

Feasibility of Identifying Pancreatic Cancer Based on Serum Metabolomics

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Abstract

Background: We postulated that the abundance of various metabolites in blood would facilitate the diagnosis of pancreatic and biliary lesions, which could potentially prevent unnecessary surgery.

Methods: Serum samples from patients with benign hepatobiliary disease ($n = 43$) and from patients with pancreatic cancer ($n = 56$) were examined by ¹H NMR spectroscopy to quantify 58 unique metabolites. Data were analyzed by "targeted profiling" followed by supervised pattern recognition and orthogonal partial least-squares discriminant analysis (O-PLS-DA) of the most significant metabolites, which enables comparison of the whole sample spectrum between groups.

Results: The metabolomic profile of patients with pancreatic cancer was significantly different from that of patients with benign disease (AUROC, area under the ROC curve, = 0.8372). Overt diabetes mellitus (DM) was identified as a possible confounding factor in the pancreatic cancer group. Thus, diabetics were excluded from further analysis. In this more homogeneous pancreatic cancer group, compared with benign cases, serum concentrations of glutamate and glucose were most elevated on multivariate analysis. In benign cases, creatine and glutamine were most abundant. To examine the usefulness of this test, a comparison was made to age- and gender-matched controls with benign lesions that mimic cancer, controlling also for presence of jaundice and diabetes ($n = 14$ per group). The metabolic profile in patients with pancreatic cancer remained distinguishable from patients with benign pancreatic lesions (AUROC = 0.8308).

Conclusions: The serum metabolomic profile may be useful for distinguishing benign from malignant pancreatic lesions.

Impact: Further studies will be required to study the effects of jaundice and diabetes. A more comprehensive metabolomic profile will be evaluated using mass spectrometry. *Cancer Epidemiol Biomarkers Prev*; 20(1): 140-7. ©2010 AACR.

Introduction

Pancreatic cancer is the fourth most common cause of cancer mortalities in North America (1). The 5-year survival rate is only 5.1% (1). The only chance of potential, long-term control of this devastating disease is

resection (2). Unfortunately, early diagnosis is difficult. The symptoms most frequently associated with pancreatic cancer (weight loss, malaise, fatigue, and pain) are vague and nonspecific. Jaundice is a later manifestation of the disease. This sign is also nonspecific, as it may be secondary to a benign biliary stricture or to gallstones. Early pancreatic cancer is invisible on routine radiographic studies. None of the radiographic findings of more advanced disease (including bile duct stricture and pancreatic mass) is pathognomonic (Fig. 1). Benign lesions mimicking pancreatic cancer include pancreatitis and pancreatic cysts. Finally, obtaining a tissue diagnosis is extremely difficult. Bile duct brushings (in the event of a biliary stricture) only have a yield of 23% to 41% (3, 4). The diagnostic rate of EUS (endoscopic ultrasound)-guided biopsies for pancreatic masses is only about 71% (5). Although their sensitivity is about 85%, negative predictive value is only about 64% (6). Therefore, negative biopsies are not particularly informative and do not aid in clinical decision making (2).

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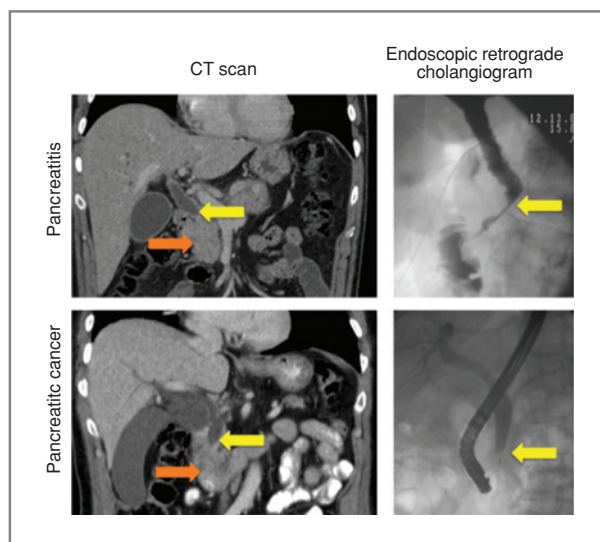


Figure 1. The clinical problem. Radiographic studies of representative patients with benign and malignant periampullary masses and biliary strictures. The features on each CT scan include a periampullary mass (orange arrow) and dilated extrahepatic bile ducts in association with a distal biliary stricture (yellow arrow). Cholangiograms demonstrate biliary dilatation secondary to a distal common bile duct stricture (yellow arrow). The findings on CT scan and cholangiogram are indistinguishable between the benign case and the malignant case. In each case, the patient underwent a pancreaticoduodenectomy.

