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Endoscopic Pancreas Fluid Collection: Methods and Relevance for Clinical Care and Translational Science

Phil A. Hart, MD[1], **Mark Topazian, MD**[2], **Massimo Raimondo, MD**[3], **Zobeida Cruz-Monserrate, PhD**[1], **William E. Fisher, MD**[4], **Gregory B. Lesinski, PhD, MPH**[1], **Hanno Steen, PhD**[5], and **Darwin L. Conwell, MD, MS**[1]

[1]Section of Pancreatic Disorders, Division of Gastroenterology, Hepatology, and Nutrition, The Ohio State University Wexner Medical Center, Columbus, Ohio, USA

[2]Division of Gastroenterology and Hepatology, Mayo Clinic Rochester, Rochester, Minnesota, USA

[3]Division of Gastroenterology and Hepatology, Mayo Clinic Jacksonville, Jacksonville, Florida, USA

[4]Michael E. DeBakey Department of Surgery, Baylor College of Medicine, Houston, Texas, USA

[5]Department of Pathology, Boston Children's Hospital and Harvard Medical School, Boston, Msaachusetts, USA

Abstract

Pancreatic secretions have an important role in the regulation of a normal nutritional state but can be altered owing to a variety of pathophysiological mechanisms in the context of exocrine pancreatic disease. The development of an endoscopic technique for collection of pancreatic fluid, termed endoscopic pancreatic function testing, has led to improved understanding of these alterations and is particularly helpful to characterize chronic pancreatitis. In addition, investigators have found endoscopically collected pancreatic fluid to be a valuable biofluid for the purposes of translational science. Techniques such as proteomic, cytokine, genetic mutation, DNA methylation, and microRNA analyses, among others, can be utilized to gain a better understanding

Correspondence: Phil A. Hart, MD, Section of Pancreatic Disorders, Division of Gastroenterology, Hepatology, and Nutrition, The Ohio State University Wexner Medical Center, 410 West Tenth Avenue, Columbus, Ohio 43210, USA. philip.hart@osumc.edu.

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of the molecular characteristics of chronic pancreatitis and other pancreatic diseases. Endoscopic collection of pancreatic fluid is safe and relatively straightforward, permitting opportunities for longitudinal analysis of these translational markers throughout the course of disease. This manuscript summarizes our current knowledge of pancreatic fluid, with an emphasis on proper techniques for sample collection and handling, its clinical utility, and preliminary observations in translational science.

INTRODUCTION

Pancreas fluid (PF), also termed pancreatic juice, has been studied by scientists for >50 years, with the goals of better understanding pancreatic physiology and pathophysiology, and improving clinical diagnosis of pancreatic diseases. Assays of PF for pancreatic enzyme and bicarbonate concentrations became mainstays of clinical diagnosis for chronic pancreatitis in the era prior to the advent of modern cross-sectional imaging and remain important in selected clinical settings today. Endoscopic collection of PF was initially introduced as a more convenient method of assessing the exocrine secretory capacity of the pancreas compared with fluoroscopically placed enteric tubes. Early studies demonstrated that the concentration of pancreatic enzymes and bicarbonate secretion correlated with direct pancreas function testing (PFT) using a tube, so most centers performing direct PFTs transitioned to endoscopic PFT (ePFT). Although the clinical utilization of this test remains somewhat limited to a small number of academic institutions, there is expanding interest in examining PF to identify pancreatic disease biomarkers and novel therapeutic targets. The key benefit of studying PF is that it is the most proximal biofluid to the pancreas containing locally secreted proteins, which may not be detectable in other more distal biofluids, such as blood or urine. Furthermore, PF is representative of the molecular changes taking place in the entire gland. However, it is necessary to utilize standardized methodology for PF collection to ensure that downstream analyses are accurate and reproducible. We set out to: (1) review the clinical role of ePFT, (2) discuss the current methodology for endoscopic PF collection, (3) review preliminary observations demonstrating opportunities for future translational research, and (4) to identify gaps in knowledge.

SEARCH METHODOLOGY

Potential references for this article were identified through a broad search of PubMed for articles published between January 1990 and March 2016, using the following terms: (pancreas fluid OR pancreas juice) AND (pancreatitis OR pancreatic cancer OR pancreatic neoplasms). Additional articles were identified through chaining, by examining the bibliographies of these selected articles as well as the authors' own files. The final references were selected based on originality and relevance to the scope of the article.

