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None

Biomarkers of Chronic Pancreatitis: A Systematic Literature Review

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Abstract

Background: Chronic pancreatitis (CP) does not have diagnostic or prognostic biomarkers. CP is the end stage of a progressive inflammatory syndrome that is diagnosed at late stages by morphologic features. To diagnose earlier stages of the disease, a new mechanistic definition was established based on identifying underlying pathogenic processes and biomarker evidence of disease activity and stage. Although multiple risk factors are known, the corresponding biomarkers needed to make a highly accurate diagnosis of earlier disease stages have not been established. The goal of this study is to systematically analyze the literature to identify the most likely candidates for development into biomarkers of CP.

Methods: We conducted a systematic review of candidate analytes from easily accessible biological fluids and identified 67 studies that compared CP to nonpancreatic-disease controls. We

then ranked candidate biomarkers for sensitivity and specificity by area under the receiver operator curves (AUROCs).

Results: Five biomarkers had a large effect size (an AUROC > 0.96), whereas 30 biomarkers had a moderate effect size (an AUROC between 0.96 and 0.83) for distinguishing CP cases from controls or other diseases. However, the studies reviewed had marked variability in design, enrollment criteria, and biospecimen sample handling and collection.

Conclusions: Several biomarkers have the potential for evaluation in prospective cohort studies and should be correlated with risk factors, clinical features, imaging studies and outcomes. The Consortium for the Study of Chronic Pancreatitis, Diabetes and Pancreas Cancer provides recommendations for avoiding design biases and heterogeneity in sample collection and handling in future studies.

Keywords

Pancreatitis; chronic pancreatitis; biomarker; PRoBE strategy; early detection

Introduction

Biomarkers are objective measures that can be indicators of normal biological or pathogenic processes or responses related to therapeutic interventions for a particular disease.[1] To date, there are no reliable diagnostic, prognostic, or therapeutic biomarkers for chronic pancreatitis (CP).[2] CP is characterized by chronic inflammation and progressive fibrosis of the pancreas, with loss of acinar cell mass. This leads to irreversible morphologic changes, loss of pancreatic function, and increased risk of pancreatic cancer.[3–5] CP, like many chronic diseases, is defined by the consensus criteria of experts as a clinicopathologic syndrome with characteristic clinical, imaging, pathological, and functional features.[6–8] Unfortunately, the detection of early-stage CP has remained elusive due to a poor understanding of the pathogenesis of CP and the nonspecific findings on endoscopic and radiologic imaging.[9–11] Although detection of early-stage CP is a research challenge, it represents an opportunity for innovative CP research and discovery in the 21st century.

Current diagnostic methods for CP are highly accurate for moderate to advanced disease and include abdominal radiographic imaging,[12] endoscopic procedures (EUS), and functional testing methods, including measurement of analytes in pancreas fluid after secretin or cholecystokinin stimulation.[13–15] However, none of these testing methods are suitable for early-stage CP diagnosis in isolation.[16] Often, a confident diagnosis of CP is not confirmed until end-stage clinical features are evident, indicating moderate to severe fibrotic changes of the pancreas gland.[13, 17–19] Therefore, the lack of accurate methods for the early diagnosis of CP impedes patient evaluation and limits the development of clinical trials of potential new CP therapies, which may alter the natural course of the disease. The development of accurate diagnostic “early-stage” CP biomarkers would create the opportunity to test the effectiveness of repurposed or new antifibrotic, antioxidant, and/or anti-inflammatory drug therapies for CP.[20–24] Furthermore, the development of accurate prognostic biomarkers could predict the development of end-stage CP complications, such as diabetes, exocrine insufficiency, bone disease, or pancreatic cancer, facilitating the development of strategies to

block, retard, or slow disease progression. Thus, the lack of successful biomarker development in CP research has remained an elusive target for decades and represents a major research gap in our knowledge.[25]

The Adult Chronic Pancreatitis Working Group of the Consortium for the Study of Chronic Pancreatitis Diabetes and Pancreas Cancer (CPDPC) established a Biospecimen Working Group, which includes a Biomarker Subcommittee devoted to addressing the research gaps related to biomarker discovery and validation in CP studies.[26] This paper represents our first step towards the development of accurate CP diagnostic and prognostic biomarkers. Our primary goal was to systematically review all promising candidate biomarkers of CP described in previous studies. For each biomarker identified, we evaluated the stage of biomarker development as defined by the prospective-specimen-collection, retrospective-blinded-evaluation (PRoBE) design method[27–30] and the biomarkers' quantitative diagnostic performance based on the area under the receiver operator curve (AUROC) and other metrics. Our secondary goal was to identify which candidate biomarkers merit further testing and validation using biospecimens (whole blood, urine, saliva, pancreas fluid, and/or pancreas tissue) that are being prospectively collected in the PROspective Evaluation of Chronic Pancreatitis for Epidemiologic and Translational Studies (PROCEED).[27] Among the types of biomarkers evaluated were adipokines, amino acids or other intermediary metabolites, lipoproteins, chemokines, cytokines, microRNAs, extracellular matrix proteins, and glycoproteins.