There are several consequences to this inherent difficulty in obtaining a confident diagnosis in lesions mimicking pancreatic cancer. Firstly, 7% to 16% (and as high as 25%) of patients who undergo a Whipple procedure or a radical pancreatectomy are found on final pathology to have benign lesions (7–11). These operations are extensive procedures associated with a high morbidity and a mortality rate. In the United States, the overall in-hospital mortality rate for pancreatic resections is 7.6% (12). Secondly, clinicians who encounter the nonspecific signs associated with pancreatic cancer are often reluctant to refer the patient for a surgical opinion because they would like to avoid the morbidity of surgery if the patient has benign disease. This conservative, expectant approach may cause a delay in treatment that can result in the loss of any opportunity for potentially curative surgery. In recent surgical series, less than 20% of patients with pancreatic cancer have resectable disease (13); although, it is difficult to discern how many of those patients are found to have unresectable disease because of delays in diagnosis. Our current inability to make a definitive and early diagnosis therefore has substantial impact on the outcomes of a significant proportion of patients.

To improve the diagnostic accuracy of pancreatic cancer, it will be important to identify diagnostic biomarkers. Although protein biomarkers represent the main focus of many groups' efforts, many proteins are undetectable in serum, due to their low abundance or due to interference by high abundance proteins. Metabolite levels may change even when protein levels

do not. *Metabolomics* describes the "quantitative measurement of time-related multiparametric metabolic responses of multicellular systems to pathophysiological stimuli or genetic modification" (14). The biomarkers of interest consist of metabolites, small molecules which are intermediates and products of metabolism, including molecules associated with energy storage and utilization, precursors to proteins and carbohydrates, regulators of gene expression, and signaling molecules. Thus, like the proteome, the metabolome represents a functional portrait of the cell or the organism. Changes in metabolism result in alterations of the abundance of groups of metabolites. Therefore, identification of the patterns of changes in metabolites would provide insight on the functional changes that occur due to any given condition. The metabolomic signature therefore represents a biomarker of considerable interest, albeit one that has been studied relatively little.

Recently, others have demonstrated the capability to distinguish malignant and nonmalignant disease states on the basis of the metabolomic profile of tissue and blood (15–17). Our intent was to determine whether the metabolomic signature in serum could be utilized to distinguish pancreatic cancer from benign hepatobiliary diseases.

Materials and Methods

Sample collection

The study was approved by the Conjoint Health Research Ethics Board at the University of Calgary. Clinically annotated serum samples were collected from patients encountered through a hepatobiliary surgery practice. Samples were obtained from patients with known pancreatic adenocarcinoma or benign pancreatic conditions (including benign masses and chronic pancreatitis). Additional controls consisted of patients undergoing elective cholecystectomy for biliary colic. Patients with any acute inflammation or sepsis were specifically excluded from this latter group. All patients provided consent to participate. Surgical pathology and follow-up data were available for all patients. This enabled more assured classification of patients to benign or malignant categories.

The collection and processing of samples was standardized as much as possible. Samples were collected by venipuncture or through a central line (taking care to exclude any heparin or any other IV infusate) in a plastic gold top Vacutainer tube (BD Biosciences) that contains a clot activator and a gel for serum separation. Samples were stored at room temperature until processing. Samples were processed within 6 hours of collection and then frozen at -20°C until the time of analysis. All samples except 2 were collected from patients in the operating room, shortly after induction of general anesthesia, prior to any surgical manipulation. Two patients (1 with pancreatic adenocarcinoma, 1 with benign disease) had samples collected under fasting conditions.

