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Association of Apolipoprotein A2 Isoform Index with Demographic, Behavioral, and Clinical Risk Factors for Pancreatic Ductal Adenocarcinoma in a Single-Center Observational Study: Prelude to a Pancreatic Cancer Screening Algorithm?

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Simple Summary

Serum apoA2-isoforms are a novel and early biomarkers for the detection of pancreatic ductal adenocarcinoma (PDAC). Blood from 265 patients presenting with abdominal pain to the San Francisco General Hospital but without PDAC were retained and tested for apoA2 isoforms and carbohydrate antigen 19-9 (CA19-9), the standard tumor marker for PDAC. Testing for apoA2 isoforms may complement CA19-9 testing because the former is indicative of pancreatic exocrine dysfunction while the latter is released with pancreatic injury. The concentrations of apoA2 isoform ratio and CA19-9 were associated with the patient's demographics, behavioral, and clinical risk factors. In a univariate analysis, apoA2 isoforms were associated with 7 of 16 risk factors studied, of which 5 remained following univariate analysis. CA19-9 was also associated with 7 of 16 risk factors, of which 3 remained following univariate analysis. Of the remaining factors, only cirrhosis/hepatitis was in common between the two markers. In addition to familial and genetic risk factors, we suggest that apoA2 isoforms be included along with CA19-9 and traditional non-genetic risk factors to screen subjects at high risk for PDAC.

Abstract

Background: Pancreatic ductal adenocarcinoma (PDAC) is associated with high mortality rates therefore early diagnosis and treatment is essential in reducing mortality. Blood tests that can identify high-risk individuals is an unmet medical need. **Methods:** In this pilot observational study, remnant samples from routine clinical lab orders were tested on 265 patients presenting with abdominal pain and/or presence of pancreatitis, pancreatic cyst, gall stones, abdominal infections, hepatic cirrhosis/hepatitis, non-pancreatic cancer, or pre-, new onset and uncontrolled diabetes. Serum apolipoprotein A2 isoforms, a novel tumor marker, and carbohydrate antigen (CA) 19-9 were correlated to 3 demographic, 4 behavioral and 9 clinical risk factors associated with PDAC. **Results:** In a univariate analysis using tumor markers as a continuous variable, males, alcohol, smoking, opioid use, pancreatitis, pancreatic cyst and hepatic cirrhosis/hepatitis were associated with an abnormal apoA2 isoforms. In a multivariate analysis, all remained significant except for males and opioid use. CA19-9 was associated with smoking, obesity, pancreatic cyst, cirrhosis/hepatitis, non-pancreatic cancer, and pre- and uncontrolled diabetes. After multivariate analysis, cirrhosis/hepatitis, non-pancreatic cancer and prediabetes remained significant. When categorizing marker data using pre-established cutoffs, patients with abnormal apoA2 had 4.4 risk factors vs. 3.2 with normal apoA2)<0.0001). No difference between normal and abnormal results

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were observed for CA19-9. **Conclusions:** Correlating known PDAC risk factors, apoA2 isoforms and CA19-9 were associated with different factors, suggesting thereby providing independent information on risk. This may justify apoA2 isoforms as part of an algorithm with CA19-9 for cancer screening, particularly for high-risk individuals (e.g., genetic risk).

Keywords: pancreatic ductal adenocarcinoma; apolipoprotein A2; carbohydrate antigen 19-9

1. Introduction

The American Cancer Society has estimated the incidence of pancreatic ductal adenocarcinoma (PDAC) risk for 2025 at nearly 70,000 individuals [1]. The 5-year survival rate is 44%, 16%, and 3% for localized (only within the pancreas), regional (spread to lymph nodes) and distant (metastatic to other organs), respectively. The diagnosis of PDAC is difficult as many patients are asymptomatic. Pancreatic cancer rates increase with increasing age starting at 50 y. The cancer incidence is higher in men versus women, smokers versus nonsmokers, and in blacks versus other racial populations [2]. Pre-existing diseases that are associated with a higher risk include pancreatitis, pancreatic cysts, prediabetes, new onset, and uncontrolled diabetes, hepatic cirrhosis, and infectious diseases especially *Helicobactor pylori*, but also hepatitis B and C, and varicella zoster [2]. There are also germ line somatic mutations such as breast cancer gene 2 (*BRCA2*) that are associated with hereditary PDAC [3].

