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The metabolic time line of pancreatic cancer: Opportunities to improve early detection of adenocarcinoma

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ABSTRACT

Background: A reliable biomarker to detect pancreatic ductal adenocarcinoma (PDAC) continues to be elusive. With employing metabolomics we hypothesize that a broader analysis of systemic blood can differentiate different stages of PDAC.
Methods: Patients undergoing pancreatic resection had plasma samples grouped by diagnosis and assayed with mass spectrometry. 10 per group [neuroendocrine (PNET), intraductal papillary mucinous neoplasm (IPMN), localized PDAC, locally advanced PDAC, and metastatic] were analyzed to assess if metabolites could delineation different stages of adenocarcinoma.
Results: Of the 215 metabolites measured, four had a stronger correlation to disease burden than CA19-9. However, none of these metabolites differentiated stepwise progression in malignancy. Principal component analysis identified five metabolic components. Each cancer cohort was characterized by a unique combination of components, two components were predictors of PDCA stages.
Conclusions: Enhanced metabolomic analysis identified metabolic pathways that may assist in differentiating PDCA stages that do not occur in a linear stepwise progression.

Introduction

Pancreatic ductal adenocarcinoma (PDAC) remains one of the most lethal solid organ malignancies.[1] One of the major reasons for dismal prognosis with PDAC is due to late detection of malignancy. The majority of patients are diagnosed with stage IV disease associated with a five year survival of less than 3%, while patients with localized disease (10% of new diagnoses) have a 10-fold increase in survival.[2] With refined surgical techniques for complex pancreatic surgery, high volume pancreatic surgery centers throughout the world can safely resect malignancies with a mortality rate ranging from 1.6 to 3%.[3] The benefits of successful resection are evident in patients with locally advanced malignancies in which patients undergoing surgical resection involving vascular reconstruction have five-year survival rates up to 23% compared to patients with aborted surgical resection with 0% survival in the same time frame.[4] Therefore, the greatest chance for improving survival in pancreatic cancer is early detection of disease, which has been a research focus for decades.

Biomarkers to assess for solid organ malignancy originated with monoclonal antibodies directed at colon cancer in the late 1970s.[5] The expansion of antibodies specific to malignancies continued over the next decade with CA19-9 emerging as the gold standard for detecting pancreatic cancer.[6] Despite the break through prognostic value of CA19-9, limitations exist and there is considerable debate if this sole biomarker is the best way to screen patients for malignancy of the pancreas and guide treatment.[7,8] There have been numerous molecular markers ranging from circulating tumor cells to small molecules assessed in conjunction with CA19-9 to improve detection of early pancreatic cancer, but these have had mixed results.[9] Metabolomics assessment as a molecular marker to assess for disease burden is of particular interest due to the altered

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mitochondrial respiration from tumor cells in a process known as the Warburg effect.[10] Previous studies have successfully differentiated patients with adenocarcinoma of the pancreas to healthy controls[11,12] and chronic pancreatsis.[13] However, none of these studies assessed the ability of metabolomics approaches to differentiate disease stages. This is of particular relevance as premalignant lesions and early pancreatic cancer, rather than patients with advanced disease, need to be identified as early as possible. We hypothesize that combinations of metabolites are associated with different PDAC stages and facilitate discriminating early from late pancreatic cancer.

Materials and methods

Patient population

Overall, 100 patients undergoing pancreatic resection for known masses had blood samples collected before surgical incision. All patients were consented with after local institutional review board approval. The demographics of the overall patient population have previously been published[14] Of this cohort, patients were stratified into five groups based on final histologic diagnosis. This included intraductal papillary mucinous neoplasm (IPMN), localized pancreatic adenocarcinoma, locally advanced pancreatic cancer with nodal disease, and un-resectcable metastatic disease determined at the time of surgery. Patients with neuroendocrine tumors served as a control group as none of these patients had any premalignant adenocarcinoma lesions of the pancreas and were in a similar age range. Patients with other cystic neoplasms of the pancreas were excluded. Ten patients were selected from each cohort to best match age and sex. Clinical data including demographics, per operative CA19-19 levels, disease recurrence, and survival were all collected from electronic medical record review.