NMR spectroscopy

All experiments were performed on a Bruker Avance 600 spectrometer (Bruker Biospin) operating at 600.22 MHz and equipped with a 5-mm TXI probe at 298 K. All 1-dimensional ^1H NMR spectra of aqueous samples were acquired using a standard Bruker *noesypr1d* pulse sequence in which the residual water peak was irradiated during the relaxation delay of 1.0 second and during the mixing time of 100 millisecond. A total of 1,024 scans were collected into 63,536 data points over a spectral width of 12,195 Hz with a 90-degree pulse width and a 5-second repetition time. A line broadening of 0.1 Hz was applied to the spectra prior to Fourier transformation, phasing, and baseline correction. Additional 2-dimensional NMR experiments were performed for the purpose of confirming chemical shift assignments, including total correlation spectroscopy (2D ^1H - ^{13}C TOCSY) and heteronuclear single quantum coherence spectroscopy (2D ^1H - ^{13}C HSQC), using standard Bruker pulse programs.

Metabolites were assigned based on comparison of both ^1H and ^{13}C chemical shifts and spin-spin coupling constants with those of model compounds in Human Metabolome Database (HMDB; ref. 18) and Chenomx NMR Suite 5.1 software (Chenomx Inc.). Metabolites were quantified using the targeted profiling approach as implemented in the Chenomx software (19).

Data analysis

Preprocessing of the raw data was carried out for each experiment by normalizing the concentration of each metabolite to a total concentration of all metabolites in the sample. Normalized metabolite concentrations were then log transformed, centered, and scaled. Data were examined for missing or influential values and for their symmetry. Descriptive statistics compared the measured clinical and patient features between the patients with benign disease and those with pancreatic cancer. Two-sample *t* tests were used for continuous variables and exact methods for categorical ones. All tests were 2-sided and statistical significance was declared for values of $P < 0.05$ unless otherwise noted.

Selection of potentially important metabolites was carried out using 2-sample *t* tests which assumed unequal variances. A value of $P < \text{threshold of } 0.3$ was used to select metabolites for inclusion in the supervised orthogonal partial least-squares discriminate analyses (O-PLS-DA).

The metabolite concentration data matrix was analyzed by pattern recognition methods within SIMCA-P (Version 12.0, Umetrics). A supervised O-PLS-DA approach was chosen (20). This allows for a direct comparison of the variance between degree of disease status (benign or malignant; *y* variable) and metabolite concentrations (*x* variables). To calculate area under the ROC curve (AUROC), specificity and sensitivity were determined on the basis of sample class prediction during the 7-fold cross validation (*Y*-predcv, predictive *Y* variables, in the

SIMCA-P software). Calculation of AUROC was performed using the GNU R ROCR package (21).

Results

Patients

Patients studied are described in more detail in Table 1. The benign group ($n = 43$) had a variety of conditions including benign pancreatic masses, pancreatitis (presenting as a mass), and gallstone disease. Six patients in the benign group had biliary colic. All patients in the malignant group ($n = 56$) had pancreatic adenocarcinoma. A resection was performed on 38 of the patients in the malignant group and the remaining had unresectable disease. Pancreatic resections were performed in 24 patients with benign disease. The average age of the 2 groups was significantly different ($P = 0.019$) with the benign group being slightly younger than the malignant group. The malignant group included more patients with a recent history of jaundice ($P < 0.00001$) and diabetes mellitus (DM; $P = 0.024$), although the gender distribution was similar in both the groups ($P = 0.52$).

Metabolic profile related to pancreatic cancer

Using ^1H NMR and 2D NMR spectroscopy, 58 metabolites were detected and verified (Supplementary Table S1). Our initial analysis of the whole group was encouraging. Twenty-four metabolites had values of $P < \text{the } 0.3$ threshold and were subsequently analyzed using both unsupervised principal component analysis (PCA) and supervised orthogonal partial O-PLS-DA. This latter method partitions variance related to the *y* variable (class separation) from unrelated factors, which are orthogonalized (20). There was a visible separation in the scores plots from patients with benign and malignant disease (Fig. 2). However, further scrutiny of the metabolomic profile associated with pancreatic cancer revealed a high degree of abundance of metabolites associated with DM (data not shown). Because incidence of overt DM was significantly greater in the malignant group, all subsequent analyses excluded patients with overt DM, so that a metabolomic profile associated with pancreatic cancer could be derived.

