ORIGINAL ARTICLE

Case Control Study

N-glycan biosignatures as a potential diagnostic biomarker for early-stage pancreatic cancer

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Abstract

BACKGROUND
Pancreatic ductal adenocarcinoma (PDAC) has a poor prognosis, with a 5-year survival rate of less than 10%, owing to its late-stage diagnosis. Early detection of pancreatic cancer (PC) can significantly increase survival rates.

AIM
To identify the serum biomarker signatures associated with early-stage PDAC by serum N-glycan analysis.

METHODS
An extensive patient cohort was used to determine a biomarker signature, including patients with PDAC that was well-defined at an early stage (stages I and II). The biomarker signature was derived from a case-control study using a case-cohort design consisting of 29 patients with stage I, 22 with stage II, 4 with stage III, 16 with stage IV PDAC, and 88 controls. We used multiparametric analysis to identify early-stage PDAC N-glycan signatures and developed an N-glycan signature-based diagnosis model called the "Glyco-model".

RESULTS
The biomarker signature was created to discriminate samples derived from patients with PC from those of controls, with a receiver operating characteristic area under the curve of 0.86. In addition, the biomarker signature combined with cancer antigen 19-9 could discriminate patients with PDAC from controls, with a receiver operating characteristic area under the curve of 0.919. Glyco-model demonstrated favorable diagnostic performance in all stages of PC. The diagnostic sensitivity for stage I PDAC was 89.66%.

CONCLUSION

In a prospective validation study, this serum biomarker signature may offer a viable method for detecting early-stage PDAC.

Key Words: Glycomics; N-glycans; Biomarkers; Pancreatic cancer; Predictive modeling

Core Tip: This study employed a patient cohort to investigate the N-glycan signature of early-stage pancreatic cancer (PC). Serum N-glycans analysis was conducted to identify the serum biomarker signature associated with early-stage pancreatic ductal adenocarcinoma (PDAC), resulting in the identification of nine early-stage PDAC N-glycan signatures. Subsequently, utilizing these biosignatures, a diagnostic model named the "Glyco-model" was developed, demonstrating promising diagnostic performance across all stages of PC. The study revealed that the diagnostic sensitivity for stage I PDAC was determined to be 89.66%. Consequently, this diagnostic model exhibits potential as a prospective strategy for the early detection of PDAC.

INTRODUCTION

Among all cancers, pancreatic cancer (PC) is the deadliest, exhibiting the lowest 5-year survival rates[1,2]. The annual incidence of PC continues to increase by approximately 1%[3]. Patients often present with non-specific symptoms such as jaundice, fatigue, changes in bowel habits, and indigestion, which makes it difficult to distinguish them from non-cancer diseases[4]. PC has a poor prognosis, primarily due to late diagnosis, with approximately 20% of patients being diagnosed at an early stage[5]. Therefore, it is imperative to accurately diagnose PC in its early stages.

Early diagnostic biomarkers for PC are lacking. Pancreatic ductal adenocarcinoma (PDAC) is the most common histological subtype of PC. Serum cancer antigen 19-9 (CA19-9), the most commonly evaluated biomarker of PDAC, has an inadequate specificity[6]. CA19-9 levels exhibit an increase in various other indications, and its absence was observed in patients with Lewisab, a condition that affects 5% of the population[7]. Therefore, CA19-9 alone is not recommended for screening[8] or as an indicator of recurrence[9], but only for disease monitoring after surgical resection[10]. Consequently, cancer diagnostics is increasingly focused on new biomarkers or analytical methods. Multiparametric analysis[11,12] combined with CA19-9[13,14] enhances sensitivity, specificity, precision, and accuracy. The combination of immunoregulatory and cancer-associated protein biomarkers has been shown to distinguish patients with late-stage III and IV PDAC from healthy controls[15]. Biomarker DUPAN-2 can be used to predict the survival outcome of patients with PC, especially in cases where CA19-9 is negative[16]. However, its diagnostic efficacy for early detection of PC is limited. Thus, biomarkers for early-stage PC diagnosis are still lacking.

The tumor markers carcinoembryonic antigen (CEA), CA19-9, and CA125 play vital roles in the diagnosis and prognosis of PC[17]. These glycoprotein alterations indicate the potential involvement of abnormal glycosylation in various biological processes[18,19]. Recent advances in glycomics have led to the discovery of unique N- and O-glycans that serve as glycobiomarkers for cancer diagnosis and treatment[20]. Various tumors have altered N-linked sugar chains [21,22]. Some N-linked glycan species and other classes of glycans are associated with PC[23]. Imaging mass spectrometry has been used to evaluate the N-glycome of the human pancreas and PC in a cohort of patients with PDAC, represented by tissue microarrays and whole tissue sections, to describe the differences between PDAC and other abdominal cancers and non-cancerous pancreatic lesions[24]. Using serum N-glycome analysis, patients with PDAC can be differentiated from healthy controls on the basis of glycosylation[25]. The previous study also revealed that the serum N-glycan profile is a promising method for detecting hepatocellular carcinoma in patients with cirrhosis, based on the log ratio of the branching α-1,3 fucosylated triantennary glycan [NA3Fb, glycan peak (GP) 9] to the bigalacto core α-1,6 fucosylated bisecting biantennary glycan (NA2FB, GP7), or the log ratio of GP9 to GP4 (a single galactic core 1,6 fucosylated biantennary glycan, NG1A2F)[26]. However, there are no specific N-glycan glycobiomarkers for the early diagnosis of PC.

