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NGAL, MIC-1 and CA19-9 in Pancreatic Juice: Pathobiological Implications in Diagnosing Benign and Malignant Disease of the Pancreas

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Abstract

Objective—Pancreatic diseases pose significant diagnostic challenge as signs and symptoms often overlap. We investigated the potential of pancreatic juice neutrophil gelatinase–associated lipocalin (NGAL), macrophage inhibitory cytokine-1 (MIC-1), and carbohydrate antigen 19-9 (CA19-9) to aid in the diagnosis of patients with symptoms suggestive of pancreatic diseases.

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Methods—105 chronic pancreatitis (CP), pancreatic cancer (PC), and non-pancreatic non-healthy (NPNH, patients with symptoms mimicking pancreatic disease but found to be free of pancreatic pathology) patients underwent endoscopic pancreatic juice collection following secretin-stimulation. NGAL and MIC-1 levels were measured by enzyme-linked immunosorbent assay while CA19-9 was measured by radioimmunoassay.

Results—NGAL, MIC-1, and CA19-9 were significantly elevated in the pancreatic juice of CP and PC patients as compared to NPNH controls ($p<0.034$). NGAL appeared most promising in differentiating diseased versus non-diseased pancreata (AUCs=0.88–0.91) while MIC-1 was found to be higher in PC than CP patients ($p=0.043$). Interestingly, MIC-1 levels in diabetic PC patients were higher than in non-diabetic PC ($p=0.030$) and diabetic CP patients ($p=0.087$). CA19-9 showed the least ability to distinguish patient groups (AUCs=0.61–0.76).

Conclusions—Pancreatic juice NGAL shows potential utility in establishing pancreatic etiology in the context of non-specific symptoms while MIC-1 may aid in differentiating PC from CP.

Keywords

Pancreatic Disease; NGAL; MIC-1; CA19-9; Pancreatic Juice

INTRODUCTION

Pancreatic disease is highly prevalent in Western countries, with a combined annual incidence of more than 2 million people in the United States alone [1–3]. Pancreatic diseases are clinically difficult to conclusively diagnose, as signs and symptoms consistent with a particular pancreatic etiology frequently overlap with multiple others, as is the case with chronic pancreatitis (CP) and pancreatic cancer (PC), to name a few. There are also myriad instances where one pancreatic disease is a risk factor for and potential driving force behind another pancreatic disease, with symptoms of both diseases often difficult or impossible to differentiate. In addition, many diseases that are not of a pancreatic etiology strongly mimic those that are, thereby increasing the time, expense, and risk of test-associated morbidity in ruling out pancreatic pathology and establishing a definitive diagnosis. This diagnostic complexity warrants the development of novel, minimally invasive methods of differentiating disease etiologies in the context of nonspecific symptoms potentially arising from one or more pancreatic pathologies. To date, serum-, plasma-, bile-, and urine-based biomarkers, as well as increasingly complex clinical evaluation criteria, have all proven disappointing in this arena.

One promising method for obtaining such a distinction is through evaluation of biomarkers in pancreatic juice. Pancreatic juice consists of biomolecules secreted by pancreatic exocrine cells as well as sloughed ductal cells and cell components that, after traveling through the ductal network of the pancreas, combine with bile in the common bile duct and are emptied into the duodenum through the sphincter of Oddi [4]. Owing to this intimate relationship with pancreatic chemical production and ductal cell status, pancreatic juice has the potential to act as a surrogate window into the cellular processes and pathologies occurring within the pancreas, allowing direct interpretability of the pancreatic environment that is surpassed only by core biopsies. Further, because it enables one to investigate disease within the entire ductal system of the pancreas, pancreatic juice analysis theoretically has more diagnostic applications than biopsy. Additionally, unlike other diagnostic methods such as bile collection via endoscopic retrograde cholangiopancreatography (ERCP), pancreatic juice collection by routine upper endoscopy is mildly invasive and carries a negligible risk of complication.