Methods

Medical literature databases (PubMed and Scopus) were searched from what was available until August 2018 using multiple search strategies, including the search terms "pancreatitis," "chronic pancreatitis," "biomarker," and "diagnosis." Based on these searches, 743 articles that potentially included biomarkers of CP were identified and underwent preliminary review. Of these, 234 articles reported CP-biomarker assays and were reviewed in detail. Sixty-seven of these articles reported quantitative results of biomarker assays in a human biological fluid (whole blood, serum, plasma, buffy coat, urine, saliva, stool, or pancreas fluid) of cases with CP compared to a normal or benign-disease control group and were included in the final analysis. (Figure 1). Animal studies and data comparing CP to pancreatic ductal adenocarcinoma (PDAC) were not included. Studies assessing tissue immunohistochemistry were included only if there were also data available regarding the performance of the same biomarker in a human biological fluid. Data abstracted from these articles included the potential biomarker being studied, the assay used to measure the biomarker of interest, type of biofluid assayed, definitions of the subject groups included in the study, sample sizes, and quantitative data regarding biomarker performance (mean, standard deviation, and/or standard error of the mean, median, or other quantiles, interquartile ranges, minimum, maximum, sensitivity, specificity, AUROC, and *p*-values from multigroup comparison).

The discrimination ability of a biomarker diagnostic test, that is, its ability to separate various phenotypic groups, arises from its different distribution among these groups and is of central importance to a diagnostic test evaluation. Although the AUROC (or sensitivity/

specificity) is widely used as a standard measure of discrimination,[31, 32] it was not reported in all the publications reviewed in this study. Even fewer studies reported the receiver operating characteristic (ROC) curve graphically. Various publications reported different summary statistics that characterize certain aspects of biomarker distributions, such as the mean, standard deviation, *p*-value from a test of means, Bayes factor, etc. Not all of these statistics have the same clinically relevant interpretability as the ROC curve or AUROC, which makes it difficult to compare biomarkers' discrimination abilities across studies. In order to compare the discrimination ability of different biomarkers, we estimated the AUROC if it was feasible to do so with the published statistics in the articles that did not report AUROC. The estimation was based on a binormal model (i.e., assuming the biomarker is normally distributed in the cases and control groups), which is widely used in diagnostic medicine.[31, 32] Normalization transformation was used when the published statistics suggested skewness in the data. The AUROC can be estimated from the binormal model when the mean and standard deviation are reported for the cases and controls or when the median, Q25 (25th quartile), and Q75 are reported. When the publication reported minimum and maximum values of the biomarker, we used them as Q10 and Q90 in the estimation. Because the estimated AUROCs are dependent on the binormal model assumption, our tables annotate them differently from the published AUROC results (estimated AUROCs are in bold font). We further classified the biomarker into one of four effect-size (discrimination ability) categories: large if the AUROC was greater than 0.96, moderate if the AUROC was between 0.96 and 0.83, modest if the AUROC was less than 0.83 and a significant *p*-value was reported, and undetermined/no effect for all others. Under a binormal model, the cutoff of 0.96 implies that the interval (Q10, Q90) of the two comparison groups does not overlap; the cutoff of 0.83 implies that the range (Q25, Q75) of the two comparison groups does not overlap (Figure 1). The level of discrimination is on a continuous spectrum, and this categorization is chosen for convenience of discussion in this paper.

Results

There was wide variation in the study populations included in the 67 articles reviewed. Most studies defined CP on the basis of unequivocal imaging and/or functional changes, but there was substantial variation in the classification systems used and in the proportion of CP cases that were attributed to alcohol. In some studies, no objective definition of CP was provided. The control groups also varied, which included healthy controls in some studies and abdominal pain patients deemed not to have pancreatic disease in others.

Overall, we analyzed the selected studies that investigated biomarker levels in human biofluids (Figure 2A) or tissues (Figure 2B) and grouped them based on their AUROCs to distinguish CP cases from healthy or benign-disease control group. We found that of the potential biomarkers analyzed from biofluids, five had a large effect size, 25 had a moderate effect size, 33 had a modest effect size, and 18 had no effect (Figure 2A). Moreover, we found that a subset of these potential biomarkers was also analyzed in tissues, for which none had a large effect size, five had a moderate effect size, and four had a modest effect size (Figure 2B). All the biomarkers analyzed from biofluids and tissues are summarized in Table 1 by analyte/biomarker category and in alphabetical order. The table provides

information regarding the following: the type of biospecimen assayed, the number of control and CP cases, the AUROC reported or imputed, the *p*-value reported in the publication, the determined effect size, any special comments regarding the comparison, and references used for the determination. In instances where we were unable to impute AUROCs or could not make an informed judgement on the effect size of a particular potential biomarker the field in the table remains blank. Since some potential biomarkers were identified in multiple papers, we provided information on all the sample types, the range of "n", AUROCs, and *p*-values provided from all the papers that mentioned each biomarker.