In this study, we designed an extensive patient cohort to identify the N-glycan signature, including patients with PDAC that were well-defined at an early stage (stages I and II). The biomarker signature was derived from a case-control study using a case-cohort design consisting of 29 patients with stage I, 22 with stage II, 4 with stage III, 16 with stage IV PDAC, and 88 controls. Serum N-glycan analysis was performed to identify the serum biomarker signatures associated with early-stage PDAC. Using a case-control study, a biomarker signature was created to discriminate between samples derived from patients with PC and those derived from controls. Moreover, the biomarker signature combined with CA19-9 could discriminate patients with PDAC from controls with a receiver operating characteristic (ROC) area under the

curve (AUC) of 0.92. The N-glycan signature discriminated patients with stage I and II PDAC from controls in this independent patient cohort, with a AUC of 0.86.

MATERIALS AND METHODS

Study designs

This retrospective study recruited 245 patients, including 93 patients with PDAC, 64 with benign pancreatic disease, and 88 healthy participants (Figure 1). This retrospective study performed on PDAC serum samples collected at the Nanjing Drum Tower Hospital was conducted according to the Standards for Reporting Diagnostic Accuracy Studies[27]. PDAC was staged according to the TNM staging system of the American Joint Committee on Cancer on Cancer (eighth edition).

Demographics of study cohorts

The study cohort comprised 93 patients with PDAC, 64 with benign pancreatic disease, and 88 healthy participants (Table 1). The patients were diagnosed using computed tomography and were histologically verified. There were 29 PDAC samples from patients with stage I, 22 with stage II, 4 with stage III, 16 with stage IV, and 22 with unknown stages (Table 1). Blood samples were collected from participants who had not received any anticancer therapy. The inclusion criteria were as follows: Patients within the age range of 18-70 years, diagnosed with PDAC, had not received any prior treatment, were recruited. Serum tumor markers CEA, CA19-9, CA125, CA242, CA724, and alpha fetoprotein (AFP) were elevated.

Study approval

The study protocol was approved by the Ethics Committee of Drum Tower Hospital, the Affiliated Hospital of Nanjing University Medical School and conformed to the ethical guidelines of the Declaration of Helsinki. Written informed consent was obtained from all patients.

Laboratory tests

Serum was collected from whole blood using a standard protocol and centrifuged at $10000 \times g$ for 4 min. Laboratory tests, including those for the serum tumor markers CEA, CA19-9, CA125, CA242, CA724, and AFP, were performed at local laboratories according to standard procedures.

Serum N-glycome profiling

Serum glycoprotein N-glycome profiling was performed on the GlyFace (Glycoprofiling by Fluorophore-Assisted Carbohydrate Electrophoresis) glycome detection technology platform provided by SysDiagno (Nanjing) Biotech Co. The results were analyzed using GeneMapper v6.0 software (Applied Biosystems). The height intensities of the nine most intense GPs were detected in all samples and normalized to the total intensity of the measured GPs.

Data analysis

Continuous variables are expressed as medians (interquartile ranges). Categorical variables are expressed as numbers (%). Continuous variables were compared using Student's *t*-test or one-way analysis of variance. ROC curve analysis and AUC values were used to evaluate the overall diagnostic performance of single markers and diagnostic models. Sensitivities and specificities were calculated using cut-off values optimally selected upon the ROC curves. The *t*-test (data conforms to a normal distribution and variance homogeneity) or Wilcoxon rank-sum test (not conforms to normal distribution and homogeneity of variance) was performed to compare the two groups. The Kruskal-Wallis test was used to compare variables among multiple groups. All statistical tests were two-sided. Statistical significance was set at $P < 0.05$. Data were analyzed using SPSS 22.0.

RESULTS

### Classifying PDAC with N-glycome profiling signature

The study cohort comprised patients with PDAC, those without PDAC, and healthy controls. The clinical characteristics were comparable between the groups (Table 1 and Supplementary Figure 1). We conducted N-GP profiling in all patients. The representative profiling patterns are shown in Figure 2A. The structures of the nine N-GPs are shown in Figure 2B. Significant changes were observed in three GPs (GP3, GP6, and GP9) in the PDAC group compared to those in the non-PDAC and control groups (Figure 2C). Furthermore, tumor marker concentrations were examined for each group. Tumor markers exhibited significant differences among the three groups (Supplementary Figure 2). The differences in N-glycans among the three groups suggest the potential use of N-glycans as diagnostic markers for PDAC.