Several clinical biomarkers are under investigation or are currently in clinical use for the diagnosis, prognostic prediction, or progression evaluation of various pancreatic diseases [3, 5–13]. Two biomarkers that are proving of increasing importance in benign and malignant pancreatic diseases, and have thus far remained unexplored in pancreatic juice are NGAL and MIC-1. NGAL is a secreted protein whose expression is elevated in multiple human cancers, including pancreatic cancer [9, 14–17]. In a previous study, we found that NGAL is a significant predictor of disease severity and outcome in patients with acute pancreatitis [1]. In a small pilot study, we also found that plasma NGAL levels were significantly higher in PC patients than in healthy controls. Another pancreatic juice biomarker, MIC-1, a member of the transforming growth factor beta superfamily, was originally identified as a gene expressed in the context of macrophage activation [18]. MIC-1 is aberrantly expressed in PC tissues, and serum MIC-1 levels have been reported to be significantly higher in patients with PC than in healthy controls and patients with chronic pancreatitis [8]. A third biomarker, CA19-9, is the only serum PC biomarker currently approved by the U.S. Food and Drug Administration; however, its utility in pancreatic juice biomarker has undergone little investigation.

The purpose of the current study was to determine whether pancreatic juice NGAL, MIC-1, and/or CA19-9 could be used to distinguish pancreatic diseases from each other as well as to differentiate patients with pancreatic diseases from those free of pancreatic pathology.

MATERIALS AND METHODS

Study Population

This prospective study was approved by the Mayo Clinic Institutional Review Board (#07-0000099). Written informed consent was obtained from all patients prior to study enrollment. 111 patients presenting with epigastric pain/discomfort warranting EUS-FNA evaluation and originally diagnosed with CP, PC, or found to have no pancreatic disease (non-pancreatic non-healthy controls, NPNH) were enrolled in the study. After initial evaluation and pancreatic juice collection, definitive final diagnoses were made.

Age, gender, body mass index (BMI), race, DM2 status, alcohol history, and smoking history were collected from each patient (Supplementary Table 1). All samples were blinded prior to analysis.

Collection of Secretin-Stimulated Exocrine Pancreatic Secretions (SSEPS)

All patients underwent upper endoscopy after an overnight fast. Patients were moderately sedated with midazolam and meperidine before the procedure. Synthetic human secretin (ChiRhoStim, ChiRhoClinInc, Burtonsville, MD) at a dose of 0.2 $\mu\text{g}/\text{kg}$ (or a total dose of 16 μg) was administered intravenously for 1 minute immediately before endoscopy intubation. Gastric fluid was aspirated before intubation of the pylorus to minimize contamination. With the endoscope positioned in the second portion of the duodenum, opposite the papilla of Vater (without cannulation), a 2.3-mm plastic aspiration catheter (Hobbs Medical, Stafford Springs, CT) was passed through the biopsy channel of the endoscope until visible on screen in the endoscopic monitor. Pancreatic juice exiting the papilla was then suctioned through the catheter for 10 minutes into a dry 20-mL tube. Approximately, 5–8 mL of fluid was collected from each patient. The collected fluid was immediately aliquoted in 2-mL vials, snap-frozen in liquid nitrogen, and stored at -80°C until assays were performed. A pan-protease inhibitor cocktail pill (Roche Diagnostic Corporation, Indianapolis, IN) was added to the collected pancreatic juice to inhibit proteolytic degradation.

Determination of Final Diagnosis

Three gastroenterologists (MR, TAW and MBW) who were blinded to the results of the assays determined the final diagnosis of the patients based on standard clinical practice. Patients with no personal history of pancreatic disease and normal pancreatic tests (ERCP, EUS, computed tomography, or magnetic resonance imaging) were deemed to have normal pancreata and thus considered to be non-pancreatic non-healthy (NPNH) controls.

NGAL Measurement

NGAL levels were measured quantitatively using the DuoSet enzyme-linked immunosorbent assay (ELISA) kit (R&D Systems, Minneapolis, MN) for human NGAL according to the manufacturer's instructions. The pancreatic juice samples were aliquoted and stored at -70°C immediately following receipt to avoid repeated freeze-thaw cycles. Standard curves were produced from standards provided with the kit and serially ($\log_2$) diluted from 4 ng/mL to 15.6 pg/mL. All samples were plated in triplicate. Samples with readings greater than that of the highest standard were diluted appropriately and the assay was repeated. ELISA plates were read at 450 nm with an absorbance correction at 540 nm. The collected data were analyzed using SOFTMAX PRO software (Molecular Devices Corp., Sunnyvale, CA).

MIC-1 Measurement

MIC-1 concentrations in pancreatic juice were measured at UNMC. MIC-1 levels were measured quantitatively using the DuoSet ELISA kit (R&D Systems) for human MIC-1 according to the manufacturer's instructions using the same methods employed for NGAL as measured at UNMC.