The morbidity and mortality of PDAC can be reduced with the existence of a biomarker that detects early disease. Unfortunately, there are no blood tests that have achieved success in this area. Carbohydrate antigen (CA) 19-9 is a cell surface glycoprotein that is expressed in the ductal cells of the pancreas and is overexpressed in PDAC [4]. As it is also found in epithelial cells of the stomach, colon, uterus and salivary glands, increased serum concentrations can be seen in benign conditions of these organs and glands. PDAC and is widely used to diagnose and monitor pancreatic cancer [5]. Unfortunately, CA19-9 is not useful for screening or for the detection of early stage PDAC [6]. Moreover among patients who are Lewis antigen negative, which occurs in 10% of PDAC cases, are unable to synthesize CA19-9 therefore the test produces a false negative test result [7]. There are other markers that have been examined including those expressed in tissues (e.g., class III β-tubulin and human equilibrative nucleoside transporter 1) [8] and DNA methylation of pancreatic tumors [9], which could have a role in predictive PDAC treatment but are not useful for early detection.

Sialylated tumor-related antigen (sTRA) are glycans that is characterized by the addition of a sialic acid and are overexpressed on cancer cells [10]. The CA199-sTRA is a sialylated isoform of CA19-9. In a trial 427 patients (236 PDAC and 191 controls at two sites), the combination of CA19-9 and CA199-sTRA demonstrated improved the sensitivity and specificity for early-stage PDAC than when CA19-9 was used alone [11]. Some of the improved performance was attributed to the detection of PDAC patients who were unable produce the CA19-9 antigen using commercial assays.

Apolipoprotein (apo) A2 isoforms were first described potential tumor markers for PDAC in 2016 [12]. ApoA2 exists as three isoforms that circulate in blood. The mature apoA2 protein is a homodimer with a molecular weight of 17,380 Daltons. It is abbreviated as apoA2-ATQ/ATQ, as the last three amino acids are alanine, threonine and glutamine. Cleavage of C-terminal amino acid on one of the two strands by carboxypeptidase A produces the second circulating isoform, apoA2-ATQ/AT. Cleavage of both C-terminal residue on both strands produces the third isoform, apoA2-AT/AT [13]. Immunoassays have been developed for the measurement of the apoA2-ATQ/ATQ and apoA2-AT/AT isoforms in blood. This enables a calculation of the apoA2 isoform index in μg/mL as:

$$\text{square root } ([\text{apoA2-ATQ/ATQ}] \times [\text{apoA2-AT/AT}]) \quad (1)$$

The apoA2 isoforms have been shown to be an early marker of PDAC [14]. Using samples obtained from seven Japanese Medical Institutions, Honda showed that apoA2 isoforms produced

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equivalent area under the receiver-operating characteristic (AUC-ROC) curve for stage I PDAC (0.939), versus the latter stages II-IV (0.957, 0.926, and 0.946, respectively)[14]. These findings are promising as stage I PDAC is associated with localized cancer and the highest 5-year survival. Two small clinical studies have shown that apoA2 isoforms combined with CA19-9 improved the value of diagnosing early-stage PDAC. The sensitivity for stage 0 was 16.7% and 33% in the two studies, which increased to 50% and 67%, respectively when the two markers were combined [15,16]. All values were higher than for CA19-9 alone. However, neither of these studies screened an enriched population, i.e., those individuals who have multiple specific demographic, behavioral, clinical, familial, or genetic risks. To date, however, the value of any single or combination of biomarkers to predict PDAC has been insufficient to warrant the expense of screening beyond high-risk subjects (e.g., hereditary pancreatic cancer) [17].

The purpose of this study is to measure apoA2 isoforms on a group of patients with abdominal pain and gastrointestinal orders. ApoA2 isoforms was correlated to the presence of medical conditions and behavioral factors known to be associated with PDAC.