CLINICAL INDICATIONS FOR PANCREATIC FLUID COLLECTION

The primary clinical value of collecting PF is to examine the concentration of various pancreatic enzymes and electrolytes following pancreatic stimulation. Historically, PF collection for clinical analysis was accomplished through the use of an enteric tube or tubes following either endogenous or exogenous stimuli. One key benefit of using multiple lumens

is that the enzyme output could be calculated using a perfusion marker (1). Although this methodology provided the foundation of our current understanding of normal pancreas secretory physiology, it became less popular clinically following the advent of high-quality cross-sectional pancreatic imaging. More recently, ePFT has been developed, which was less cumbersome and did not require fluoroscopy (2). Although the stimuli for pancreatic secretion remained similar (typically cholecystokinin and/or secretin), the method of fluid collection was through the suction channel of the endoscope rather than an enteric tube.

The most common use of ePFT currently is for evaluation of suspected early chronic pancreatitis (CP). For patients with advanced CP, this test is generally not necessary because the disease can be readily diagnosed by computed tomography and/or magnetic resonance imaging. As fibrosis progresses in the early stages of disease, there is a concomitant decrease in exocrine function of both duct and acinar cells. Because subtle functional changes occur at an early stage of fibrosis, direct PFTs may be the most sensitive diagnostic tests for early CP. The most commonly utilized ePFT involves the measurement of peak bicarbonate concentration following secretin stimulation over either 45 or 60 min. Even though this measurement is more directly a measure of pancreatic duct epithelial cell function than acinar cell function, previous studies have demonstrated a correlation between the peak bicarbonate and lipase concentrations in pancreatic fluid (3). For the evaluation of CP, measurement of peak bicarbonate concentration (following secretin stimulation) has improved discrimination compared with peak lipase or amylase concentrations following cholecystokinin stimulation (4). Notably, a retrospective follow-up study demonstrated that the negative predictive value of a normal bicarbonate response to secretin stimulation to rule out CP is high (97%), while the positive predictive value (45%) is poor (5). Thus, although ePFT may be useful to rule out CP in those with chronic abdominal pain and equivocal or indeterminate imaging findings, this test is currently inadequate to establish the diagnosis in isolation of other diagnostic criteria.

A STANDARDIZED PROTOCOL FOR ENDOSCOPIC COLLECTION AND ASSESSMENT OF PANCREATIC FLUID

Patient preparation

During the routine consent process for the procedure, patients are also notified that they will be administered intravenous secretin, a synthetic, human-derived protein. Common side effects include self-limited nausea and asymptomatic flushing. Uncommon side effects include reports of secretin-induced acute pancreatitis and aspiration pneumonia (6).

The patient is preferentially positioned in the left lateral decubitus position with the head of the bed elevated at least 45°. The purpose of this position is to promote pooling of any gastric fluids and secretions into the fundus and avoid passage into the duodenum. Preferential pooling in the gastric fundus may be further facilitated by rotating the right shoulder toward the supine position, which causes the fundus to fall posteriorly; however, this position may be less favorable for control of oral secretions. The test is typically performed using either a standard (10 mm) or thin (6 mm) upper endoscope; however, it can

also be performed in combination with an echoendoscope (i.e., EUS) or side-viewing duodenoscope (i.e., ERCP).

Pancreatic fluid stimulation and collection

Prior to the induction of sedation, a test dose of secretin (0.2 μg intravenously) (ChiRhoStim synthetic human secretion from ChiRhoClin (Burtonsville, MD)) is administered in the endoscopy suite with continuous monitoring (Figure 1). This practice is recommended in the current packaging insert due to anaphylactic reactions to biologically derived porcine secretin; however, allergic reactions have not been reported from the currently utilized synthetic human secretin. Nevertheless, this is carried out prior to starting the endoscopy to ensure that the ePFT can be safely completed. A standard diagnostic exam is performed with minor modifications. First, any residual gastric fluid is completely aspirated and discarded. Upon passage into the duodenum, an initial fluid collection is attempted with the tip of the endoscope positioned in the postbulbar duodenum. The initial 3–5 cc of fluid suctioned should be discarded to ensure that residual gastric contents in the suction channel of the endoscope have been removed; discarding the initial aspirate is recommended prior to all subsequent collections as well. Next, duodenal fluid is collected until a volume of 3–5 cc is reached; this fluid is labeled as bottle 1 (time 0; baseline) and immediately placed on ice. Despite the possible increase in pancreatic secretions in response to the secretin test dose, the baseline fluid collection typically only yields a small volume.

Careful endoscopic collection techniques are important to minimize mucosal injury, because blood products may skew test results. This is accomplished by using low suction with partial decompression of the suction button, or alternatively, the biopsy channel port can be removed and held lightly over the suction channel. Some elect to suction fluid with a plastic catheter, which is passed through the accessory channel until it is endoscopically visible (Figure 2); however, it is uncertain if this causes mucosal injury with serial sample collections (7,8). Following the baseline fluid collection, the endoscope may be withdrawn into the gastric lumen, where it is allowed to rest until the subsequent fluid collection. Leaving the tip in the duodenal lumen is an alternative but may create a mechanical scenario in which PF can reflux into the stomach and then return to the duodenum. Between fluid collections, we typically advise resting the scope handle on the patient’s bed or the guardrail, secured by tape or an assistant.