CA19-9 Measurement

Concentrations of CA19-9 in pancreatic juice were measured at UNMC by a solid phase radioimmunoassay (Centocor, Malvern, PA or Fujiribio), according to the manufacturer's instructions. All samples were analyzed in duplicate and expressed in arbitrary units (U/mL) with 1 unit of activity corresponding to approximately 0.8 ng of purified antigen [19].

Statistical Analysis

Continuous variables were summarized using sample medians, 25th percentiles, and 75th percentiles. Mann-Whitney tests were used to make pair-wise comparisons in biomarker expression between CP patients, PC patients, and NPNH patients. Receiver-operating characteristic (ROC) curves were constructed and AUCs and 95% confidence intervals (CIs) were estimated to assess each biomarker's ability to differentiate between the three patient groups. Sensitivity, specificity, and corresponding 95% CIs were estimated for each biomarker. For ease of presentation, these quantities are presented only at the marker concentration at which both sensitivity and specificity were simultaneously maximized. P-values < 0.05 were considered statistically significant.

A secondary analysis to explore patient demographic and clinicopathologic information as potentially confounding factors was performed comparing biomarker expression between the patient subgroups. The focus of this exploratory analysis was to descriptively summarize biomarker expression in the various patient subgroups; however, p-values resulting from Mann-Whitney tests are presented in the tables. No adjustment for multiple testing was made owing to the exploratory nature of this analysis. Statistical analyses were performed using SAS software version 9.2 (SAS Institute Inc., Cary, NC) and S-Plus software version 8.0.1 (Insightful Corporation, Seattle, WA).

RESULTS

One hundred eleven patients presenting with epigastric pain/discomfort warranting EUS-FNA evaluation and diagnosed with CP, PC, or being free of pancreatic disease (non-pancreatic non-healthy controls, NPNH) were enrolled in the study. Six patients were lost to screening failure and thus were excluded from the analysis, leaving 105 patients for analysis: CP (n=24), PC (n=58) and NPNH (n=23).

Differentiating Non-Pancreatic Non-Healthy Patients from Chronic Pancreatitis Patients

All marker concentrations were significantly higher in CP patients than in NPNH controls ($P = 0.034$, Table 1, Figure 1). ROC curves for each marker reveal that NGAL (AUC (95% CI) = 0.88 (0.78–0.98)) differentiated NPNH patients from CP patients more effectively than MIC-1 or CA19-9 (AUC (95% CI) = 0.76 (0.62–0.90) and 0.67 (0.51–0.82) respectively) (Figure 2, Table 1). NGAL also showed the greatest sensitivity (75%, 95% CI = 53–90%) and specificity (74%, 95% CI = 52–96%) in differentiating CP from NPNH patients (Table 1). In contrast, the sensitivity and specificity (95% CI) of MIC-1 and CA19-9 were 71% (49–87%) and 74% (52–90%), and 63% (41–81%) and 65% (43–84%) respectively.

Differentiating Non-Pancreatic Non-Healthy Patients from Pancreatic Cancer Patients

All marker concentrations were significantly higher in PC patients than in the NPNH group (all $P < 0.001$, Table 1, Figure 1). ROC curves for each marker indicate that NGAL (AUC (95% CI) = 0.91 (0.84–0.98)), and MIC-1 (AUC (95% CI) = 0.83 (0.73–0.93)) differentiated NPNH from PC patients more effectively than CA19-9 (AUC (95% CI) = 0.76 (0.65–0.88)) (Figure 2, Table 1). NGAL showed the greatest sensitivity (79%, 95% CI = 67–89%) and specificity (83%, 95% CI = 61–95%) in differentiating PC from NPNH patients (Table 1). In contrast, the sensitivity and specificity (95% CI) was 78% (65–87%) and 78% (56–93%), and 84% (73–93%) and 52% (31–73%) for MIC-1 and CA19-9 respectively.

Differentiating Chronic Pancreatitis Patients from Pancreatic Cancer Patients

MIC-1 was the only marker for which there was a statistically significant difference in concentration between PC and CP patients ($P = 0.043$, Table 1, Figure 1). No noticeable differences were observed in NGAL or CA19-9 ($P = 0.110$) (Figure 2).

Further, we investigated whether combining MIC-1 with either NGAL or CA19-9 would enhance the ability to distinguish the two groups. MIC-1 levels were evaluated with each of the other biomarkers to identify direct or inverse relationships between them that might improve its diagnostic utility but no obvious trends were observed (Supplementary Figure 1).