In this more defined group (consisting of 43 patients with cancer and 41 patients with benign disease), 22 metabolites had values of $P < 0.3$. The scores plot from PCA is depicted in Figure 3A. Because metabolites were selected on the basis of 2-sample *t* tests, the PCA model could be considered semi-supervised. Next, a supervised classification model was built using disease status, age, and gender as *Y*-predcv. Cross-validated O-PLS-DA scores indicated that the age and disease status were important ($P = 0.016$ and $P = 1.3 \times 10^{-5}$, respectively) whereas gender had little influence on the model ($P = 0.99$). Scores plots comparing the metabolomic profiles of samples from patients with benign disease were visibly distinguishable from those of patients with pancreatic adenocarcinoma (Fig. 3B).

Table 1. Patient characteristics by diagnosis

	Malignant (n = 56)	Benign (n = 43)	P
Age, y	65 ± 10	58 ± 15	0.019
Gender			0.52
Male	31 (55)	21 (49)	
Female	25 (45)	22 (51)	
Jaundice at time of sample collection	10 (18)	2 (5)	0.046
Recent history of jaundice	35 (63)	6 (14)	<0.00001
Diabetes mellitus	11 (20)	2 (5)	0.024
Diagnosis			
Pancreatic adenocarcinoma	56	0	
Autoimmune pancreatitis	0	2	
Gallstone pancreatitis	0	3	
Chronic pancreatitis NOS	0	5	
Pancreatic pseudocyst	0	6	
Benign pancreatic mucinous neoplasm	0	9	
Serous cystadenoma	0	4	
Cholelithiasis, biliary colic	0	8	
Choledocholithiasis	0	2	
Choledochal cyst	0	1	
Other benign neoplasms	0	3	
Procedures			
Pancreaticoduodenectomy	31	9	
Other pancreatic resection	7	15	
Palliative bypass ^a	15	1	
Bile duct resection	0	1	
Drainage of pancreatic pseudocyst	0	1	
Pancreatic drainage procedure	0	1	
Cholecystectomy	0	13	
Laparotomy	1	1	
Nonoperative treatment	2	1	

Abbreviation: NOS, not otherwise specified.

^aHepaticojejunostomy and/or gastroenterostomy.

The degree of differential abundance of individual metabolites is summarized in Figure 3C for the predictive component distinguishing benign from malignant disease. As the effect of age was also considered in the model (Fig. 3D), we were able to distinguish metabolites that are strongly related to disease alone (elevated glutamate, acetone, and 3-hydroxybutyrate) and those related to disease and age (elevated glucose, phenylalanine, formate, and mannose). In the case of benign disease, glutamine, ethanol, and asparagine were related to pathology alone; creatine, proline, and glycerol were related to both pathology and age.

Model validation and AUROC

One important aspect of the data-modeling procedures lies in the predictive ability in terms of specificity and sensitivity in distinguishing benign from malignant pancreatic disease. The multivariate O-PLS-DA modeling

procedures employed here incorporated a 7-fold cross-validation step. In this case, 7 models were built with exactly one seventh of the data excluded from each model and each sample excluded a single time. The ability of the models to predict those samples not involved in the modeling provided a measure of the overall predictive ability of the metabolite profiling. Using these values (Y -predcv), we were able to generate a receiver-operating characteristic (ROC) curve. The AUROC was 0.8372 (Fig. 4). Values greater than 0.8 indicate excellent predictive ability, and the values obtained here are clearly superior to any current clinical markers.