After 1 min of observation following the test dose of secretin, the full dose of intravenous secretin (0.2 mcg/kg body weight) can be administered as a slow, continuous intravenous push over 60 s. The time at which the full dose of secretin is completed is referenced as time 0 for subsequent collections. When the goal is assessment of bicarbonate concentration (termed endoscopic pancreatic function testing), the fluid within the duodenum is collected every 15 min for 60 min in total (bottles 2 at 15 min, 3 at 30 min, 4 at 45 min, and 5 at 60 min); there are reported variations in the frequency and total duration of fluid collection for bicarbonate testing. In the classic descriptions of ePFT, the scope is advanced back to the postbulbar duodenum 15 min following the secretin administration and the second collection is started after discarding the initial 3–5 cc of fluid. Aspiration of duodenal fluid is

continued intermittently during the next 4–5 min, ideally reaching a goal of 15–20 cc to permit additional molecular studies. All collected PF is immediately placed on ice.

For patients undergoing ePFT for clinical evaluation of suspected CP, specimens are subsequently collected 30, 45, and 60 min after secretin administration with a minimum volume of 3–5 cc per collection period (Figure 1). When translational studies are being considered, we would encourage collection of 15–20 cc of PF at 15 and 30 min. Samples that are not immediately analyzed are placed on ice (or alternatively frozen immediately with dry ice or liquid nitrogen) in the endoscopy room, transported to an appropriate laboratory where they are aliquoted, and then stored at −80 °C for future use. Removing particulate fluid prior to freezing by centrifugation is an option; however, the impact of this step for various biomarkers merits further investigation.

Assessment of analyte concentrations

In routine clinical practice, PF may be readily analyzed for pancreatic enzyme and electrolyte concentrations; however, only the latter yields readily usable clinical data. Physiologically, cholecystokinin is a strong stimulus for secretion of pancreatic enzymes, while secretin is only a weak stimulus for the acinar cells (9). Thus, if one is interested in evaluating the pancreatic enzyme output, cholecystokinin is the preferred stimulant. One considerable drawback of endoscopic-assisted PF collection is that pancreatic enzyme output cannot be accurately assessed.

An alternate clinical use of PF is the assessment of peak bicarbonate concentration following secretin stimulation. In addition to bicarbonate, the concentration of other electrolytes involved in PF secretion (i.e., chloride, sodium, and potassium) are measured to confirm the accuracy of fluid analysis and to help identify spurious lab values. A standard, clinical chemistry autoanalyzer can be used to determine the bicarbonate concentration rather than the tedious and time-consuming back titration method without compromising accuracy (10). A peak bicarbonate >80 mEq/l is normal and is associated with a high negative predictive value for the diagnosis of CP (5). A value ≤80 mEq/l is abnormal, but although this finding may be supportive, it is not diagnostic in isolation of other evidence for CP (further discussion below) (11).

Alternate methods of pancreatic fluid collection

There are at least two alternate methods of PF collection. First, PF can been aspirated during direct cannulation of the pancreatic duct at the time of ERCP using either a nasopancreatic tube or direct cannulation for fluid aspiration (12–15). Fluid collection during ERCP has not gained widespread popularity. Although one can avoid contamination with gastric or duodenal secretions, only a limited volume of fluid can be collected and there is an unacceptably high risk of post-ERCP pancreatitis for this indication (15). Also, direct puncture of the pancreatic duct with aspiration of PF using a sterile syringe can be performed intraoperatively (16). This technique avoids contamination with gastric and duodenal secretion; however, contamination with blood is relatively common (16). A key benefit of this sample collection method is the availability of paired surgical pathology specimens for comparison to PF. For both clinical and translational purposes, endoscopically

assisted collection of PF in the duodenum remains the most versatile method. Upper gastrointestinal endoscopy is minimally invasive, there are few associated risks, and samples can be collected longitudinally in an individual subject during different stages of their disease as well as prior to and following a targeted intervention. Another key benefit of endoscopic-assisted PF collection is that samples can be analyzed in healthy controls without subjecting them to unacceptable risks of the other tests.

POTENTIAL CONFOUNDING VARIABLES

Sedation

Although secretin produces pancreatic stimulation, the effect may potentially be blunted by analgesia. There are data demonstrating the potential blunting effects on pancreatic secretion in a pig model; however, these results have not been replicated in humans (17). In constrast, human studies have shown either no effect or increased bicarbonate concentration in PF with mode-rate sedation (18,19). However, the effect of deep sedation (or general anesthesia) on pancreatic secretion is unknown and may be potentially relevant considering many patients may require this level of sedation owing to concurrent medication usage for chronic abdominal pain. Thus we would recommend sedation type and dosages are recorded for an individual's endoscopic PFT to permit future analyses for confounding.