Exploratory Correlation of Pancreatic Juice NGAL, MIC-1, and CA19-9 with Patient Demographics

Exploratory analyses of pancreatic juice biomarker concentrations based on patient demographics are shown in Table 2. No difference in pancreatic juice biomarker levels were observed based on patient age, BMI, gender, smoking history, or current alcohol use for NPNH or CP patients. While no differences between CA19-9 or NGAL levels based on demographic information were observed in PC patients, we found that PC patients aged ≥ 68 years had elevated MIC-1 levels as compared to those <68 years old (median level (IQR) = 1.26 (0.59–2.30) ng/mL and 0.49 (0.20–1.16) ng/mL respectively, $P = 0.03$). MIC-1 levels were also higher in men than in women with PC (median level (IQR) = 1.26 (0.36–2.86) ng/mL and 0.57 (0.17–0.98) ng/mL respectively, $P = 0.03$). However, BMI, smoking history, and current alcohol use did not correlate with pancreatic juice MIC-1 levels in PC patients.

Exploratory Correlation of Pancreatic Juice NGAL, MIC-1, and CA19-9 with Clinicopathologic Characteristics

Patient history of type-2 diabetes (DM2) was singled out for additional analysis due to its strong correlation with PC diagnosis as well as the mounting evidence that it may be an early, non-specific symptom of PC development [20]. Due to the fact that only three NPNH patients used in this study had a positive history of DM2, this group was omitted from this analysis. In patients with CP, no difference was observed in any of the measured biomarkers based on DM2 status ($P = 0.23$, Table 3). In PC patients, however, MIC-1 was found to significantly correlate with a past diagnosis of DM2 (median value for patients with positive DM2 status was 1.81 ng/mL while it was 0.61 ng/mL for those with a negative DM2 history, $P = 0.03$), while no significant differences were found in NGAL or CA19-9 levels ($P = 0.17$, Table 3). Further, in comparing CP patients with a history of DM2 to their PC counterparts, it was found that CA19-9, NGAL-UNMC, and MIC-1 were all elevated in the PC group with moderate significance ($P = 0.067$ – 0.082 , Table 4). NGAL-MDACC was also found to be higher in PC patients with a history of DM2 as compared to DM2-positive CP patients (189 ng/mL vs. 86 ng/mL), though this difference was found to be non-significant ($P = 0.16$, Table 4).

In determining whether measurement of NGAL, MIC-1, or CA19-9 in pancreatic juice has potential utility in the staging of newly diagnosed PC, initial staging by EUS was used to stratify PC patients as having resectable or non-resectable tumors. Pathological staging was evaluated to confirm true tumor resectability in patients with tumors deemed to be resectable by EUS. Fourteen patients had EUS-determined resectable tumors and forty-four had unresectable tumors. Statistical analysis revealed no difference in pancreatic juice NGAL, MIC-1, or CA19-9 levels with regard to tumor resectability ($P = 0.12$ – 0.93 , Supplementary Table 2).

Pancreatic juice marker levels were also analyzed for correlation with overall survival in PC patients to determine their prognostic potential. None of the markers was correlated with survival time (Supplementary Table 3); however, this result is not conclusive as the number of PC patients was insufficient, precluding optimal analysis.

DISCUSSION

We found that NGAL, MIC-1, and CA19-9 levels in pancreatic juice were significantly higher in CP and PC patients than in their NPNH counterparts ($P < 0.034$). Of these biomarkers, NGAL appears to hold the most individual promise in differentiating patients with pancreatic disease from those without, giving sensitivities of 79% and 75%, specificities of 83% and 74%, and areas under the curve (AUCs) of 0.91 and 0.88 when comparing PC and CP, respectively, to NPNH patients. MIC-1, however, also shows clinical potential when comparing PC to NPNH patients (sensitivity 78%, specificity 78%, AUC: 0.83). Furthermore, MIC-1 showed a significant difference in levels between PC and CP patients ($P = 0.043$), with no noticeable difference observed for NGAL or CA19-9 ($P > 0.10$). Interestingly, these results are similar to available data from previously published studies evaluating these markers in patient sera (Supplementary Table 4) [8, 9, 21–23].