Sample analysis

Blood samples from patients were collected in citrated tubes and spun to separate plasma. Resulting plasma samples were flash frozen and stored at −80 °C until analysis. For metabolomics analysis, plasma samples were thawed on ice and extracted in an ice-cold solution of methanol, acetonitrile, and water (5:3:2, v/v/v) as described.[15] Supernatants were analyzed on a Thermo Vanquish UHPLC (San Jose, CA) coupled online to a Thermo Q Exactive mass spectrometer (Bremen, Germany). Metabolites were separated using a 3 min isocratic run (as previously described[16] and a 4 min C18 gradient of 5–95% acetonitrile at 450 μL/min and a column temperature of 45 °C in positive and negative ion modes (total of 4 runs per sample). Quality control, metabolite assignments, and peak area measurements were performed as described previously.[16,17]

Statistical analysis

SPSS version 23 (IBM, Armonk, NY, USA) and SAS 9.4 (SAS Institute Inc. Cary, NC) were used for statistical analysis. Clinical and metabolomic measurements are presented as median and 25th to 75th interquartile (IQR). The correlation of pancreatic disease burden was ranked from PNET (no adenocarcinoma), IPMN (premalignant), to local, locally advanced, and metastatic adenocarcinoma. Three different methods were used to assess all metabolites that were analyzed. These methods assessed for the association of metabolites with different disease states with pancreatic malignancy. 1) Ordinal rank was used for correlation to metabolites using spearman's Rho test, and considered a significant biomarker if had a higher correlation than the gold standard CA19-9. 2) Concentrations of metabolites were contrasted between each progressive ordinal group using Mann Whitney *U* test and determined to be significant. This was first conducted only with the variables that had a higher correlation to cancer stage than with CA19-9. An additional analysis was also completed in a nondiscriminatory fashion, analyzing all 215 metabolites between each progressive stage of disease and considered significant if $p < 0.0125$ based on a Bonferroni adjustment. 3) Variable reduction with principal components analysis (PCA). For PCA the metabolites were combined into uncorrelated principal components (PC). PCA is a statistical approach for unsupervised variable reduction through finding correlations between multiple variables and grouping them. In simple terms, PCA consists of an automated, systematic examination of correlations among measured variables, aimed at identifying underlying latent components. The first PC is a line with the minimum possible distance from all the data points from several variables and explains the most variance; PC2 is a second line, perpendicular to the first line oriented in such a way as to explain the greatest amount of variation not explained by PC1. The process is repeated with subsequent individual components explaining lesser variance than the previous ones. The end result is that the original set of N variables is replaced by a smaller group of uncorrelated linear, weighted combinations of the original variables. PCs were considered significant for eigenvalues greater than or equal to 1.0; factor loadings (which can be roughly interpret like correlation coefficients) were considered significant for coefficients greater than or equal to 0.5. Subsequently, we examined the predictive power of the PCs for the distinct PDAC stages through logistic regression; performance of the logistic regression model was assessed by c-statistics.

Results

Patients

The median age of the 50 patients included in the analysis was 66 years and 30% were female. The majority of patients had the preoperative plan to undergo pancreaticoduodenectomy (82%) with the remaining 18% undergoing distal pancreatectomy. All patients without metastatic disease had an R0 resection. The overall patient cohort had a median follow up time of 20 months with a disease-specific mortality rate of 32%. There was a higher rate of disease recurrence in patients with locally advanced diseases ($p < 0.001$) compared to the other non-metastatic cohort patient (Table 1) and expectedly differed based on cohort with only 20% of patients with metastatic disease alive ($p < 0.001$). The characteristics of patients in the different groups is listed in Table 1.

