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Metabolomic-derived novel cyst fluid biomarkers for pancreatic cysts: glucose and kynurenine

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Abstract

Background—Better pancreatic cyst fluid biomarkers are needed.

Objective—To determine whether metabolomic profiling of pancreatic cyst fluid would yield clinically useful cyst fluid biomarkers.

Design—Retrospective study.

Setting—Tertiary-care referral center.

Patients—Two independent cohorts of patients (n = 26 and n = 19) with histologically defined pancreatic cysts.

Intervention—Exploratory analysis for differentially expressed metabolites between (1) nonmucinous and mucinous cysts and (2) malignant and premalignant cysts was performed in the first cohort. With the second cohort, a validation analysis of promising identified metabolites was performed.

Main Outcome Measurements—Identification of differentially expressed metabolites between clinically relevant cyst categories and their diagnostic performance (receiver operating characteristic [ROC] curve).

Results—Two metabolites had diagnostic significance—dglucose and kynurenine. Metabolomic abundances for both were significantly lower in mucinous cysts compared with nonmucinous cysts in both cohorts (glucose first cohort $P = .002$, validation $P = .006$; and kynurenine first cohort $P = .002$, validation $P = .002$). The ROC curve for glucose was 0.92 (95% confidence interval [CI], 0.81–1.00) and 0.88 (95% CI, 0.72–1.00) in the first and validation cohorts, respectively. The ROC for kynurenine was 0.94 (95% CI, 0.81–1.00) and 0.92 (95% CI, 0.76–1.00) in the first and validation cohorts, respectively. Neither could differentiate premalignant

from malignant cysts. Glucose and kynurenine levels were significantly elevated for serous cystadenomas in both cohorts.

Limitations—Small sample sizes.

Conclusion—Metabolomic profiling identified glucose and kynurenine to have potential clinical utility for differentiating mucinous from nonmucinous pancreatic cysts. These markers also may diagnose serous cystadenomas.

Pancreatic cysts have an estimated prevalence of 2% in the general population,^{1,2} which is more common than was previously believed. Their clinical management currently relies on clinical monitoring, imaging data, and in many cases EUS with FNA. EUS-derived cytology and carcinoembryonic antigen (CEA) analysis, however, have diagnostic limitations.^{3,4}

To identify novel cyst fluid biomarkers, we used a metabolomic approach to identify uniquely expressed metabolites in clinically relevant pancreatic cyst types. Within the “-omics” cascade of discovering differences among different disease states, genomics focuses on what can happen, proteomics focuses on what makes it happen, and metabolomics focuses on what has happened and is happening⁵. Metabolomic analysis can reveal a great deal about the physiologic state of a tissue. However, the extreme differences in physicochemical properties make it impossible to accurately measure changes in all metabolites with a single analytic method.

In this study, we used a recently developed Dansyl [5-(dimethylamino)-1-naphthalene sulfonamide] derivation method⁶ and liquid chromatography with mass spectrometry (LC/MS) analysis to robustly analyze changes in many metabolites in pancreatic cyst fluid aspirates. The dansyl derivation method increases metabolite detection sensitivity by 10 to 1000-fold and improves metabolite identification. It enables changes in many metabolites to be evaluated in an unbiased fashion. This semi-targeted method was used to profile the metabolites in pancreatic cyst fluid obtained from 2 cohorts of individuals with pancreatic cysts defined by histology.

METHODS

Pancreatic cyst fluid collection and clinical cohorts

An institutional review board–approved bio-repository for pancreatic cyst fluid has been maintained at the Stanford University Medical Center since July 2008. Cyst fluid samples were obtained from patients with pancreatic cysts that were evaluated at Stanford Hospital and Clinics by EUS or surgery. All procedures and sample collections were performed after informed consent was obtained according to an institutional review board–approved protocol. The cyst fluid that was obtained during EUS and/or surgical procedures and not needed for clinical care was immediately placed on ice, divided into aliquots, and stored at –80°C. All samples were frozen within 30 minutes of collection. No samples underwent more than 2 freeze-thaw cycles before evaluation. All included cysts were defined by histology from surgery (n = 40) or positive cytology (n = 5). We defined cancer to include high-grade dysplasia, pancreatic adenocarcinomas with cystic degeneration, and intraductal papillary mucinous neoplasm (IPMN)–associated cancers.