Few studies have investigated the role of pancreatic juice in diagnosing pancreatic disease. One study found that the levels of certain heavy metals, such as chromium, were significantly elevated in the pancreatic juice of PC patients as compared to healthy controls and may have diagnostic significance [24]. Another study yielded a set of genes from the cells present in pancreatic juice that accurately differentiated healthy participants from those with PC [25]. A comparison of the proteome of pancreatic juice from PC patients to that of patients with other pancreatic diseases yielded pancreatitis-associated protein-1 (HIP/

PAP-1) that, when validated by ELISA, showed significant elevation in PC, but not in non-malignant pancreatic diseases, as compared to healthy controls [26]. Another recent study identified 20 proteins that are significantly elevated ($p < 0.05$) at least 2-fold or greater in pancreatic juice collected from three patients with preneoplastic PanIN-3 lesions compared to a control sample comprising pooled pancreatic juice from five patients with benign pancreatic diseases (sphincter of Oddi dysfunction and CP), some of which have also been previously shown to be elevated in PanIN tissues [27]. Human telomerase reverse transcriptase (hTERT) expression analysis in pancreatic juice from patients with PC and IPMN revealed that a positive staining for hTERT was nearly 85% sensitive and over 80% accurate in distinguishing benign from malignant cells, results far superior to those allowed by cytology [28]. One study comparing the proteome of pooled pancreatic juice from 9 PC samples and 9 control samples (including CP, gallstone induced pancreatitis, benign cystic neoplasm, and cystic fibrosis) found 3 proteins—matrix metalloproteinase-9, oncogene DJ-1, and α -1B-glycoprotein precursor—to be differentially upregulated in PC patients [29]. Additionally, several studies have analyzed the diagnostic abilities of pancreatic juice CA19-9 in the context of PC and CP as compared to healthy controls with results that are in general highly similar to those reported in this study, showing that pancreatic juice CA19-9 values are higher in PC than in CP and give a sensitivity for PC ranging between 70–90% [25, 30, 31]. One study in particular indicated that the sensitivity of elevated CA19-9 in pancreatic juice was 90% and 66% for PC and CP respectively, comparable to our values of 84% and 63% [31]. Interestingly however, data regarding pancreatic juice CA19-9 levels in the context of chronic pancreatitis has been inconsistent, with values ranging from normal to elevated and indistinguishable from PC [25, 30, 31].

Despite the general promise of these studies, several factors limit their clinical interpretability. Use of pooled samples prevents the direct comparison of individual groups and unblinded sample and/or biomarker studies could lead to bias. Small sample sizes and failure to investigate the consistency of biomarker level measurements across various medical centers and testing methods also curbs interpretability. Further, some platforms used in previous studies are not easily converted to the clinical setting which is problematic as protein elevation detectability is often inconsistent between different platforms.

In comparison, the current study has several strengths. This investigation recruited a sufficient number of patients to enable in-depth and powerful statistical analyses. Further, ample pertinent clinicopathologic and demographic patient data were obtained, allowing us to evaluate potential confounders while providing the ability to better determine the true clinical relevance of these tests.

Importantly, no difference in pancreatic juice NGAL, MIC-1, or CA19-9 levels was found in NPNH or CP patients based on our exploratory analysis of patient demographics (Table 2). In PC patients, however, pancreatic juice MIC-1 levels correlated with patient age and gender. Consequently, though no multivariate analysis could be performed due to lack of sufficient patient numbers in each group, we believe the biomarker differences found between NPNH and CP patients are indeed due to the respective disease states. We are less confident, however, that data skewing due to confounders was not a factor in comparisons of MIC-1 levels in PC patients to those in other groups, though the matched patient age to the NPNH and CP patients and the matched gender distribution to the CP patients reduce this likelihood.

In our exploratory analyses, we also found that MIC-1 is higher in DM2-positive PC patients than in DM2-positive CP patients which approached statistical significance ($P = 0.082$) (Table 4). This point is of particular importance as MIC-1 serum levels have been previously found to be elevated in DM2 patients as compared to healthy controls, a comparison that

could not be evaluated in pancreatic juice through this study [32]. Additionally, DM2 and PC appear to have a potential synergistic effect on pancreatic juice MIC-1 levels as DM2-positive PC patients were found to have significantly higher levels than DM2-negative PC patients (Table 3). These results suggest that pancreatic juice MIC-1 levels might be useful in differentiating DM2 patients with PC from DM2 patients without PC ($P = 0.030$). The ability to make such a differentiation is of increasing importance as the link between DM2 and PC becomes better established.