Ultimately, a diagnostic test for pancreatic cancer should be able to distinguish malignant and benign pancreatic lesions in patients under consideration for surgery. Our control group included patients with conditions that are not typically confused with pancreatic cancer, and there was some imbalance in age and inci-

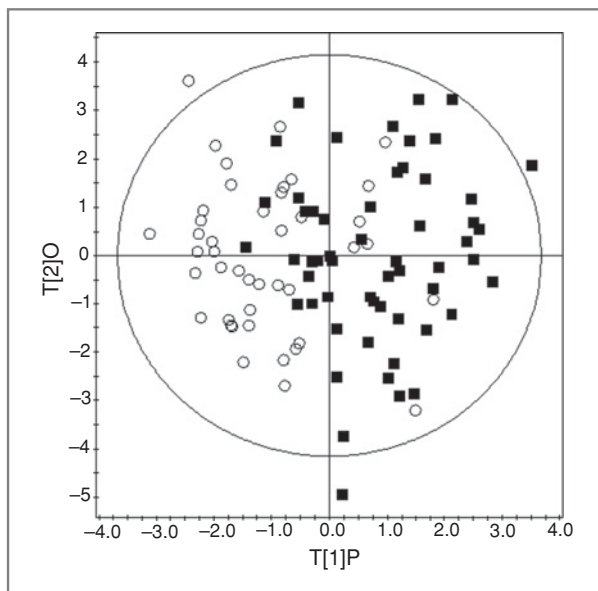


Figure 2. Cluster scores plot of supervised O-PLS-DA of all patients with pancreatic cancer and benign pancreatobiliary disease. Benign samples are represented by white circles and malignant samples are depicted as black squares.

dence of jaundice. Therefore, as a final proof of feasibility, we queried whether the metabolomic profile associated with malignancy could discriminate between benign and malignant diagnoses in a group of patients with biliary strictures and pancreatic masses matched by age, jaundice, and incidence of diabetes. This subgroup is described in Supplementary Table 2. The AUROC in this subset of 14 matched pairs (28 samples total) was similar to that of the larger dataset (0.8308).

Discussion

Clinically, distinguishing benign biliary strictures and pancreatic masses from pancreatic cancer and other periampullary malignancies is a challenge. The clinician is often faced with a decision on whether to recommend resection or another treatment directed at a presumed malignancy without a definitive diagnosis. Resection is associated with substantial morbidity and a risk of mortality (12). On the other hand, a delay in diagnosis may result in a missed opportunity to administer definitive treatment. We have demonstrated that it is feasible to distinguish pancreatic cancer from benign hepatobiliary disease using ^1H NMR spectroscopy to obtain a metabolomic profile. With further development, it is possible that a diagnostic test can be derived that will aid in clinical decision making.