Most of the studies compared CP cases to control cases as defined in the study. However, several studies included control cases with non-ulcer dyspepsia or chronic upper abdominal pain that were deemed by the investigators not to have pancreatic disease.[33–39] Between the biofluids and tissue biomarkers, we identified 30 potential biomarkers with a moderate effect size and five potential biomarkers with a large effect size. In addition to the effect size of the biomarkers, we examined other features of the published studies against the PRoBE study design, such as the presence of a validation set. Among the studies reviewed, we found that only one included a validation set.[40] Additionally, after careful analysis of the study methodologies used in the articles, we determined that all the studies fell into the Phase 1 category (initial discovery studies), that is, the first of the five biomarker-development phases defined by the PRoBE design.[29]

We found several studies that used the Bayes factor as the measure of distribution difference between cases and controls. The Bayes factor can be viewed as the Bayesian equivalent of the frequentist *p*-value. It is a positive number, defined as the ratio of the likelihoods under the null and alternative hypotheses. A Bayes factor that deviates from 1 indicates departure from the null hypothesis. In two studies, a Bayes factor greater than 10 was used as the criterion for differential protein expression between the comparison groups.[12, 41] The Bayes factor can be used with sample sizes that are even smaller than those typically required by two-sample tests and that are hence suitable for small, pilot Phase 1 studies. The Bayes factor generates initial evidence for differential biomarker distributions among the comparison groups but does not provide the same clinically relevant interpretation as sensitivity and specificity. Because these studies were relevant to our analysis and their Bayes factors could not be converted into *p*-values or AUROCs, we could not directly compare them to the studies represented in Table 1; they are listed in Supplemental Table 1. In addition, we excluded one study of potential proteomic biomarkers that reported biomarker performance characteristics using a "leave-one-out" methodology.[40] This study reported a biomarker panel AUROC of 1.0, suggesting perfect sensitivity and specificity. We were unable to determine the effect of the statistical methodology used on potential future clinical reporting of biomarker values. However, it will be of interest to validate the methodology reported in this study following the PRoBE design.

Discussion

The diagnosis of earlier stages of CP remains difficult because current diagnostic tests are specific for CP only when morphologic features are more apparent on imaging from late stages of CP or there is loss of pancreatic function.[10] The aim of this systematic literature

review was to identify potential biomarkers in the medical literature that merit further investigation for their ability to diagnose definitive CP as defined by advanced to moderate-severe changes on imaging. In contrast to other published reviews of CP biomarkers,[42] this review focused on a quantitative analysis of available data to identify promising biofluid biomarkers worthy of further development and validation.

All the studies we identified fit the definition of Phase 1 biomarker development as per the PRoBE strategy.[29] This means that the studies were exploratory in nature and focused on biomarker discovery, not on validation of the proposed biomarker.[40] No proposed human CP biomarkers have been adequately validated using a clinical assay in separate discovery and validation cohorts (Phase 2) or tested for their ability to diagnose early or even preclinical disease using the PRoBE strategy (Phase 3).[30] Although the current literature contains only Phase 1 studies, we were able to identify promising biomarkers on the basis of their apparent relative effect sizes. Below, we highlight the five biomarkers that had a large effect size using biofluids; these merit further investigation in additional Phase 1 studies utilizing rigorous sample collection and processing techniques and Phase 2 studies. Although these biomarkers had a large effect size, it may still be worthwhile to investigate the potential biomarkers that had a moderate effect size, particularly in combination with other potential biomarkers.

Adenosine:

Adenosine is a metabolite of ATP hydrolysis and, as such, is elevated in conditions of metabolic stress caused by disease. In patients with CP, adenosine levels were significantly increased in the urine compared to healthy controls in a study comparing urinary metabolomics using a ^{1}H-NMR (proton nuclear magnetic resonance) assay.[43] In addition to CP, this study included a group of patients with mild acute pancreatitis (AP). Although the data indicated that urine metabolites could not differentiate between AP and CP, the groups were small ($n = 5$) and the risk of a type II error was high. Therefore, validation of adenosine as a CP-specific biomarker needs to include comparisons between healthy controls, CP, and AP with larger cohorts.

Adiponectin:

Adiponectin is an anti-inflammatory adipokine that is secreted mainly from adipocytes and can reduce the secretion of many pro-inflammatory cytokines.[44] However, the adiponectin data reviewed had heterogeneous results; that is, in terms of differentiating CP from healthy controls, adiponectin was not effective in some studies.[45–47]Gasiorowska et al.[47] found that plasma adiponectin levels were significantly elevated in both CP and PDAC patients compared to normal controls, but no difference was found between the CP and PDAC groups. Another study found that serum levels of adiponectin were higher only in PDAC patients, compared to CP and control.[48] Validation of adiponectin should include a PDAC comparison group to determine its ability to differentiate between healthy controls, CP, and PDAC cases.