Based on the results of our study, NGAL, MIC-1 and CA19-9 remain situationally promising markers in pancreatic juice as they may help narrow the potential etiologies of diseases with non-specific gastroenterological symptoms to being of pancreatic origin. Such utility carries high clinical relevance as it could allow patients to be more effectively stratified according to their risk of pancreatic and non-pancreatic diseases, thereby aiding in diagnostic decision making regarding employment of invasive tests for definitive diagnoses. However, it is unlikely that these biomarkers could be used to specifically distinguish between pancreatic diseases.

Despite the strengths of this study design, several weaknesses prevail. While the patient population considered as NPNH was clinically relevant in that all NPNH patients had symptoms resulting in pancreatic pathology being considered on their respective differential diagnoses, it is doubtless that not all diseases presenting with symptomatology mimicking pancreatic disease were represented in this group. As such, the true clinical specificity of pancreatic juice NGAL, MIC-1, and CA19-9 in diagnosing pancreatic disease cannot be determined. Additionally, any definitive conclusions regarding biomarker levels with respect to DM2 patients are hindered by the lack of ability to include the NPNH patient group in this analysis. Further, patient sera were not available for comparative analysis of the diagnostic utility of these markers across the two body fluids.

Per our present findings, future study using larger patient groups is warranted for the development of pancreatic juice NGAL, MIC-1 and CA19-9 testing in the differentiation of pancreatic disease and to further determine the existence of any correlation with patient diabetic status. Additionally, efforts should be made to compare the levels of these biomarkers in pancreatic juice to their respective levels in patient serum. In this manner, pancreatic juice may be evaluated for its superiority to serum as a source for biomarkers in the context of pancreatic disease and it can be determined if serum levels of the biomarkers reflect their levels in juice. Further, the results of this study should be combined with those of future studies assessing additional pancreatic juice biomarkers that are more closely associated with PC cells, such as mucins, in an attempt to improve the specificity of pancreatic juice biomarker testing in the context of pancreatic disease.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. Distribution of biomarkers analyzed in the pancreatic juice of non-pancreatic non-healthy, chronic pancreatitis, and pancreatic cancer patients

Distribution of pancreatic juice macrophage inhibitor cytokine-1 (MIC-1; A); neutrophil gelatinase–associated lipocalin (NGAL; B); and carbohydrate antigen 19-9 (CA19-9; C) of non-pancreatic non-healthy (NPNH) patients, chronic pancreatitis (CP) patients, and pancreatic cancer (PC) patients. NGAL and MIC-1 were measured by enzyme-linked immunosorbent assay (ELISA), while CA19-9 was measured by radioimmunoassay. MIC-1 and NGAL values are given in ng/mL, CA19-9 values are given in U/mL.

Figure 2. Comparison ROC curves examining the ability of tested biomarkers to discriminate between chronic pancreatitis, pancreatic cancer, and non-pancreatic non-healthy patients
 Receiver-operating characteristic curves examining the ability of macrophage inhibitor cytokine-1 (MIC-1); neutrophil gelatinase-associated lipocalin (NGAL); and carbohydrate antigen 19-9 (CA19-9) to differentiate A) non-pancreatic non-healthy (NPNH) patients from pancreatic cancer (PC) patients; B) chronic pancreatitis (CP) patients from PC patients; and C) CP patients from NPNH patients. Both NGAL and MIC-1 were found to be superior to CA19-9 in differentiating patients with pancreatic disease (PC and CP) from their NPNH counterparts.