Subsequently, we assessed the discriminatory potential of the N-glycan signature in distinguishing between various clinical subgroups of patients diagnosed with PC and those diagnosed with benign pancreatic disease. Nine N-GPs were analyzed in plasma samples from 64 patients with benign pancreatic cysts (benign group) and 93 patients with pancreatic adenocarcinoma (Table 1 and Supplementary Figure 3). While GP1-GP8 did not differ significantly between patients with benign disease and patients with PDAC, patient with PDAC samples displayed a markedly higher level of the GP9 signal

Table 1 Patient demographics and clinical characteristics of the study cohort

Characteristics	PDAC (*n* = 93)	Non-PDAC (*n* = 64)	Control (*n* = 88)
Men, *n* (%)	59 (63.4)	35 (54.7)	38 (43.2)
Women, *n* (%)	34 (36.6)	29 (45.3)	50 (56.8)
Age (yr)	64 (54.74)	54 (40.68)	57 (43, 71)
CEA, *n* (%)	93 (100)	64 (100)	82 (93.18)
CA19-9, *n* (%)	93 (100)	64 (100)	69 (78.41)
CA125, *n* (%)	93 (100)	63 (98.44)	42 (47.73)
CA242, *n* (%)	93 (100)	63 (98.33)	0 (0.00)
CA724, *n* (%)	93 (100)	63 (98.44)	0 (0.00)
AFP, *n* (%)	93 (100)	64 (100)	82 (93.18)
Stage I, *n* (%)	29 (31.18)	-	-
Stage II, *n* (%)	22 (23.66)	-	-
Stage III, *n* (%)	4 (4.30)	-	-
Stage IV, *n* (%)	16 (17.20)	-	-
Unknown, *n* (%)	22 (23.66)	-	-

PDAC: Pancreatic ductal adenocarcinoma; AFP: Alpha fetoprotein; CEA: Carcinoembryonic antigen; CA19-9: Cancer antigen 19-9.
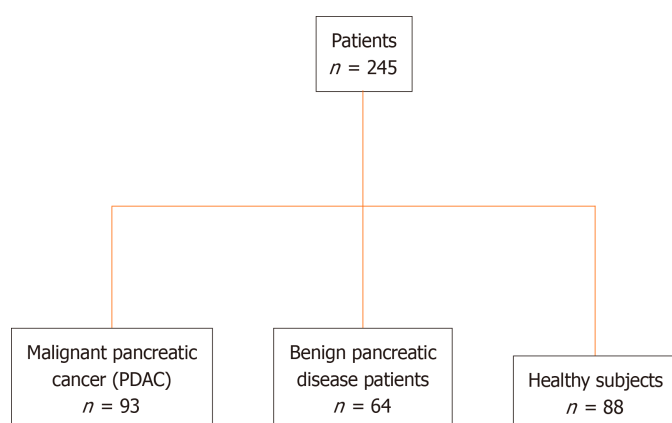
Figure 1 Overview of study design and patient cohort. PDAC: Pancreatic ductal adenocarcinoma.

(Figure 3A). Furthermore, as a control, we investigated the efficacy of tumor markers in distinguishing between malignancies of pancreatic origin and benign pancreatic cysts. We found that CA125, CA19-9, and CA242 could distinguish PC from non-PC (Figure 3B). This implies that there are differences in biomarkers between different types of non-PDAC and PDAC, indicating the potential of N-glycans to differentiate various non-PDAC subtypes from PDAC.

Validation of N-glycan signature as a diagnostic biomarker for early-stage PC in serum from retrospective cohorts of patients with PDAC

To distinguish early-stage PC, the potential of GPs as diagnostic markers in patient serum was verified in a 71-patient cohort. The participants' clinical information regarding age and sex is shown in Supplementary Figure 4. We conducted GPs analyses in blood samples of patients with early stages I-II and advanced clinical stages III-IV to confirm the expression pattern of N-glycans. Both GP2 and GP7 distinguished patients with early- from late-stage (Figure 4A). We also compared the ability of tumor biomarkers to differentiate between patients with early-stage and advanced PDAC. The levels of CA19-9 and CA 242 were capable of distinguishing between patients with early- and late-stage PDAC (Figure 4B). This result implies that N-glycans GP2 and GP7 could be used as biomarkers to differentiate early-stage (I-II) from advanced PDAC (III-IV).