Des-Leu albumin:

Des-Leu albumin is a truncated form of serum albumin that lacks the C-terminal leucine residue, likely due to the action of pancreatic carboxypeptidase-A. The des-Leu form of albumin was found to comprise 68% of circulating albumin in patients hospitalized with CP versus 5% in control patients. This form of albumin appears to have a longer serum half-life; however, samples were obtained from patients hospitalized with acute flare-ups of pancreatitis.[49] Therefore, studies of albumin and its truncated variants as a potential biomarker for CP should focus on determining specificity for CP in stable outpatients, should include clinically relevant control groups, and need to assess for potential confounding factors due to acute inflammation.

Interleukin 6 (IL-6):

IL-6 is a pro-inflammatory cytokine produced by many cell types, including macrophages and adipocytes. IL-6 levels are often elevated secondary to infection, acute or chronic inflammation, and cancer. Results of IL-6 as a CP biomarker were varied, as shown in Table 1. Heterogeneity may have been introduced due to varying definitions of cases and controls, confounding from acute inflammation (e.g., acute pancreatitis), different detection limits of IL-6 assays, and IL-6 gene polymorphisms.[50] Circulating IL-6 levels are also influenced by PDAC and acute alcohol ingestion, so these variables should be considered in future analyses.[51, 52]

Oxidized fatty acids:

Oxidized fatty acids are generated in response to increased oxidative stress and may also play a role in the pathogenesis of CP. One small study using serum samples from 16 subjects (six with mild CP, five with severe CP, and five controls) and pancreatic fluid samples from 18 subjects (nine with mild CP, nine controls) identified elevated levels of several oxidized fatty acids in patients with mild and severe CP.[33] In our analyses, the large effect observed came from findings related to differentiating severe CP from healthy controls, which is not helpful for the diagnosis of early-stage CP. These levels also correlated with the severity of the EUS findings. Large differences between CP and healthy controls were reported for the arachidonic-acid-based 5-HETE:AA, 11-HETE:AA, and 15-HETE:AA. Because this was a small pilot study, a larger validation study would be needed, and it is unclear whether oxidized fatty acids could distinguish early CP and from relevant controls.

As outlined above, several promising CP-biomarker candidates that warrant further investigation were identified in the literature. Some of the biomarkers found to have moderate effect sizes could also be explored further. However, many of the articles we reviewed had methodological limitations that limit the certainty and generalizability of their findings. For example, there was a large variation in the sample size used in each study. Since many of these studies did not provide a sample size power calculation, the actual value of each biomarker for the detection of CP still needs to be determined through validation studies. Clinical definitions of CP varied across most of the studies and often relied on the judgement of the investigators rather than the guidelines of an established professional society.[5, 10, 53] Baseline phenotypic definitions and characteristics for the control and CP cases were provided in only a fraction of the studies. In addition, many studies included only

limited information regarding the characteristics of the control groups and did not match controls to CP subjects in terms of age and/or gender. Most studies did not indicate the percentage of patients suffering from episodes of acute pancreatitis or the length of time between the most recent episode of acute pancreatitis and the time point of the biospecimen acquisition. The biospecimen collection protocols often did not have a standard operating procedure that included important information such as the time from collection to processing and storage, centrifuge speeds and times, duration of sample storage, or the number of freeze–thaw cycles. In some cases, neither the AUROC nor biomarker diagnostic specificity and sensitivity were reported (see Supplemental Table 1), and in some reports no quantitative data was provided, with results presented in graphical form only. Many of the statistical tests used in the studies reviewed, such as *t*-tests, compared the mean biomarker levels between cases and controls. However, a difference in the means, regardless of statistical significance, does not necessarily translate into adequate discrimination. Finally, only a few studies reported results from benign-disease control groups that represent important differential diagnoses for early CP, such as patients with abdominal pain, peptic ulcer disease, non-ulcer dyspepsia, and functional bowel disease.

To successfully identify and develop CP biomarkers, best practices and standardized guidelines should be followed regarding study design, sample selection, sample size determination, analytical methods,[30, 54] and presentation of results, even in exploratory studies. This will minimize common research biases and reduce the likelihood of false-positive findings in the early phases of biomarker discovery studies. We recommend that researchers clearly state case definitions of CP and control groups studied, using sample sizes of matched disease and controls large enough to adequately power the study and achieve reasonably precise confidence intervals.[55, 56] A biomarker's diagnostic performance should be reported in terms of the sensitivity, specificity, AUROC, and ROC curve. Moreover, data should be presented in both quantitative and graphical formats, ideally depicting median, interquartile range, and outliers.[57–59] Biospecimens should be collected and processed following a standard operating procedure and should be well annotated with clinical information. The PRoBE strategy is a useful guide for performing the tasks described above.[27–30]

