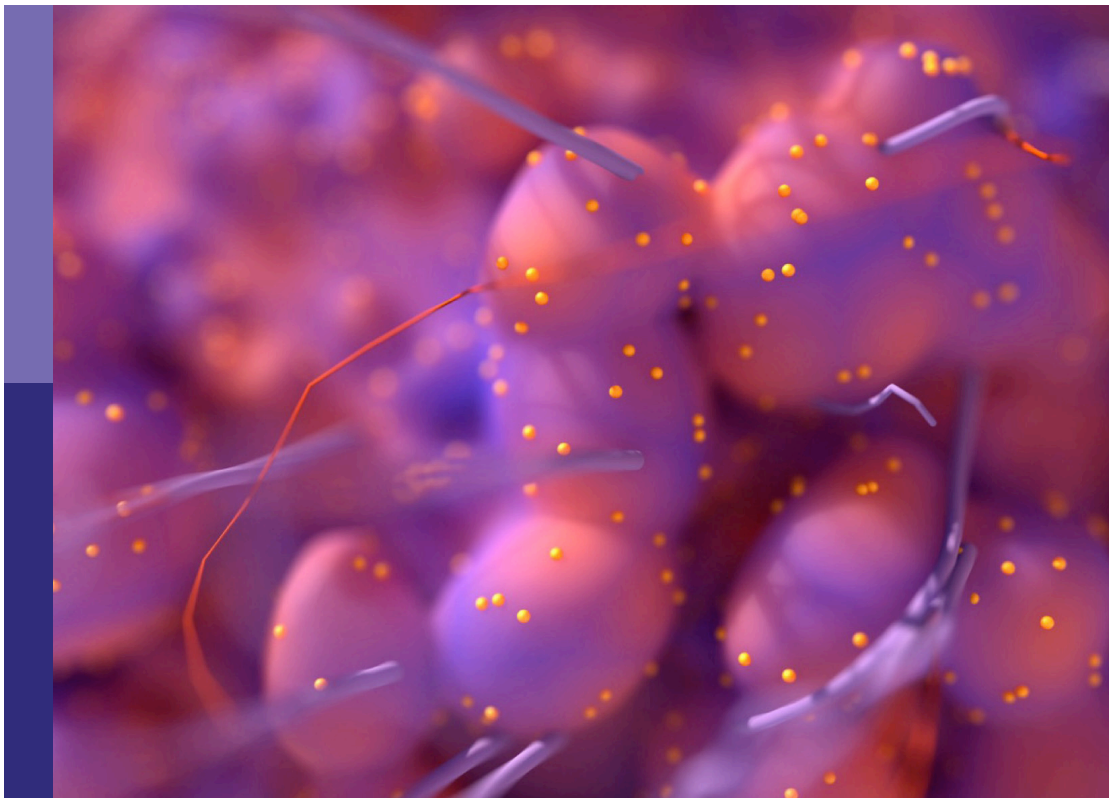
frontiers | Research Topics

Perioperative optimization of patients undergoing pancreatic surgery

Edited by
Xiaodong Tian, Menghua Dai and Jorg Kleeff

Published in
Frontiers in Oncology



ISSN 1664-8714
ISBN 978-2-83251-951-6
DOI 10.3389/978-2-83251-951-6

About Frontiers

Frontiers is more than just an open access publisher of scholarly articles: it is a pioneering approach to the world of academia, radically improving the way scholarly research is managed. The grand vision of Frontiers is a world where all people have an equal opportunity to seek, share and generate knowledge. Frontiers provides immediate and permanent online open access to all its publications, but this alone is not enough to realize our grand goals.

Frontiers journal series

The Frontiers journal series is a multi-tier and interdisciplinary set of open-access, online journals, promising a paradigm shift from the current review, selection and dissemination processes in academic publishing. All Frontiers journals are driven by researchers for researchers; therefore, they constitute a service to the scholarly community. At the same time, the *Frontiers journal series* operates on a revolutionary invention, the tiered publishing system, initially addressing specific communities of scholars, and gradually climbing up to broader public understanding, thus serving the interests of the lay society, too.

Dedication to quality

Each Frontiers article is a landmark of the highest quality, thanks to genuinely collaborative interactions between authors and review editors, who include some of the world's best academicians. Research must be certified by peers before entering a stream of knowledge that may eventually reach the public - and shape society; therefore, Frontiers only applies the most rigorous and unbiased reviews. Frontiers revolutionizes research publishing by freely delivering the most outstanding research, evaluated with no bias from both the academic and social point of view. By applying the most advanced information technologies, Frontiers is catapulting scholarly publishing into a new generation.

What are Frontiers Research Topics?

Frontiers Research Topics are very popular trademarks of the *Frontiers journals series*: they are collections of at least ten articles, all centered on a particular subject. With their unique mix of varied contributions from Original Research to Review Articles, Frontiers Research Topics unify the most influential researchers, the latest key findings and historical advances in a hot research area.

Find out more on how to host your own Frontiers Research Topic or contribute to one as an author by contacting the Frontiers editorial office: frontiersin.org/about/contact

Perioperative optimization of patients undergoing pancreatic surgery

Topic editors

Xiaodong Tian — Peking University, China

Menghua Dai — Peking Union Medical College Hospital (CAMS), China

Jorg Kleeff — University Hospital in Halle, Germany

Citation

Tian, X., Dai, M., Kleeff, J., eds. (2023). *Perioperative optimization of patients undergoing pancreatic surgery*. Lausanne: Frontiers Media SA. doi: 10.3389/978-2-83251-951-6

Table of contents

frontiers | Frontiers in Oncology

TYPE Editorial
PUBLISHED 03 March 2023
DOI 10.3389/fonc.2023.1170409

OPEN ACCESS

EDITED AND REVIEWED BY
Liang Qiao,
Westmead Institute for Medical Research, Australia

*CORRESPONDENCE
Jorg Kleeff
joerg.kleeff@uk-halle.de

SPECIALTY SECTION
This article was submitted to Gastrointestinal Cancers: Hepato Pancreatic Biliary Cancers, a section of the journal Frontiers in Oncology

RECEIVED 20 February 2023
ACCEPTED 27 February 2023
PUBLISHED 03 March 2023

CITATION
Kleeff J, Klose J, Rebelo A and Ronellenfitsch U (2023) Editorial: Perioperative optimization of patients undergoing pancreatic surgery. *Front. Oncol.* 13:1170409. doi: 10.3389/fonc.2023.1170409

Editorial: Perioperative optimization of patients undergoing pancreatic surgery

Jorg Kleeff*, Johannes Klose, Artur Rebelo and Ulrich Ronellenfitsch

Department of Abdominal, Vascular and Endocrine Surgery, Martin-Luther-University Halle-Wittenberg, University Medical Center Halle (Saale), Halle (Saale), Germany

KEYWORDS

pancreatic surgery, pancreatic surgery complications, pancreatic surgery outcomes, pancreatic cancer, perioperative care

Editorial on the Research Topic
Perioperative optimization of patients undergoing pancreatic surgery

Pancreatic surgery has gained in volume in many parts of the world in recent years. This is mainly caused by the rising incidence of pancreatic cancer, for which resection is the only potentially curative treatment modality. There are also indications for pancreatic surgery in specific scenarios of benign diseases such as chronic pancreatitis. For example, the number of pancreatic resections carried out in Germany in 2021 was about 12,000, which equals a rate of roughly 15 per 100,000 inhabitants (1). Notwithstanding this rise in volume, pancreatic resections still bear a relevant risk of complications and death. While the benchmark for mortality is assumed 2% for pancreatic head resection and 1% for distal pancreatectomy, the benchmark for morbidity is between 50% and 60% for both surgeries (2). Yet, in broad clinical practice, these targets are not always reached, with mortality depending on a variety of factors such as hospital and surgeon volume (3). Thus, it is evident that optimal selection, preparation and intra- and postoperative treatment of patients is required to increase the likelihood of a favorable postoperative outcome.

The appropriate indication for surgery in patients with pancreatic cancer is paramount. Interdisciplinary tumor boards play an important role in weighing expected benefits against risks of the available treatments and can recommend a treatment on an evidence basis. Even if technical resectability is given, patients with a high risk of subsequent tumor recurrence and thus poor oncological prognosis will most likely not benefit from upfront surgery. While as of now there is no unanimously accepted modality to predict recurrence risk and survival, a number of potential parameters deserve consideration and further validation. In this special issue, Yang et al. have demonstrated that high serum concentrations of Gremlin 1 (GREM1), a regulator of bone morphogenetic protein signaling, predict shorter survival. This makes it a promising candidate to be potentially used as an adjunct to carbohydrate antigen 19-9 (Ca 19-9), which so far is the only biomarker routinely employed to evaluate the prognosis of patients with pancreatic cancer, but is not without limitations in its applicability (4). In fact, Ca 19-9 serum levels are affected by cholestasis. Wu et al.'s study suggests that Ca 19-9 levels should be adjusted for total bilirubin levels and clinical stage to enhance their prognostic value. Nomograms are a good instrument to assess the prognosis based on several factors. Guo et al. have developed and validated such a

nomogram incorporating age, tumor size, leukocyte count, lymphocyte/monocyte ratio and albumin for predicting lymph node metastases. While local lymph node metastases do not pose a contraindication to resection, they indicate more advanced disease and might influence the decision for or against neoadjuvant therapy. Finally, the study by Cheng et al. suggests that the level of folate receptor-positive circulating tumor cells could predict recurrence and survival in patients with pancreatic adenocarcinoma.

Most patients with cancers located in the pancreatic head or periampullary region are jaundiced upon diagnosis. There is an ongoing debate if preoperative biliary drainage should be aimed for, with improved hepatic function as an argument in favor and the risk of interventional complications and infection of the pancreato-biliary duct system as arguments against it (5). Pattarapuntakul et al. show in their series of patients undergoing pancreatic head resections for periampullary lesions, the majority of which were pancreatic adenocarcinoma, ampullary adenocarcinoma, and cholangiocarcinoma, that preoperative bile drainage was not associated with 1-year survival, but with a lower risk of intraoperative bleeding and bile leakage. Based on their analyses, they recommend drainage for severely jaundiced patients, suggesting a threshold of 14.6 mg/dL.