Gas insufflation

There have been no studies evaluating the effects of using air vs. carbon dioxide gas during endoscopic PF collection. The primary consideration is that using carbon dioxide may alter the bicarbonate concentration in the duodenal lumen. Considering the endoscopic technique for ePFT is straightforward, we propose using air insufflation to avoid this potential problem when samples will be analyzed for bicarbonate concentration.

Fluid contamination

It is understood that secretin-stimulated PF collected endoscopically or with an enteric tube is most precisely a combination of secretions from the stomach, bile fluid, duodenum, and pancreas. In both methods, all fluid is aspirated from the gastric and duodenal lumens prior to stimulation and the initial 3–5 cc from each collection are discarded to minimize contamination. Moreover, any remaining fluids are progressively diluted by the protein-rich secretin-stimulated pancreatic secretions. Accordingly, we recommend not using the baseline fluid collection for translational purposes as the risk for contamination is relatively higher. In the absence of gastric, duodenal, or liver disease, the PF analytes should reflect the biochemistry of the pancreas, which can confirm the purity of the sample.

Genetic factors

Patients with a deleterious mutation in the cystic fibrosis transmembrane regulator secretory pathway are expected to have an impaired bicarbonate response to secretin. Therefore, in patients with a normal secretory response the gene status is irrelevant; however, mutations may potentially explain an abnormal bi carbonate response that could be falsely attributed to CP. Thus, in patients with an abnormal ePFT (related to bicarbonate response), it is most ideal to know the cystic fibrosis transmembrane regulator status to accurately interpret the

results. Unfortunately, there are multiple barriers for pursuing genetic screening, so these data are not always available for interpretation of ePFT test results.

Cigarette smoking

In a similar manner, based on indirect evidence, it has been suggested that cigarette smoking may lead to cystic fibrosis transmembrane regulator dysfunction altering the pancreatic duct cell secretion (20). Although the mechanism is not fully elucidated in the pancreas, it has been observed that a current or previous history of cigarette smoking is associated with a lower peak bicarbonate concentration at baseline and likely following secretin stimulation in some subjects (21–23). The effects of cigarette smoking may persist even when the patient is not continually exposed to smoking, so a subject's smoking status should be considered when analyzing results from ePFT, specifically the bicarbonate response to secretin stimulation.

In summary, there are multiple factors that may influence the volume of pancreatic fluid collected and the electrolyte composition. When possible, all efforts should be made to avoid or control for these known confounding factors (Supplementary Table online). Aside from the potential for contamination with non-pancreatic fluids, these variations are not expected to alter the composition of pancreatic fluid as it is related to potential biomarker targets.

THE EXPANDING ROLE OF PANCREATIC FLUID IN TRANSLATIONAL SCIENCE

The analysis of pancreatic enzyme and electrolyte concentrations represents a small fraction of information that can be obtained from PF. Specifically, investigations of PF provide rich opportunities for translational science, including identification of novel disease biomarkers and therapeutic targets. Pancreatic fluid is an attractive body fluid to expand our understanding of pancreatic diseases, because this is the most proximal body fluid to the pancreas. Resultantly, the protein composition is less complex compared with more systemic biofluids, such as blood or urine. As a consequence, pancreas-specific proteins and other markers are expected to occur in greater magnitudes and with more specificity than in more distal body fluids. In contrast to direct pancreatic tissue analysis, multiple PF samples can be collected longitudinally to assess for changes over time. Although a variety of molecular analyses can be performed using PF, additional experiments are needed to further guide specimen handling and storage prior to large-scale efforts to explore all potentially relevant end points.

Proteomic analysis

The most mature area of translational research using PF involves proteomic analysis. The ultimate goal of these analyses is to identify a protein (or group of proteins) that are differentially expressed in the disease state compared with health or in various stages of the same disease. Early studies examined differences in protein levels using protein-specific assays. Presently, proteomics primarily utilizes mass spectrometry, which enables investigators to perform comprehensive characterization of a vast number of proteins simultaneously. Proteomic analysis of PF has been most promising for the diagnosis of early

CP but may also be relevant in patients with pancreatic cancer and/or pancreatic cystic neoplasms. The quality of proteomic results is dependent on the methodology of handling and preparation of the biospecimens, so it is important to use a standardized approach to minimize heterogeneity of samples and maximize protein extraction, using validated methods, when available.