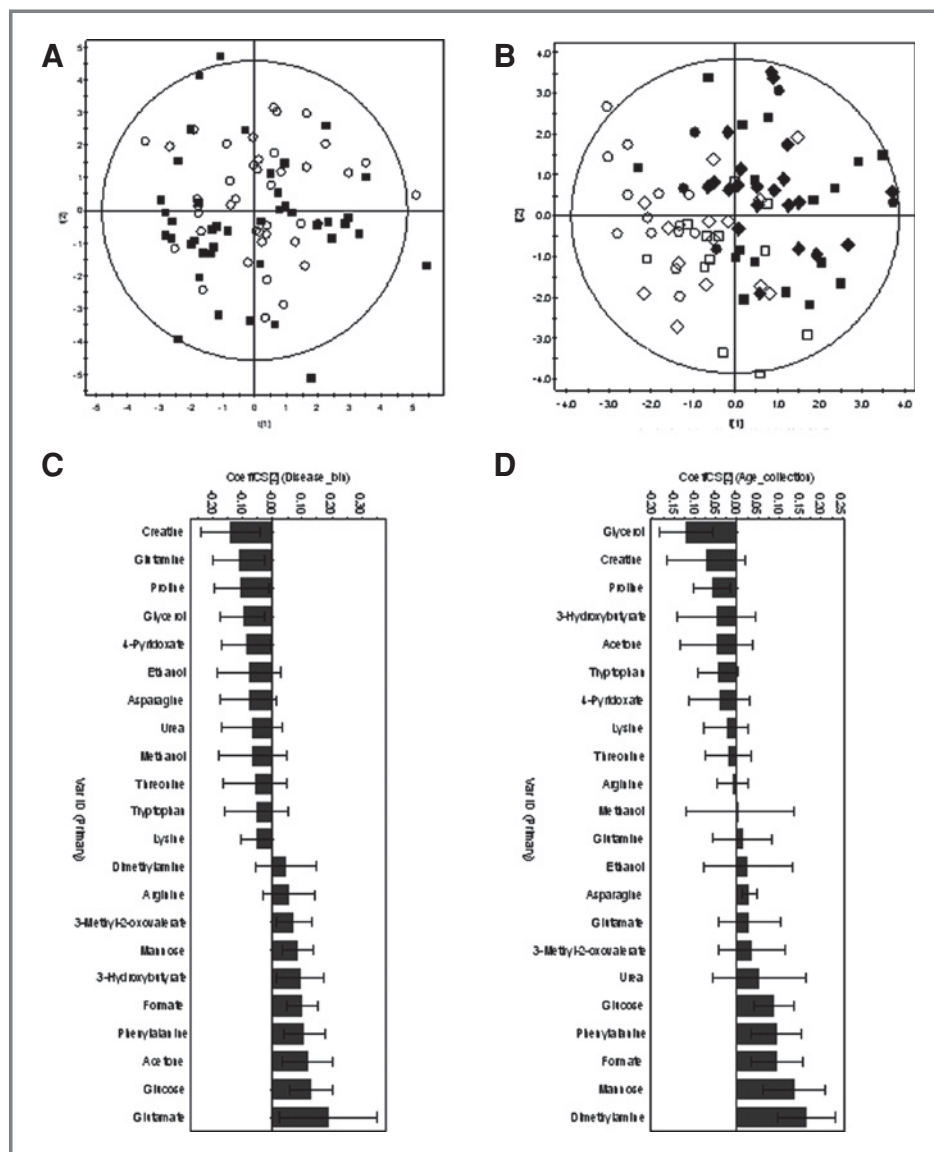
Presently, a clinician who encounters a patient with a biliary stricture or a pancreatic/periampullary mass must make decisions based on radiographic studies, serum tumor markers, and the availability of any tissue samples. Radiographic findings occur late in the course of pancreatic cancer and findings are not specific (Fig. 1).

Serum levels of CA19-9 have a sensitivity of 50% to 80%, although specificity is about 90% (22–25). Serum carcinoembryonic antigen (CEA) is less frequently elevated. Finally, obtaining a reliable tissue diagnosis is extremely difficult. Bile duct brushings only have a yield of 23% to 41% (3, 4). The diagnostic rate of biopsies for pancreatic masses is only about 71% (5). Moreover, although the sensitivity of biopsies is about 85%, negative predictive value is only about 64% (6). Therefore, negative biopsies are not particularly informative and do not aid in clinical decision making (2). Despite the availability of each of these tests, it is extremely difficult to accurately identify patients harboring a malignancy. That is, of all patients subjected to a pancreaticoduodenectomy or a radical pancreatectomy, 7% to 16% (and as high as 25%) are found on final pathology to have benign lesions (7–11). Therefore, there is a need for a more accurate, clinically feasible method of distinguishing patients with benign or malignant disease.

We considered that a metabolomic approach would be useful for a number of reasons. Pancreatic cancer is well known to have associated metabolic changes. The prevalence of DM in pancreatic cancer is reported as 40% to 47%, often preceding the diagnosis within less than 2 years (26–28). Hyperinsulinemia and peripheral insulin resistance are typical in pancreatic cancer, whereas chronic pancreatitis (which also may be associated with a pancreatic mass) is accompanied by islet cell destruction and impaired insulin production (29, 30). Serum lactate levels tend to be higher in patients with periampullary malignancies than healthy controls and patients with benign periampullary lesions (31). In animal models of pancreatic cancer (32), metabolomic profiles associated with disease progression have been demonstrated. Others have reported that the metabolomic profile of bile can discriminate benign and malignant strictures (31, 33). However, bile is generally inconvenient to sample. Recently, it was demonstrated in a small number of patients with pancreatic cancer that the salivary metabolomic profile was significantly different from normal controls (34). Finally, the plasma metabolomic profiles of 5 patients (incidence of diabetes and jaundice unknown) were significantly different from normal controls (35). These early results spurred our interest in more fully exploring the feasibility of using serum metabolomics to improve the diagnosis of pancreatic cancer.