Construction and validation of a diagnostic model to distinguish early-stage PDAC

A multivariate logistic regression analysis was performed to build a diagnostic model using GP1, GP2, GP3, GP4, GP5,
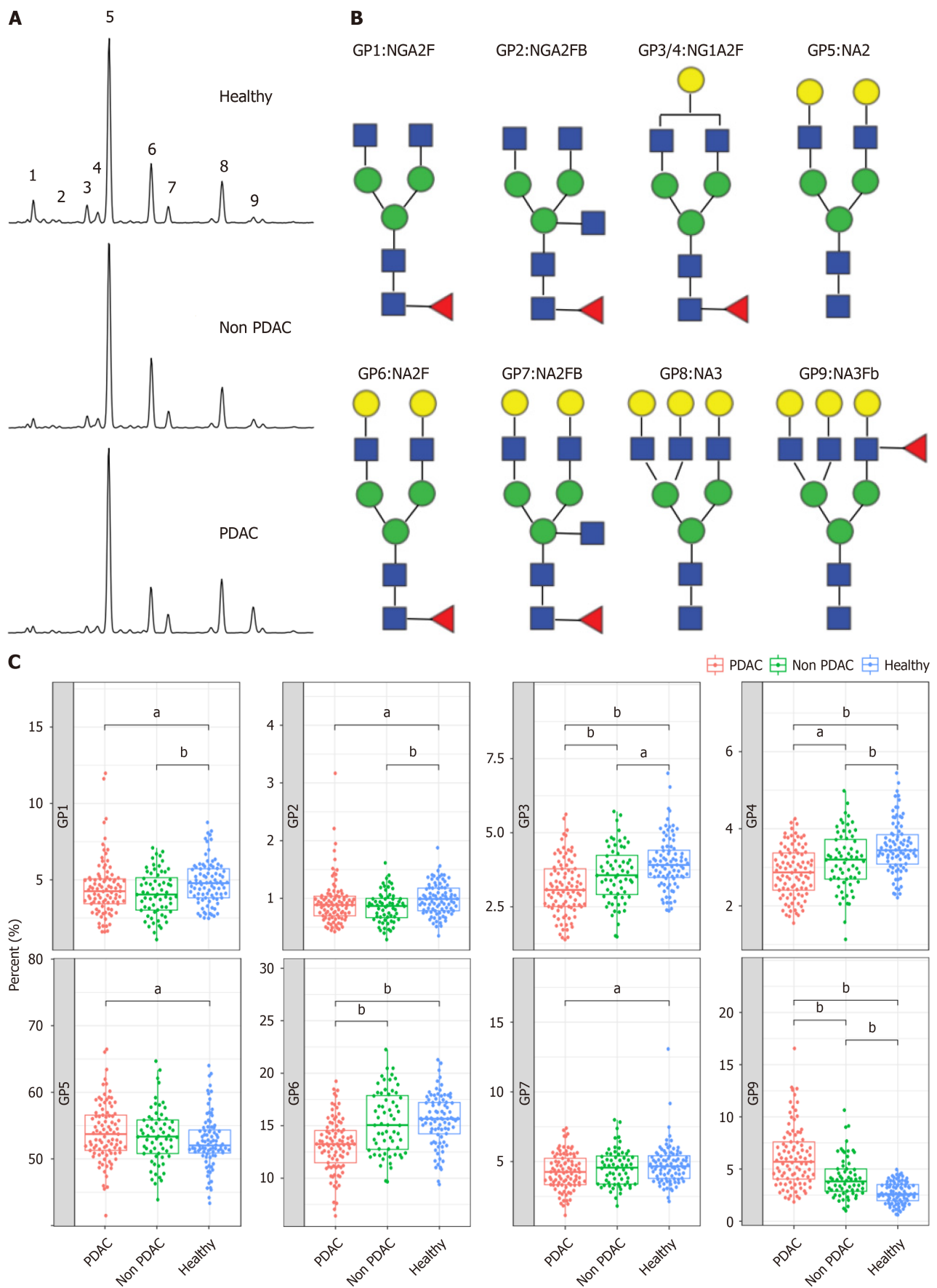
Figure 2 N-glycome profile from desialylated serum. A: Typical desialylated N-glycan profiles from the total serum protein in patients with pancreatic ductal adenocarcinoma (PDAC), patients without PDAC, and healthy participants; B: Structure of nine N-glycan peaks (GPs). GP1 indicates an agalacto core α-1,6 fucosylated biantennary glycan (NGA2F), GP2 indicates an agalacto core α-1,6 fucosylated bisecting bian tennary glycan (NGA2FB), GP3 and GP4 indicate a single

agalacto core α-1,6 fucosylated biantennary glycan (NG1A2F), GP5 indicates a bigalacto biantennary glycan (NA2), GP6 indicates a bigalacto core α-1,6 fucosylated biantennary glycan (NA2F), GP7 indicates a bigalacto core α-1,6 fucosylated bisecting biantennary glycan (NA2FB), GP8 indicates a triantennary glycan (NA3), and GP9 indicates a branching α-1,3 fucosylated triantennary glycan (NA3Fb); C: N-glycan analysis in the three groups of patients. [a]P < 0.05, [b]P < 0.01. GPs: Glycan peaks; PDAC: Pancreatic ductal adenocarcinoma.