2. Methods

2.1. Samples

In this pilot observational study, a total of 276 remnant serum and plasma samples left over from routine medical testing was retained from the clinical laboratory at the Zuckerberg San Francisco General Hospital. The protocol was reviewed and approved by the Institutional Review Board at the University of California, San Francisco, who deemed that informed consent was not required. Figure 1 summarizes the flow of patients recruited for this study. Samples were selected from patients admitted to the emergency department and inpatient wards and outpatient clinics with complaints of abdominal pain and/or presence of an increased serum lipase. Using results from serial hemoglobin A1c measurement, we also enrolled individuals who had prediabetes, a new onset diabetes (less than 3 years), and uncontrolled diabetes. These inclusion criteria were meant to enrich recruitment of subjects that may suffer from disorders that put them at risk for PDAC. Medical records were reviewed for the patient's demographics including age, gender, and ancestry, behavioral factors such as alcohol, smoking history, obesity (body mass index >30 kg/m^2) and opioid use and a history of gastrointestinal diseases. Patient ages between 60 and 80 y were considered at higher risk than patients who were younger or older than these ages. An individual who self-disclosed as being black race was considered as the higher risk factor for PDAC than other ancestries [2]. In addition to lipase, other clinical laboratory results typically measured for patients presenting with abdominal conditions included total bilirubin, alanine and aspartate aminotransferase (Siemens Atellica, Tarrytown, NY). The hemoglobin A1c was used to assess glucose control (Bio-Rad Laboratories, Hercules, CA). An A1c result between 5.7 to 6.4% was considered as pre-diabetic, ≥6.5 considered as diabetic, and >10% as uncontrolled diabetes. Not all clinical laboratory tests were available on all subjects. Medical and clinical laboratory records were reviewed for discharge diagnoses using the following search terms: "pancreas, cyst, cirrhosis, hepatitis, gall stones, H. pylori, herpes, and diabetes."

2.2. ApoA2 Isoforms and CA19-9 Testing

Samples frozen and sent to Toray Molecular Oncology Laboratory (Brisbane, CA) for analysis of apoA2-ATQ and apoA2-AT isoforms using enzyme linked immunosorbent assay (ELISA). After thawing samples were diluted and tested in duplicate for apoA2-ATQ/ATQ and apoA2-AT/AT. These assays have a limit of quantification of 5.75 and 3.25 μg/mL, respectively, and a day-to-day precision for both assay ranging 3-7% for the low, medium, and high concentrations. There are no assay interferences from hemoglobin, lipids, bilirubin, or human anti-mouse antibodies. The apoA2 isoform index was calculated based on the mean of duplicate results. In previous reports using a prototype polyclonal assay, a cutoff of 59.5 μg/mL was used [16,18] based on a reference range

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study of 2000 healthy Japanese subjects [13]. However the manufacturer of the reagents have determined that for predicting PDAC risk, an index value of <44 μg/mL is more appropriate, with values between 45 and 59.5 μg/mL being considered as intermediate risk. For the purposes of this study, a cutoff <44 μg/mL was used. For CA19-9 testing, the AccuBind assay was used (Monobind Inc., Lake Forest, CA). This assay has a dynamic range of 0.19 to 500 U/mL and a between assay imprecision 3.4% at a level of 54 U/mL and 9.2% at 3.7 U/mL. The normal range cutoff for this assay is ≤40 U/mL.

2.3. Statistics

Data was entered into Excel spread sheets to calculate means and standard deviations (SD) for various groups. A one-way analysis of variance (ANOVA) was used to determine univariate significance for apoA2 isoforms and CA19-9 as dependent continuous variables and the presence or absence for other factors as the dependent variable. Multivariate analysis was conducted using an analysis of covariance (ANCOVA). Results of routine testing was not available for all of the clinical laboratory tests, and these subjects were omitted in the statistical analysis. Statistical significance were established at $p<0.05$ and were calculated using MedCalc (ver. 19.6.4, Ostend, Belgium).

3. Results

The demographics of the patients enrolled in this study are shown in Table 1 broken down by abnormal apoA2 isoform index (cutoff <44 μg/mL) and serum CA19-9 results (cutoff >40 U/mL). For apoA2 isoforms, there was no statistical difference race, sex, age, or body mass index (except there were more white subjects and less young patients in the high-risk group). For CA19-9, there were more Asians in the high-risk group and less Hispanics, and more in the >80 year age group. Table 2 compares the results of commonly ordered serum laboratory tests. The proportion of patients in the high-risk apoA2 isoform risk group were higher for lipase, total bilirubin, and aspartate aminotransferase ($p<0.05$). For CA19-9, increases were seen in hemoglobin A1c, total bilirubin, aspartate and alanine aminotransferase, and alkaline phosphatase ($p<0.05$) but not for lipase.