Table 1

Biomarker Concentrations and Comparisons in NPNH, CP, and PC Patients

Median marker concentration (25th percentile, 75th percentile)								
Marker	NPNH (n=23)	CP (n=24)	PC (n=58)					
MIC-1 (ng/mL)	0.0 (0.0, 0.3)	0.5 (0.2, 0.8)	0.9 (0.3, 2.2)					
NGAL (ng/mL)	61 (34, 133)	173 (126, 307)	266 (144, 557)					
CA19-9 (U/mL)	0 (0, 3363)	3340 (116, 10111)	6471 (1171, 14207)					
Group Comparisons (ROC Curves)								
Groups	Marker	AUC (95% CI)	P value ^a	Sensitivity		Specificity		
				% (Fraction)	95% CI	% (Fraction)	95% CI	
CP vs. NPNH	MIC-1 (ng/mL)	0.76 (0.62–0.90)	<0.001	0.21	71% (17/24)	49%–87%	74% (17/23)	52%–90%
	NGAL (ng/mL)	0.88 (0.78–0.98)	<0.001	132.8	75% (18/24)	53%–90%	74% (17/23)	52%–90%
	CA19-9 (U/mL)	0.67 (0.51–0.82)	0.034	758.8	63% (15/24)	41%–81%	65% (15/23)	43%–84%
PC vs. NPNH	MIC-1 (ng/mL)	0.83 (0.73–0.93)	<0.001	0.29	78% (45/58)	65%–87%	78% (18/23)	56%–93%
	NGAL (ng/mL)	0.91 (0.84–0.98)	<0.001	138	79% (46/58)	67%–89%	83% (19/23)	61%–95%
	CA19-9 (U/mL)	0.76 (0.65–0.88)	<0.001	2221.7	84% (49/58)	73%–93%	52% (12/23)	31%–73%
PC vs. CP	MIC-1 (ng/mL)	0.63 (0.50–0.76)	0.043	0.63	57% (33/58)	43%–70%	58% (14/24)	37%–78%
	NGAL (ng/mL)	0.61 (0.48–0.74)	0.110	220.49	59% (34/58)	45%–71%	58% (14/24)	37%–78%
	CA19-9 (U/mL)	0.61 (0.48–0.74)	0.110	5868.2	55% (32/58)	42%–68%	58% (14/24)	37%–78%

AUC, area under the curve; CI, confidence interval; MIC-1, macrophage inhibitor cytokine-1; NGAL, neutrophil gelatinase-associated lipocalin; CA19-9, carbohydrate antigen 19-9; NPNH, non-pancreatic non-healthy; CP, chronic pancreatitis; PC, pancreatic cancer.

^aMann-Whitney U test.

^bCut-off value is the marker level that maximized the sensitivity and specificity averages.

NOTE. For estimation of sensitivity, chronic pancreatitis patients were considered to have disease and non-pancreatic non-healthy patients were considered to have no disease.

Table 2

Biomarker Concentrations in Pancreatic Juice Based on Patient Demographics

Characteristic	CA19-9 (U/mL)		NGAL-UNMC (ng/mL)		MIC-1 (ng/mL)	
	Median (IQR)	P value	Median (IQR)	P value	Median (IQR)	P value
NPNH patients (n = 23)						
Age, years ^a (median: 65)		0.26		0.30		0.66
< Median (n = 11)	581 (0–6621)		61 (34–78)		0.00 (0.00–0.29)	
Median (n = 12)	0 (0–3328)		54 (27–136)		0.03 (0.00–0.24)	
BMI ^a (median: 27)		0.18		0.76		0.72
< Median (n = 12)	0 (0–596)		50 (27–82)		0.03 (0.00–0.24)	
Median (n = 10)	918 (0–6549)		62 (45–134)		0.00 (0.00–0.05)	
Sex ^b		0.44		0.29		0.30
Male (n = 6)	918 (0–6549)		71 (59–134)		0.00 (0.00–0.05)	
Female (n = 17)	0 (0–3293)		56 (20–93)		0.02 (0.00–0.27)	
History of smoking ^b		0.58		0.77		0.57
No (n = 15)	0 (0–3363)		62 (34–133)		0.00 (0.00–0.27)	
Yes (n = 8)	506 (0–4921)		57 (24–102)		0.02 (0.00–0.24)	
Current alcohol use ^b		0.55		0.56		0.97
No (n = 9)	0 (0–6549)		63 (45–133)		0.02 (0.00–0.21)	
Yes (n = 14)	581 (0–1256)		56 (20–78)		0.01 (0.00–0.29)	
CP patients (n = 24)						
Age, years ^a (median: 64)		0.28		0.49		0.67
< Median (n = 12)	5144 (0–10111)		168 (111–269)		0.52 (0.19–0.72)	
Median (n = 12)	2634 (462–8975)		210 (141–367)		0.55 (0.11–0.92)	
BMI ^a (median: 27)		0.65		0.95		0.65
< Median (n = 11)	1387 (0–9070)		178 (137–250)		0.61 (0.25–0.76)	
Median (n = 10)	6266 (0–11153)		242 (90–419)		0.49 (0.14–1.54)	
Sex ^b		0.64		0.35		0.88
Male (n = 16)	3923 (603–8828)		208 (126–367)		0.49 (0.18–0.83)	
Female (n = 8)	1940 (0–13416)		153 (118–229)		0.58 (0.15–1.11)	