The first (derivation) cohort was developed by choosing consecutive samples with available histology of each cyst type with a goal of making the sample as balanced as possible of different cyst types. Because the clinical goal is to not operate on benign cysts like serous cystadenomas (SCA) and pseudocysts, it was difficult to achieve equal numbers of nonmucinous and mucinous cysts. The validation cohort was developed after the derivation cohort by using the same consecutive selection method.

Metabolomic analysis

Four volumes of acetonitrile, methanol, and acetone (1:1:1 by volume) were added to 1 volume (50 μ L) of pancreatic cyst fluid then incubated at -20°C for 1 hour. The dansyl derivation method was performed by using a modification of the procedures developed by Guo and Li.⁶ A half volume of 0.1 M sodium tetraborate buffer was added to 1 volume of the metabolite extract and then combined with 1 volume of 50 mM dansyl chloride and mixed using a vortex mixer. The mixture was incubated at room temperature for 30 minutes before addition of 1 volume of 0.5% formic acid to stop the reaction. The supernatant of the reaction mixture was then placed into an autosampler vial. All samples were then analyzed on an Agilent (Santa Clara, Calif) accurate mass Q-TOF 6520 coupled with an Agilent ultra high-performance liquid chromatography infinity 1290 system. Ultra high-performance liquid chromatography is a high-pressure chromatography technique for separating a mixture of compounds for the purposes of identifying and quantifying individual components. The chromatography runs were performed by using a Phenom-enex (Torrance, Calif) Kinetex reversed-phase C18 column (dimension 2.1×100 mm, 2.6 mm particles, 100Å pore size). Solvent A was high-performance liquid chromatography water with 0.1% formic acid, and solvent B was LC/MS grade acetonitrile with 0.1% formic acid. A 30-minute gradient at 0.5 mL/minute was as follows: $t = 0.5$ minute, 5% B; $t = 20.5$ minutes, 60% B; $t = 25$ minutes, 95% B; $t = 30$ minutes, 95% B. The column was balanced at 5% B for 5 minutes. All data were acquired by positive electrospray ionization with Masshunter acquisition software (Agilent Technologies, Santa Clara, CA, USA). Molecular feature extraction on all data was performed by using Masshunter qualitative software. The metabolite abundance, which is a measure of the metabolite concentration in an extract, was determined by integration of the peak area for the indicated metabolite on the extracted ion chromatogram for each sample.

Glucose assay

Based on metabolite abundance results, cyst fluid glucose levels were measured by using an adaptation of the hexokinase-glucose-6-phosphate dehydrogenase spectrophotometric method, which was performed on a Dimension RxL analyzer (Siemens Healthcare Diagnostics, Deerfield, Ill).⁷ The reportable range for this assay is 5 to 500 mg/dL, with an intra-assay ($n = 20$) and interassay ($n = 20$) coefficient of variation of 0.6% and 1.2% at 88 mg/dL and 0.3% and 1.3% at 276 mg/dL, respectively. To minimize metabolism, all samples were processed within 15 minutes after complete thawing. Each sample (50 μ L) was measured twice, and the average was used for analysis. When the measured glucose value in the cyst fluid glucose was below 5 mg/dL, the actual value was less precisely determined, and a 5 mg/dL value was used in these analyses.

Statistical analyses

The Kruskal-Wallis and Wilcoxon rank sum tests were used to evaluate quantitative differences in cyst fluid glucose and kynurenine among cyst types and between non-mucinous and mucinous cysts and between mucinous noncancerous cysts and cancerous cysts. The chi-square test was used for comparing proportions between the 2 cohorts when appropriate. A 2-sample t test with unequal variance was used on the log-scale of the data to compare the abundance measured by mass spectrometry. To compare the diagnostic accuracy of combining 2 biomarkers to each alone, a conditional binomial test was applied. Statistical analysis was performed by using STATA 11.0 (College Station, Tex).

Principle component analysis was used to investigate the pattern of metabolite changes in an unbiased and un-supervised manner. For this analysis, the metabolomic data in the 5 different cyst categories (SCA, pseudocysts, mucinous cystic neoplasms [MCN], IPMN, and cancer) was obtained, and all metabolites that were present in more than 1 sample were

included. The minimum threshold abundance was empirically set to 1000. If a metabolite was undetected in a sample (ie, the abundance was less than the threshold), it was then assumed that its true abundance was between 0 and 1000, and metabolite abundance in that sample was assumed to be half of the threshold value. The data then underwent a $\log_{10}$ -based transformation, and principle component analysis⁸ was used to display the data. The principle component analysis was performed in the R programming language (<http://www.r-project.org>).