Advances are being made in the field of pancreatic disease, in part due to recent NIH-sponsored grants, workshops, and symposia focused on outlining research gaps and defining funding opportunities for innovative investigators and collaborative teams.[20, 60–62] In regard to standardizing CP definitions, the following mechanistic definition of CP has been developed by Whitcomb and colleagues: "chronic pancreatitis is a pathologic fibro-inflammatory syndrome of the pancreas in individuals with genetic, environmental, and/or other risk factors who develop persistent pathologic responses to parenchymal injury or stress."[5] With this new definition, early diagnosis may be possible based on a combination of risk factors and selected biomarkers of disease activity and/or progression, once those biomarkers have been validated. Currently, the best method of validating biomarkers for diagnosis of early CP is to prospectively collect biospecimens from a large group of patients with suspected CP who lack definitive imaging or functional findings and to obtain a chronological follow-up of the cohort. This will permit retrospective identification of patients who progressed to unequivocal CP. Testing these subjects' stored biospecimens can

then be performed to identify biomarkers of early disease. Identifying mechanistic dysfunctional pathways can help define or stratify populations in which various biomarkers might have more utility. Thus, utilization of a CP-biomarker test of even moderate accuracy in these risk-stratified groups might markedly enhance diagnostic accuracy, improve the precision of pre- and post-test probabilities of having disease, and free diagnostic testing from relying solely on advanced imaging criteria.[5]

In regard to standardization in biospecimen collection, the NIH-sponsored PROCEED study[27] within the CPDPC is currently collecting biospecimens from patients with known or suspected CP and nonpancreatic-disease controls, utilizing detailed, published standard operating procedures.[28] PROCEED is the first prospective, longitudinal observational cohort study of CP in the United States. The study is innovative in several ways: it enrolls subjects representing the complete clinical spectrum of acute to chronic pancreatitis, and it establishes a robust biorepository of longitudinally collected samples consistent with the accepted principles of the PRoBE strategy to support translational studies, including biomarker testing.[29, 30] At the time of this writing, PROCEED has enrolled over 1,350 subjects. One of the main goals of PROCEED is to develop a platform for conducting biomarker studies using clinical information and longitudinally collected biospecimens. The detailed phenotyping of the PROCEED cohort, along with its stringent biospecimen collection and handling procedures, addresses many of the methodological limitations and biases that frequently hamper biomarker studies in the current literature. Developing large, well-defined biorepositories like this will allow for robust and efficient validation (Phase 2) and diagnostic (Phase 3) biomarker studies.[56]

In conclusion, the detection of earlier stages of CP has remained elusive due to a poor understanding of pathogenic mechanisms and the dependence on obvious morphologic changes on radiologic and endoscopic imaging.[60] Moreover, numerous methodological issues have hampered the search for CP biomarkers. Recent advances in our understanding of the etiologies, risk factors, genetic alterations, and fibro-inflammatory changes observed in CP has clarified our understanding of “at risk” patient populations and mechanistically defined CP phenotypes.[5] The NIH-funded PROCEED study developed by the Chronic Pancreatitis Working Group of the CPDPC has established a robust biorepository of well-annotated CP samples in alignment with the guiding principles of the PRoBE strategy.[27, 28] The platform has now been established for the pancreas community to conduct robust investigations in CP-biomarker discovery and validation.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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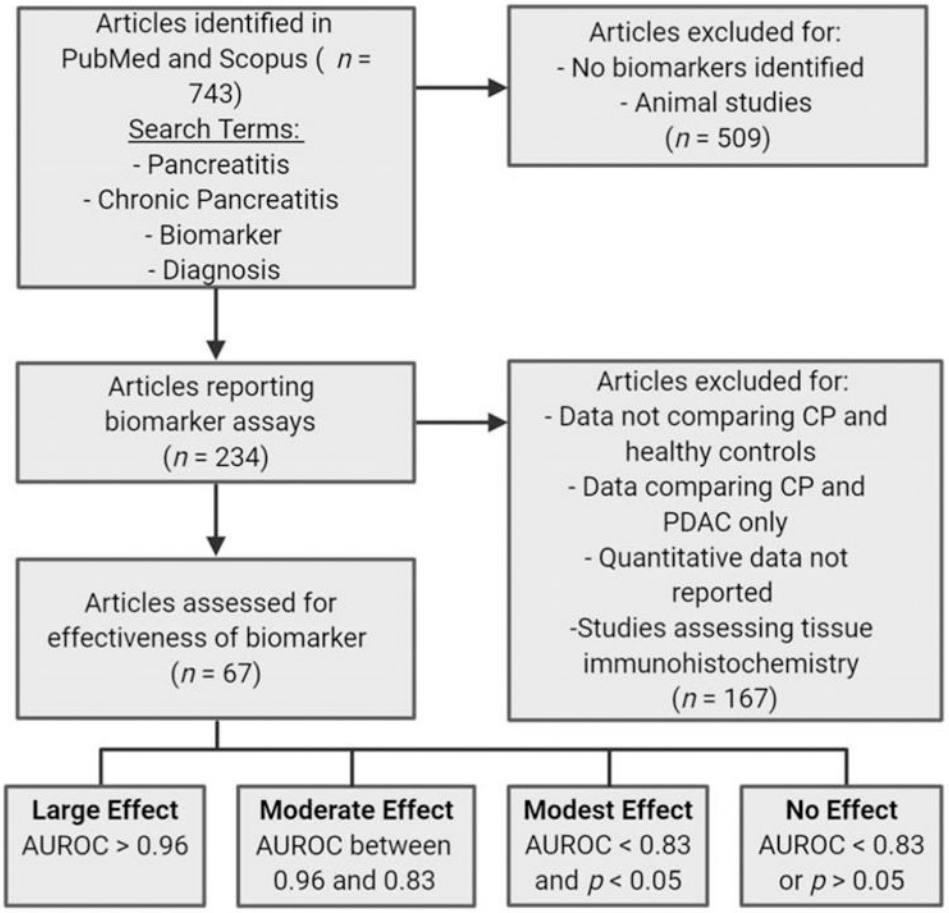