GP6, GP7and GP9. The diagnostic formula is as follows: Glyco-model = exp(10.696 + 11.368 × GP1 + 121.372 × GP2 - 46.884 × GP3 -66.918 × GP4 - 15.329 × GP5 - 21.862 × GP6 + 1.177 × GP7 + 47.976 × GP9)/1 + exp(10.696 + 11.368 × GP1 + 121.372 × GP2 - 46.884 × GP3 - 66.918 × GP4 - 15.329 × GP5 - 21.862 × GP6 + 1.177 × GP7 + 47.976 × GP9).

Eighty-eight normal samples, 64 non-PDAC, and 93 PDAC samples were used to construct and validate a diagnostic model for discriminating PDAC from non-PDAC and healthy individuals. To reduce the negative influence of overfitting on the PDAC predictive power, we performed a 10-fold cross-validation on the modeling training set. The training set was further divided into ten folds, of which nine were used for modeling and the remaining were used for validation. Finally, the AUC values representing the optimal diagnostic power from one of the ten-fold cross-validations, AUCROC, were 0.854-0.875 (Figure 5A). Using a cut-off value of 0.28, this model achieved good diagnostic efficiency with a validation AUROC of 0.863 (sensitivity, 84.90%; specificity, 73.00%), indicating that this Glyco-model is a promising method for detecting patients with PDAC (Figure 5B). A comparative analysis was performed to evaluate the diagnostic efficacy of the Glyco-model and tumor markers in discriminating patients with PDAC from those of non-PDAC and healthy individuals, as illustrated in Supplementary Figure 5. The results revealed that N-GP biomarkers had a significantly higher diagnostic AUROC than the tumor markers (Supplementary Figure 5 and Table 2). The concomitant utilization of N-glycans and tumor markers can substantially augment the diagnostic precision for PDAC, as shown in Supplementary Table 1. The combined use of N-glycans and CA19-9 to diagnose PC yielded an AUC value exceeding 0.919.

The potential clinical value of the proposed model is evaluated based on its diagnostic performance. We then compared the diagnostic performance of the Glyco-model at different clinical stages of PDAC. The results showed that the Glyco-model demonstrated favorable diagnostic performance in all PC stages, with sensitivities ranging from 77.27%-90.00% (Table 3). The diagnostic sensitivity for patients with stage I PDAC was 89.66%. These results show that the Glyco-model can achieve high diagnostic ability and potentially benefit patients by enabling an early diagnosis of PC.

In addition, we have conducted a comparative analysis of the diagnostic efficacy of the model across diverse non-PDAC (healthy control and patients with chronic pancreatitis) cohorts to verify the accuracy and sensitivity of the model. The N-glycan biomarker yielded a specificity of 79.6% for distinguishing patients with PC from healthy controls (Table 4).

We then examined the positivity rate for N-glycan (GP-9) in patients with PDAC who tested negative for various tumor markers. Patients with PDAC who tested negative for each tumor marker showed a higher positive rate of N-glycans (Table 5). The sensitivity of N-glycan features exceeded 80%. This suggests that the inclusion of N-glycans in the negative detection of tumor markers may decrease the rate of missed diagnoses in clinical settings.

DISCUSSION

The primary finding of this study was that N-glycome analysis can differentiate individuals with early-stage (stages I and II) PDAC from the control group. The utilization of such a test in the monitoring of: (1) High-risk patients, including those with hereditary PDAC, PC, *etc.*; (2) Patients with late-onset diabetes who have an elevated risk of developing PDAC within the first 3 years of diabetes; and (3) Patients with ambiguous abdominal symptoms may have clinical benefits. The World Health Organization posits that a significant number of patients with cancer can be spared from untimely mortality if timely diagnosis and treatment are administered. Therefore, the development of more sophisticated diagnostic techniques could facilitate the early detection of PDAC.

Differentiating PDAC from pancreatitis can pose a challenge; however, the current investigation demonstrates that the N-glycan analysis model effectively discriminates PDAC from various pancreatic inflammatory conditions. Currently, a comprehensive biological rationale for the use of N-glycans as PC biomarkers remains elusive. However, cancer progression is characterized by incremental modifications in the tumor microenvironment, which may indicate alterations in the blood biomarker profiles. Hence, clinical data were utilized to identify markers that exhibit altered expression patterns during stage progression, specifically displaying varying levels in samples obtained from patients with early or advanced PDAC.