RESULTS

Clinical cohorts

Clinical and relevant imaging characteristics of 2 independent clinical cohorts for metabolite analysis are displayed in Table 1. There were no significant differences in age, sex distribution, or cyst size between these 2 cohorts. The mean age was 62 years in the first cohort and 59 years in the validation cohort, with a slight predominance of men. The median cyst size was 3.0 cm in the first cohort and 3.2 cm in the validation cohort. As a reason for surgical resection, 62% of patients in the first cohort had 1 of the following high-risk features: main duct dilation, solid component, or associated symptoms. Associated symptoms included abnormal weight loss, jaundice, or acute pancreatitis. In the validation cohort, 58% of patients had 1 of the previously stated high-risk features. There was no significant difference in high-risk features between the 2 cohorts ($P = .8$).

Table 2 displays the type and frequency of pancreatic cysts in each cohort. The first cohort of 26 individuals included 6 nonmucinous (4 SCA and 2 pseudocysts) and 20 mucinous (4 MCN, 6 IPMN, and 10 cancer) cysts. The validation cohort of 19 individuals included 8 nonmucinous (4 SCA and 4 pseudocysts) and 11 mucinous (1 MCN, 8 IPMN, and 2 cancer) cysts. Of the 5 patients with positive cytology, 4 had cysts with adenocarcinoma identified from intracystic nodules, and 1 had a pseudocyst.

Metabolomic analysis

A total of 506 metabolites were detected in the first cohort. Principal component analysis indicated that nonmucinous (SCA and pseudocysts) and mucinous (MCN, IPMN, and cancer) cysts could be separated based on the measured metabolite abundances (Appendix Fig. A1, available online at www.giejournal.org). Mucinous cysts could not be separated out from those cysts harboring cancer. Among the total detected metabolites, 10 were differentially abundant in the mucinous and nonmucinous cysts, by using a threshold cutoff of a fold-change >2.0 and P value $< .05$. Four of these 10 metabolites were also differentially abundant in the validation cohort, and the identities of 2 of these were determined to be glucose and kynurenine (Appendix Table A1, available online at www.giejournal.org). The remaining 8 metabolites could not be matched to any known metabolite, and their abundance was very low. Despite several attempts to identify them by tandem mass spectrometry (MS/MS) analysis, we could not obtain a sufficient amount to enable their characterization.

Reduced glucose levels in mucinous cysts

Table 2 shows that in the first and validation cohorts, the glucose abundance as measured by LC/MS analysis was significantly reduced in mucinous relative to nonmucinous cysts ($P = .0001$ and $P = .005$, respectively). The area under the receiver operating characteristic (ROC) curve was 0.92 (95% confidence interval [CI] 0.81–1.00) and 0.88 (95% CI, 0.72–1.00) in the first and validation cohorts (Fig. 1).

To confirm these observations, we used a spectrophotometric hexokinase assay for glucose (used at Stanford clinical laboratory) to measure cyst glucose levels.⁷ The median (interquartile range [IQR]) cyst glucose level in mucinous cysts (5 mg/dL [5–18]) was more than 16-fold below that in nonmucinous cysts (82 mg/dL [66–105]) in the first cohort ($P = .002$) (Table 2). In the validation cohort, the median cyst glucose level in mucinous cysts (5 mg/dL [5–21]) was 10-fold below that in nonmucinous cysts (58 mg/dL [20–130]) ($P = .01$) (Table 2). Cyst fluid glucose levels could not differentiate mucinous, premalignant cysts from cancerous cysts.

Combining data from both cohorts, the ROC curve was 0.88 (95% CI, 0.76–0.99). The highest diagnostic accuracy was observed by using a cutoff of 66 mg/dL for differentiating nonmucinous from mucinous cysts. With this threshold, cyst fluid glucose had a sensitivity and specificity of 94% and 64%, respectively, for classifying mucinous and nonmucinous cysts (Fig. 2).

