procedure report. Based on the location and type of the lesion, the participating endosonographer determines the necessity of performing an FNA. If FNA deemed necessary, the patient will be included in the study.

- 5) Randomization of needle order. The first needle to be used will be selected by randomization using block randomization in envelopes (FNA first, FNB first) by tumor location (1 block for head tumors, 1 block for body/tail tumors).
- 6) Conventional FNA will be alternated with FNB (22 gauge for all sites) in the usual fashion for obtaining histological material. A minimum of 2 passes (1 with each needle) will be obtained from all lesions. Conventional FNA will be performed with a standard 25g needle (our standard needle) in the usual fashion using back and forth passes for 30 seconds. Negative pressure will be applied for both needle types using the “capillary” technique of withdrawing the stylet slowly (5-10cm per second) during the to and fro movement of the needle. All FNA material will be expressed onto a glass slide. Visible core samples will be removed and placed in formalin after an initial “touch prep” (light touch of tissue to slide). The presence or absence and length (in mm) of grossly visible each core will be recorded by the cytotechnologist/study personnel. Standard passes (alternating with each needle) will be repeated in pairs until an adequate sample is obtained or the endoscopist feels that no further sampling is warranted. Then, a 2nd and 3rd set of passes (one with each needle) will be performed to collect material for biobanking and DNA and RNA extraction. The sample will be expressed into a sealed biospecimen container and flash frozen in liquid nitrogen, then stored at -80C. If no material is obtained, the research pass will be repeated up to a maximum of 2 passes for each needle.
- 7) After each pass of needle, the on-site cytotechnologist will evaluate the adequacy and the degree of the pathological changes in the obtained material. Based on the information provided by the cytotechnologist, the endosonographer will repeat the FNA until enough material is obtained to confirm the clinical diagnosis. The initial determination of adequacy will be made by the onsite cytotechnologist. The final determination of cytological adequacy will be made by the study pathologist (AN) based on the criteria below.
- 8) Separate sets of slides and formalin jars will be produced in the GI suite from the tissue obtained from each type of the needle. Each pass will be placed on separate slides and

formalin jars with labelling (A, B). 9) In order for the pathologist to remain blinded of the needle type that the tissue was obtained with, two separate slide sets will be generated and each set will be labeled as “*Slide Set A or B*”. Slides that are prepared from the FNB will be labeled as “*Slide Set B*” depending on the parity of the assigned SNI number. Slides that are prepared from the conventional FNA needle will be labeled as “*Slide Set A*” to complete each pair10) Pathology interpretation for clinical purposes will be done by the on-call pathologist of the day, using all material available. For study purposes a single study pathologist (AN) will interpret all of the slides. The pathologist will evaluate the slides for cell quantity (adequate vs. scanty/acellular), grade of dysplasia (atypical vs. malignant cells), and presence of core samples.

- 11) Each slide set (A or B) will be evaluated and reported separately for research purposes and accumulatively to generate the patient’s pathology report for medical records.
- 12) Quarterly, the available data will be reviewed retrospectively and the rate of anticipated complications will be calculated. Anticipated complications of EUS with FNA include, but are not limited to post-procedure pancreatitis, bleeding, perforation, and infection. Serious adverse events from FNA are rare but include life threatening events, inpatient hospitalization, prolongation of existing hospitalization or any unanticipated complication including death as a result of the procedure will be reported to Mayo Clinic IRB.
- 13) DNA extraction and quantification: One or two biopsy tissues are first digested, then DNA extractions will be performed using the silica membrane-based column DNA extraction method with the QIAamp DNA Mini Kit. This column based extraction method provides purified DNA which is free of protein, nucleases and other contaminants or inhibitors. This kit is used for fresh or frozen tissue, cells, and blood. If larger tissue samples are obtained; the Puregene DNA extraction will be used and is a salt precipitation method. Tissues are lysed and DNA is precipitated from the cells. This method allows for purification of high molecular weight DNA for downstream use.

For DNA quantification, the Trinean DropSense96 spectrophotometer will be used which is a multichannel spectrophotometer for quick and precise UV/VIS spectral analysis of microliter droplets of DNA. This method allows for the measurement of total and double stranded DNA as well as purity and quality ratios (A260/280 and A260/230).

For whole exome sequencing, a minimum of 1.1 ug of DNA is needed (personal communication, Joshua Gorman; Department of Laboratory Medicine). Thus for this study purpose, 1.1 ug of DNA will be considered a “sufficient” sample. Whole exome sequencing, using a minimum of 1.1 ug of DNA, sequencing will be performed using sequence type: Exome Sure Select v5 + UTR (71MB) at 50% on target (PE 101 base) on the Version 4 HiSeq instrument.

In order to validate that WES can be performed a subset of 5 samples from each needle will be evaluated by whole exome sequencing using Illumina standard methods. Only samples with at least 5 micrograms of DNA will be evaluated. The WES will be performed in a de-identified manner and will not be used clinically for the care of the patient.

14. All the pathological data collection will done by following guidelines from the Papanicolaou Society of Cytopathology Guidelines^{21, 22}.

8.3 9. STATISTICAL CONSIDERATIONS

9.1 Statistical Analysis

This is paired sample study of FNB vs FNA for whole exome sequencing and genomic profiling. For descriptive analyses, continuous variables will be reported as a mean \pm SD or median (interquartile range) and comparison between two groups will be done by Paired *t*- test. Categorical variables will be reported as frequencies with percentages and compared using chi-square test or Fisher’s exact test. Two sided P values less than 0.05 will be considered statistical

significance. Descriptive analysis will be performed in terms of DNA yield per sample, histology and cytology yield per sample. Sample size calculation is done after interim analysis. **Sample**

size calculation:

From our preliminary data from 5 PDAC patients and using the Foundation Medicine adequacy criteria, 2 (40%) had an adequate sample of DNA with FNA, while all 5 (100%) patients had adequate DNA with FNB. Although this is a very small sample size it is consistent with the notion that FNB may be much superior to FNA. Based on this preliminary data, it seems reasonable to expect that the difference in proportions of adequate samples by the two methods might be 25% or more. Assuming we have 50 patients who all are sampled with both FNA and FNA, then with a McNemar's test we will have 80% or higher power to detect a difference in adequacy proportions between FNA and FNB (at the 5% significance level) of $\geq 25\%$ if $\leq 38\%$ of patients have discordant results for FNA and FNB, or a difference of $\geq 20\%$ if $\leq 25\%$ are discordant. The following are just 3 of many possible examples of scenarios whereby we will have sufficient power with a sample size of 50 patients, all of which are consistent with the findings in the preliminary data:

Example A (25% difference):

		<u>FNA adequate?</u>		
		Yes	No	
<u>FNB adequate?</u>	Yes	$\geq 58.5 \%$	$\leq 31.5 \%$	90 %
	No	$\leq 6.5 \%$	$\geq 3.5 \%$	10 %
		65 %	35 %	

Example B (25% difference):

		<u>FNA adequate?</u>		
		Yes	No	
<u>FNB adequate?</u>	Yes	$\geq 48.5 \%$	$\leq 31.5 \%$	80 %
	No	$\leq 6.5 \%$	$\geq 13.5 \%$	20 %
		55 %	45 %	

Example C (20% difference):

		<u>FNA adequate?</u>		
		Yes	No	
<u>FNB adequate?</u>	Yes	$\geq 68\%$	$\leq 22\%$	90 %
	No	$\leq 2\%$	$\geq 8\%$	10 %
		70 %	30 %	

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