Caution is advised when interpreting our findings because of several limitations of the study design. First, N-glycan marker ascertainment was developed through case-control studies, thereby precluding the knowledge of its efficacy in a surveillance or therapeutic context until a prospective validation study was conducted. Second, because all patient samples were obtained at diagnosis, the behavior of N-glycan markers in patients following surgical tumor removal remains unpredictable. Furthermore, it should be noted that our study included individuals with an established disease status in both the patient and control groups. Although the obtained AUC values were elevated, it is imperative to acknowledge that this does not necessarily translate to an equivalent performance of the marker in pre-diagnostic samples.
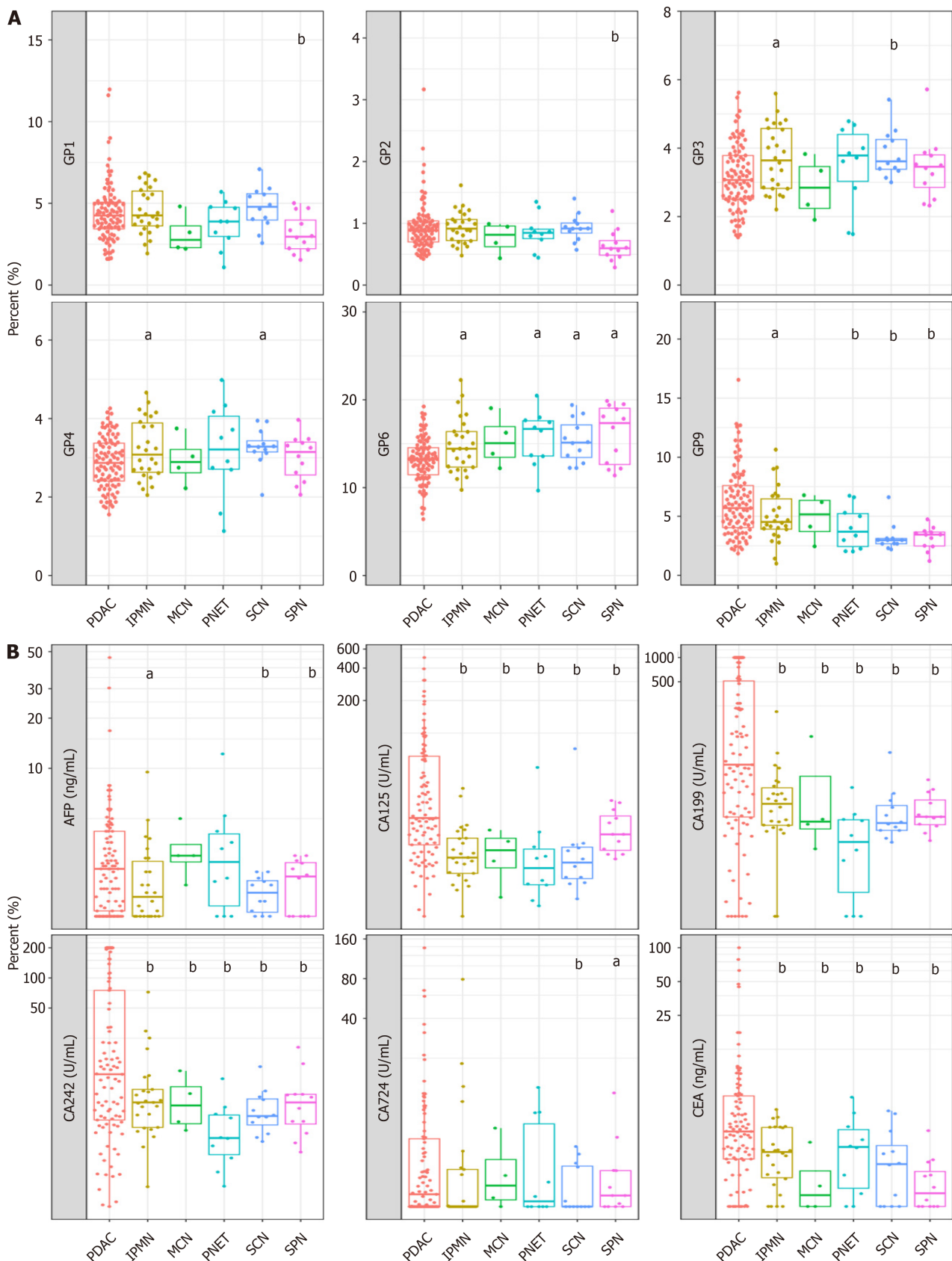
Figure 3 Verification of N-glycan peaks for distinguishing malignant pancreatic cancer (pancreatic ductal adenocarcinoma) from non-pancreatic ductal adenocarcinoma cases. A: N-glycan analysis in pancreatic ductal adenocarcinoma (PDAC) and non-PDAC (intraductal papillary mucinous neoplasm, mucinous cyst neoplasm, pancreatic neuroendocrine tumor, serous cyst adenoma, solid pseudopapillary neoplasm) groups; B: Tumor markers analysis in PDAC and non-PDAC groups. [a]*P* < 0.05, [b]*P* < 0.01. GPs: Glycan peaks; PDAC: Pancreatic ductal adenocarcinoma; SPN: Solid pseudopapillary neoplasm; PNET: Pancreatic neuroendocrine tumor; SCN: Serous cyst adenoma; IPMN: Intraductal papillary mucinous neoplasm; MCN: Mucinous cyst neoplasm; AFP: Alpha fetoprotein; CEA: Carcinoembryonic antigen; CA19-9: Cancer antigen 19-9.