Serous cystadenomas have elevated glucose levels

Among nonmucinous cysts, the median glucose levels of SCAs were higher than those of pseudocysts in both cohorts. When we combined the cohorts, the median (IQR) cyst glucose level of SCAs ($n = 8$) was 98 mg/dL (67–157) compared with that of pseudocysts ($n = 6$), which was 23 mg/dL (20–96) ($P = .07$). When cyst glucose levels of SCAs were compared with those of lesions that were not SCAs (pseudocysts, IPMN, MCN, and cancer), the median cyst glucose level was significantly elevated (98 mg/dL vs 7 mg/dL) ($P = .0001$), with an ROC curve of 0.93 (95% CI, 0.86–1.0). The highest diagnostic accuracy was obtained at a cutoff of 66 mg/dL, with a sensitivity and specificity for differentiating SCAs from lesions that were not SCAs of 88% and 89%, respectively.

Glucose performs similarly to CEA in differentiating mucinous from nonmucinous cysts

Because cyst fluid CEA data were available for 31 of the 45 samples when we combined the cohorts, we could compare the relative diagnostic performance of cyst fluid glucose and CEA as diagnostic markers for differentiating mucinous from nonmucinous cysts. The median (IQR) CEA levels in nonmucinous ($n = 9$) and mucinous ($n = 22$) cysts were 1.7 ng/mL (0.9–69) and 985 ng/mL (173–5797), respectively, which was significantly different ($P = .0005$). Among the 22 mucinous cysts, the median CEA level for premalignant cysts ($n = 15$) was 319 ng/mL (IQR 171–5797), which was not significantly different ($P = .323$) from that of malignant cysts ($n = 7$) (median 2298 ng/mL; IQR 319–11962). When we used the standard cutoff of 192 ng/mL, CEA had a diagnostic accuracy, sensitivity, and specificity of 77%, 73%, and 89%, respectively.

In this sample, glucose, at a cutoff of <66 mg/dL, had a diagnostic accuracy, sensitivity, and specificity for diagnosing mucinous from nonmucinous cysts of 84%, 95%, and 56%, respectively. Requiring both CEA > 192 ng/mL and glucose < 66 mg/dL as combined criteria for differentiating mucinous from nonmucinous cysts did not improve the diagnostic accuracy (74%) relative to either marker alone. Using either CEA > 192 ng/mL or glucose < 66 mg/dL to differentiate mucinous from nonmucinous cysts showed a trend of improved diagnostic accuracy (87%) compared with CEA or glucose levels alone, but this was not statistically significant.

Mucinous cysts have reduced kynurenine abundance

In the first cohort, the kynurenine abundance in the mucinous cysts (median 12,954) was significantly reduced ($P = .0006$) relative to benign nonmucinous cysts (median 195,686). In the validation cohort, the kynurenine abundance in mucinous cysts (median 1435) also was significantly below ($P = .002$) that in nonmucinous cysts (median 95,660) (Table 2).

Differences in extraction efficiency and detection sensitivity for each LC/MS run used to evaluate metabolite levels led to different absolute abundance levels observed in the 2 different cohorts. No significant difference was observed between mucinous premalignant cysts and cancerous cysts.

Data from each cohort were separately analyzed to evaluate the performance of kynurenine as an indicator of whether a cyst was mucinous or nonmucinous. The ROC curve for kynurenine was 0.94 (95% CI, 0.81–1.00) and 0.92 (95% CI, 0.76–1.00) in the first and validation cohorts, respectively (Fig. 3). In the first cohort, the maximum diagnostic accuracy was observed at a cutoff abundance of 185,650, providing a sensitivity and specificity of 100% and 80%, respectively. In the validation cohort, an abundance level of 34,000 provided the maximum diagnostic accuracy, providing a sensitivity and specificity of 90%, and 100%, respectively. In the absence of an established assay for kynurenine, direct comparison with CEA and glucose was not performed at this time.

SCAs have elevated kynurenine abundance

For the purposes of distinguishing SCA lesions among lesions that were not SCAs, the kynurenine abundance was compared in both cohorts. In the first cohort, SCA lesions had a significant kynurenine abundance (median [IQR] 189,236 [113,892–378,913]) compared with lesions that were not SCAs (median [IQR] 21,043 [1805–79,297]) ($P = .038$). In the validation cohort, SCA lesions also had a significant kynurenine abundance compared with lesions that were not SCAs (median [IQR] 124,564 [75,152–179,259] vs 3029 [732–54,862]) ($P = .035$). The area under the ROC curve was 0.83 (95% CI, 0.63–1.0) and 0.85 (95% CI, 0.66–1.0) for the first and validation cohorts, respectively.