Table 3 tabulates the univariate and multivariate associations of various demographic, behavioral and clinical risk factors associated for PDAC with apoA2 isoforms and CA19-9. Of the 16 risk factors studied, male gender, alcohol, smoking, opioid use, pancreatitis (acute and chronic), pancreatic cyst, and hepatic cirrhosis or hepatitis were statistically significant for apoA2 isoforms. For CA19-9, smoking, obesity, pancreatic cyst, non-pancreatic cancer, hepatic cirrhosis and hepatitis, and both pre-diabetes and uncontrolled diabetes were significantly associated, although for the latter two factors, lower values were associated with risk. New onset diabetes was not associated with either marker. Only smoking, the presence of a pancreatic cyst and hepatic cirrhosis or hepatitis were significant for both apoA2 isoforms and CA19-9. Interestingly, gall stones was not associated with either marker. For the multivariate analysis, male gender and opiate use dropped out of the regression for apoA2, while smoking, obesity, pancreatic cyst, and uncontrolled diabetes dropped out for CA19-9. Taken together, only hepatic cirrhosis/hepatitis remained in both multivariate models. ApoA2 isoforms was sensitive towards pancreatic diseases while CA19-9 was inversely sensitive towards prediabetes.

Table 4 shows the breakdown of risk factors compared to apoA2 and CA19-9 as a dichotomous variable (i.e., against the pre-established cutoff concentrations). For apoA2, results were largely consistent than when the data was expressed as a continuous variable. For CA19-9, many of the risk factors dropped out when a cutoff concentration of 40 U/mL except for cirrhosis/hepatitis and prediabetes. Of the 16 risk factors studied, patients with an abnormal apoA2-isoforms had on the average of 4.4 factors (SD=1.7) vs. 3.2 (SD=1.7) for a normal apoA2 isoform ($p<0.005$). There was no difference in the number of risk factors present when results were divided by high between abnormal and normal CA19-9 results (3.6 ±1.6) and 3.5 (±1.6) respectively (p=NS).

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4. Discussion

There are many demographic, behavioral, and clinical risk factors that are associated with the development of PDAC [19]. Many of but not all variables have been examined in this study. A limitation of this study was the absence of family history of PDAC and testing for relevant genetic markers [3]. Among the demographic factors, male gender, increasing age, and the black race have higher rates of PDAC than among females, younger subjects and other races.

Behavioral factors such as smoking, alcohol use, obesity, and diet have been recognized as risk factors for PDAC [20]. Active smoking and alcohol use was associated with apoA2 isoform index ($p<0.0001$ and <0.0010, respectively, Table 3). A meta-analysis showed that obesity has been mildly associated with PDAC [21]. A subsequent study showed that abdominal obesity is more important than overall obesity for PDAC risk [22]. In our study, only obesity was correlated with the presence of CA19-9 and dropped out with a multivariate analysis. An assessment as to distribution of obesity was not available in this study. Diet has been studied as a risk factor for PDAC [23], but was not included in the patient's medical record and therefore not assessed here. The role of a sedentary lifestyle has been examined for pancreatic cancer risk, but results have been equivocal, and not investigated [24].

Among the pre-existing non-malignant conditions, a history of pancreatitis and pancreatic cysts are well described risk factors to PDAC. The most common cause of chronic pancreatitis is alcohol abuse. Each of these variables are statistically linked to a high PDAC risk for apoA2-isoform results (Tables 3 and 4) and higher serum lipase levels (Table 2). The correlation of apoA2 isoforms to pancreatitis progression has been described previously [18]. These investigators opined that the apoA2 may be an indicator for pancreatic exocrine dysfunction. No significant difference in the incidence of pancreatitis was observed using stratification based on CA19-9 results. Benign liver disease such as hepatic cirrhosis or hepatitis is associated with abnormalities in both apoA2-isoforms and CA19-9. This is reflected in higher levels of some liver function tests (e.g., AST, ALT, total bilirubin and alkaline phosphatase) for patients at high risk for either ApoA2-i index or CA19-9. These lab results were similar to the study by Singh et al. [25] who concluded that CA19-9 had poor specificity and was not useful as an early tumor marker. These investigators did not opine as to the role of CA19-9 as a predictor of PDAC.