It is important to recognize that fluid collected prior to secretin stimulation most precisely represents a combination of gastroduodenal and basal pancreatic secretions, rather than pure pancreatic secretions. When compared within the same subject, there are >300 differentially detected proteins in a baseline fluid sample (i.e., gastroduodenal fluid) and following secretin stimulation (i.e, pancreatic secretion) (24). Using ERCP-assisted PF collection, the proteome was compared in three subjects prior to and following secretin administration (25). Although the concentration of proteins was greater prior to secretin administration, the spectrum of proteins was unchanged. When comparing the density of all proteins measured, the greatest levels were identified between 15 and 30 min following secretin stimulation (26). Thus, the baseline sample should not be used for proteomic analysis. We propose that the 15 and 30 min time collection points are the earliest time windows to provide a focused assessment of acinar, ductal, and islet cells while avoiding confounding and suggest using them for future proteomic analysis.

A series of experiments has been completed to inform the methodology of specimen handling and preparation, with the goal of minimizing auto-digestion and maximizing protein expression. Auto-digestion of proteins is highly prevalent following PF collection, particularly in the presence of activated trypsin. One of the key observations is that incubation with ice minimizes proteolysis following fluid collection (27). Thus, when PF samples are collected endoscopically, they must be immediately placed on ice. We ensure that at least two tubs of ice are available in the endoscopy suite prior to beginning the procedure. Once collected, samples are centrifuged (3,000 *g* for 15 min at 4 °C) and particulate matter extracted prior to evaluation (27). Then the supernatant is divided into at maximum 1-ml aliquots and frozen at −80 °C. With regards to chemical preparation of the sample, multiple options have been examined, and it was demonstrated that acidification, in particular precipitation with trichloroacetic acid maximizes protein yield from PF (27). In addition to immediate acid-induced denaturation and inactivation of proteases, this step also concentrates and desalts the proteins. Although protein extraction using ultracentrifugation, rather than trichloroacetic acid, requires less sample handling, only 40% of the proteins identified in trichloroacetic acid–precipitated PF could be identified using this alternative technique (28). The addition of a protease inhibitor cocktail only results in a small change in analyzable proteins and may potentially confound proteomic analysis depending on the inhibitor used, so this is not routinely used when proteomic analysis is the primary objective. However, it should be noted that protease inhibitor cocktails have been used by several groups outside of the context of proteomic analysis (7,29). Importantly, samples should not be pooled for proteomic analysis if they were handled differently with regards to treatment with a protease inhibitor. In summary, endoscopic-collected PF should be immediately placed on ice and then transported to the laboratory for additional preparation prior to long-term storage. Samples should be aliquoted within 2 h of placing on ice, because protein degradation begins to become detectable at this point (27). Alternate chilling methods

include dry ice and liquid nitrogen for snap freezing. These alternatives should theoretically decrease sample degradation compared with ice, but require an additional freeze thaw cycle for sample preparation; it remains unclear whether or not these alternate methods ultimately improve sample quality.

Using these methods, a variety of proteins have been shown to be differentially expressed in CP and controls without pancreatic disease. These candidate biomarkers for early diagnosis are being further explored in a larger validation cohort. Multiple investigators have also examined the proteome of patients with pancreatic cancer and identified a variety of potential targets (16,30–32). However, these preliminary findings also need further validation.

Cytokine profiling

Considering the chronic fibroinflammatory state associated with CP and pancreatic cancer, there is a viable role for investigation of locally secreted cytokines that may stimulate pancreatic inflammation and/or fibrosis and are detectable in PF. An early study demonstrated the feasibility of cytokine profiling using a multiplexed microarray assay (33). A subsequent study in 118 subjects investigated a panel of cytokines measurable by enzyme-linked immunosorbent assay (interleukin (IL)-6, IL-8, inter cellular adhesion molecule-1, and transforming growth factor-β) to evaluate for different profiles in subjects with abdominal pain owing to CP, pancreatic cancer, or a non-pancreatic etiology (7). On multivariate analysis, only IL-8 levels discriminated between pancreatic disease (CP or pancreatic ductal adenocarcinoma (PDAC)) and no pancreatic disease. Another study demonstrated neutrophil gelatinase-associated lipocalin and macrophage inhibitory cytokine 1 also discriminate between these pancreatic diseases and controls as well as differentiating subjects with PDAC with and without a history of diabetes mellitus, respectively (29). Other potentially relevant cytokines that have been investigated in animals include fractalkine (CX3CL1) and connective tissue growth factor (or CCN2) (34–37).

Prostaglandin E2 is an example of a candidate diagnostic marker. In a recent study, Abu Dayyeh *et al.* (38) demonstrated that levels of prostaglandin E2 are differentially expressed in early and advanced CP in contrast to healthy controls, with areas under the curve (AUC) of 0.62 and 0.9, respectively. When used in combination with the duodenal bicarbonate concentration, the AUC for diagnosis of early and advanced CP were 0.94 and 1.0, respectively. Comprehensive analysis of other cytokines and the use of PF cytokine levels for monitoring disease activity after therapeutic interventions may be further enlightening.