Intraoperative techniques play an important role with regard to postoperative morbidity. While in pancreatoduodenectomy, the pancreatic anastomosis is a crucial element with postoperative pancreatic fistula (POPF) being one of the most impacting complications (6), in distal pancreatectomy the closure of the resection margin is of relevance to avoid POPF. In 2011, the multi-centre randomized DISPACT trial failed to show superiority of stapler versus suture closure of the remnant (7). Consequently, there is no uniform standard for margin closure. In a propensity-matched analysis, Tian et al. show a considerably lower incidence of POPF for lockstitch-enforced staple line closure compared with staple line closure alone. This result requires verification in randomized trials. Drain placement and timing of removal remain issues of controversy in pancreatic surgery and particularly so in pancreatoduodenectomy. In line with the results of recent meta-analyses on the topic, Xie et al. show in a propensity-matched analysis that drain removal on postoperative day three is safe.

After resection of pancreatic cancer and successful recovery of the patient, estimating the risk of recurrence is important to decide about the expected benefit from generally recommended adjuvant treatment and to guide follow-up. Tong et al. developed and validated a nomogram for predicting the risk of subsequent hepatic metastases incorporating postoperative Ca-125 level, tumor differentiation and size, lymph node ratio and venous invasion.

In summary, pre- intra- and postoperative management of patients undergoing pancreatic resections is crucial to achieve the best outcomes both in terms of postoperative morbidity and mortality and in terms of oncological outcomes for patients with cancer. The studies contained in this special issue have suggested some promising approaches, which require and deserve further validation in prospective studies.

Author contributions

All authors have drafted the manuscript, provided intellectual content, and approved of the final version of the manuscript.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

1. Destatis Statistisches Bundesamt. *Hospital statistics based on rates per case (DRG statistics). operations and procedures of and patients in hospitals* (2021). Available at: https://www.destatis.de/DE/Themen/Gesellschaft-Umwelt/Gesundheit/Krankenhaeuser/Publikationen/Downloads-Krankenhaeuser/operationen-prozeduren-5231401217014.pdf?__blob=publicationFile.

2. Probst P, Hüttner FJ, Meydan Ö, Abu Hilal M, Adham M, Barreto SG, et al. Evidence map of pancreatic surgery-a living systematic review with meta-analyses by the international study group of pancreatic surgery (ISGPS). *Surgery* (2021) 170 (5):1517–24. doi: 10.1016/j.surg.2021.04.023

3. Balzano G, Guarneri G, Pecorelli N, Paiella S, Rancoita PMV, Bassi C, et al. Modelling centralization of pancreatic surgery in a nationwide analysis. *Br J Surg* (2020) 107(11):1510–9. doi: 10.1002/bjs.11716

4. Khomiak A, Brunner M, Kordes M, Lindblad S, Miksch RC, Öhlund D, et al. Recent discoveries of diagnostic, prognostic and predictive biomarkers for pancreatic cancer. *Cancers (Basel)* (2020) 12(11):3234. doi: 10.3390/cancers 12113234

5. Blacker S, Lahiri RP, Phillips M, Pinn G, Pencavel TD, Kumar R, et al. Which patients benefit from preoperative biliary drainage in resectable pancreatic cancer? *Expert Rev Gastroenterol Hepatol* (2021) 15(8):855–63. doi: 10.1080/17474124.2021. 1915127

6. Bassi C, Marchegiani G, Dervenis C, Sarr M, Abu Hilal M, Adham M, et al. The 2016 update of the international study group (ISGPS) definition and grading of postoperative pancreatic fistula: 11 years after. *Surgery* (2017) 161(3):584–91. doi: 10.1016/j.surg.2016.11.014

7. Diener MK, Seiler CM, Rossion I, Kleeff J, Glanemann M, Butturini G, et al. Efficacy of stapler versus hand-sewn closure after distal pancreatectomy (DISPACT): a randomised, controlled multicentre trial. *Lancet* (2011) 377(9776):1514–22. doi: 10.1016/S0140-6736(11)60237-7

frontiers | Frontiers in Oncology

TYPE Original Research
PUBLISHED 29 July 2022
DOI 10.3389/fonc.2022.966256

OPEN ACCESS

EDITED BY
Xiaodong Tian,
First Hospital, Peking University, China

REVIEWED BY
Guopei Luo,
Fudan University, China
Ren Lang,
Beijing Chaoyang Hospital, Capital Medical University, China

*CORRESPONDENCE
Bole Tian
hxtbl0338@163.com

SPECIALTY SECTION
This article was submitted to Gastrointestinal Cancers: Hepato Pancreatic Biliary Cancers, a section of the journal Frontiers in Oncology

RECEIVED 10 June 2022
ACCEPTED 11 July 2022
PUBLISHED 29 July 2022

CITATION
Wu Z, Zhao P, Wang Z, Huang X, Wu C, Li M, Wang L and Tian B (2022) Adjusting CA19-9 values with clinical stage and bilirubin to better predict survival of resectable pancreatic cancer patients: 5-year-follow-up of a single center.
Front. Oncol. 12:966256.
doi: 10.3389/fonc.2022.966256

Adjusting CA19-9 values with clinical stage and bilirubin to better predict survival of resectable pancreatic cancer patients: 5-year-follow-up of a single center

Zuowei Wu, Pengcheng Zhao, Zihe Wang, Xing Huang, Chao Wu, Mao Li, Li Wang and Bole Tian*

Department of Pancreatic Surgery, West China Hospital, Sichuan University, Chengdu, China

Background: Pancreatic cancer mortality is growing every year, and radical resection is the most essential therapy strategy. It is critical to evaluate the long-term prognosis of individuals receiving radical surgery. CA19-9 is a biomarker for patient recurrence and survival, however obstructive jaundice has a significant impact on this index. Researchers have attempted to modify the index using various modification methods, but the results have been unsatisfactory. In this study, we adjusted CA19-9 values based on clinical stage and bilirubin and found that it provided better prediction than CA19-9 alone in assessing patients.

Methods: We analyzed over 5 years follow-up records of patients who underwent radical pancreatic cancer surgery between August 2009 and May 2017 in a single center. We investigated the association of risk factors with overall survival (OS) as well as disease-free survival (DFS) after surgery. Threshold values for high-risk features associated with poor prognosis in resectable pancreatic cancer were determined. The hazard ratios of the indicators were eventually examined under the stratification of patients' clinical stages.

Results: A total of 202 patients were involved in the study. The optimum cut-off values for CA19-9 and CA19-9/TB for predicting overall survival were 219.4 (p = 0.0075) and 18.8 (p = 0.0353), respectively. CA19-9>219.4 increased the risk of patient mortality by 1.70 times (95% CI 1.217-2.377, p = 0.002), and tumor poor differentiation raised the risk by 1.66 times (95% CI 1.083-2.553, P = 0.02). Based on clinical stage stratification, we found discrepancies in the predictive efficacy of CA19-9 and CA19-9/TB. CA19-9 was a better predictor in clinical stage 1 (HR = 2.056[CI 95%1.169-3.616], P = 0.012), whereas CA19-9/TB indications were better in stages 2 (HR = 1.650[CI 95%1.023-2.662], P = 0.040) and 3 (HR = 3.989[CI95%1.145-13.896], P = 0.030).

Conclusions: CA19-9, CEA, and tumor differentiation are predictors for patients with resectable PDAC. CA19-9 values can be adjusted based on clinical stage and bilirubin levels to better predict overall survival in patients with resectable PDAC. CA19-9>219.4 predicted poor survival in individuals in clinical stage 1, whereas CA19-9/TB>18.8 predicted poor survival for individuals in stages 2 and 3.

KEYWORDS

carcinoma, pancreatic ductal, pancreatectomy, prognosis, biomarkers, CA19-9 antigen, bilirubin

Introduction

The morbidity and mortality of pancreatic cancer are rising quickly over the world (1). Non-surgical treatments for pancreatic cancer are unsatisfactory (2). The only method to achieve a radical cure is by surgical resection. Whether or not the operation is performed influences the patient's overall prognosis and treatment strategy (3). Therefore, it is important to investigate the prognostic indicators of pancreatic cancer surgery.

Many studies have been undertaken in order to identify the best serum biomarkers for predicting the prognosis of pancreatic ductal adenocarcinoma (PDAC) patients. Previous research has shown that carbohydrate antigen 19-9 (CA19-9) is a biomarker for recurrence and survival (4). Bilirubin, on the other hand, has an effect on the level of CA19-9. Under normal conditions, bilirubin is generated from the hemoglobin of senescent erythrocytes (5). Bilirubin levels rise dramatically when the obstruction is caused by malignant disease. CA19-9 levels are higher in hyperbilirubinemia patients, resulting in a lower specificity of CA19-9 in predicting patients' survival. This interferes with the prognostic value of CA19-9 in individuals with pancreatic cancer who have obstructive jaundice. Researchers use a variety of adjustment formulae to optimize the indicators to increase the prediction accuracy of CA19-9. The clinical stage of the patient can indicate the size of the tumor and may suggest the degree of biliary obstruction (4). In the light of this hypothesis, we expect that adjusting CA19-9 values with clinical staging and bilirubin provides a better accurate prognostic expectation than CA19-9 alone.

In this study, we reviewed the patient data of our hospital and investigated the correlation between indicators and the overall survival (OS) as well as disease-free survival (DFS) of patients in different clinical stages. It is favorable to the future clinical application of these indicators in order to improve the surgeon's ability to predict PDAC patients prior to surgery. In compliance with the STROBE reporting checklist, we provide the following article (6).

Materials and methods

Patients

Between August 2009 and May 2017, we analyzed the data of PDAC patients who underwent radical surgical treatment at our institution (West China Hospital of Sichuan University, Sichuan, China). The hospital's medical record system was used to collect patient information, laboratory and pathological features for this study. All patients did not get preoperative pretreatment but received postoperative adjuvant chemotherapy (based on S1 or gemcitabine). The patient's latest follow-up was in April 2022. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). This study was approved by the Ethics Committee of West China Hospital of Sichuan University, and written informed consent was obtained from all patients before surgery.

Eligibility criteria: (I) patients with PDAC who underwent radical surgical treatment between August, 2009 to May 2017; (II) no restriction was imposed on age and gender; (III) histopathological diagnosis of pancreatic ductal adenocarcinoma with intact pathological samples.