Infectious disease that are associated with PDAC include *H. pylori* which causes gastric ulcers, and herpes simplex. The incidence of a high-risk apoA2-i isoforms was higher in a patient with H. pylori infection, although results did not reach statistical significance (data not shown). No difference was observed for these infections when testing for CA19-9.

Diabetes is a recognized risk factor for PDAC. Mellenthin et al. stated at the time of PDAC onset, many patients have either prediabetes or developed diabetes within the prior three years [26]. They suggested that for patients in these two groups, a PDAC screening program could be developed using an algorithm that included age, family history, and medical history of gall stones and pancreatitis.

CA19-9 is found in normal pancreatic and biliary ductal cells and is overexpressed in PDAC, reflecting pancreatic inflammation, obstruction, and injury [20,27]. For patients who are Lewis antigen negative, and therefore do not express CA19-9 [28], it may be optimal to include or substitute sTRA testing, as this biomarker is not affected by the absence of this antigen [11].

Results from this study show that apoA2 isoforms and CA19-9 are independent and complement each other as predictors for the presence of PDAC risk factors. In particular the incidence of risk factor presence statistically increased for patients with abnormal apoA2 compared to normal results (4.4 vs. 3.2 ($p<0.0001$). Abnormal apoA2 isoform processing is indicative of dysregulation of carboxypeptidase, and therefore a marker of exocrine pancreatic dysfunction. This data may provide the basis for the development of an algorithm used to predict PDAC, including serum CA19-9 levels, which alone has not been shown to be useful as an early PDAC screening test [29].

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This study did not correlate the risk factors on blood from patients with PDAC or on subjects months or years before cancer development. Such studies require testing of prospective sample banks such as the ongoing Pancreatic Cancer Early Detection Consortium (PRECEDE, https://www.clinicaltrials.gov/study/NCT04970056). The inclusion criteria include those with a family history of PDAC and individuals who have germline variants in genes linked to PDAC risk [30]. Serial samples from these cohorts are extremely valuable and testing for novel biomarkers can only be justified if preliminary studies show promise, such as suggested in this report. If baseline biomarker results are available prior to the onset of PDAC, a change in results that exceeds the biomarker's biological variation is a better indicator for early cancer, than the use of a population-based reference interval [31]. Erdin et al. showed that CA19-9 has low within- and high between individual biological variation to indicate that the use of serial change in results is more effective than a single result used in cross-section [32].

In general, the effective use for any multi-marker analysis requires that the individual tests provide complementary information. ApoA2-i index along with the established CA19-9 has promise because abnormalities in the two tests are due to different pathophysiological mechanisms. ApoA2 isoform hyper- or hypo-processing occurs in pancreatic exocrine dysfunction.

5. Conclusions

This study showed that an abnormal apoA2 isoform index was associated with known clinical and behavioral risk factors for PDAC than a normal index. This marker is unspecific for PDAC, as abnormal apoA2 isoform results are seen in diseases and conditions not associated with PDAC risk. However, its value may be in the inclusion of a multi-marker screening panel to predict PDAC development for individuals identified to be a PDAC risk. A prospective study will be required to validate the value of apoA2 isoforms for screening among asymptomatic subjects at high risk.