Metabolomic correlation to adenocarcinoma disease burden

Of the 215 metabolites that were measured by mass spectrometry, all 215 were included in the study analysis. Four metabolites had a higher correlation to disease state than CA19-9 which are listed in Table 2 with their relative concentrations between different conditions. While all of these metabolites and CA19-9 had good correlation between overall disease states, they could not effectively discriminate patients between cohorts in a stepwise fashion. Lysine, C5-acylcarnitine and dodecanedioic acid had different concentrations between IPMN and PNET but could not differentiate between additional groups. Propionyl-carnitine, despite having a good correlation overall could not differentiate between groups, while only CA19-9 was different between local and locally advanced disease. However, when reevaluating the entire metabolic profile, specific metabolites were identified that differed between groups, but could not sequentially separate multiple groups (Table 3). The number of metabolites that differed

Table 1
Patient demographics and outcomes.

Group	Age	Female	Pancreatic Head	Tumor Size	Recurrence	Disease Specific Mortality
PNET	64 (58–68)	20%	60%	2.1 (1.6–4.0)	10%	0%
IPMN	66 (54–72)	40%	60%	3.9 (2.3–7.1)	0%	0%
Local	63 (58–74)	60%	100%	2.2 (1.8–3.5)	40%	30%
Locally Adv	70 (67–73)	40%	100%	3.1 (2.5–3.6)	70%	50%
Metastatic	64 (61–71)	40%	70%	4.3 (1.2–6.5)	na	80%

PNET = pancreatic neuroendocrine tumor, IPMN = intraductal papillary mucinous neoplasm, Adv = advanced.

Table 2
Correlation between CA19-9 and metabolites to disease burden of PDAC.

	CA19-9	C5- acylcarnitine (10^6 AU)	Propionyl-carnitine (10^6 AU)	Lysine (10^6 AU)	Dodecanedioic Acid (10^6 AU)
Rho	0.450	−0.577	−0.482	−0.485	0.488
Correlation	P = 0.002	P = 0.001	P < 0.001	P < 0.001	P < 0.001
PNET	15 (10–30)	1.24 (1.04–1.40)	0.83 (0.76–1.15)	7.5 (6.6–8.4)	0.16 (0.11–0.21)
IPMN	12 (2–19)	0.95* (0.64–1.23)	0.78 (0.47–1.07)	6.3* (5.9–6.7)	0.24* (0.19–0.27)
Local	18 (2–81)	0.84 (0.70–0.94)	0.61 (0.43–0.67)	5.3 (4.6–5.9)	0.27 (0.21–0.39)
Locally Adv	118* (68–483)	0.85 (0.58–0.93)	0.64 (0.47–0.74)	5.6 (4.5–6.2)	0.28 (0.23–0.55)
Metastatic	214 (4–51,590)	0.69 (0.55–0.87)	0.57 (0.44–0.69)	5.5 (4.4–6.3)	0.33 (0.28–0.39)

AU = arbitrary units, * = p < 0.05 compared to group above, Adv = advanced, PNET = pancreatic neuroendocrine tumor, IPMN = intraductal papillary mucinous neoplasm.

Table 3
Heatmap of differences of metabolites between groups.

	PNET vs IPMN	IPMN vs Local	Local vs Locally Adv	Locally Adv vs Metastatic
Lysine	*			
IDP	*			
Glyceraldehyde 3-phosphate	*			
Spermidine	*			
Acetamidobitanoate	*			
Cysteate	*			
Methyleneoxindole	*			
Acyl-C5	*			
Dodecanedioic Acid	*			
Cortisol	*			
7αHydroxy3oxo4 cholestenoate	*			
PG D2	*			
Asparagine		*		
Threonine		*		
Creatine		*		
Guanidinoacetate		*		
C12-acylcarnitine		*		
Methylenediurea		*		
Oxalomalate		*		
N6-Methyl-Lysine		*		
Hydroxydecanoic acid		*		
Homomethionine		*		
CA19-9			*	
Serine			*	
Hypoxanthine			*	
C6-acylcarnitine			*	
Tetradecanoic Acid			*	
11 HETE			*	
Catechin			*	
Thromboxane B2				*

PG = prostaglandin, PNET = pancreatic neuroendocrine tumor, IPMN = intraductal papillary mucinous neoplasm, PDAC = pancreatic ductal adenocarcinoma, PC = principal component.

between group decreased with progressive disease were as follows: 12, 10, 6 and 1 for PNET vs IPMN, IPMN vs local disease, local vs locally advanced and locally advanced vs metastatic respectively.