Exclusion criteria: (I) metastasis was found at the initial operation; (II) R2 resection; (III) data on clinical, laboratory characteristics, treatments, outcomes and follow-up are not available; (IV) died within 30 days; (V) lost to follow-up within two years after operation.

The OS was defined as from the dates of surgery to the dates of death. The DFS was calculated from the interval between the dates of surgery and the first recurrence or metastasis. If there was no recurrence or metastasis at the time of the patient's death, the DFS and OS dates were the same.

Risk factor analysis

We investigated the correlation between risk factors and the prognosis of patients in different clinical stages. The potential

indicators such as age, gender, pain, degree of tumor differentiation, vascular invasion, nerve invasion, cutting edge status, tumor site, CA19-9, Carcinoembryonic antigen (CEA), total bilirubin (TB), CA19-9/TB, and clinical stage, etc. were used to identify the univariate risk factors affecting resectable PDAC patients' OS and DFS, respectively. The eighth edition of the American Joint Committee on Cancer (AJCC) guidelines for pancreatic cancer is used to determine Tumor-Node-Metastasis (TNM) and clinical stage of malignancies (7).

On the basis of univariate test, Cox survival regression analysis was further carried out to find the risk factor and the hazard ratio (HR). The analysis is based on the premise that the Kaplan–Meier survival curve does not cross. We only investigated variables that were statistically significant after initial screening, on which HR values and P values were built.

Correlation analysis and linear regression analysis

We performed Pearson correlation to examine the relationship between CA19-9 and tumor pathological parameters such as tumor maximum diameter, lymph node metastases, tumor site, differentiation, vascular invasion, and nerve invasion in order to improve the accuracy of CA19-9 in predicting OS. In addition, a linear regression analysis was applied to see if the indicators and CA19-9 have a linear correlation.

Statistical methods

Continuous variables were stratified using the X-tile software (8) (version 3.6.1, Yale University, Connecticut, USA) to determine ideal cut-off values according to the minimum P values from log-rank, chi-square statistics, and convert into classified variables. Univariate risk factors were performed by Log-rank analyses. "Backward: Conditional" of Cox proportional hazards model was used for multivariate analysis and the hazard ratios were obtained. Pearson test and linear regression analysis is used to analysis the correlation between continuous variables. The Kaplan–Meier method was used to analyze survival duration. These statistical analyses were performed using IBM SPSS Statistics for Windows, version 22.0 (IBM Corp., Armonk, NY, USA). A P value of < 0.05 was considered statistically significant. All P values are derived from two-tailed tests.

Results

Patient characteristics

The study comprised 216 individuals who had radical excision of pancreatic ductal adenocarcinoma between August 11, 2009 and May 16, 2017. Ten patients with PDAC oligometastasis concurrent resection were eliminated, four patients with R2 resection verified by intraoperative and postoperative pathology were excluded, and no patients died within 30 days. As a result, 202 patients were included in the research. Seven patients who lived for 24-60 months but were unable to contact effectively in the most recent follow-up were included in the research as the censored value. Table 1 summarizes the patients' characteristics.

Cut-off value of continuous variables

Using the X-tile program, continuous variables such as CA19-9, CEA, TB, and CA19-9/TB were stratified (Figure 1). OS was used as the dependent variable. Then, using the optimal cut-off value, an accurate and vital survival analysis was performed by subgroups for OS and DFS, respectively. The analysis results revealed that the appropriate cut-off values for CA19-9, CEA, TB and CA19-9/TB, were 219.4 ($p = 0.008$), 5.8 ($p = 0.138$), 200 ($p = 1.000$) and 18.8 ($p = 0.035$), respectively. The survival curves showed that patients with higher CA19-9 and CA19-9/TB values had a shorter OS and DFS.

Risk factors analysis

The Kaplan–Meier analysis was carried out to screen the univariate indicators affecting OS and DFS (Table 2). CA19-9 was found to be statistically significant in predicting OS in a univariate survival analysis. The survival curves of the indicators with statistically significant differences were recorded in Figure 2. CA19-9>219.4 increased the risk of death by 1.70 times (95% CI 1.217-2.377, $P = 0.002$) in the Cox proportional hazards model, and Tumor low differentiation increased the risk of death by 1.66 times (95% CI 1.083-2.553, $P = 0.02$).

Correlation analysis between CA19-9 and tumor characteristics

CA19-9 has a correlation with both the maximum diameter of the tumor ($P = 0.022$) and the positive lymph nodes ($P = 0.033$) according to linear regression analysis. There is no multicollinearity between the two indicators (variance inflation factor (VIF) = 1.000). The Dubin Watson (DW) test was used to determine that the independent variables had no autocorrelation (DW value = 1.804, Adjusted R2 value=0.038). Based on the linear correlation between CA19-9 and tumor information, the correction formula CA19-9 = 160.562+46.685*d+39.654*ln (d = tumor maximum diameter, ln=lymph nodes metastases number) was developed.

10.3389/fonc.2022.966256

TABLE 1 Patient characteristics and baseline information.

Characteristic		Patients	[%]	Clinical Stage						Statistics	
		All (N=202)		I (N=80)		II (N=100)		III (N=22)		X^2	P
Sex	Male	120	[59.4]	47	[58.8]	64	[64.0]	9	[40.9]	4.011	0.135
	Female	82	[40.6]	33	[41.2]	36	[36.0]	13	[59.1]		
Age, (range), year		60	(30-84)	59	(34-84)	62	(30-77)	56	[43-74]	4.873	0.087
Abdominal/back pain		121	[59.9]	51	[63.7]	57	[57.0]	13	[59.1]	0.850	0.654
Tumor site	Head	143	[70.8]	56	[70.0]	71	[71.0]	16	[72.7]	0.066	0.967
	Body & Tail	59	[29.2]	24	[30.0]	29	[29.0]	6	[27.3]		
CA19-9 level, (IQR), U/mL		187.4	(44.3-639.8)	139.0	(37.6-498.3)	256.1	(50.5-796.2)	203.2	(81.6-1000.0)	5.640	0.060
CEA level, (IQR), ng/mL		3.0	(1.8-5.2)	2.2	(1.6-4.1)	3.8	(2.1-5.8)	2.5	(1.5-5.8)	4.506	0.105
Total Bilirubin level, (IQR), μmol/L		21.8	(11.2-184.5)	21.5	(12.2-182.2)	24.3	(10.4-183.7)	17.3	(11.1-224.5)	1.300	0.522
Surgery	Pancreaticoduodenectomy	135	[66.8]	55	[68.7]	67	[67.0]	13	[59.1]	6.282	0.392
	Distal pancreatectomy	52	[25.7]	22	[27.5]	25	[25.0]	5	[22.7]		
	Total pancreatectomy	15	[7.4]	3	[3.8]	8	[8.0]	4	[18.2]		
Cutting edge status	R0	144	[71.3]	61	[76.3]	70	[70.0]	13	[59.1]	2.642	0.267
	R1	58	[28.7]	19	[23.7]	30	[30.0]	9	[40.9]		
T	Maximum diameter of tumor, (range), centimeter	3.5	(1.0-8.5)	3.0	(1.5-4)	4.3	(1.0-8.5)	4.0	[2.0-6.0]	NA	
	1	16	[7.9]	12	[15.0]	4	[4.0]	0	[0.0]		
	2	109	[54.0]	68	[85.0]	35	[35.0]	6	[27.3]		
	3	62	[30.7]			61	[61.0]	1	[4.5]		
	4	15	[7.4]					15	[68.2]		
N	Node, (range), number	0	(0-8)	0	(0-0)	1	(0-3)	1	(0-8)	NA	
	0	131	[64.9]	80	[100.0]	40	[40.0]	11	[50.0]		
	1	64	[31.7]			60	[60.0]	4	[18.2]		
	2	7	[3.5]					7	[31.8]		
Vascular Invasion		98	[48.5]	10	[12.5]	74	[74.0]	14	[63.6]	24.390	<0.001
Nerve Invasion		112	[55.4]	36	[45.0]	62	[62.0]	14	[63.6]	5.870	0.053
Differentiation	Low	151	[74.8]	57	[71.3]	81	[81.0]	13	[59.1]	5.447	0.066
	Intermediate/High	51	[25.2]	23	[28.7]	19	[19.0]	9	[40.9]		
Recurrence		37	[18.3]	14	[17.5]	19	[19.0]	4	[18.2]	0.067	0.967
Metastasis	Abdominal	45	[22.3]	16	[20.0]	23	[23.0]	6	[27.3]	0.587	0.746
	Chest	31	[15.3]	7	[8.8]	21	[21.0]	3	[13.6]	5.189	0.075
	Multiple	15	[7.4]	8	[10.0]	6	[6.0]	1	[4.5]	1.332	0.514
OS	median (range)	31.5	(1-120)	40	(4-120)	24	(1-94)	20.5	(8-76)	8.168	0.017
	1-year-survivor	166	[82.2]	71	[88.7]	75	[75.0]	20	[90.9]		
	3-year-survivor	119	[58.9]	56	[70.0]	52	[52.0]	11	[50.0]		
	5-year-survivor	58	[28.7]	31	[38.8]	22	[22.0]	5	[22.7]		
DFS	median (range)	19.0	(1-120)	26	(1-120)	17	(1-94)	11	(4-76)	7.293	0.026
	1-year-survivor	129	[63.9]	58	[72.5]	61	[61.0]	10	[45.5]		
	3-year-survivor	73	[36.1]	36	[45.0]	31	[31.0]	6	[27.3]		
	5-year-survivor	53	[26.2]	30	[37.5]	18	[18.0]	5	[22.7]		

OS, Overall Survival; DFS, Disease Free Survival; CA19-9, Carbohydrate Antigen 19-9; CEA, Carcinoembryonic Antigen; IQR, interquartile range.