DISCUSSION

With increasing recognition of the prevalence of pancreatic cysts and the premalignant potential in a substantial proportion of them, better diagnostic tools are needed. In recent years, there has been growing interest in cyst fluid–based biomarkers, with reports of potential clinical utility by using DNA, RNA, and cytokine expression profiling methods.^{9–13} In this study, we described the potential clinical utility of metabolite profiling for identifying novel pancreatic cyst fluid biomarkers.

Metabolomic profiling for the identification of disease biomarkers in serum has had very limited success, which is (at least in part) related to the effects of diet and other confounding factors as well as the ultra-complex pattern of metabolites present in serum. In this study, we focused on pancreatic cyst fluid, which is a relatively isolated space, and we hypothesized that tissues immediately surrounding the cyst may have a relatively stronger effect on the metabolites present in the cyst fluid. By using a semi-targeted approach, we identified glucose and kynurenine as metabolites that were differentially abundant by clinically relevant cyst categories in 2 independent cohorts. The identity of glucose and kynurenine was confirmed by MS/MS analysis and by comparison to chemical standards.

Because glucose is a commonly measured analyte, we were able to rapidly validate the observations of our metabolomic profile by using a widely available assay found in most clinical laboratories. Cyst fluid glucose levels were significantly decreased in mucinous cysts compared with in nonmucinous cysts, providing a diagnostic accuracy, sensitivity, and specificity of 84%, 94%, and 64%, respectively, when we used a threshold value of 66 mg/dL. Of further clinical relevance, SCA lesions had uniquely elevated glucose levels when compared to the other cyst types. Cyst fluid glucose could differentiate SCAs from lesions that were not SCAs with a diagnostic accuracy, sensitivity, and specificity of 89%, 88%, and 89% respectively, when we used a similar threshold of 66 mg/dL.

Based on the diagnostic performance of glucose, we compared it to CEA—the 1 biomarker currently accepted and widely used in clinical practice to differentiate mucinous from nonmucinous cysts. CEA performed similarly in differentiating mucinous from nonmucinous cysts to that reported in the literature.^{4,14} Glucose had a diagnostic accuracy similar to that of CEA (84% vs 77%). Using either CEA >192 ng/mL or glucose <66 mg/dL to differentiate mucinous from nonmucinous cysts did not significantly improve the diagnostic accuracy (87%) compared with CEA or glucose alone.

These observations of glucose in pancreatic cysts are clinically meaningful and warrant further validation. Analyzing glucose required a very small amount of cyst fluid (50 µL), and it was done rapidly in our hospital laboratory. In contrast, CEA analysis for many centers requires sending from 300 to 500 µL of cyst fluid out to a reference laboratory, with a consequent delay in results. Further, the high diagnostic accuracy of glucose for SCA lesions may minimize the number of patients who require imaging surveillance for indeterminate pancreatic cysts.

Kynurenine plays an important role in pancreatic cancer and immune biology, so it was of great interest to observe a differential abundance between mucinous and nonmucinous cysts.^{15–19} We observed decreased kynurenine abundances associated with mucinous cysts compared with nonmucinous cysts in 2 independent cohorts, with a diagnostic accuracy of approximately 95%. Similar to increased levels of glucose, we observed that kynurenine levels were significantly elevated in SCA lesions when compared to lesions that were not SCAs. Further analysis combining glucose and CEA with kynurenine was not performed at this time because of the lack of an available standardized assay for kynurenine.

There are several limitations that should be considered when evaluating the results of this study. A significant limitation includes the relatively small sample size of each cohort, which limits our ability to consider potential confounding factors (such as infection, metabolic and endocrine disorders, and medication use) as well as to identify other real differences correctly. Furthermore, this study did not include less common types of pancreatic cysts, such as cystic neuroendocrine tumors. To ensure a clear criterion standard, we included patients with a histologic diagnosis only. The vast majority of individuals underwent surgery because of cysts with recognized high-risk features, which also could introduce a bias in this cohort that differs from the larger population of individuals with pancreatic cysts.