Figure Captions

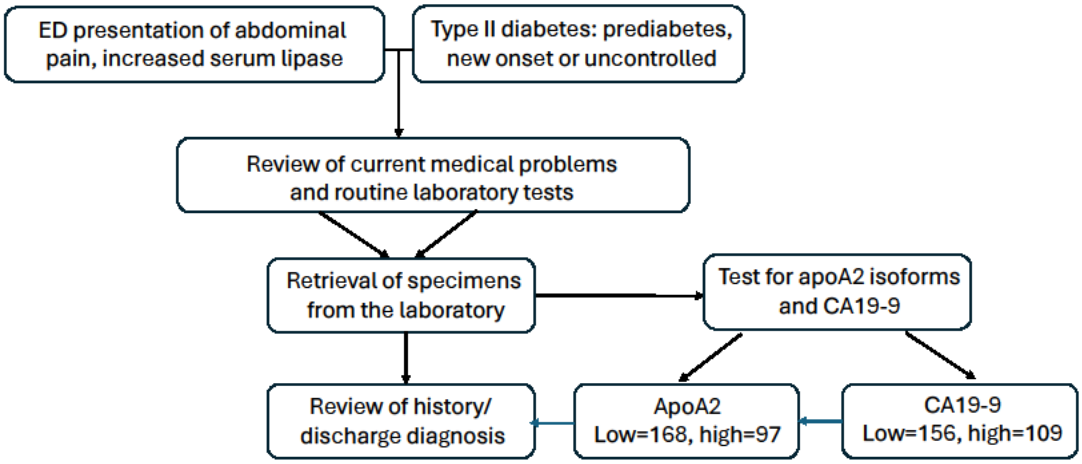

Figure 1. Recruitment strategy for retention and testing of serum samples for apoA2 isoforms and CA19-9.

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Table 1. Demographics of patients enrolled according to apoA2-isoform and CA19-9 risk groups

	ApoA2≥44 Low risk	ApoA2<44 High risk	P-value	CA19-9<40 Low risk	CA19-9≥40 High risk	P-value
Number of subjects	168.00	97		156	109	
Race						
White	12.8%	24.0%	<0.005	1.2.2	21.2%	NS
Black	15.9%	17.4%	NS	18.2%	11.5%	NS
Hispanic	50.6%	45.4%	NS	54.7%	38.5%	<0.05
Asian	20.7%	15.2%	NS	14.8%	28.8%	<0.010
Male sex	78.0%	66%	NS	60%	62%	NS
Age, y						
20-30	10.1%	1.0%	<0.05	8.9%	4.6%	NS
31-40	14.2%	15.5%	NS	17.2%	11.0%	NS
41-50	14.8%	25.8%	NS	19.7%	17.4%	NS
51-60	21.3%	15.5%	NS	20.0%	16.5%	NS
61-70	34.8%	19.6%	NS	19.1%	23.8%	NS
71-80	10.1%	16.7%	NS	10.2%	14.7%	NS
>80	17.4%	3.5%	NS	3.8%	11.9%	<0.05
All ages, y	51±16	56±16	NS	51±16	56±17	NS

NS=not significant at p>0.050
Statistical significance in bold

Table 2. Correlation of clinical laboratory results to ApoA2-isoform risk groups

	ApoA2≥44 Low risk	ApoA2<44 High risk	P value	CA19-9<40 Low risk	CA19-9≥40 High risk	P value
Number of subjects	168	97		156	109	
Hemoglobin A1c, %	8.4±2.5	7.8±3.3	NS	8.7±2.7	7.7±2.9	<0.005
Lipase, U/L	110±90	138±122	<0.05	113±96	131±113	NS
Total bilirubin, mg/dL	0.8±0.6	2.8±4.6	<0.0001	0.9±0.9	2.5±4.4	<0.0001
Aspartate aminotransferase, U/L	74±130	129±200	<0.05	71±133	131±192	<0.0001
Alanine aminotransferase, U/L	62±103	76±117	NS	54±94	86±125	<0.05
Alkaline phosphatase, U/L	136±138	152±109	NS	115±77	181±168	<0.0001
AT value, µg/mL	6.49±3.97	2.90±3.39	<0.0001	5.44±4.26	4.82±3.97	NS
ATQ value, µg/mL	10.0±4.80	3.99±4.87	<0.0001	9.12±5.33	6.1±4.89	NS
ApoA2-index, µg/mL	72±21	19.8±13.4	<0.0001	57.3±30.8	45.4±30.4	NS
CA19-9	44±64	99±133	<0.0001	19±17	130±128	<0.0001

NS=not significant at p>0.050
Statistical significance in bold
Lab results not available on all patients

Table 3. Univariate of apoA2 isoforms and CA19-9 fordemographic, behavioral and clinical risk factors