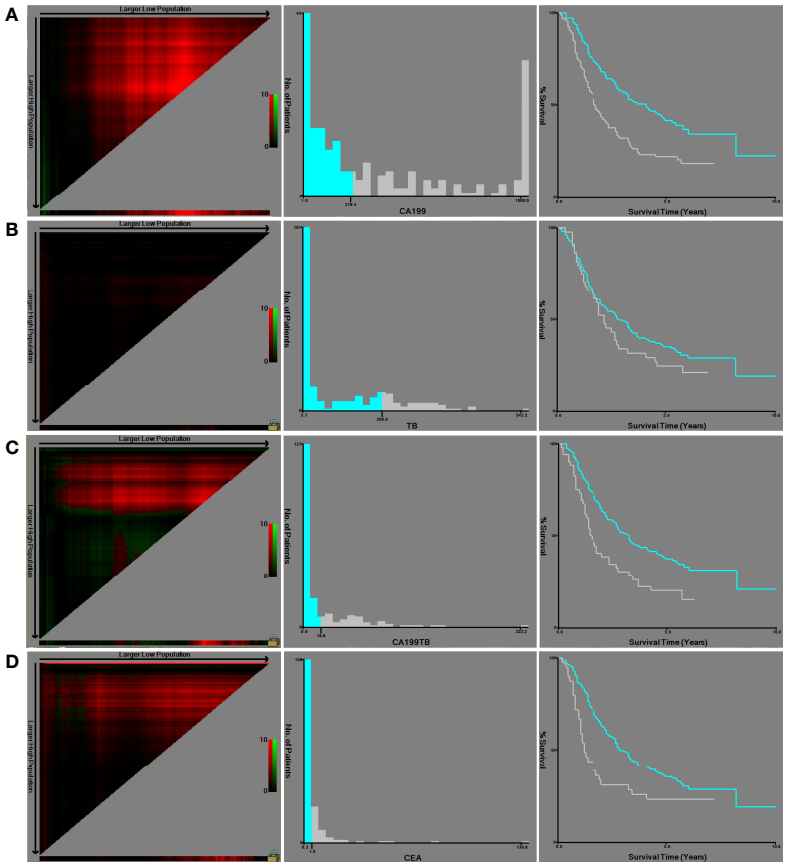


FIGURE 1
X-tile software was used to obtain the cut-off values of continuous variables and to perform survival analysis: **(A)** CA19-9; **(B)** TB; **(C)** CA19-9/TB; **(D)** CEA.

TABLE 2 Risk factors affecting survival.

Variables	OS Log-Rank	P	Cox(HR)	95%CI	P	DFS Log-Rank	P	Cox(HR)	95%CI	P
Age(>65)	1.079	0.299				1.178	0.278			
Sex(Male)	2.228	0.135				2.508	0.113			
Pain	0.272	0.602				0.074	0.786			
Differentiation(Low)	7.293	0.007	1.663	1.083-2.553	0.020	6.72	0.01	1.591	1.049-2.413	0.029
Vascular Invasion	0.042	0.838				0.038	0.846			
Nerve Invasion	2.652	0.103				3.411	0.065			
R0	0.247	0.619				0.228	0.633			
Site	2.238	0.135				4.328	0.037			
CA19-9>219.4	12.096	0.001	1.701	1.217-2.377	0.002	12.242	<0.001	1.696	1.215-2.365	0.002
CA19-9/TB>18.8	10.074	0.002				12.502	<0.001			
CEA>5.8	5.421	0.02				3.763	0.052			
TB>200	1.445	0.229				0.618	0.432			
Clinical stage	8.168	0.017				7.293	0.026			

OS, Overall Survival; DFS, Disease Free Survival; CA19-9, Carbohydrate Antigen 19-9; CEA, Carcinoembryonic Antigen; TB, Total Bilirubin.

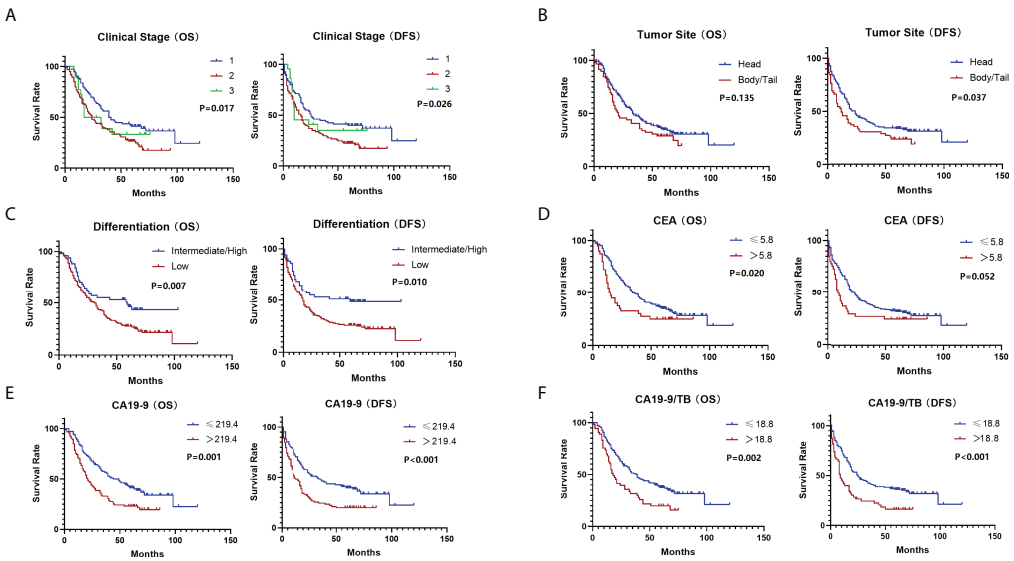


FIGURE 2
The survival curves (OS & DFS) of the indicators after primary screening: **(A)** Clinical stage; **(B)** Tumor site; **(C)** Differentiation; **(D)** CEA; **(E)** CA19-9; **(F)** CA19-9/TB.

Survival analysis with adjusted CA19-9

According to the findings, tumor stage has a significant impact on patients' overall survival. We divided patients into three groups based on their clinical stage to reduce the interference of tumor stage. The ability of CA19-9 and CA19-9/TB to predict disease progression was then tested at different stages. In clinical stage 1, the HR of CA19-9 was higher than CA19-9/TB (HR=2.056 [CI 95% 1.169-3.616], P=0.012 vs HR=1.513[CI 95% 0.802-2.856], P=0.201), while lower in stage 2 (HR=1.381[CI 95% 0.880-2.166], P=0.160 vs HR=1.650[CI 95% 1.023-2.662], P=0.040) and stage 3(HR=2.359[CI 95% 0.812-6.854], P=0.115 vs HR=3.989[CI 95% 1.145-13.896], P=0.030).

Discussion

In this study, we first looked into the factors that affect OS and DFS, and found that CA19-9, CEA, and low-differentiation were mostly predictors for poor patient outcomes. Meanwhile, using correlation analysis, we found that CA19-9 had a possible linear association with tumor dimensions and the positive lymph nodes. CA19-9 levels may be connected to tumor growth or metastatic burden, according to this research. Despite the fact that this linear association is modest, it gives us fresh ideas. These two indications are, interestingly, closely tied to TNM staging. We hypothesized that the patient's TNM stage could be effective in predicting the indicators' predictive effect. We divided patients into groups based on their clinical stage based on this idea. CA19-9 and CA19-9/TB were investigated for their prognostic accuracy in range of clinical stages. CA19-9 was found to have better predictive qualities for patients' OS when stratified by clinical stage and adjusted with TB. Briefly, we found using CA19-9>219.4 in clinical stage 1 and CA19-9/TB>18.8 in clinical stages 2 and 3 has a better effect on dividing patients' OS as well as DFS and indicates a worse prognosis. More importantly, TNM staging is broadly assessable from pre-operative radiographic indicators. It is helpful for clinicians to make preoperative judgments on patient prognosis.

Pancreatic cancer has a dismal overall prognosis, however patient response to treatment varies substantially. Surgical operation is an important aspect of the patient's treatment process. Patients with resectable pancreatic cancer have a significantly better prognosis than those with unresectable cancer (9). There is no doubt that patient treatment could be improved if clinicians could predict a patient's long-term prognosis prior to surgery rather than thereafter *via* pathology. However, predicting a patient's survival before surgery is challenging. Using CA19-9 to predict the long-term prognosis of resectable patients is a convenient and efficient method (10). It also serves as a signal for recurrence surveillance and chemotherapy sensitivity. However, CA19-9 is not specific enough because of the interference of raised bilirubin due to biliary obstruction (11). Previous investigators directly used bilirubin-corrected CA19-9 without considering clinical staging, which may be flawed (12). A bile duct obstruction, for example, would be unusual in a patient with a tiny tumor. Using

CA19-9/TB directly may lead to a lower predictive value and diverge from the primary purpose in this situation.

Previously, researchers employed CA19-9 or CA19-9/TB as an indication of prognosis or tumor malignancy in earlier. Others have developed more complex compositions, such as CA19-9 +Bilirubin+CA19-9/(Bilirubin^{-1}) (4). To reduce the effect of biliary blockage on CA19-9, Zhao et al. developed a correction formula for CA19-9 following bile duct draining (13). Xu et al. found that tumor volume influenced CA19-9, and that volume-corrected CA19-9 may be employed as an independent risk factor influencing PDAC prognosis (14). It is apparent that utilizing more sophisticated formulas or adding more markers may increase predictive accuracy, but whether this is beneficial for clinical usage and popularization is debatable. We should find a new balance between a simple rough calculation and a complex precise one. We believe that using clinical staging to stratify patients is reasonable in this situation. Preoperative radiography can provide a rough estimate of clinical staging for patients with resectable PDAC based on tumor diameter and lymph node morphology in actual clinical practice, especially following the 8th revision of AJCC staging (15, 16). We believe that staging, in addition to the serum index CA19-9, can incorporate more preoperative information to improve patient prognosis prediction.