Characteristic	CA19-9 (U/mL)		NGAL-UNMC (ng/mL)		MIC-1 (ng/mL)	
	Median (IQR)	P value	Median (IQR)	P value	Median (IQR)	P value
History of smoking ^b		0.88		0.35		0.98
No (n = 8)	2634 (257–8501)		276 (118–716)		0.67 (0.01–0.92)	
Yes (n = 16)	4398 (116–10111)		164 (126–266)		0.46 (0.23–0.78)	
Current alcohol use ^b		0.98		0.34		0.47
No (n = 14)	2634 (514–7487)		223 (137–413)		0.61 (0.22–0.86)	
Yes (n = 10)	5693 (0–11955)		153 (114–250)		0.37 (0.04–0.76)	
PC patients (n = 58)						
Age, years ^a (median: 68)		0.86		0.11		0.03
< Median (n = 29)	6230 (1453–11921)		226 (143–477)		0.49 (0.20–1.16)	
Median (n = 29)	6627 (368–14207)		304 (157–876)		1.26 (0.59–2.30)	
BMI ^a (median: 26)		0.36		0.90		0.07
< Median (n = 30)	7418 (1163–26086)		266 (143–535)		1.11 (0.36–2.82)	
Median (n = 24)	6429 (1447–11577)		266 (173–642)		0.52 (0.17–1.18)	
Sex ^b		0.40		0.53		0.03
Male (n = 35)	8100 (1453–14059)		282 (144–557)		1.26 (0.36–2.86)	
Female (n = 23)	4557 (70–18248)		214 (111–600)		0.57 (0.17–0.98)	
History of smoking ^b		0.71		0.24		0.76
No (n = 27)	8111 (1139–18248)		331 (119–1310)		0.76 (0.18–2.30)	
Yes (n = 29)	6314 (1626–11234)		225 (144–337)		0.98 (0.36–1.81)	
Current alcohol use ^b		0.76		0.16		0.37
No (n = 30)	7247 (1171–13423)		306 (122–1310)		0.68 (0.17–2.08)	
Yes (n = 26)	7207 (1453–21033)		223 (144–337)		0.98 (0.43–2.1)	

CA19-9, carbohydrate antigen 19-9; NGAL, neutrophil gelatinase associated lipocalin; MIC-1, macrophage inhibitor cytokine-1; IQR, interquartile range;

BMI, body mass index; NPNH, non-pancreatic non-healthy; CP, chronic pancreatitis; PC, pancreatic cancer.

^aP values obtained using Kendall's correlation test.

^bP values obtained using Wilcoxon rank sum test.

Table 3

Pancreatic Juice Biomarker Levels in CP and PC Patients with a History of Diabetes Mellitus Type 2 (DM2)

DM2 status	CA19-9 (U/mL)		NGAL (ng/mL)		MIC-1 (ng/mL)	
	Median (IQR)	P value ^a	Median (IQR)	P value ^a	Median (IQR)	P value ^a
CP patients (n = 24)		0.23		0.67		0.97
No (n = 18)	4937 (692–11153)		193 (114–314)		0.52 (0.14–0.86)	
Yes (n = 6)	373 (0–5046)		158 (144–238)		0.55 (0.22–0.76)	
PC patients (n = 58)		0.17		0.25		0.03
No (n = 47)	5898 (1171–14059)		256 (138–557)		0.61 (0.18–1.64)	
Yes (n = 11)	11234 (6314–26086)		297 (219–910)		1.81 (0.76–3.08)	

Table 4

Pancreatic Juice Biomarker Levels in CP and PC Patients with a History of Diabetes Mellitus Type 2 (DM2)

Marker	CP (n = 6)	PC (n = 11)	P value ^a
CA19-9 (U/mL)	373 (0, 0, 5047, 11955)	11234 (0, 6314, 26086, 85573)	0.067
NGAL-UNMC (ng/mL)	158 (90, 144, 238, 419)	297 (143, 219, 910, 3495)	0.082
MIC-1 (ng/mL)	0.55 (0.00, 0.22, 0.76, 2.31)	1.81 (0.32, 0.76, 3.08, 16.64)	0.082

NOTE. Data are presented as the sample median (minimum, 25th percentile, 75th percentile, maximum

CA19-9, carbohydrate antigen 19-9; NGAL, neutrophil gelatinase associated; MIC-1, macrophage inhibitor cytokine-1.

^aWilcoxon rank test.