Table 2 Diagnostic performance of N-glycans, tumor markers in distinguishing patients with pancreatic ductal adenocarcinoma

Biomarker	AUC	Cut-off	Sensitivity	Specificity
N-glycan	0.86	0.28	84.90%	73.00%
CA19-9	0.75	37	55.91%	95.49%
CEA	0.72	5.0	22.58%	100.00%
CA125	0.76	35	32.26%	98.10%
CA242	0.67	30	30.11%	98.41%
CA724	0.56	6.9	20.43%	87.30%

AUC: Area under the curve; CEA: Carcinoembryonic antigen; CA19-9: Cancer antigen 19-9.

Table 3 Sensitivity of the Glyco-model in detecting patients with pancreatic ductal adenocarcinoma of different stages

Subgroup	Sensitivity
Overall	84.95% (79/93)
Stage I	89.66% (26/29)
Stage II	77.27% (17/22)
Stage III & IV	90.00% (18/20)
Unknown	81.82% (18/22)

Table 4 Sensitivity of the N-glycan model in non-pancreatic ductal adenocarcinoma individuals

Subgroup	Specificity
Overall	73.03% (111/152)
Healthy	79.55% (70/88)
SPN	83.33% (10/12)
PNET	50.00% (5/10)
SCN	91.67% (11/12)
IPMN	50.00% (13/26)
MCN	50.00% (2/4)

SPN: Solid pseudopapillary neoplasm; PNET: Pancreatic neuroendocrine tumor; SCN: Serous cyst adenoma; IPMN: Intraductal papillary mucinous neoplasm; MCN: Mucinous cyst neoplasm.

Table 5 The positivity rate of Glyco-model among patients with pancreatic ductal adenocarcinoma with tumor markers-negative

Tumor marker negative	Glyco-model positivity rate
CEA ≤ 5 (72)	87.50% (63/72)
CA19-9 ≤ 37 (41)	82.93% (34/41)
CA125 ≤ 35 (63)	88.89% (56/63)
CA242 ≤ 30 (65)	83.08% (54/65)
CA724 ≤ 6.9 (74)	86.49% (64/74)

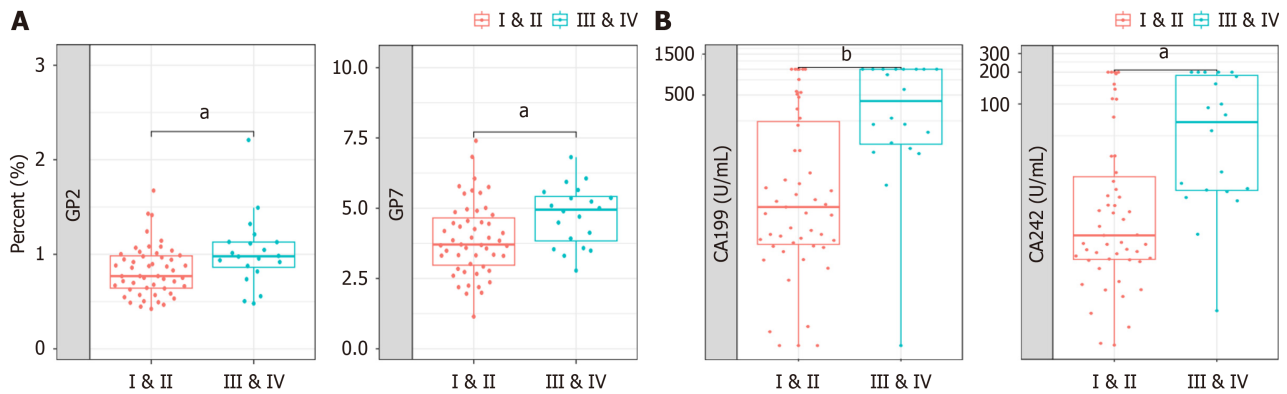CEA: Carcinoembryonic antigen; CA19-9: Cancer antigen 19-9.