Principal component analysis

Five principal components (PC) were identified with eigenvalue greater than or equal to 1. The metabolites with a loading >|0.50| in each PC are listed in supplemental digital component -Table SDC-1. PC-1 included 47 metabolites with loading >|0.50| and explained 36% of the variance. A common theme to PC-1 was the amino acid structure, (7 of the 9 essential amino acids with high loading). PC-2 represented 24% of the variance with 28 metabolites related to fatty acid metabolism with high loading PC-3 represented 14% of the variance and hwith TCA metabolites with high loadings. Finally, PC-4 and PC-5 both represented 13% of the variance each, with 4 having a theme of methionine and uric acid metabolism, while PC-5 loaded polyamine metabolites. The PCs created unique metabolic

profiles which can be visualized in the radar plot in Fig. 1. Specifically, PNET was associated with high PC-4, IPMN with high PC-1, locally advanced PDAC with both high PC-2 and high PC-5, and metastatic PDAC with high PC-3, while local PDAC showed no predominance of specific PCs. Logistic regression confirmed the visual inspection depicted in Fig. 1 (Table 4), although some PCs did not reach significance possibly due to the small sample size.

Discussion

Metabolomic analysis of patients undergoing pancreatectomy for a known mass, demonstrates that several metabolites and the gold standard biomarker of pancreatic cancer CA19-9 correlate to disease burden of adenocarcinoma of the pancreas. However, the inability of these metabolites and CA19-9 to discriminate between each step of disease burden was evident. CA19-9 was only significantly different between local and locally advanced disease, but could not differentiate IPMN versus localized cancer and locally advanced from metastatic disease. Metabolites that correlated to disease burden on the other hand, were able to differentiate PNET from IPMN, but not IPMN from adenocarcinoma or advanced disease. No single metabolite of the 215 analyzed could delineate more than one stepwise progression of disease burden. Reevaluation of the entire metabolomic pathways using principal component analysis demonstrated that each patient cohort harbored distinct metabolic differences when assessing a global proteome picture, but even clustered metabolites failed to differentiate each step of disease burden.

Previous investigations into metabolites as biomarkers for pancreatic cancer have identified a number of metabolites that differ between healthy controls and patients with pancreatic adenocarcinoma.[11,12,18] In these studies (n = 40–59 cancer patients) metabolites can differentiate a healthy volunteer from a patient with pancreatic cancer with a high degree of confidence. The ability of metabolites to discriminate malignant and healthy control has multiple confounders, and investigation into contrasting patients with nonmalignant pathology is more challenging. In a study with over a 100 subjects a cluster of metabolites had an area under the curve of 0.97 to predict malignancy when including healthy controls, but was reduced to 0.76 between benign and malignant hepatobiliary disease.[19] A different study used a meta-analysis to select 10 candidate metabolites to discriminate between healthy volunteers and patients with pancreatic cancer, and used these metabolites in an analysis of their own study subjects with pancreatic cancer, diabetes, and colorectal cancer.[20] The meta-analysis focusing on the 10 most common metabolites that differed between PDAC and health controls, but only 3 were consistent across studies and within the groups own analysis (threonine, alanine, and tyrosine). Threonine was different between patients with IPMN and local PDAC in our study while alanine fit in to PCA group 1, and tyrosine was not found to be significant in any of our analysis. When comparing metabolites to differentiate between colorectal cancer and PDAC the AUC was 0.653 vs 0.992 when contrasting PDAC to healthy controls.[20] These data reflect the limitations of using healthy controls for finding biomarkers specific to pancreatic malignancy.