	Apo-A2 isoforms		CA19-9	
Univariate analysis	**P value-univariate**	**P value-multivariate**	**P value-univariate**	**P value-multivariate**
Demographic factors				
Middle age (60-80 y)	NS	x	NS	x
Male sex	<0.001	NS	NS	x
Black race	NS	x	NS	x
Behavioral factors				
Alcohol use	<0.001	<0.05	NS	x
Smoking	<0.001	<0.10	<0.05	NS
Obesity (BMI>30 kg/m2)	NS		<0.05	NS
Opioid use	0.007	NS	NS	x
Clinical factors				
Pancreatitis (acute and chronic)	<0.001	<0.05	NS	x
Pancreatic cyst	<0.05	<0.05	<0.05	NS
Gall stones	NS	x	NS	x
Abdominal infection	NS	x	NS	x
Hepatic cirrhosis/hepatitis	<0.001	<0.05	<0.05	<0.05
Non-pancreatic cancer	NS	x	<0.001	<0.010
Prediabetes	NS	x	<0.05	<0.05
New onset diabetes	NS	x	NS	x
Uncontrolled diabetes	NS	x	<0.05	NS

x=not included in the multivariate model
NS=not significant at p>0.050
Significance in bold

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Table 4. Demographic, behavioral and clinical risk factors according to apoA2 isoform index and CA19-9 cutoffs

	ApoA2≥44	ApoA2<44	p	CA19-9<40	CA19-9≥40	p
	Low risk	High risk	Low vs high	Low risk	High risk	Low vs high
Number of subjects	168	97		156	109	
Age (60-80 y)	28.4%	21%	NS	30.1%	38.5%	NS
Male sex	39.6%	39%	NS	40.4%	39.4%	NS
Race (black)	16.0%	11%	NS	16.0%	11.0%	NS
Alcohol	44.4%	73.5%	**<0.0010**	53.8%	60.6%	NS
Smoking	26.6%	57.1%	**<0.0001**	36.5%	45.9%	NS
Obesity (BMI>30 kg/m2)	44.4%	27.6%	NS	32.7%	22.0%	NS
Opioid use	25.5%	25.5%	NS	16.0%	21.1%	NS
Pancreatitis (acute or chronic)	16.6%	46.9%	**<0.0001**	31.4%	31.8%	NS
Pancreatic cyst	2.4%	12.2%	**<0.010**	5.8%	7.3%	NS
Gall stones	6.5%	4.1%	NS	6.4%	3.7%	NS
Abdonimal infection	23.1%	36.7%	**<0.05**	32.1%	26.4%	NS
Hepatic cirrhosis/hepatitis	24.9%	41.8%	**<0.005**	24.4%	44.5%	**<0.001**
Non-pancreatic cancer	9.5%	15.3%	NS	10.3%	12.8%	NS
Prediabetes	14.8%	7.1%	NS	15.4%	7.3%	**<0.05**
New onset diabetes	4.2%	8.2%	NS	4.5%	5.5%	NS
Uncontrolled diabetes A1c>9.0	18.3%	15.3%	NS	21.2%	15.6%	NS
Mean ±SD number of risk factors	3.2±1.7	4.4±1.7	**<0.0001**	3.5±1.6	3.6±1.6	NS

NS=not significant at p>0.050
Statistical significance in bold

Author Contributions: Conceptualization, G.Y., and A.H.W.; methodology, G.Y., and A.H.W.; data curation; M.A., Z.C., C.M.O., C.W., A.H.W.; writing-original draft preparation, A.H.W., writing-review and editing, G.Y., and A.H.W.; Supervision, A.H. Wu. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: This study was conducted in accordance with the Declaration of Helsinki and approved by the Institutional Review Board of the University of California (25-44393, 8 July 2025).

Informed Consent Statement: Informed consent was not deemed necessary by the UCSF IRB.

Data Availability Statement: The data presented in this study are available from the corresponding author. Thie data are not publicly available due to the ethics approval agreement.

Acknowledgments: None.

Conflict: of Interest: CW and GJ are employees of Toray International America.

Abbreviations

The following abbreviations are used in this manuscript.

ALT	Alanine aminotransferase
ANOVA	Analysis of variance
ANCOVA	Analysis of covariance
ApoA2	Apolipoprotein A2
AST	Aspartate aminotransferase
ATQ	Amino acids alanine, threonine, and glutamine
AUC-ROC	Area under the receiver operating characteristic
BMI	Body mass index
CA19-9	Carbohydrate antigen
ELISA	Enzyme-linked immunosorbent assay
NS	Not statistically significant
PDAC	Pancreatic ductal adenocarcinoma
PRECEDE	Pancreatic Cancer Early Detection
SD	Standard deviation

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