Other clinical and pathological variables, such as pain, cutting edge status (R0 vs. R1), vascular invasion, and nerve invasion, were found to be of limited utility in assessing prognosis in our study. Pain, as demonstrated by Xu et al., cannot be used alone to predict PDAC (5). The two most common causes of stomach and/or back pain produced by pancreatic cancer are chronic pancreatitis and tumor invasion of nerves. Due to a lack of pancreatitis symptoms, we simply looked at the relationship between pain and nerve invasion, however there was no statistical significance (P=0.566). Previous research have been divided on the impact of R0/R1 resection on patient prognosis, and there is conflicting evidence on whether R1 resection has a similar prognosis to R0 resection (17). Because this was a retrospective analysis with a long follow-up period, we concentrated on the actual outcome of patients following R1 resection who did not receive neoadjuvant chemotherapy. The findings indicated that R0 and R1 resection had little effect on patient prognosis. Datta et al. explained that this may be a surrogate for biologic aggressiveness that is unlikely to be mitigated by the extent of surgical resection (18). We believe that more studies are needed to verify the new outcomes in response to current neoadjuvant therapies. In contrast, there was an association between the lower differentiation and worse prognosis. Unfortunately, 74.8% of patients had a low differentiated tumor pathology result. In contrast, if a patient's postoperative pathology is intermediate/high differentiated, the patient has a considerably better survival rate. Although CEA had predictive effect on OS in this study, it was not as powerful as CA19-9 and was not predictive on DFS (19). Therefore, we did not incorporate further correction for CEA. Also, we found an interesting result that patients with pancreatic body or tail tumors had a shorter DFS and were more likely to have disease recurrence but have similar OS. Erning's study also stated that pancreatic body tail cancer is more likely to metastasize (20). However, Zheng et al. reviewed previous research and found that pancreatic head cancer, particularly in stage II, appears to have a significantly worse prognosis (21). It is uncertain if this phenomena is caused by anatomy or molecular biology. Further study is needed to elucidate the underlying mechanisms for these disparities.

The limitation of this study is that it was a retrospective study conducted at a single center. Since the cut-off value only comes from the population in this study, the cut-off value mainly shows the trend in the sample data. A bigger sample size is required for an accurate cut-off value. The time span is long, and the level of experience of surgeons varies. Patients were mostly from 5 years ago, therefore current adjuvant and neoadjuvant therapy were not used. In additional to this, we did not consider CA19-9-Low&Lewis (+) pancreatic cancer patients, which may have an uncertain impact on accuracy (22). Furthermore, we did not distinguish the tumor site because the sample size would be further lowered. Because the constraints listed above may cause bias in the results, further data from other centers is required for further validation of our findings.

Through this study, we found that CA19-9, CEA and tumor low-differentiated are markers of a poor prognosis in resectable PDAC patients. CA19-9 has a modest linear correlation with tumor maximum diameter and positive lymph nodes. Following clinical staging, we used CA19-9 to assess clinical stage 1 patients and CA19-9/TB to assess stage 2,3 patients, respectively. CA19-9>219.4 or CA19-9/TB>18.8 suggested a poor long-term prognosis for patients.

Conclusions

CA19-9, CEA and tumor low-differentiated are predictors that affect the prognosis of resectable PDAC patients. CA19-9 values adjusted with clinical stage and bilirubin could better predict overall survival in patients with resectable PDAC. For patients in clinical phase 1, CA19-9>219.4 indicates a worse chance of survival. CA19-9/TB>18.8 predicts a worse OS in patients in stages 2 and 3. This study will help clinicians comprehend patients with high preoperative CA19-9 levels and simply probability of patient survival.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving human participants were reviewed and approved by West China Hospital of Sichuan University. The patients/participants provided their written informed consent to participate in this study.

Author contributions

ZWW, PZ, CW, and BT conceived and designed the study. ZWW, PZ, XH, and ZHW were responsible for the collection and assembly of data, data analysis, and interpretation. ZWW, ML, and LW were involved in writing the manuscript. ZWW and BT revised the manuscript. All authors contributed to the article and approved the submitted version.

Funding

The authors declare that the research was conducted in the absence of any funding including commercial or financial relationships that could be construed as a potential conflict of interest.

Acknowledgments

The authors thank all medical staff who contributed to the maintenance of medical records and access to follow-up records.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

1. Siegel RL, Miller KD, Fuchs HE, Jemal A. Cancer statistics, 2021. *CA Cancer J Clin* (2021) 71(1):7–33. doi: 10.3322/caac.21654

2. Schizas D, Charalampakis N, Kole C, Economopoulou P, Koustas E, Gkotsis E, et al. Immunotherapy for pancreatic cancer: A 2020 update. *Cancer Treat Rev* (2020) 86:102016. doi: 10.1016/j.ctrv.2020.102016

3. Huang J, Lok V, Ngai CH, Zhang L, Yuan J, Lao XQ, et al. Worldwide burden of, risk factors for, and trends in pancreatic cancer. *Gastroenterology* (2021) 160 (3):744–54. doi: 10.1053/j.gastro.2020.10.007

4. Boyd LNC, Ali M, Kam L, Puik JR, Rodrigues SMF, Zwart ES, et al. The diagnostic value of the Ca19-9 and bilirubin ratio in patients with pancreatic cancer, distal bile duct cancer and benign periampullary diseases, a novel approach. *Cancers* (2022) 14(2):344. doi: 10.3390/cancers14020344

5. Xu WL, Wang J, Lyu SC, Zhou L, He Q, Lang R. Ratio of Ca19-9 level to total bilirubin as a novel prognostic indicator in patients with pancreatic head carcinoma following curative resection. *Gland Surg* (2021) 10(3):980–91. doi: 10.21037/gs-20-720

6. Cuschieri S. The strobe guidelines. *Saudi J Anaesth* (2019) 13(Suppl 1):S31–S4. doi: 10.4103/sja.SJA_543_18

7. van Roessel S, Kasumova GG, Verheij J, Najarian RM, Maggino L, de Pastena M, et al. International validation of the eighth edition of the American joint committee on cancer (Ajcc) tnm staging system in patients with resected pancreatic cancer. *JAMA Surg* (2018) 153(12):e183617. doi: 10.1001/jamasurg.2018.3617

8. Camp RL, Dolled-Filhart M, Rimm DL. X-Tile: A new bio-informatics tool for biomarker assessment and outcome-based cut-point optimization. *Clin Cancer Res* (2004) 10(21):7252–9. doi: 10.1158/1078-0432.CCR-04-0713

9. Perri G, Prakash L, Katz MHG. Defining and treating borderline resectable pancreatic cancer. *Curr Treat Options Oncol* (2020) 21(9):71. doi: 10.1007/s11864-020-00769-1

10. Ye C, Sadula A, Ren S, Guo X, Yuan M, Yuan C, et al. The prognostic value of Ca19-9 response after neoadjuvant therapy in patients with pancreatic cancer: A systematic review and pooled analysis. *Cancer Chemother Pharmacol* (2020) 86 (6):731–40. doi: 10.1007/s00280-020-04165-2

11. Tsen A, Barbara M, Rosenkranz L. Dilemma of elevated Ca 19-9 in biliary pathology. *Pancreatology* (2018) 18(8):862–7. doi: 10.1016/j.pan.2018.09.004

12. Distler M, Pilarsky E, Kersting S, Grützmann R. Preoperative cea and Ca 19-9 are prognostic markers for survival after curative resection for ductal adenocarcinoma of the pancreas – a retrospective tumor marker prognostic study. *Int J Surg* (2013) 11(10):1067–72. doi: 10.1016/j.ijsu.2013.10.005

13. Zhao B, Cheng Q, Cao H, Zhou X, Li T, Dong L, et al. Dynamic change of serum Ca19–9 levels in benign and malignant patients with obstructive jaundice after biliary drainage and new correction formulas. *BMC Cancer* (2021) 21(1):517. doi: 10.1186/s12885-021-08204-w

14. Xu D, Wang J, Liu T, Huang Z, Luo J, Chen Y, et al. Quantitative definitions of pain, Ca19-9, and tumor size as high-risk features of resectable pancreatic cancer: A single-center retrospective cohort study. *Gland Surg* (2021) 10(2):770–9. doi: 10.21037/gs-20-877

15. Takahashi D, Kojima M, Morisue R, Sugimoto M, Kobayashi S, Takahashi S, et al. Comparison of morphological features in lymph node metastasis between pancreatic neuroendocrine neoplasms and pancreatic ductal adenocarcinomas. *Pancreatology* (2020) 20(5):936–43. doi: 10.1016/j.pan.2020.05.013

16. Marmor S, Burke EE, Portschy PR, Virnig BA, Jensen EH, Tuttle TM. Lymph node evaluation for treatment of adenocarcinoma of the pancreas. *Surg Oncol* (2015) 24(3):284–91. doi: 10.1016/j.suronc.2015.06.006

17. Rau BM, Moritz K, Schuschan S, Alsfasser G, Prall F, Klar E. R1 resection in pancreatic cancer has significant impact on long-term outcome in standardized pathology modified for routine use. *Surgery* (2012) 152(3 Suppl 1):S103–11. doi: 10.1016/j.surg.2012.05.015

18. Datta J, Willobee BA, Ryon EL, Shah MM, Drebin JA, Kooby DA, et al. Contemporary reappraisal of intraoperative neck margin assessment during pancreaticoduodenectomy for pancreatic ductal adenocarcinoma: A review. *JAMA Surg* (2021) 156(5):489–95. doi: 10.1001/jamasurg.2020.5676

19. Luo G, Liu C, Guo M, Long J, Liu Z, Xiao Z, et al. Ca19-9-Low&Lewis (+) pancreatic cancer: A unique subtype. *Cancer Lett* (2017) 385:46–50. doi: 10.1016/j.canlet.2016.10.046

20. van Erning FN, Mackay TM, van der Geest LGM, Groot Koerkamp B, van Laarhoven HWM, Bonsing BA, et al. Association of the location of pancreatic ductal adenocarcinoma (Head, body, tail) with tumor stage, treatment, and survival: A population-based analysis. *Acta Oncol* (2018) 57(12):1655–62. doi: 10.1080/0284186X.2018.1518593

21. Zheng Z, Wang M, Tan C, Chen Y, Ping J, Wang R, et al. Disparities in survival by stage after surgery between pancreatic head and Body/Tail in patients with nonmetastatic pancreatic cancer. *PLoS One* (2019) 14(12):e0226726. doi: 10.1371/journal.pone.0226726

22. Luo G, Liu C, Guo M, Cheng H, Lu Y, Jin K, et al. Potential biomarkers in Lewis negative patients with pancreatic cancer. *Ann Surg* (2017) 265(4):800–5. doi: 10.1097/SLA.0000000000001741

frontiers | Frontiers in Oncology

TYPE Original Research
PUBLISHED 26 August 2022
DOI 10.3389/fonc.2022.968610

OPEN ACCESS

EDITED BY
Xiaodong Tian,
First Hospital, Peking University, China

REVIEWED BY
Liang Liu,
Fudan University, China
Chunhui Yuan,
Peking University Third Hospital, China