Genetic mutations

The identification of genetic mutations in PF is an especially promising strategy for risk stratification of patients with pancreatic cystic neoplasms and early detection of PDAC. Multiple groups have demonstrated a high frequency of detectable mutations in p53 (43%) and K-ras (73–81%) in the PF; however, studies have not focused on those with an early stage of malignancy (14,39). The utility of these mutations is limited owing to the low prevalence of p53 mutations in pancreatic cancer and the observation of K-ras mutations in approximately 20% of those with CP and controls (14,39). Additionally, genetic mutation

concentrations (e.g., K-ras and GNAS) are significantly lower in secretin-stimulated PF than when the fluid is directly collected from the pancreatic duct (40). However, the risk of post-ERCP pancreatitis is unacceptably high if the sole indication for the procedure if PF collection. Thus, the successful utilization of genetic mutations from secretin-stimulated PF will require highly sensitive detection methods as well as further studies to characterize the chronology of early genetic mutations in pancreatic cancer compared with CP and other high-risk patient groups. There is no special handling or processing requirements for the assessment of genetic mutations in PF; however, we recommend avoiding the use of the baseline PF fluid collection for genetic analysis owing to the increased likelihood of fluid contamination.

DNA methylation markers

DNA methylation is a process that primarily occurs at the CpG residues, leading to transcriptional silencing of various genes, particularly tumor-suppressor genes. This epigenetic process can be measured, and differential levels and patterns of methylation have been investigated in various body fluids for a host of malignant diseases. In addition to serum and stool, DNA methylation markers have been investigated in PF from subjects with PDAC and compared with both disease and healthy controls.

In a recent series of experiments, Kisiel *et al.* (41) identified a group of six candidate markers (CD1D, KCNK12, CLEC11A, NDRG4, IKZF1, and PKRCB) using DNA sequencing results from tissue. This group of markers and KRAS were then evaluated in PF from subjects with pancreatic cancer (n =61), CP (n =22), and healthy controls (n =19). The AUC values for pancreatic cancer compared with normal pancreas ranged from 0.92 to 0.75, and the values were similar for pancreatic cancer compared with CP. Additionally, it has been shown that methylation status of a series of mucins (MUC1, MUC2, and MUC4) detected in the PF matches the respective mucin production in tissue (12). Importantly, the mucin methylation status in PF may assist in the diagnosis of pancreatic cancer and subtyping (intestinal type vs. gastric type) of intraductal papillary mucinous neoplasms (IPMNs). The DNA methylation status of these mucins resulted in good sensitivity/specificity for pancreatic cancer (80%/87%), intestinal-type IPMN (88%/100%), and gastric-type IPMN (77%/88%). Other potential candidates demonstrating aberrant hypermethylation include ppENK, NPTX2, QMSP, Cyclin D2, FOXE1, p16, and TFPI2 (42–45). These studies suggest a potential role for DNA methylation markers to diagnose pancreatic cancer and subtypes of IPMNs, while additional studies investigating aberrant methylation in patients with an increased risk for pancreatic cancer (such as in CP with diabetes mellitus) are warranted.

MicroRNAs (miRNAs)

miRNAs represent a group of non-coding strands of RNA, which regulate gene expression and translation at the posttranscriptional level and have important roles in both healthy and disease states. Differences in miRNA expression can be detected at various locations, including tissue, serum, and PF. For the analysis of miRNA expression in PF, the sample collection and processing of fluid samples remains unchanged from the previously discussed

methodology. Initial studies have primarily focused on the role of miRNAs for diagnosis of pancreatic cancer.

Based on the observation that miR-21 and miR-155 are overexpressed in the tissue of those with pancreatic cancer compared with CP, expression levels were subsequently evaluated in PF (13,46). In a series including 16 subjects with pancreatic cancer and 5 with CP, overexpression of miR-21 and miR-155 was observed in both formalin-fixed paraffin-embedded tissue samples and PF (13). In a separate study, investigators evaluated the potential of miR-196a to determine the subtype of IPMN, based on Aso *et al.* (47). In 36 subjects, the miR-196a level was elevated in those with intestinal-type IPMN (vs. non-intestinal), resulting in an AUC of 0.85.