Indeed, we were successful in demonstrating a serum metabolomic profile that reflects the presence of pancreatic cancer. Interestingly, it was observed that 3-hydroxybutyrate and acetone (end products of ketogenesis) were associated with pancreatic cancer (but not other clinical factors). This was particularly intriguing because patients with overt DM were specifically excluded from that analysis. This may reflect the presence of latent diabetogenic changes perhaps resulting from pancreatic cancer.

Figure 3. Comparison of the metabolic profiles associated with benign pancreatobiliary disease and pancreatic adenocarcinoma, with diabetic patients excluded. A, cluster scores plot of unsupervised PCA, with benign samples as open circles and malignant samples as black squares. B, cluster scores plot of supervised O-PLS-DA with disease status (colored as in A), and symbols according to age (<50 years, circles; 50–64 years, diamond; >64 years, square). C, histogram demonstrating changes in PLS coefficients of individual metabolites related to disease. Bars to the left (negative) illustrate which metabolites were more abundant in benign disease; those to the right (positive) demonstrate metabolites more abundant in pancreatic cancer. D, histogram demonstrating changes in O-PLS-DA coefficients of individual metabolites related to age. Bars to the left illustrate which metabolites were more abundant in young patients.



Although our approach to diagnosing pancreatic cancer is novel and promising, there were a number of limitations to this study. We were successful in demonstrating that it was possible to distinguish benign from malignant pancreatic lesions. A better understanding of the metabolic changes associated with diabetes and hyperbilirubinemia (in patients with benign and malignant lesions) will be required. The specificity of the metabolic profile must also be evaluated by comparing with samples from patients with other periampullary and hepatobiliary malignancies. Thus, although promising results were obtained, the metabolomic profiles we have so far obtained cannot yet be considered definitive or diagnostic.

In addition to addressing the above limitations, which will aid in refining the metabolomic profile diagnostic for pancreatic cancer, it may be beneficial to make a more

comprehensive metabolomic assessment. This could be done using gas chromatography–mass spectrometry (GC-MS) and liquid chromatography–mass spectrometry (LC-MS), which may enable the detection of more metabolites (15). A more comprehensive metabolomic profile may improve diagnostic accuracy. In addition, it is possible that we could derive a better understanding of the underlying metabolic processes associated with pancreatic cancer.

Once all discovery phase studies have been completed, validation of the metabolomic profiles will entail testing of samples from an independent patient cohort, preferably consisting of patients undergoing surgery for a pancreatic or periampullary mass, or a biliary stricture. Given the need for further discovery phase studies, it is premature to speculate on the sample size that would be required to validate the metabolomic biomar-

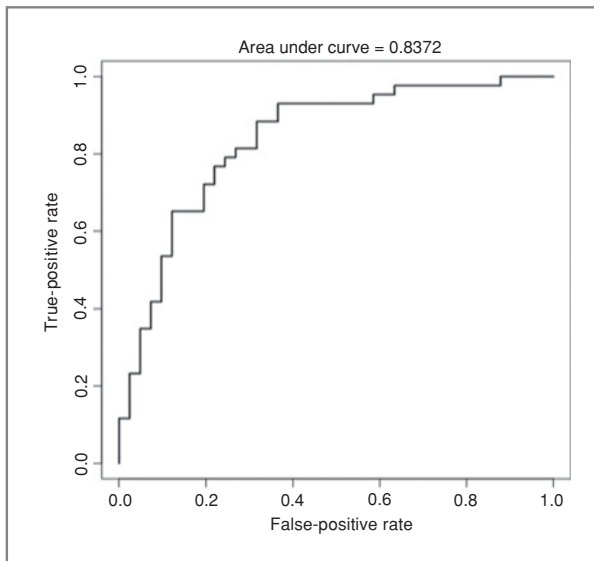


Figure 4. AUROC distinguishing malignant from benign pancreaticobiliary disease in the data set which excluded diabetic patients.

kers in this heterogeneous population. Finally, determination of the clinical utility of the diagnostic test will be essential. That is, it will be important to determine how the diagnostic test impacts the clinical management of clinicians. Ideally, this would entail a randomized controlled trial comparing the clinical management in patients who have and have not been tested. To power such a trial, more information will be required to understand the determinants of clinicians' current management decisions.