Figure 4 Comparative analysis of early-stage and advanced pancreatic ductal adenocarcinoma. A: Serum glycan peak (GP)2 and GP7 levels differ significantly between early (stage I and II) and advanced (stage III and IV) pancreatic ductal adenocarcinoma; B: Serum cancer antigen (CA)19-9 and CA242 levels differ significantly between early (stage I and II) and advanced (stage III and IV) pancreatic ductal adenocarcinoma. $^{a}P < 0.05$, $^{b}P < 0.01$. GPs: Glycan peaks; CA19-9: Cancer antigen 19-9.
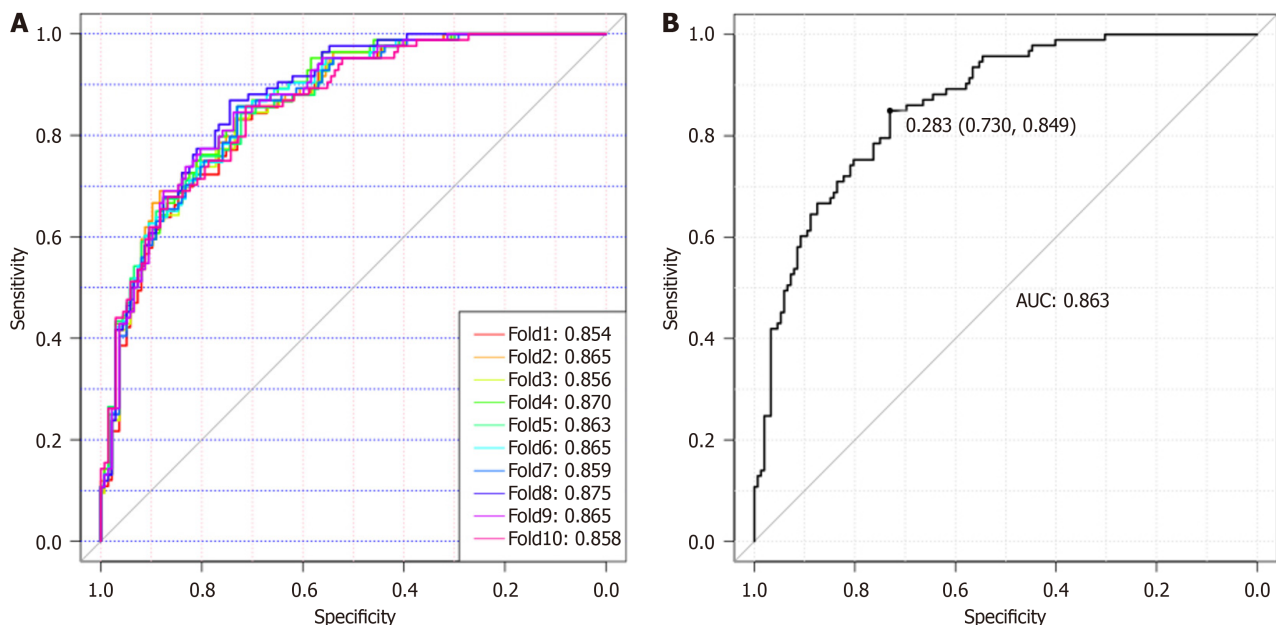

Figure 5 Glyco-model for detecting patients with pancreatic ductal adenocarcinoma. A: Diagnostic model for detecting patients with pancreatic ductal adenocarcinoma (PDAC) using the 10-fold cross-validation diagnostic model; B: The diagnostic values for Glyco-model to distinguish patients with PDAC from those of non-PDAC. AUC: Area under the curve.

CONCLUSION

In summary, we conducted a comprehensive case-control study of patients with PDAC, resulting in the identification and validation of an N-glycan biomarker signature. These results indicate that this biomarker signature exhibited a high level of accuracy in detecting blood samples from patients with stage I and II PDAC.

ARTICLE HIGHLIGHTS

Research background

The absence of diagnostic biomarkers for pancreatic cancer (PC) poses challenges in achieving early detection.

Research motivation

The aim of this study is to identify novel diagnostic markers for the early detection of PC.

Research objectives

The identification of novel glycan markers holds the potential to differentiate early-stage PC, while the development of corresponding models can facilitate the early diagnosis of PC as well as other pancreatic ailments.

Research methods

Serum N-glycan analysis performed to identify the serum biomarker signatures associated with early-stage pancreatic ductal adenocarcinoma (PDAC). A multivariate logistic regression analysis was performed to build a diagnostic model.

Research results

The biomarker signature was created to discriminate samples derived from patients with PC from those of controls. Glyco-model demonstrated favorable diagnostic performance in all stages of PC.

Research conclusions

The serum N-glycan biosignatures and the "Glyco-model" offer a viable method for detecting early-stage PDAC.

Research perspectives

There is a desire to develop additional biomarkers that exhibit heightened sensitivity and specificity for PDAC, in order to facilitate early detection.

FOOTNOTES

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Co-corresponding authors: Cui-Ying Chen and Jian He.

Author contributions: All authors contributed to the study conception and design. Wen YR and Lin XW contributed equally to this work; Lin XW and He J collected the samples and conducted the data analysis; Zhou YW, Xu L, Zhang JL, and Chen CY performed N-glycans analysis; Wen YR and He J designed the research, analyzed data and wrote the manuscript. He J and Chen CY are the co-corresponding authors of this study. He J and Chen CY were involved in the experimental design and revision of the article. Specifically, He J assumed responsibility for the overall design of the subject, Chen CY focused on the experimental design of the N-glycan analysis.

Institutional review board statement: The study protocol was approved by the Ethics Committee of Drum Tower Hospital, the Affiliated Hospital of Nanjing University Medical School and conformed to the ethical guidelines of the Declaration of Helsinki.

Informed consent statement: Written informed consent was obtained from all patients.

Conflict-of-interest statement: All the authors report no relevant conflicts of interest for this article.

Data sharing statement: Technical appendix, statistical code, and dataset available from the corresponding author at hjxueren@126.com.

STROBE statement: The authors have read the STROBE Statement-checklist of items, and the manuscript was prepared and revised according to the STROBE Statement-checklist of items.

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