We utilized patients with neuroendocrine tumors as non PDAC controls, as they were well matched in age, tumor location, and have pathology confirming no adenocarcinoma in their pancreatic lesion. Other investigators have also utilized a more targeted control group to obtain a better match to patients with pancreatic cancer. Recently, a large cohort of patients with pancreatic cancer were contrasted to patient with chronic pancreatitis and other surgical patients.[13] This study ultimately tested a patient cohort of 79 patients with PDAC vs 80 patients with chronic pancreatitis vs 80 non PDAC surgical patients with a metabolomic panel to predict PDAC vs CA19-9. The metabolic panel had a higher area under the curve than CA19-9 with a strong negative predictive value. The

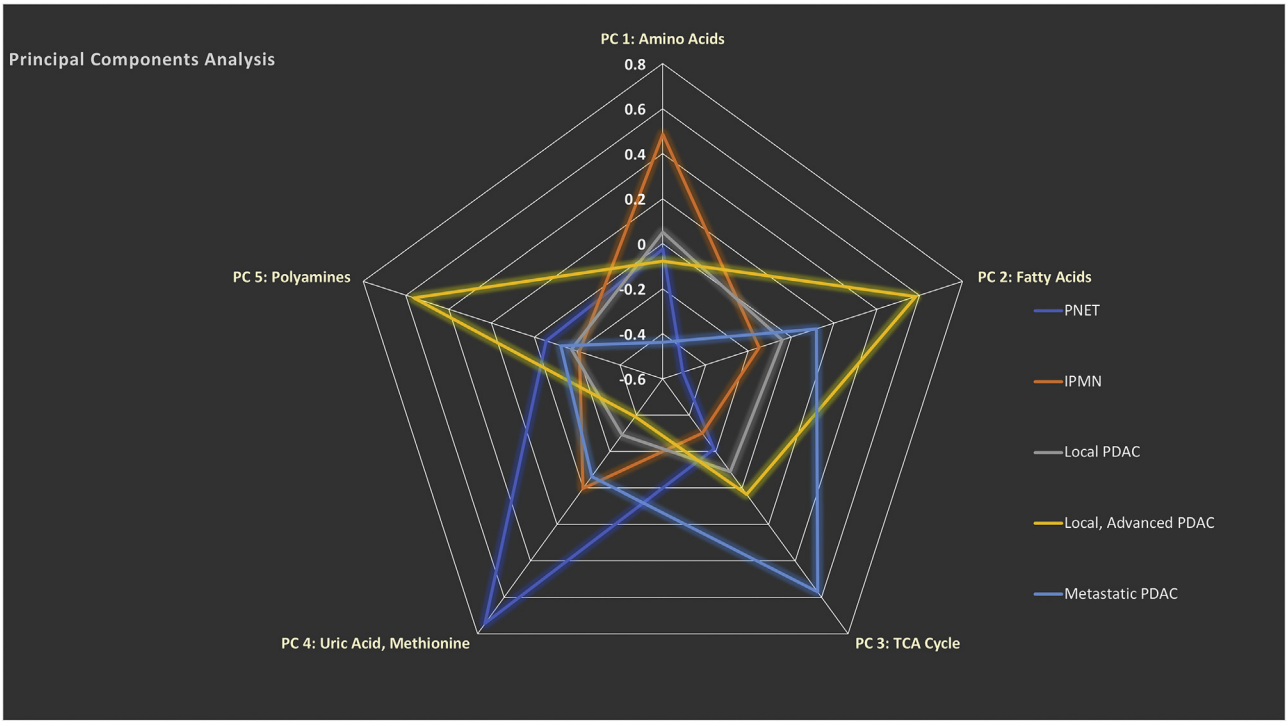

Fig. 1. Radar Plot of Metabolite Clusters Differing Between Cancer Groups. PNET = pancreatic neuroendocrine tumor, IPMN = intraductal papillary mucinous neoplasm, PDAC = pancreatic ductal adenocarcinoma, PC = principal component.

Table 4
Principal component (PC) scores as predictors of PDAC stages expressed as odds ratios (OR) and 95% confidence intervals of each specific stage (versus all other stages).