Figure 1.
Flow diagram of literature search strategy.

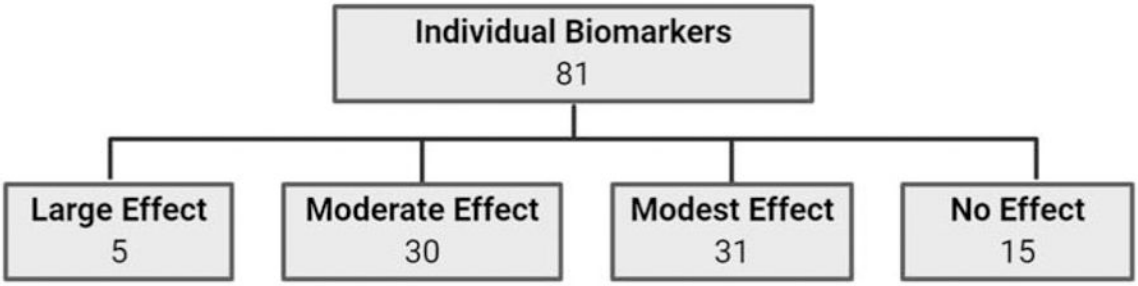

Figure 2.
Summary of effect sizes of individual biomarkers based on AUROCs. The highest effect size is used for biomarkers with heterogeneity across studies.

Table 1.

The determined diagnostic biomarker effect size for distinguishing between control and CP cases in biological fluids. The *p*-values presented were reported in the original publications from various statistical tests for between-group difference. The AUROC values, shown in **bold font**, were imputed from published data, as described in the methods section.

Biomarker	Biospecimen Type	n (Control)	n (CP)	AUROC	p-value	Effect Size	Comments	Ref(s)
Adipokines								
Adiponectin	Plasma, serum	13–30	27–44	**0.514–0.994**	NS–<0.0003	None - Large	Heterogeneity across study results: two studies of serum with null results, one study with plasma showing large effect size	45, 46, 48
Leptin	Serum	16–30	30–44	**0.788–0.803**	<0.05-<0.01	Modest		45, 48
Neutrophil gelatinase-associated lipocalin (NGAL)	Pancreatic juice	23	24	0.88	<0.001	Moderate		63
Resistin	Serum	16–78	23–81	**0.865**	<0.001	Moderate	May distinguish between RAP and CP	64, 65
Chemokines								
Chemokine ligand 5 (CCL5)	Plasma, serum	20	20	0.92	<0.0001	Moderate		66
Chemerin	Serum	40	68	**0.746 (DM), 0.787 (non-DM)**	<0.01	Modest		67
C-reactive protein (CRP)	Serum	28–70	14–45	**0.513 –0.828**	<0.05	Modest		68, 69
Fractalkine	Serum	15–116	78–109	**0.898**	0.011 to <0.0001	Modest - Moderate	May be elevated in earlystage disease	70, 71
Fractalkine (CX3CR1)	Pancreatic tissue	21	61	**0.736**	<0.001	Modest	Correlates with pain and degree of inflammation	72
Monocyte chemoattractant protein-1 (MCP-1)	Plasma, serum, whole blood	15–88	78–142	**N/A–0.943**	NS-<0.001	None – Moderate	Heterogeneity across study results	64, 70, 73
Platelet basic protein (PPBP)	Plasma, serum	20	20	0.92	<0.001	Moderate		66
Cytokines								
Cytokine array	Serum	30	16	0.71	-	-		74
Endothelin-1	Serum	13–26	24–39	**0.711–0.720**	NS	None	Correlated with tobacco use (AUROC 0.7) rather than CP	47, 75
Interleukin-1β (IL-1β)	Plasma, serum	18–31	27–33	N/A to **0.777**	NS-<0.001	None – Modest	Heterogeneity across study results	47, 76
Interleukin-6 (IL-6)	Pancreatic juice	3–41	3–39	N/A to 0.64	NS–0.01	None – Modest	AUC is for study with larger *n*	34, 77