Wang *et al.* (48) used a different methodological approach by performing a comprehensive microarray analysis on PF from subjects with pancreatic cancer compared with non-pancreatic diseases. Using a hierarchical clustering analysis, they identified four potential biomarkers (miR-205, miR-210, miR-492, miR-1427). In the subsequent validation set using PF from subjects with pancreatic cancer (n =44), CP (n =19), and controls (n =13), the presence of any of these markers had high sensitivity and specificity (87 and 88%, respectively) for PDAC. Moreover, elevated levels of these circulating miRNAs were associated with shortened overall survival in the pancreatic cancer group. The use of a panel of miRNA markers improves diagnostic accuracy and may potentially enable clinicians to develop more tailored surveillance programs for pancreatic cystic neoplasms and assist in diagnosis and assessment of prognosis in pancreatic cancer.

Microbiome

Emerging evidence suggests that variation in our “other genome”—the collective genome of the microorganisms inhabiting our body, known as the microbiome—may have an even greater role than the human genome variations in the pathogenesis of disease. Recent discoveries suggest that bacteria are likely to influence inflammatory processes such as CP and even cancer by activating immune receptors and perpetuating cancer-associated inflammation. Very little is currently known about the microbiome of human PF and its role in the pathophysiology of CP.

Other molecules

There have also been preliminary observations regarding components of the extracellular matrix (ECM). Specifically, hyaluronan is a glycosaminogen that is ubiquitously present in the ECM. Lohr *et al.* (49) assessed the concentrations of both hyaluronan as well as an ECM protein named laminin in PF from those with CP and controls without pancreatic disease. The concentration of hyaluronan in those with CP was higher in PF compared with a paired serum sample. Additionally, the PF hyaluronan levels were increased in CP compared with the controls, suggesting increased production of this ECM component in the setting of chronic inflammation. Whether or not this may be used as a marker of disease severity or to determine a response to therapeutic interventions is uncertain.

GAPS IN KNOWLEDGE

Although advancements have been made in our understanding of pancreatic disorders by studying PF, there are many remaining clinical questions that may be answered through this approach. To adequately address these questions, though, there are gaps in our knowledge of PF that should be clarified. In brief, investigators need to determine and further refine optimal methods for PF collection, processing, and storage. This will be helpful to optimize the balance between utilization of resources (both personnel and equipment) and prevention of sample degradation. Importantly, the optimal handling of samples may vary depending on the translational marker under investigation.

The measurement of overall protein expression levels are most helpful at 15 and 30 min (as discussed above); however, the optimal time points following secretin administration should be further examined for other translational areas and may also need to be varied depending on the analyte being examined. For example, examination of the cellular debris in the pancreatic ducts should likely occur shortly after secretin is administered. Although we propose using air insufflation to avoid confounding from carbon dioxide, it is unclear if this is necessary. The utilization of carbon dioxide for insufflation is attractive for maintaining patient comfort, particularly when the 60-min ePFT is performed, but may alter the PF bicarbonate concentration. Another important area for further examination is to determine the optimal chilling method in the endoscopy suite to minimize sample degradation, with options including ice, dry ice, liquid nitrogen, or immediate freezing in a −80 °C freezer. There is wide heterogeneity between various investigators regarding chilling; however, it is clear that at minimum samples should be placed on ice immediately after collection. Finally, the value of adding of a protease inhibitor and/or DNAase to samples (or aliquots) to impair degradation should be further explored.