Cyst fluid used for this analysis was acquired during surgery in 14 of 26 cases (54%) in the first cohort and 15 of 19 cases (79%) in the validation cohort. The remaining cases had cyst fluid collected preoperatively by EUS. The different method of cyst fluid acquisition theoretically could have influenced the metabolite results. We compared CEA and glucose levels between surgically collected and EUS-collected samples by cyst category and did not observe a significant difference. A recent study comparing EUS and surgically collected cyst fluid also showed no difference.²⁰

In this study, we defined cancer to include mucinous cysts with high-grade dysplasia and carcinoma. Among invasive carcinomas, we included cases in which we could not determine clearly whether the cyst was a consequence of tumor degeneration or malignant transformation of a mucinous cyst. Although the biology may be different between these 2 types of cysts, we chose to include them because differentiating them in clinical practice can be difficult. We did not observe a significant difference in glucose levels between presumed IPMN cancers and adenocarcinomas with cystic degeneration.

Although metabolomic profiling may shed insight into the pathophysiology of pancreatic cysts, it does not provide a mechanism for differential metabolite expression. Individual

hypotheses regarding the mechanism for different glucose and kynurenine levels between the different types of cysts exist based on current understanding of pancreatic tumor biology, and these warrant further investigation. The potential of metabolomic profiling to identify other biomarkers remains because the semi-targeted derivation method used here labels only a restricted set of metabolites with certain chemical features (primary and secondary amines and a few other functionalities). Other labeling methods could be used to identify other metabolomic markers, particularly those that differentiate mucinous, premalignant lesions from cancerous cysts.

In conclusion, we used a novel metabolomic profiling approach on 2 separate histologically defined pancreatic cyst cohorts and discovered glucose and kynurenine to have promise as clinically useful cyst biomarkers. Although they may differentiate mucinous from nonmucinous cysts, they may actually be more specific biomarkers for SCAs. Such a biomarker would have significant clinical utility. Several limitations of this study have been noted and will be addressed in future studies.

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Abbreviations

CEA	carcinoembryonic antigen
CI	confidence interval
IQR	interquartile range
IPMN	intraductal papillary mucinous neoplasm
LC/MS	liquid chromatography with mass spectrometry
MCN	mucinous cystic neoplasm
MS	mass spectrometry
ROC	receiver operating characteristic
SCA	serous cystadenoma

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Appendix

Figure A1. Principle component analysis of metabolomic data. The x- and y-axis represent the 1st and 2nd principle components (PC), and the numbers in parenthesis indicate the proportion of the total variance explained by the corresponding PC. The shape indicates the type of cyst, and the color indicates whether it is in group 1 (non-mucinous, red) or group 2 (mucinous, blue). The pattern indicates that the metabolite levels can distinguish the mucinous and non-mucinous cysts.

Table A1

Differentially abundant metabolites in mucinous and non-mucinous pancreatic cyst fluids obtained from 2 independent clinical cohorts. The accurate mass, retention time (RT), metabolite abundance ($\pm$ standard error of the mean), fold change (FC), and p-value (calculated using a 2-sample t-test with unequal variance on log-transformed data) for the four metabolites that were differentially abundant in the 2 cohorts are shown. The abundances of the 2 un-identified metabolites were too low to enable their identification.

First Cohort (n=26 samples)						
Metabolite	Mass	RT	FC	Mucinous	Non-Mucinous	P value
Glucose	413.1159	7.257	-2.2	96716 $\pm$ 39743	625669 $\pm$ 168585	0.015
Kynurenine	441.1352	11.234	-24.8	30115 $\pm$ 8656	483315 $\pm$ 330377	0.002
Unknown #1	772.2558	13.243	-30.1	4782 $\pm$ 1962	23161 $\pm$ 11469	0.033
Unknown #2	726.2774	13.035	-174.4	2919 $\pm$ 1447	34255 $\pm$ 15871	0.007
Validation Cohort (n=19 samples)						
Metabolite	Mass	RT	FC	Mucinous	Non-Mucinous	P value
Glucose	413.1143	7.257	-7.2	47439 $\pm$ 17800	161759 $\pm$ 37245	0.004
Kynurenine	441.1356	11.159	-179.8	20881 $\pm$ 17295	109825 $\pm$ 21233	0.002
Unknown #1	772.2560	12.558	-808.1	1550 $\pm$ 1300	12221 $\pm$ 5579	8×10^{-5}
Unknown #2	726.2774	13.035	-505.4	2870 $\pm$ 799	4430 $\pm$ 1729	5×10^{-5}

Take-home message

- A metabolomic profiling of pancreatic cyst fluid was performed and identified glucose and kynurenine to be differentially expressed between nonmucinous and mucinous pancreatic cysts.
- This study supports metabolomic profiling as a novel approach to identifying clinically useful biomarkers for the diagnosis and management of pancreatic cysts.