Biomarker	Biospecimen Type	n (Control)	n (CP)	AUROC	p-value	Effect Size	Comments	Ref(s)
Interleukin-6 (IL-6)	Plasma, serum	8–72	8–56	**0.507–0.997**	NS-<0.001	None – Large	Heterogeneity across study results: only one study shows more than a modest effect size.	47, 50–52, 68, 78, 79
Interleukin-8 (IL-8)	Serum	45	49	-	<0.05	Modest		79, 80
Interleukin-8 (IL-8)	Pancreatic juice	3–41	3–39	0.82	NS–0.011	Modest	AUC is from study with larger *n*	34, 77
Interleukin-8 (IL-8)	Pancreatic tissue	4	4	-	<0.05	Modest	Unable to estimate AUROC from data provided	80
Interleukin-10 (IL-10)	Serum	30	39	**0.614**	0.058	None		81
Interleukin-18 (IL-18)	Serum	30	29	**0.764**	<0.005	Modest		81
Cytokine array	Serum	30	16	0.71	-	-		74
Macrophage inhibitory cytokine 1 (MIC-1)	Plasma	24–500	23–50	**0.508–0.875**	NS-<0.001	None – Moderate	Heterogeneity across study results	63, 82
Platelet-derived growth factor-AA (PDGF-AA)	Serum	28	61	**0.612**	0.071	None		83
Platelet-derived growth factor-BB (PDGF-BB)	Serum	35–40	40–60	**0.732**	<0.01-<0.001	Modest		67, 84
Transforming growth factor-α (TGF-α)	Pancreatic juice	3	3	-	0.176	None	CP subjects had end-stage clinical disease	77
Transforming growth factor-α (TGF-α)	Pancreatic tissue	5	12	-	<0.01	Modest	Unable to estimate AUROC from data provided	85
Transforming growth factor-β1 (TGF-β1)	Pancreatic juice	41	39	0.55	0.4	None		34
Transforming growth factor-β1 (TGF-β1)	Plasma, serum	11–116	10–109	**0.513–0.913**	0.3-<0.0001	None – Moderate	Heterogeneity across study results	51, 64, 69–71, 83, 84
Tumor necrosis factor-α (TNF-α)	Plasma, serum	100	71–100	**0.654–0.665**	<0.014	Modest		69, 86
Vascular endothelial growth factor (VEGF)	Serum	24–50	10–72	**0.735**	0.031-<0.001	Modest		87, 88
Extracellular Matrix Proteins								
Hyaluronic acid	Pancreatic juice	20	20	**0.95**	<0.01	Moderate		89
Hyaluronic acid	Serum	15–78	78–81	**0.688–0.733**	<0.001	Modest		64, 70
MAC-2 binding protein (M2BP)	Serum	30–59	74–162	0.727-**0.788**	<0.001-<0.0001	Modest	Levels correlated with morphologic severity of CP and were highest in patients with severe disease; AUC is for mild CP group	90, 91
Glycoproteins								

Biomarker	Biospecimen Type	n (Control)	n (CP)	AUROC	p-value	Effect Size	Comments	Ref(s)
Intercellular adhesion molecule 1 (ICAM1)	Plasma, serum	20	20	0.92	<0.001	Moderate		66
Lactotransferrin (LTF)	Plasma	41	52	0.73–092	0.084-<0.0001	None - Moderate		66
Thrombospondin 1 (THBS1)	Plasma, serum	20	20	0.92	<0.001	Moderate		66
Tissue inhibitor of metalloproteinases 1 (TIMP-1)	Plasma, serum	10–86	23–48	**0.503**	0.98-<0.05	None - Modest	Heterogeneity across study results; imputed AUROC is from study with largest *n*	38, 92, 93
Lipoproteins								
Apolipoprotein 2 (APOA2)	Plasma	41	52	0.65	0.199	None		66
High density lipoprotein-c (HDLc)	Serum	40	48	**0.647**	0.004	Modest		94
Paraoxonase 1 (PON1)	Plasma	132	186	**0.586**	<0.001	Modest		95
Zinc-α−2-glycoprotein (AZGP1)	Plasma, serum	20	20	0.9292	<0.0001	Moderate		66
Metabolites								
4-hydroxynonenal (4-HNE)	Plasma	27	105	-	<0.05	Modest	Twofold elevation in CP; unable to impute AUROC from presented data	96
Citrate	Urine	5	5	**0.948**	0.048	Moderate		43
Malondialdehyde (MDA)	Plasma	27	105	-	<0.05	Modest	Six fold elevation in CP; unable to impute AUROC from presented data	96
Methionine	Plasma	48	90	**0.807–0.878**	<0.001	Modest - Moderate	Mean plasma methionine levels almost two times lower in CP	65
Oxidized fatty acid 13:HODE:LA	Serum	5	5	**0.959**	0.008	Moderate	Values shown for “severe CP” vs. control	33
Oxidized fatty acids: 5-HETE:AA, 11-HETE:AA, 15HETE:AA, 9-HODE:LA, 9oxoODE:LA, 13-oxoODE:LA	Serum	5	5	**0.877–1**	0.03-<0.001	Moderate – Large	Values shown for “severe CP” vs. control	33
Phosphatidylcholine 18:2n-6	Plasma	108	96	**0.875**	<0.001	Moderate	Fatty acid deficiencies also occur in luminal GI diseases and PDAC	97
Phosphatidylcholines 16:1n–7, 18:1n–9, 18:1n–7, 18:3n6, 22:4n6, 22:5n–6, 22:6n–3, D9D16, D5Dn6	Plasma	108	96	**0.586–0.750**	<0.001	Modest		97
Thiobarbituric acid-reactive substances (TBARS)	Serum	28	57	**0.865**	0.001	Moderate	10-fold differences in median values	83
Metal Binding Proteins								