	PNET			IPMN			Local			Locally Advanced			Metastatic		
	OR	95% CI		OR	95% CI		OR	95% CI		OR	95% CI		OR	95% CI	
PC-1	1.48	0.47	4.64	2.06	0.95	4.45	1.05	0.53	2.07	0.96	0.45	2.02	0.47	0.17	1.28
PC-2	0.21	0.04	0.98	0.85	0.39	1.83	0.96	0.48	1.94	2.18	1.01	4.72	1.19	0.56	2.56
PC-3	0.39	0.05	2.84	0.29	0.05	1.77	0.84	0.33	2.12	1.01	0.40	2.54	1.93	0.82	4.53
PC-4	6.74	1.36	33.50	1.13	0.52	2.46	0.69	0.33	1.41	0.55	0.24	1.26	1.02	0.46	2.31
PC-5	1.13	0.49	2.57	0.33	0.05	2.32	0.64	0.15	2.79	1.97	0.80	4.88	0.85	0.35	2.07
C-statistic	0.83			0.75			0.62			0.80			0.69		

metabolic panel included 9 metabolites which had the largest overlap with principal component group 1 in our study. PCA group 1 was the highest in patients with IPMNs and lowest in patients with metastatic disease. The authors from this large study[8] state that the performance of the test was equivalent in patients with unresectable disease versus resectable disease and claim that tumor burden had no effect on metabolomic panel, but did not provide proof within their own data that stratifying patients by disease stage would preserve the performance of their assay.

A recent study from China was conducted matching 260 patients based on demographics, smoking habits, and BMI with and without PDAC.[21] Patients with PDAC in this study had 10 metabolites consistent with PDAC that had overlap with PCA group 2 from our study related to fatty acid metabolism including tetracosanoic acid, which was also significantly different between patients with locally advanced PDAC in our study compared to localized disease. This study also failed to stratify patients by disease burden when assessing metabolites. Another small clinical study assessing 40 patients with pancreatic cancer also identified altered fatty acid metabolism as a marker of pancreatic cancer, but did not find differences when stratifying patients by stage.[18]

In our analysis of individual metabolites and their correlation to cancer stage, components of fatty acid metabolism were appreciated all four metabolites that had a stronger correlation than CA19-9. Carnitine is derived from lysine[22] which was negatively correlated with cancer stage. Carnitine mobilizes fatty acids into the mitochondria where beta oxidation occurs and produces smaller chain acyl-carnitine chains,[23] in which we appreciated C5-acylcarnitine was negatively correlated with cancer stage. This was also seen with Propionyl-carnitine, which is an end fatty acid oxidation product that goes into the TCA cycle as an energy source.[24] Propionyl-carnitine and C5-acylcarnitine can be derived from precursor amino acids to serve as energy, but also have biological activity including anti-inflammatory properties.[24] Decreased lysine levels in patients with pancreatic cancer have previously been reported to be lower than patients with pancreatitis, but have not demonstrated the downstream depletion of beta oxidation byproducts.[25] A potential mechanism for lysine depletion could be related to increased metabolic demand of the tumor and the need for mobilization of more energy sources, which would include fatty acids and amino acids. A recent pilot analysis in a mixed population of patients with malignancy found carnitine deficiency to be present in 18% of the population analyzed, which is rare in a healthy population.[26] However, more work is needed to elaborate the role of carnitine in pancreatic cancer. Even within our own data set, carnitine on its own did not correlate pancreatic cancer stage, and components of metabolites had better predictability in determining a stepwise progression in pancreatic cancer (Fig. 2).

Dodecanedioic Acid was the only metabolites that had a positive correlation to pancreatic cancer, of the four metabolites which had a higher correlation than CA19-9. This 12 chain fatty acid has been proposed to be an intermediate metabolite that serves as an energy pool during impaired glycolysis.[27] Dodecanedioic acid has been demonstrated to promote beta oxidation for increased metabolic respiration in the presence of parenteral carbohydrates.[28] This fatty acid has also been demonstrated to provide a direct energy substrate to pancreatic islet cells in rats.[29] However, an association between dodecanedioic acid and pancreatic cancer has not previously been described. Several potential caused for an elevation in this metabolite exist, once of which is pancreatic tumor cells are increasing production for energy utilization, or the complete opposite in that pancreatic tumors have altered metabolism and cannot use this fatty acid for energy. We are not able to determine the cause for increased levels in patients with advanced pancreatic cancer, but fatty acid metabolism is a promising area for future research in this patient population.