*CORRESPONDENCE
Qiaofei Liu
qfliu@aliyun.com
Quan Liao
lqpumc@126.com

†These authors have contributed equally to this work

SPECIALTY SECTION
This article was submitted to Gastrointestinal Cancers: Hepato Pancreatic Biliary Cancers, a section of the journal Frontiers in Oncology

RECEIVED 14 June 2022
ACCEPTED 26 July 2022
PUBLISHED 26 August 2022

CITATION
Yang S, Zhang Y, Hua Y, Cui M, Wang M, Gao J, Liu Q and Liao Q (2022) GREM1 is a novel serum diagnostic marker and potential therapeutic target for pancreatic ductal adenocarcinoma. *Front. Oncol.* 12:968610. doi: 10.3389/fonc.2022.968610

GREM1 is a novel serum diagnostic marker and potential therapeutic target for pancreatic ductal adenocarcinoma

Sen Yang[1†], Yalu Zhang[1,2†], Yuze Hua[1], Ming Cui[1], Mengyi Wang[1], Junyi Gao[1], Qiaofei Liu[1*] and Quan Liao[1*]

[1]Department of General Surgery, State Key Laboratory of Complex Severe and Rare Diseases, Peking Union Medical College Hospital, Chinese Academy of Medical Science and Peking Union Medical College, Beijing, China, [2]Department of General Surgery, Anhui Provincial Hospital, The First Affiliated Hospital of USTC, Division of Life Science and Medicine, University of Science and Technology of China, Hefei, China

Objective: Pancreatic ductal adenocarcinoma (PDAC) is a highly malignant neoplasm with rising incidence worldwide. Gremlin 1 (GREM1), a regulator of bone morphogenetic protein (BMP) signaling, fine-tunes extensive biological processes, including organ morphology, cellular metabolism, and multiple pathological developments. The roles of GREM1 in PDAC remain unknown.

Methods: Varieties of public databases and online software were employed to analyze the expressions at transcription and protein levels of GREM1 in multiple malignant neoplasms including PDAC, and in addition, its potential pro-tumoral functions in PDAC were further evaluated. A total of 340 serum samples of pancreatic disease, including PDAC, low-grade malignant pancreatic neoplasm, benign pancreatic neoplasm, pancreatitis, and 132 healthy controls, were collected to detect GREM1. The roles of serum GREM1 in the diagnosis and prediction of survival of PDAC after radical resection were also analyzed.

Results: Bioinformatics analyses revealed that GREM1 was overexpressed in PDAC and predicted a poorer survival in PDAC. A higher protein level of GREM1 in PDAC correlated with stroma formation and immunosuppression by recruiting varieties of immunosuppressive cells, including T regulatory cells (Tregs), M2 macrophages, myeloid-derived suppressor cells (MDSCs), and exhaustion T cells into the tumor microenvironment. A higher level of serum GREM1 was observed in PDAC patients, compared to healthy control ($p < 0.001$). Serum GREM1 had a good diagnostic value (area under the curve (AUC) = 0.718, $p < 0.001$), and its combination with carbohydrate antigen 199 (CA199) achieved a better diagnostic efficacy (AUC = 0.914, $p < 0.001$), compared to CA199 alone. The cutoff value was calculated by receiver operating characteristic (ROC) analysis, and PDAC patients were divided into two groups of low and high GREM1. Logistic analyses showed serum GREM1 positively correlated with tumor size (hazard ratio (HR) = 7.097, $p = 0.032$) and

histopathological grades (HR = 2.898, p = 0.014). High-level serum GREM1 (1,117.8 pg/ml) showed a shorter postoperative survival (p = 0.0394).

Conclusion: Higher intra-tumoral expression of GREM1 in PDAC contributes to tumor stroma and immunosuppressive tumor microenvironment, presenting its therapeutic potential. High-level serum GREM1 predicts poorer survival after resection. A combination of serum CA199 and GREM1 shows a stronger diagnostic efficacy in PDAC.

KEYWORDS

gremlin 1 (GREM1), pancreatic adenocarcinoma (PDAC), tumor microenvironment, diagnosis, marker

Introduction

Pancreatic cancer, mainly referred to as pancreatic ductal adenocarcinoma (PDAC), is the fourth leading fatal neoplasm, with a deteriorating tendency in the next decade (1). The relatively low incidence is coupled with disproportionately high mortality, with an average annual incidence rate of 12.5 per 100,000 population in the United States but with a 5-year survival rate of approximately 10% (2). Surgical therapy is currently the only curable approach. However, most patients with PDAC are diagnosed with advanced stage, due to the predicament of early diagnosis, and most patients relapse after surgical treatment (3). Furthermore, the median survival of patients with metastatic disease is only 3 months (4, 5). PDAC is featured as the conspicuous chronic inflammation, where massive inflammatory signaling cascade and abundant immune cells occur. Chaos in external environmental signals is comprehensively advantageous to tumor cell survival and proliferation (1). Furthermore, the inactivation of antitumor immunity and prevalence of pro-tumor immunity symbolize the pancreatic tumor microenvironment, leaving PDAC as one of the refractory malignant diseases (6). All in all, early diagnosis and effective therapeutic methods are urgently needed in clinical practice. The most commonly used serum marker to diagnose PDAC is carbohydrate antigen 199 (CA199). However, nearly 30% of the PDAC patients have a normal level of CA199; in addition, in the early stage of PDAC, the positive rate of CA199 was even lower; therefore, novel markers that could improve the diagnostic roles of CA199 are urgently needed (7).

The bone morphogenetic protein (BMP) was firstly reported by Marshall Urist in 1965, which is a demineralized bone matrix with significant osteoinductive activity (8). As the accumulation of research advances, the formidable signaling has revealed its participation beyond osteogenesis and bone remodeling, in multiple biological processes such as embryonic development, angiogenesis, iron metabolism, inflammation, and sexual reproduction (9). BMPs belong to the transforming growth factor β (TGF-β) family, delivering signals *via* type I and type II serine–threonine kinase receptors and intracellular downstream effectors. Furthermore, BMP signals are fine-tuned by various agonists and antagonists (10). GREM1, as an antagonist, is predominantly expressed in stromal cells and encodes the generation of the secreted glycosylated protein to combine with BMP-2, BMP-4, and BMP-7 to typically form homo- and heterodimers, binding to selective BMPs to prevent ligand–receptor interactions and subsequent downstream signaling (11). Several studies have reported the overexpression of GREM1 by cancer-related stromal cells, promoting tumor cell proliferation, which suggests that GREM1 is responsible for the specialized tumor microenvironment (12). GREM1 enhances the TGF-β-mediated epithelial-to-mesenchymal transition (EMT) as the result of BMP maintaining epithelial integrity by antagonizing TGF-β. Moreover, GREM1 production by cancer-associated fibroblasts (CAFs) expedited the fibrogenic activation and facilitated breast cancer cell intravasation and extravasation in co-injection xenograft zebrafish models (13).

As far as we know, the roles of GREM1 in PDAC remain unknown. Considering its regulatory roles in tumor stroma and inflammatory cells, in this study, the different expression levels at transcription and protein levels and potential pro-tumoral roles were evaluated by using varieties of online public databases. Further, the diagnostic and predictive roles of serum GREM1 were analyzed by using a large cohort of patients.

Materials and methods

Ethics statement

The study was approved by the Ethics Committee of Peking Union Medical College Hospital. All patients enrolled in our

study provided written informed consent for the scientific research use of the samples.

Patients and serum samples

Serum from 340 patients and 132 healthy controls (HCs) were collected from the Clinical Biobank of Medical Science Research Center of Peking Union Medical College Hospital, including 128 cases of PDAC, 39 cases of intraductal papillary mucinous neoplasm (IPMN), 47 cases of pancreatic solid pseudopapillary neoplasm (SPN), 54 cases of pancreatic neuroendocrine tumor (pNET), 31 cases of serous cystadenoma (SCN), 26 cases of mucinous cystadenoma (MCN), and 15 cases of chronic pancreatitis (CP) and pancreatic pseudocyst (PPC). The characteristics of patients are summarized in Table 1. A total of 117 patients receiving radical resection operations, from September 2013 to December 2017, were conducted in a follow-up cohort, with the end time up to 19 November 2019, and ultimately 82 patients acquired the survival status and overall time. The male-to-female ratio was 72:56. The age of the patients ranges from 36 to 79 years with a mean age of 61.0 ± 9.1 years old and a median age of 62 years. Of the 82 cases, 52 patients died, and 30 were alive to the end time of follow-up. The median survival time of follow-up was 512 days. The diagnosis and staging were based on the 8th edition of the American Joint Committee on Cancer (AJCC). Inclusion criteria were as follows: 1) older than 18 years, 2) pathologically diagnosed with PDAC, and 3) receiving radical operation. Exclusion criteria were as follows: 1) pathological specimens could not be obtained and 2) refused follow-up.

Detection of serum GREM1 levels

Serum samples from 340 patients and 132 healthy people were collected and underwent enzyme-linked immunosorbent assay (ELISA). Serum GREM1 levels were quantitatively detected using a GREM1 ELISA Kit (MM-60567H1, Meimian, Jiangsu, China), according to the manufacturer's protocol.

Gene expression profiling interactive analysis

Gene Expression Profiling Interactive Analysis (GEPIA) (http://gepia.cancer-pku.cn/) is an online web tool that explores cancer and normal gene expression profiling and interactive analyses based on The Cancer Genome Atlas (TCGA) and Genotype-Tissue Expression (GTEx) databases (14). With the use of the GEPIA database, the gene expression profile of GREM1 was analyzed across 33 tumor samples and paired with normal tissues by the module 'Expression DIY', selecting ANOVA as the differential method. The prognostic values, overall survival (OS), and disease-free survival (DFS) of GREM1 were analyzed by using the function module 'Survival Plot' in several tumor types, and the 'Median' was arranged as the 'Group Cutoff'. The correlation function was used to predict the influence of the GREM1

TABLE 1 Basic characteristics of 340 patients with pancreatic diseases.

