

**A Molecular Based Assay to Distinguish Benign from  
Pre-Malignant Cystic Lesions of the Pancreas**

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## **A. Study Purpose and Rationale:**

Pancreatic adenocarcinoma is a disease with staggering statistics. Approximately 30,000 new patients are diagnosed with pancreatic adenocarcinoma each year, with less than 100 of them surviving the 5 year benchmark for disease remission, making it the 5<sup>th</sup> leading cause of cancer mortality (1, 2). The reasons for this morbid outcome are multifactorial, encompassing deficiencies in every aspect of our understanding of the disease. The initial diagnosis of pancreatic adenocarcinoma is typically made in a patient presenting with jaundice, weight loss, and abdominal pain. Unfortunately, a clinical diagnosis made at this stage often corresponds to a malignant tumor that is symptomatic for the first time as a result of rapid growth and physical obstruction of the common bile duct. Diagnostic testing is often only confirmatory, and the newest techniques of multidetector CT acquisition and MRI cholangiopancreatography are most useful in the staging of a tumor once it has presented clinically (3, 4). Currently, the only curative option for pancreatic adenocarcinoma is the combined surgical removal of the identified mass, the head, neck, and uncinate process of the pancreas (body and tail, also, if the tumor is left-sided), a portion of the duodenum, and the gallbladder and biliary duct tree. However, even such extensive surgery provides only an 18 month median survival rate (5). The almost certain fatal outcome of this disease highlights the lack of, and beckons the need for effective screening and prevention regimens to identify disease early and alter its ultimate clinical course.

Efforts to identify patients at risk for the development of pancreatic cancer have identified several predisposing conditions, including hereditary pancreatitis, Peutz-Jehghers syndrome, hereditary breast and ovarian cancer with BRCA2 mutations, and familial atypical multiple mole syndrome (2, 6, 7). Unfortunately, these conditions often affect well-defined families, and population based genetic screening would provide little added benefit in the detection of sporadic pancreatic adenocarcinoma. There are few recognized precursor conditions for sporadic pancreatic adenocarcinoma (akin to the adenomatous polyp), but 2 specific cystic pancreatic lesions; intraductal papillary mucinous neoplasm (IPMN), and mucinous cystic neoplasm, appear to fill that role (1).

Cystic lesions of the pancreas have become increasingly recognized as a clinical entity, both because of their increased incidental detection, and because of their potential for malignant progression (8). The prevalence of these lesions has been estimated at between 0.5-1% of the general population, a rate much higher than that of pancreatic adenocarcinoma. However, this prevalence encompasses all categories of cystic pancreatic lesions, including the malignant ductal adenocarcinoma with a cystic component, the pre-malignant IPMN and mucinous cystic neoplasm, and the benign entities of serous cystadenoma, simple cyst, and retention cysts. The clinical dilemma they create is the balancing act of safely removing premalignant lesions while avoiding the high risk, high morbidity

surgery in those with truly benign lesions. At present, clinical decision-making is guided by recommendations from various professional societies and the clinical experience of high volume centers. The professional recommendations do little to define when to operate vs. when to watch, but do suggest that all cystic lesions be investigated because of their malignant potential. The clinical experience of a few investigators, therefore, is currently guiding the worldwide management of cystic lesions of the pancreas. These investigators have identified certain high risk features such as the appearance of septations within a cyst, size > 2 cm, main duct involvement, and elevated cyst fluid CEA levels as indicators of likely pre-malignant lesions (9). However, even when these recommendations are followed, up to 50% of patients undergoing surgery have benign lesions, while in short-term follow up, at least 20% of premalignant lesions were initially missed (10, 11). The imbalance of sensitivity and specificity in our current diagnostic approach to benign vs. premalignant cysts, represents an opportunity for the implementation of improved diagnostic methods. Ideally, efforts to more reliably separate pre-malignant from benign cystic lesions would take advantage of our molecular understanding of the genetic lesions needed to drive the normal pancreas towards tumorigenesis.