Our study has identified a potential explanation for the discordances found in previous studies contrasting PDAC to healthy volunteers.[20] Patients with different stages of pancreatic cancer appear to harbor unique metabolomic profiles. By using a composite of patients with PDAC at different stages, the variance of each of these metabolites differs based on the percentage of patients with local vs locally advanced vs metastatic disease. This is consistent with our results in which PCA groups matched metabolites in studies implicating different metabolites with PDAC including lipids,[11–13,21] amino acids,[8,20] and polyamines.[30] A more pragmatic approach using metabolomics in screening for pancreatic cancer should start with at risk patients with known pancreatic masses. Recent analysis of an international cohort of patient with hereditary predisposition for pancreatic cancer demonstrated the efficacy of close long-term monitoring with imaging to improve survival.[33] However, this is a resource intensive endeavor including invasive imaging with endoscopic ultrasound and serial expensive imaging modalities such as MRI with intervals that can be as close as 3 months. Employing metabolite profiles in this patient cohort is appealing as it would be more cost effective and less intensive on resources. Many of the metabolites of interest can be assessed by sending clinical samples to specialized laboratories that perform comprehensive newborn metabolic screening that appear to have a cost comparable to CA19-9.

While our study was limited to 50 patients, an important finding was the burden of PDAC did not have a strong correlation with any specific metabolite or CA19-9. This has implications for future research into biomarkers for pancreatic cancer. While in vitro and animal models have shown a step wise progression in KRAS mutations starting with pancreatic intraepithelial neoplasms (PanIN) to metastatic PDAC,[34] these changes are confined to the tumor and not reflected in circulating blood. The same is true in regards to the Warburg metabolic changes of tumor, with a transition to anaerobic metabolism[10] in local tumor metabolism but not the systemic circulation. The progression from premalignant to systemic disease involves complex biological processes impacting which metabolites and proteins enter the circulation. Unfortunately, these changes do not appear to occur in a linear progression, with different pathways altered unequally during disease progression.