	PDAC	IPMN	SPN	pNET	SCN	MCN	CP+PPC
Total number	128	39	47	54	31	26	15
Age (years)	61.0 ± 9.1	59.3 ± 10.6	31.6 ± 10.4	50.1 ± 11.1	54.5 ± 13.1	47.5 ± 13.4	51.9 ± 15.2
Gender							
Male	56 (43.8%)	16 (41%)	35 (74.5%)	32 (59.3%)	25 (80.6%)	23 (88.5%)	3 (20%)
Female	72 (56.2%)	23 (59%)	12 (25.5%)	22 (40.7%)	6 (19.4%)	3 (11.5%)	12 (80%)
Surgery							
TPS	3 (2.3%)	6 (15.4%)	0 (0)	1 (1.9%)	0 (0)	0 (0)	0 (0)
PD	62 (48.4%)	14 (35.9%)	5 (10.6%)	6 (11.1%)	7 (22.6%)	2 (7.7%)	4 (26.7%)
PPPD	6 (4.7%)	6 (15.4%)	0 (0)	4 (7.4%)	3 (9.7%)	0 (0)	1 (6.7%)
DP	2 (1.6%)	3 (7.7%)	14 (29.8%)	5 (9.3%)	7 (22.6%)	9 (34.6%)	1 (6.7%)
DPS	41 (32%)	6 (15.4%)	13 (27.7%)	17 (31.5%)	6 (19.4%)	13 (50%)	3 (20%)
LR	1 (0.8%)	2 (5.1%)	15 (31.9%)	21 (38.9%)	8 (25.8%)	2 (7.7%)	6 (40%)
Biopsy	13 (10.2%)	2 (5.1%)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Tumor location							
Head and neck	86 (67.2%)	29 (74.4%)	22 (46.8%)	29 (53.7%)	19 (61.3%)	5 (19.2%)	9 (60%)
Body and tail	42 (32.8%)	10 (25.6%)	25 (53.2%)	25 (46.3%)	12 (38.7%)	21 (80.8%)	6 (40%)
Neoadjuvant chemotherapy	2 (1.6%))	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)

PDAC, pancreatic ductal adenocarcinoma; IPMN, intraductal papillary mucinous neoplasm; SPN, solid pseudopapillary neoplasm; pNET, pancreatic neuroendocrine tumor; SCN, serous cystadenoma; MCN, mucinous cystadenoma; CP, chronic pancreatitis; PPC, pancreatic pseudocyst; TPS, Total pancreatectomy and splenectomy; PD, Pancreaticoduodenectomy; PPPD, Pylorus-preserving pancreaticoduodenectomy; DP, Distal pancreatectomy; DPS, Distal pancreatectomy and slenectomy; LR, Local rescetion.

expression on the genes of the T-cell exhaustion state, selecting the 'Spearman' as the 'correlation coefficient'.

Oncomine

Oncomine (https://www.oncomine.org/) is a gene chip-based database that contains substantial tumor microarray datasets (15). The log2-transformed form was utilized to represent the transcriptional levels of GREM1, and the inclusion criteria were designed as the 'Fold change > 2' and '*p*-value < 0.05'.

The human protein atlas

The Human Protein Atlas (HPA) database (https://www.proteinatlas.org/) is an interactive data-mining platform, integrating substantial distribution information of human protein from more than 20 kinds of cancer at the cellular and histopathological levels. The immunohistochemical (IHC) staining images of GREM1 in normal pancreas tissue and PDAC, the protein expression, and the survival plot were all collected from the HPA database.

Kaplan-Meier plotter

Kaplan-Meier plotter (http://kmplot.com/) is a potent public online database for survival analysis of 21 tumor types in the basement of substantial RNA-seq and next-generation sequencing. The OS of GREM1 under the different conditions of immune cell infiltration was evaluated by the Kaplan-Meier plotter database. Hazard ratio (HR) and 95% confidence interval (CI) were calculated automatically according to 'Auto select best cutoff'.

LinkedOmics

LinkedOmics (http://www.linkedomics.org/) is an available web portal for users to analyze multi-omics data on the basement of 32 cancer types (16). Functional enrichment and prediction of GREM1 were performed using gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis from the database, containing biological process (BP), cellular component (CC), and molecular function (MF).

GeneMANIA

GeneMANIA (http://www.genemania.org) is a potent and convenient website tool to predict gene function, analyze gene lists, and perform functional assays (17). We used this tool to manufacture the gene–gene interaction network of GREM1 and the top 20 correlated genes.

STRING

STRING (https://www.string-db.org/) is a useful database predicting protein–protein interactions by physical and functional association. A protein–protein interaction (PPI) network of GREM1 was implemented to scan correlated genes for GREM1 function prediction.

TISIDB

TISIDB (http://cis.hku.hk/TISIDB/index.php) is a public portal for tumor and immune system interaction, as well as the integration of numerous heterogeneous data types (18). Detailed analysis of immune infiltration of GREM1 in PDAC was performed using analyzing high-throughput data in the database.

Tumor immune estimation resource

Tumor Immune Estimation Resource (TIMER) (https://cistrome.shinyapps.io/timer/) is a user-friendly web server for systematic and comprehensive analysis of immune infiltration across various tumor types *via* inputting function-specific parameters (19). The immune infiltration of GREM1 in PDAC tissues was estimated using the TIMER database (Spearman's correlation) in their different conditions (None, Tumor Purity, and Age).

Statistical analysis

Statistical analysis and graphs were performed and plotted by SPSS v.25.0 (IBM Corp., Armonk, NY, USA) and GraphPad Prism 6.0 (La Jolla, CA, USA). Receiver operating characteristic (ROC) curves were conducted, and area under the curve (AUC), sensitivity, and specificity were calculated to compare the diagnostic value of GREM1 and other markers. Based on the ROC results, the cutoff of GREM1 was obtained to achieve the division of the low- and high-GREM1 groups. Meanwhile, different cutoff values were calculated by the X-tile program to explore the overall survival of the low- and high-GREM1 groups. Logistic regression analysis by SPSS software and Kaplan–Meier analysis by GraphPad Prism were performed in the two groups. Comparisons between two groups were conducted using a two-tailed Student's t-test. For comparisons of three or more groups, the one-way ANOVA with post-hoc Dunnett's test or Tukey's test was utilized. Continuous data were presented as the mean ±

SD and analyzed using Student's t-tests. Statistical significance was indicated as a p-value <0.05.

Results

Aberrant transcriptional levels of GREM1 in human cancers

We initially analyzed the mRNA expression of GREM1 in human cancers using the database GEPIA. Its elevation in transcriptional level could be observed in multiple cancers, incorporating breast invasive carcinoma (BRCA), cholangial carcinoma (CHOL), lymphoid neoplasm diffuse large B-cell lymphoma (DLBC), lung adenocarcinoma (LUAD), glioblastoma multiforme (GBM), pancreatic ductal adenocarcinoma (PDAC), rectum adenocarcinoma (READ), thymoma (THYM), and stomach adenocarcinoma (STAD), whereas there were negative expressions in some neoplasms, including kidney renal clear cell carcinoma (KIRC), adrenocortical carcinoma (ACC), skin cutaneous melanoma (SKCM), and kidney chromophobe (KICH), compared with the corresponding normal tissues (Figures 1A–C). Notably, higher expression of GREM1 in PDAC tissues was reconfirmed in the Oncomine database (Figure 2A), and four patient cohort studies revealed its upregulation in PDAC (Figures 2B–E). Thus, the above results showed the differential expression of GREM1 between normal tissue and tumor, suggestive of an important regulatory role in tumor progression.

GREM1 gene expression negatively correlated with poor prognosis in various cancer

By analyses of the GEPIA database, the upregulation of GREM1 gene expression in human cancers was of vital significance in the prognosis of multiple cancers. The analysis from the GEPIA database presented the poor OS in multiple tumors as the high expression of *GREM1* gene, such as the ACC, KIRC, lung squamous cell carcinoma (LUSC), PDAC, and uveal melanoma (UVM) (Figure 3A). Among these solid tumors, the difference in DFS was only PDAC (Figure 3B), predicting that the upregulation of GREM1 contributed to high relapse probability following treatment. These results manifested the potent capacity of GREM1 to forecast the prognosis of PDAC.

Translational levels of GREM1 in pancreatic ductal adenocarcinoma

GREM1 protein was investigated in PDAC by IHC staining, and the results found that GREM1 at the protein level was elevated in tumor tissues in contrast with normal pancreatic tissues (Figure 4A). From the results of IHC, GREM1 protein concentrated in interstitial space and executed its function in an exocellular environment. The protein level of GREM1 in PDAC was also higher than in the normal pancreas from the analyses of TCGA data (Figure 4B). According to the HPA database, GREM1 protein was reckoned as a type of secreted protein to exocellular stroma (Figure 4C), implicating that this message molecule might be involved in the signaling transmission and intercellular communication. Survival analysis revealed that patients with high-level GREM1 protein had shorter survival (Figure 4D). The results illuminated that GREM1 was overexpressed in PDAC and might promote PDAC progression.

The pro-tumoral role of GREM1 correlated with stroma formation

The gene–gene interaction network for GREM1 in GeneMANIA exhibited 20 related functional genes, including KDR, GREM2, and BMP2 (Figure 5A). These molecules have been proved by previous studies as pro-tumoral factors in tumor progression (20). A PPI network of GREM1 through STRING analysis demonstrated the intimate relationship between GREM1 and BMP family, and its regulation could be implemented *via* a BMP-related pathway (Figure 5B). The results indicated that the function of GREM1 was linked to stroma formation, suggesting a significant role in tumor microenvironment constitution. The top 50 genes positively and negatively related to GREM1 in PDAC are shown in heatmaps (Figures 5C, D) (positively and negatively correlated gene lists are provided in Supplementary Table 1). Then, the top 200 genes positively related to GREM1 were used for GO and KEGG methods to predict the correlated signaling pathways and diverse biological functions (the top 200 positively correlated gene lists are provided in Supplementary Table 2; Figures 5E, F). The top 10 significant terms of BP, MF, and CC analytic results were obtained by the David database (Figure 5E). These data illustrated that GREM1 mainly acted in an exocellular environment and worked from cell to cell. In terms of BP, its function was associated with extracellular matrix organization, cell adhesion, and collagen catabolic. In terms of CC, the most correlated significant functions were extracellular matrix, proteinaceous extracellular matrix, and extracellular region. In terms of BP, its related functions were extracellular matrix structural constituent, collagen binding, and integrin binding. They all pointed to the regulation of exocellular matrix and were possibly engaged in exocellular signaling pathway transmission and integrate modulation of tumor external environment. These results revealed that GREM1 participated in exocellular environment constitution and it might play an important and extensive role in multiple biological effects, which was of