CONCLUSIONS

The endoscopic PFT is a valuable clinical test in the evaluation of suspected early CP to rule out CP. Moreover, endoscopically collected PF is a potential translational research “highway”, with data emerging from the areas of proteomics, genomics, cytokines, genetic mutations, and micro-RNAs. We have presented a standardized approach to PF collection highlighting the need for proper specimen handling. Additional experiments are needed to further optimize handling and storage procedures to ensure sample validity and reproducibility of results for the various molecular techniques. This body fluid is easily collected, permits longitudinal assessments of natural history and therapeutic interventions, and will hopefully provide greater understanding of exocrine pancreatic diseases.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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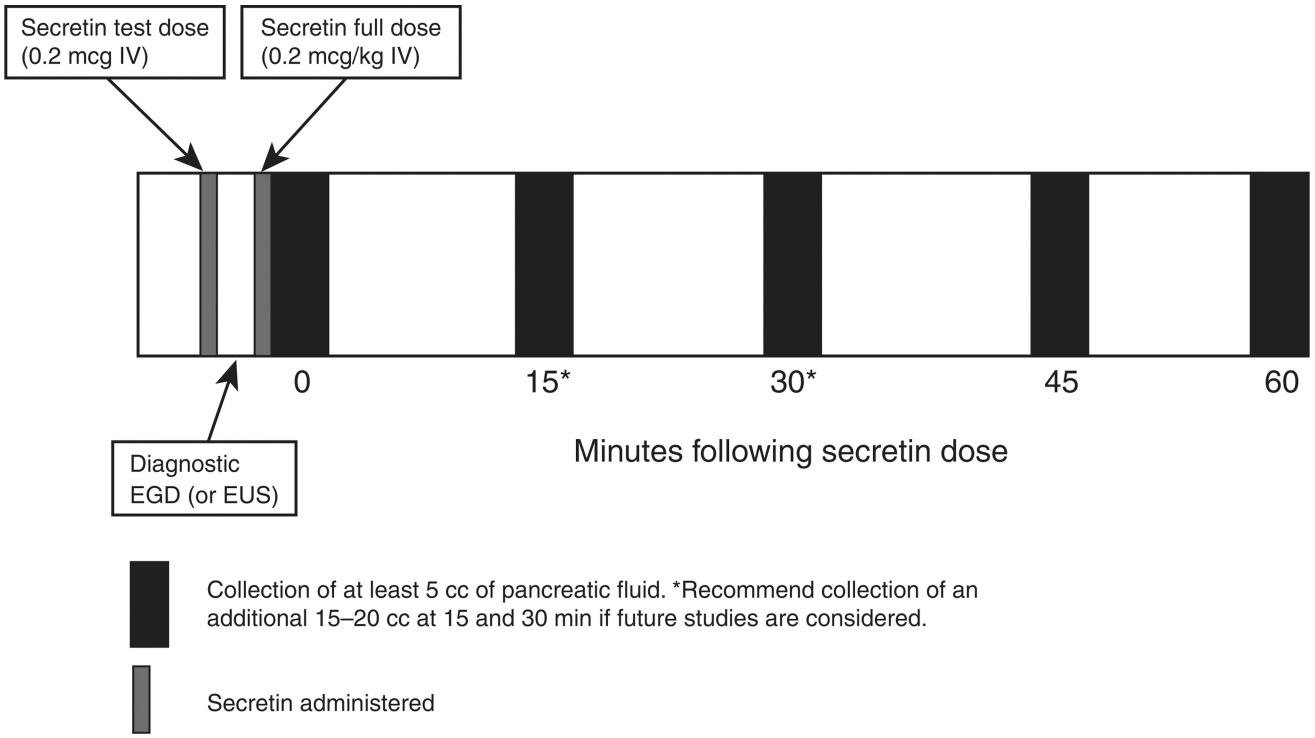

Figure 1.

Representative timeline of endoscopically collected pancreatic fluid for pancreatic function testing and translational research.

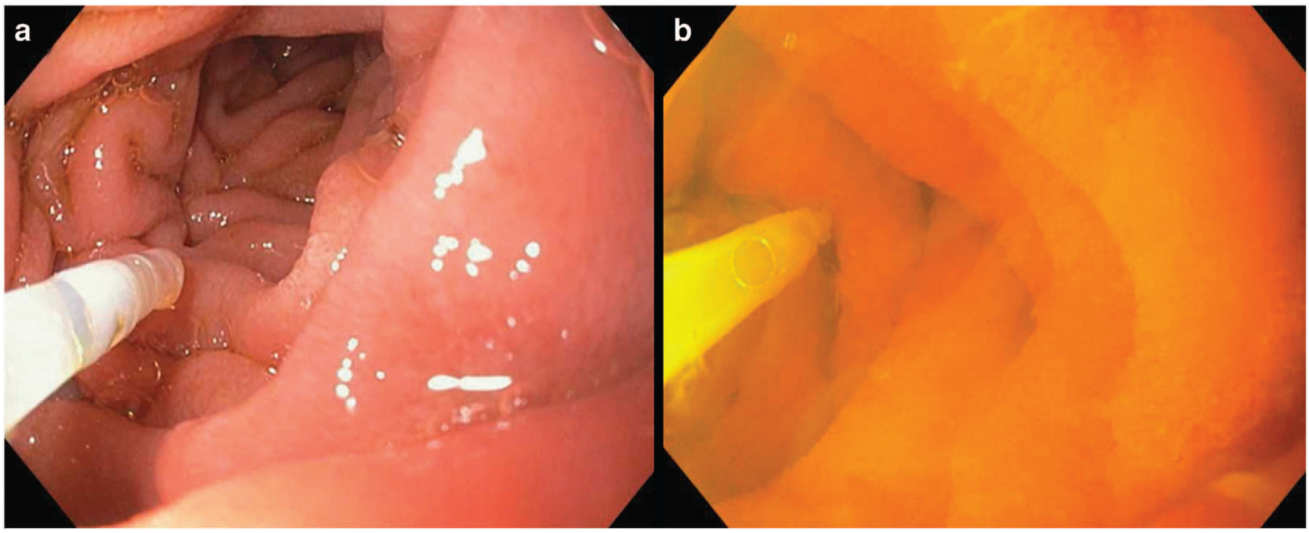

Figure 2.

Endoscopic images from the duodenal lumen **(a)** prior to and **(b)** following secretin administration demonstrating the alternative collection technique of using an aspiration catheter.