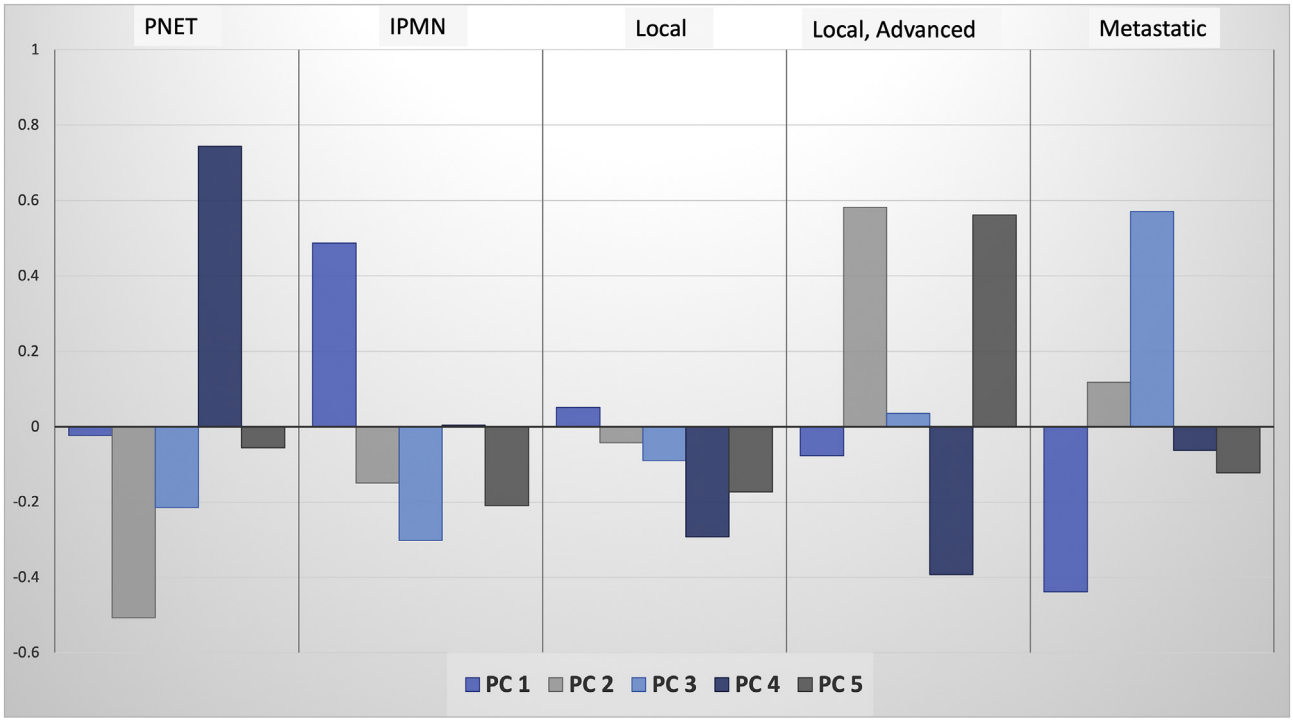

Fig. 2. Changes in Principal Component Groups Through Stepwise Progression of Pancreatic Cancer. y axis represents the relative weight of each principal component group as cancer progression moved from left to right on the X axis. Rather than a linear trend of metabolic clusters with cancer progression, there are unique changes in each step of pancreatic cancer, with up and down regulation of each PC group with disease progression.
PNET = pancreatic neuroendocrine tumor, IPMN = intraductal papillary mucinous neoplasm, PC = principal component.

This is the shortcoming of using CA19-9 as a screening assay as elevated levels predominantly occur in late stage disease decreasing its sensitivity for detecting early lesions.[35]

Principal component analysis was used as an analytic strategy to improve the ease in interpretation of clusters of metabolites. PCA is one of the oldest and commonly used strategies to implement single value decomposition to simplify large data sets without losing variability that can discriminate differences between the study population.[36] In our analysis, we utilized correlations between metabolites to form principal component analysis groups, creating descriptive groups within our cohort without consideration of the patient's cancer status. These clusters tended to follow specific metabolic pathways, but did include metabolites from other pathways. PCA strategies can be further refined with additional methodologies such as rotational and adding a restraint, but at the cost of making interpretation of results more complex.[36] We avoided utilizing these alternative strategies to avoid additional confusion to the readers, and these strategies can introduce bias[37] if there is no prior hypothesis for which cluster of metabolites has a more critical role. Mathematic modeling, taking into consideration tumor burden, biomarker shed, background normal circulating proteins/metabolites, and biomarker clearance supports that component analysis is a feasible methodology to detect pancreatic cancer.[38] However, producing a reliable set of biomarkers is dependent on knowing all of these factors as they change in the individual over time, which is a limitation of our study. This is a key area for future research in pancreatic cancer, which will predominantly be limited to monitoring patients from premalignant IPMN lesions to local pancreatic cancer.

Conclusions

Based on our analysis, we believe that metabolomics for the diagnosis in PDAC has the potential to be used in identifying patients transitioning between specific stages of disease. Metabolomic analysis is unlikely to be successful in screening healthy patients for pancreatic malignancy, but may have utility in monitoring patients with pancreatic lesions over time, and determining if patients are amenable to resection in combination with CA19-9 with advanced disease. These data reflect the inconsistencies between prior metabolomic analysis in contrasting patients with variable stages of pancreatic cancer with healthy volunteers. Future research is needed for a more in-depth analysis of the different stages of pancreatic cancer including PanIN (1–3) followed by the individual stage of cancer (Ia -IV) to validate these findings and assess for inflection points of the different pathways for disease progression to optimize screening/surveillance strategies with metabolomics and pancreatic cancer.

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Appendix A. Supplementary data

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