Pancreatic adenocarcinoma is a carcinoma composed of aggressive duct-like lesions that infiltrate the pancreatic parenchyma (1). A series of molecular studies has allowed for the generation of a pancreatic adenocarcinoma progression model that defines unique stages of morphological change within this duct epithelium, and correlates those changes with the acquisition of defined genetic lesions known to exist in advanced adenocarcinoma. This classification system, termed Pancreatic Intraepithelial Neoplasia (PanIN)(12) proposes the advancement of pancreatic ducts from normal cuboidal epithelial morphology with round to oval lumens to the first stage in this progression, PanIN-1A through a true carcinoma in situ stage with advanced nuclear atypia and luminal shedding of the hyperproliferative duct lining termed PanIN-3. The definition of these morphological stages has allowed for the generation of a temporal map of the incurrence of the genetic lesions manifest in advanced pancreatic adenocarcinoma, and a framework for understanding the role of each of these lesions in the initiation and development of pancreatic adenocarcinoma. The earliest, most conserved mutation to occur in the generation of pancreatic adenocarcinoma is the activation of the K-Ras protooncogene via mutation of codon 12. K-Ras activation is achieved at PanIN-1 and is maintained in over 90% of fully developed pancreatic adenocarcinomas (1, 12). However, progression through the more advanced stages of the PanIN model is associated with the acquisition of further genetic lesions. The loss of the tumor suppressor p16<sup>INK4a</sup>, for example, first occurs in PanIN-2, while the tumor suppressor DPC4/Smad4 is lost later within this sequence during PanIN-3. The knowledge of this defined progression provides an opportunity to diagnose pre-malignant and malignant lesions of the pancreas at an earlier stage of progression (13).

Given our current understanding of the molecular basis of pancreatic

tumorigenesis, we propose that analysis of the genetic lesions present within pancreatic cystic lesions will enhance our ability to detect truly pre-malignant lesions; thereby increasing our diagnostic sensitivity. Our specific aim is to correlate the presence of either < 2, or 2 or more such genetic lesions with the pathologic diagnosis arrived at upon surgical resection. To achieve this aim, we will utilize a commercially available molecular diagnostic testing service provided by RedPath Integrated Pathology. This service assesses the cells available within cyst aspirates for the presence of K-Ras mutations, and LOH over 16 different alleles, encompassing all those identified within the pancreatic progression model. In this pilot study, it is our prediction that 2 or more known genetic lesions identified in the cystic aspirate will increase the diagnostic sensitivity for pre-malignant lesions by approximately 50%, and may warrant further validation studies in the future.

## **B. Study Design and Statistical Analysis:**

Fifty-two consecutive patients presenting to the section of Digestive Diseases at CUMC for endoscopic ultrasound with fine needle aspiration (EUS-FNA) of a cystic lesion of the pancreas will be enrolled in the study prior to the procedure. All patients will receive conscious sedation, followed by EUS-FNA examination by a skilled endoscopist according to standard clinical protocols. The cyst contents will be sent to the CUMC clinical/pathology laboratories for cytology, CEA, and amylase levels, as is the current standard of care. Only if greater than 3 mL of cyst contents are aspirated (the minimum amount to successfully complete the above tests), 1 mL of the remaining cyst aspirate will be shipped on ice to the RedPath Integrated Pathology labs for analysis. There, the sample will be microdissected, and using proprietary technology, DNA will be amplified and subjected to an analysis of K-Ras mutation status, as well as a LOH analysis, as previously described (14). A report including a K-Ras mutation analysis, and a LOH analysis over 16 alleles (each including a known tumor suppressor implicated in the progression model of pancreatic cancer), will be categorized as containing 2 or more mutations, or less than 2 mutations. This data will then be correlated with the results of surgical pathology, which itself will be divided into benign vs. premalignant. For the purposes of this study, benign cystic lesions will be defined as serous cystadenomas, benign cysts, retention cysts, or pseudocysts. Pre-malignant cystic lesions will include IPMN, MCN, and adenocarcinomas with a cystic component. All other definitions, or cases of inconclusive pathology will be excluded.

The study as proposed is powered at 80%, and testing at  $P=0.05$  to detect a 50% difference in the presence of 2 or more genetic lesions in those patients whose pathology reveals a pre-malignant cystic lesion vs. a benign cystic lesion. To arrive at the final number needed for the study, certain assumptions were made based on the available literature. As illustrated in the studies by Spinelli KS, et. al., and Lee SH, et. al., the expected ratio of benign to pre-malignant lesions

found at surgical resection based on the current diagnostic modalities is approximately 1:1. We therefore assume an even division of pre-malignant vs. benign lesions among our study participants. Pilot studies on the RedPath system of mutation analysis have shown a 100% prevalence of > 2 mutations in a mixed population of cystic and solid pancreatic lesions (15). Furthermore, in retrospective studies of pancreatic brush cytology specimens stored in less than ideal permanent fixatives, more than 2 mutations were detected in 45% of samples tested, with follow up pathology in less than half the samples tested (16). We therefore made the assumption that in adequately collected and stored cystic fluid, one would expect to find 2 or more mutations in 70% of the patients with a confirmed pre-malignant lesion by pathology with 30% of confirmed pre-malignant lesions showing less than 2 mutations. With these assumptions, we chose the smallest difference of clinical interest to be an effect of 0.5. Preliminary modeling was done to determine if a smaller effect size would still be clinically relevant, but such a lowering would have the undesired effect of creating more false positives (thus subjecting a greater proportion of patients to unneeded surgery, should this analysis be validated as a diagnostic test). Therefore, using the Chi square test for power analysis we obtained:

$$N = 8 [(0.3)(0.7) + (0.8)(0.2)/0.5^2] + (2/0.5) + 2$$

where N = the number of participants needed in each group

p1 = 0.7  
p2 = 0.2  
effect = 0.5

The total number needed in this analysis is, therefore, 2N = 34. Assuming that 1/3 of all patients evaluated for pancreatic cysts undergo continued observation rather than surgery, a total of 52 patients will be enrolled to achieve the above calculated power.

### C. Study Procedures:

**Clinical:** Patients will undergo EUS-FNA by an experienced endoscopist as per the standard protocol. This study does not require any modification to the accepted method of performing EUS-FNA. A single tube of blood, 2mL, will additionally be obtained from the patients at the time of endoscopy for use as a positive control in the mutation analysis.

**Laboratory:** > 3mL, up to the maximum amount obtainable as determined by the endoscopist, of cyst fluid will be collected from each patient's pancreatic cyst. The first 3 mL of fluid obtained will be sent to the CUMC clinical and pathology laboratories for cytology analysis, CEA, and amylase levels as per standard operating procedures. Excess fluid, at least 1 mL, no

maximum, will be stored in a company provided sealed vial, and placed on ice. At the end of each procedure, the collected study specimen will be packed in ice, along with 2mL of the patient's peripheral blood, and shipped to RedPath Integrated Pathology via overnight shipping. If overnight pickup is not obtainable, the samples will be stored in the specimen refrigerator in the procedure room between 2°-8°C in accordance with the company's instructions, until it can be sent via the next available overnight shipping.

**D. Study Drugs:**

N/A

**E. Medical Device:**

N/A

**F. Study Questionnaires**

N/A

**G. Study Subjects**

**a. Inclusion Criteria**

- Adults ages 18-80
- Cystic Pancreatic Lesion > 1cm
- Otherwise undergoing EUS-FNA as part of the diagnostic evaluation
- Referred for surgical resection of the pancreatic cyst

**b. Exclusion Criteria**

- Acute Pancreatitis within the past 6 months
- INR > 2
- Known malignancy of any tissue origin
- Less than 3 mL's of cyst contents aspirated.

**H. Recruitment of Subjects**

Potential subjects will be identified by the interventional endoscopists in CUMC's division of Digestive Diseases, a large tertiary referral division.

**I. Confidentiality of Study Data**

Subjects will be coded at the time of specimen collection. All data will be deidentified and stored in a database including results of the mutational analysis and final pathologic diagnosis.

**J. Potential Conflict of Interest**

The investigators declare no potential conflicts of interest

**K. Location of the Study**

Patients will all undergo EUS-FNA in the procedure suite of the Digestive

Diseases Division of CUMC, located in the Atchley Pavillion, 13<sup>th</sup> floor. Mutational analysis will occur at RedPath Integrated Pathology in Pittsburgh, PA.

**L. Potential Risks**

The potential risks to the patient are the same as those incurred during the course of any EUS-FNA. These included gastrointestinal perforation, aspiration and respiratory failure, bleeding, infection, damage to dentition, and failure to make a definitive diagnosis.

**M. Potential Benefits**

This is a prospective observational study, and the current participants will not benefit from the study protocol. The potential benefit to society is more accurate diagnosis of benign vs. pre-malignant pancreatic cystic lesions, leading to improved survival from pancreatic cancer and less unneeded operations for benign cysts.

**N. Alternative Therapies**

The alternative to participating in this study is to not participate. Such a decision will not effect the ongoing medical/surgical care provided for the management of the patient's pancreatic cyst.

**O. Compensation to Subjects**

Subjects will not be compensated for this study.

**P. Costs to Subjects**

Billing for mutation analysis will be performed by RedPath. The costs of this analysis will be incurred by the patient's insurance provider. If the patient is insured by Medicare, Medicaid, or a third-party payor, RedPath will accept the reimbursement provided. If the patient is uninsured, a medical needs program is available through the testing company.

**Q. Radiation or Radioactive Substances**

N/A

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