In summary, we have demonstrated that it is possible to distinguish the metabolomic profile of pancreatic cancer from that associated with benign hepatopancreaticobiliary diseases in sera. Our future work will involve the refinement of the metabolomic profile obtained, as well as obtaining a more comprehensive profile. Although we have determined internal validity of our findings, further validation in an independent patient cohort will be essential. Ultimately, we will have to determine the clinical utility of the new diagnostic test, determining the effects of the test on clinical decision making.

Table 2. Features of matched groups—arranged in pairs

Age, y	Gender	Presentation	Diagnosis	Jaundice	DM	Procedure
66	Male	Solid mass	Pancreatic adenocarcinoma	No	No	Palliative bypass
68	Male	Solid mass	Serous cystadenoma	No	No	Distal pancreatectomy
60	Male	Solid mass	Pancreatic adenocarcinoma	No	No	Pancreaticoduodenectomy
64	Male	Solid mass	Autoimmune pancreatitis	No ^a	No	Hepaticojejunostomy
64	Male	Solid mass	Pancreatic adenocarcinoma	No	No	Pancreaticoduodenectomy
47	Male	Cystic mass	Pancreatic pseudocyst	No	No	Cystgastrostomy
49	Male	Stricture	Pancreatic adenocarcinoma	Yes	No	Pancreaticoduodenectomy
50	Male	Solid mass	Autoimmune pancreatitis	Yes	No	Pancreaticoduodenectomy
57	Female	Solid mass	Pancreatic adenocarcinoma	No	No	Pancreaticoduodenectomy
57	Female	Cystic mass	Pancreatic pseudocyst	No	No	Distal pancreatectomy
63	Female	Solid mass	Pancreatic adenocarcinoma	No ^a	No	Palliative bypass
62	Female	Cystic mass	Mucinous cystadenoma	No	No	Distal pancreatectomy
71	Female	Solid mass	Pancreatic adenocarcinoma	No	No	Pancreaticoduodenectomy
71	Female	Cystic mass	Serous cystadenoma	No	No	Distal pancreatectomy
58	Male	Solid mass	Pancreatic adenocarcinoma	No	No	Distal pancreatectomy
44	Male	Cystic mass	Pancreatic pseudocyst	No	No	Distal pancreatectomy
68	Male	Solid mass	Pancreatic adenocarcinoma	No ^a	No	Pancreaticoduodenectomy
71	Male	Solid mass	Pancreatitis NOS	No	No	Treated nonoperatively
57	Female	Solid mass	Pancreatic adenocarcinoma	No ^a	No	Pancreaticoduodenectomy
45	Female	Solid mass	Pancreatitis NOS	No	No	Distal pancreatectomy
66	Female	Solid mass	Pancreatic adenocarcinoma	No ^a	No	Pancreaticoduodenectomy
77	Female	Solid mass	Duodenal lipoma	No	No	Other pancreatectomy
76	Female	Solid mass	Pancreatic adenocarcinoma	No	No	Pancreaticoduodenectomy
76	Female	Cystic mass	Serous cystadenoma	No	No	Pancreaticoduodenectomy
63	Female	Mass	Pancreatic adenocarcinoma	No	No	Distal pancreatectomy
53	Female	Cystic mass	Pancreatic pseudocyst ^b	No	No	Distal pancreatectomy
76	Male	Solid mass	Pancreatic adenocarcinoma	No	No	Palliative bypass
79	Male	Solid mass	Pancreatitis NOS	No	No	Pancreaticoduodenectomy

^aHistory of jaundice, anicteric at time of collection.

^bContained histologic features of mucinous neoplasm.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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