Figure 1.

A, The area under the ROC curve for glucose to differentiate mucinous from nonmucinous cysts in the first cohort was 0.92 (95% CI, 0.81–1.00). **B**, The area under the ROC for glucose to differentiate mucinous from nonmucinous cysts in the validation cohort was 0.88 (95% CI, 0.72–1.00). ROC, receiver operating characteristic; *CI*, confidence interval.

Figure 2. Scatter plots of cyst fluid glucose levels by nonmucinous and mucinous pancreatic cysts in 2 independent cohorts. The hexokinaseglucose- 6-phosphate dehydrogenase spectrophotometric method was used. Mucinous cysts have significantly reduced glucose levels in both cohorts. The dashed line indicates the glucose level with the maximum empirical diagnostic performance (66 mg/dL) for distinguishing mucinous and nonmucinous cysts.

Figure 3.

A, The area under the ROC curve for kynurenine to differentiate mucinous from nonmucinous cysts in the first cohort was 0.94 (95% CI, 0.81–1.00). **B**, The area under the ROC curve for kynurenine to differentiate mucinous from nonmucinous cysts in the validation cohort was 0.92 (95% CI, 0.76–1.00). *ROC*, receiver operating characteristic; *CI*, confidence interval.

Table 1

Clinical characteristics of cohorts

	First cohort	Validation cohort	<i>P</i> value
Total patients	26	19	
Age, mean (range), y	62 (33–83)	59 (30–78)	.49
Male/female, no. (%)	14 (54)/12 (46)	11 (58)/8 (42)	.78
Cyst size, median (IQR), cm	3.2 (2.0–6.1)	3.0 (2.0–5.4)	.69
High-risk features, %	62	58	.8
Main duct dilation	15	26	.36
Solid component	31	16	.24
Associated symptoms *	42	37	.71

IQR, Interquartile range

* Weight loss, jaundice, pancreatitis

Table 2

The median and interquartile range cyst glucose levels (measured by using a standard hexokinase assay) and the LC/MS-determined abundance of glucose and kynurenine for the first and validation cohorts

First cohort	Glucose assay	Metabolite levels (LC/MS-determined abundance)	
	Glucose, median, (IQR) mg/dL	Glucose, median (IQR)	Kynurenine, median (IQR)
Nonmucinous (n = 6)	82 (66–105)	516,398 (104,255–844,052)	195,686 (185,655–565,007)
SCA (n = 4)	86 (67–162)	690,415 (516,398–1,423,682)	189,236 (113,892–378,913)
Pseudocyst (n = 2)	61 (25–96)	175,405 (104,255–246,555)	3,210,834 (198,553–6,223,115)
Mucinous (n = 20)	5 (5–18)	22,875 (7885–122,083)	12,954 (1376–53,566)
MCN (n = 4)	7 (5–19)	55,432 (15,269–154,376)	36,830 (11,424–53,092)
IPMN (n = 6)	5 (5–5)	7784 (1102–32,264)	3949 (1–7882)
Cancer (n = 10)	16 (5–38)	27,202 (10,113–155,926)	46,805 (1376–90,310)
Validation cohort	Glucose, median, (IQR) mg/dL	Glucose, median (IQR)	Kynurenine, median (IQR)
Nonmucinous (n = 8)	58 (20–130)	151,709 (55,034–265,430)	95,660 (46,531–143,193)
SCA (n = 4)	103 (58–157)	214,618 (151,709–369,758)	124,564 (75,152–179,259)
Pseudocyst (n = 4)	20 (13–82)	55,034 (43,748–56,275)	64,978 (46,531–114,289)
Mucinous (n = 11)	5 (5–21)	31,204 (3187–56,275)	1435 (1–6825)
MCN (n = 1)	16	43,551	6825
IPMN (n = 8)	5 (5–8)	19,923 (2882–43,740)	967 (1–1,471)
Cancer (n = 2)	23 (21–25)	73,304 (33,836–112,771)	12,348 (3029–21,666)

LC/MS, Liquid chromatography with mass spectrometry; IQR, interquartile range; SCA, serous cystadenoma; MCN, mucinous cystic neoplasm; IPMN, intraductal papillary mucinous neoplasm.