Biomarker	Biospecimen Type	n (Control)	n (CP)	AUROC	p-value	Effect Size	Comments	Ref(s)
Core-fucosylated haptoglobin	Serum	59	159	0.897	0.0001	Moderate		98
Matrix metalloproteinase 3 (MMP-3)	Plasma, serum	120	120	**0.56**	0.10	None		99
Matrix metalloproteinase 7 (MMP-7)	Serum	150	100	**0.559**	0.34	None		100
Matrix metalloproteinase 9 (MMP-9)	Plasma, serum	100	71	**0.938**	<0.001	Moderate		39
mRNAs								
miIR-106b	Plasma, exosomes	6–46	11–37	**0.578–0.713**	0.851–0.092	None		35, 36
miR-10b	Plasma, exosomes	3–46	3–37	**0.551–0.591**	1.0-< 0.001	None - Modest		35, 36, 101
miR-124	Serum	47	28	0.61	0.074	None		102
miR-148	Pancreatic tissue	16	19	0.92	0.022	Moderate		103
miR-155	Plasma	46	37	**0.682**	0.558-< 0.001	Modest		36
miR-181a	Whole blood, plasma, exosomes	6–33	11–38	**0.626**–0.8	0.316 -< 0.01	None – Modest	Best results with exosomes or cell pellet	35, 103
miR-182	Pancreatic tissue	16	19	0.95	0.005	Moderate		103
miR-1826	Whole blood	33	38	0.81	< 0.01	Modest		103
miR-192	Pancreatic tissue	16	19	0.93	0.028	Moderate		103
miR-194	Pancreatic tissue	16	19	0.91	0.033	Moderate		103
miR-200a	Plasma	11	31	**0.861**	< 0.001	Moderate		104
miR-200c	Plasma	11	31	**0.731**	0.004	Modest		104
miR-20a	Plasma, exosomes	6	11	**0.544–0.623**	0.8–0.321	None		35
miR-21	Plasma, exosomes	6	11	0.558–0.542	0.828–0.719	None		35
miR-212	Plasma	46	37	**0.630**	0.578	None		36
miR-215	Pancreatic tissue	16	19	0.96	0.028	Moderate		103
miR-217	Pancreatic tissue	27	26	-	<0.05	Modest	Unable to estimate AUROC from data provided	37, 105
miR-30c	Plasma, exosomes	6–38	48	**0.5–0.706**	0.999–0.126	None		35, 36
miR-320a	Blood, plasma	44	69	0.83–**0.848**	<0.01–0.001	Moderate		103, 104
miR-375	Plasma	11	31	**0.823**	<0.001	Modest		104
miR-378	Whole blood	33	38	0.80	<0.01	Modest		103
miR-letA	Plasma, exosomes	6	11	**0.764–0.782**	**0.025–0.017**	Modest		35

Biomarker	Biospecimen Type	n (Control)	n (CP)	AUROC	p-value	Effect Size	Comments	Ref(s)
Others								
Adenosine	Urine	5	5	**0.971**	0.048	Large		
Anti-carbonic anhydrase antibody	Serum plasma	40	48	**0.560**	0.315	None		106
Circulating T-helper cells	Peripheral blood	50	50	-	<0.01	Modest	Unable to impute AUROC	107
Cysteine	Plasma	48	90	**0.862–0.918**	<0.001	Moderate	Mean plasma cysteine levels four times lower in CP than normal	65
Heat shock protein 27	Serum	102	44	**0.799**	<0.001	Modest		108
Substance P	Serum	8	30	**0.752**	0.006	Modest	About half of CP patients had marked rise compared to controls	109
Proteomic panels	Serum	14–30	9–16	0.96	<0.001	Moderate	Distinct biomarker panels described for acute, chronic, and autoimmune pancreatitis 75% sensitivity / 100% specificity	40, 74
Relative microbial abundance	Saliva	38	27	-	<0.05	Modest	Granulicatella adiacens (increased abundance) and S mitis (decreased abundance) were validated using independent samples and showed significant variation ($p < 0.05$, qPCR) between CP samples and controls; unable to impute AUROC from published data	110
Des-Leu albumin	Plasma, serum	34	9	**0.996**	-	Large	Measured in patients hospitalized with acute flare-ups; no *p*-value presented in article	49
Receptors								
Serum TNFR-p75 (TNF receptor)	Serum	28	34	**0.832**	<0.05	Moderate		111
Soluble IL-2 receptor	Serum	72	24	**0.808**	<0.05	Modest		78