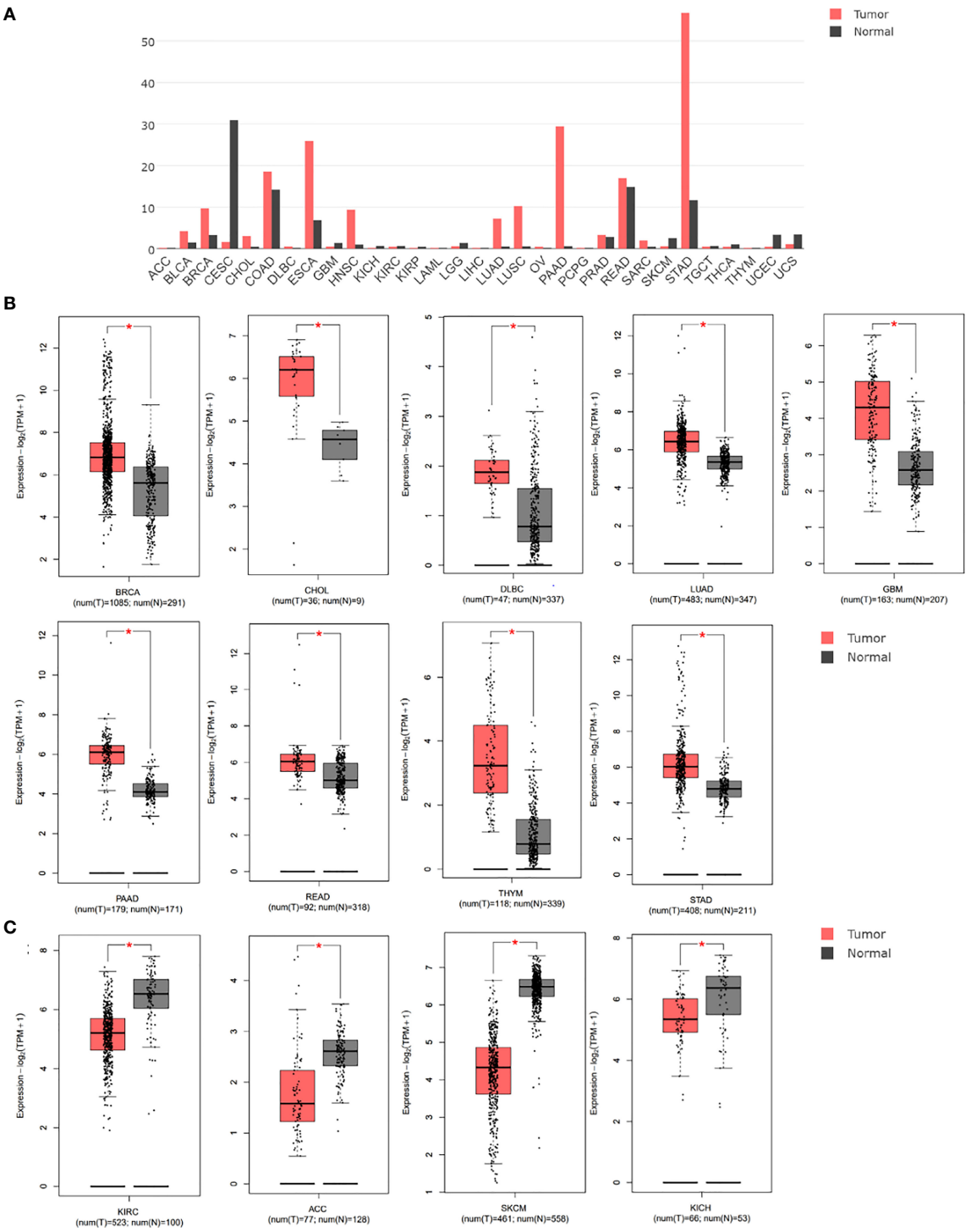


FIGURE 1
Gene expression differences of GREM1 were analyzed using the GEPIA database based on TCGA and GTEx databases. **(A)** Expression profile of GREM1 expression in 31 tumor types. **(B)** Higher expression of GREM1 in breast invasive carcinoma (BRCA), cholangial carcinoma (CHOL), lymphoid neoplasm diffuse large B-cell lymphoma (DLBC), lung adenocarcinoma (LUAD), glioblastoma multiforme (GBM), pancreatic ductal adenocarcinoma (PDAC, equal to PAAD used by bioinformatics databases), rectum adenocarcinoma (READ), thymoma (THYM), and stomach adenocarcinoma (STAD). **(C)** Lower expression of GREM1 in kidney renal clear cell carcinoma (KIRC), adrenocortical carcinoma (ACC), skin cutaneous melanoma (SKCM), and kidney chromophobe (KICH). GEPIA, Gene Expression Profiling Interactive Analysis; TCGA, The Cancer Genome Atlas; GTEx, Genotype-Tissue Expression. * means p-value < 0.05.

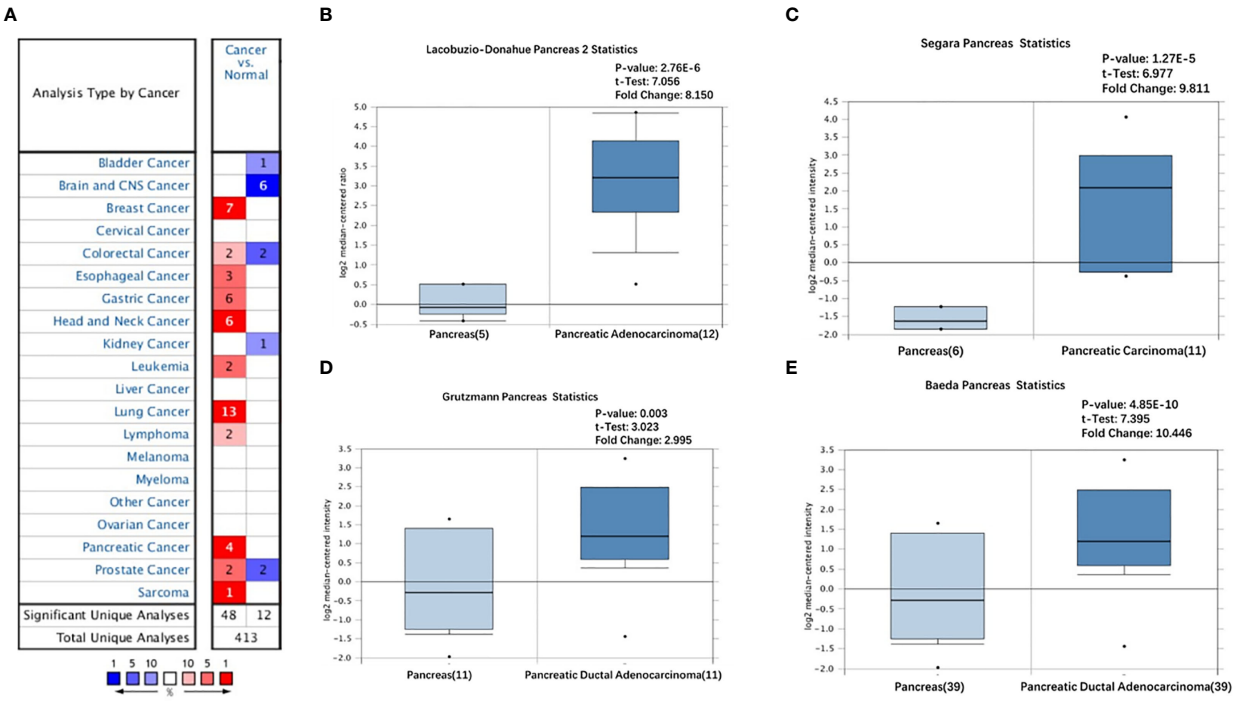


FIGURE 2
Overexpression of GREM1 in PDAC based on Oncomine database. **(A)** Different expression of GREM1 in multiple tumors (fold change > 2, *p*-value < 0.05). **(B–E)** The increase of GREM1 mRNA in four studies. PDAC, pancreatic ductal adenocarcinoma.

great potential in the integrated modulation of the tumor microenvironment.

Immune modulation roles of GREM1 in pancreatic ductal adenocarcinoma

Immune cell infiltration in the tumor microenvironment of PDAC is exhibited in Figure 6A, showing the complicated alterations of immune components in PDAC. We observed that the immunosuppressive cells were enhanced by GREM1 expression, including T regulatory cells (Tregs), macrophages, myeloid-derived suppressor cells (MDSCs), $CD4^+$ T cells, and Th2 cells (Figure 6B), in agreement with previous studies that reported that these cells were positively correlated with immunosuppression (21, 22). NK, NKT, and DC were positively related to GREM1 expression (Figure 6B), associated with tumor cell killing, but a recent study revealed the functional silence in the tumor microenvironment of PDAC and thus facilitated tumor progression. Therefore, the specific roles of these cells needed more investigation. Interestingly, methylation of GREM1 (GREM1 downregulation) induced the opposite results in these cells in Figures 6B, D, implying that low methylation of GREM1 might trigger immune cell infiltration. We further explored the infiltration of immune cells with the prognosis of PDAC. From the subgroup analysis of Figures 7A, B, the pro-tumor role of GREM1 could be embodied in CD8+ memory T cell, Treg, and Th2 cell enriched groups, and B cell, CD4+ memory T cell, macrophage, Treg, and Th1 decreased groups, which illustrated the prognostic prediction of GREM1 in such immune cells increased or decreased infiltration conditions. The correlation between GREM1 expression and a variety of T-cell subtypes was proved by the TIMER database (Table 2). T-cell exhaustion was detected in the tumor microenvironment of PDAC with GREM1 expression (Figure 8). A series of markers symbolized T-cell exhaustion, such as CTLA4, PD-1, PD-L1 (CD244), LAG3, TIM3(HAVCR2), BTLA, 2B4(CD244), and TIGIT, were positively associated with GREM1 expression. These results demonstrated that GREM1 had potent immune modulation in PDAC and promoted tumor progression by sustaining immunosuppression.

Clinical significance of serum GREM1 in pancreatic ductal adenocarcinoma diagnosis and prognosis

Serum GREM1 levels in PDAC and other pancreatic neoplasms were obtained at varying degrees of elevation, compared to the HC group (Figure 9A, $p < 0.05$). We could

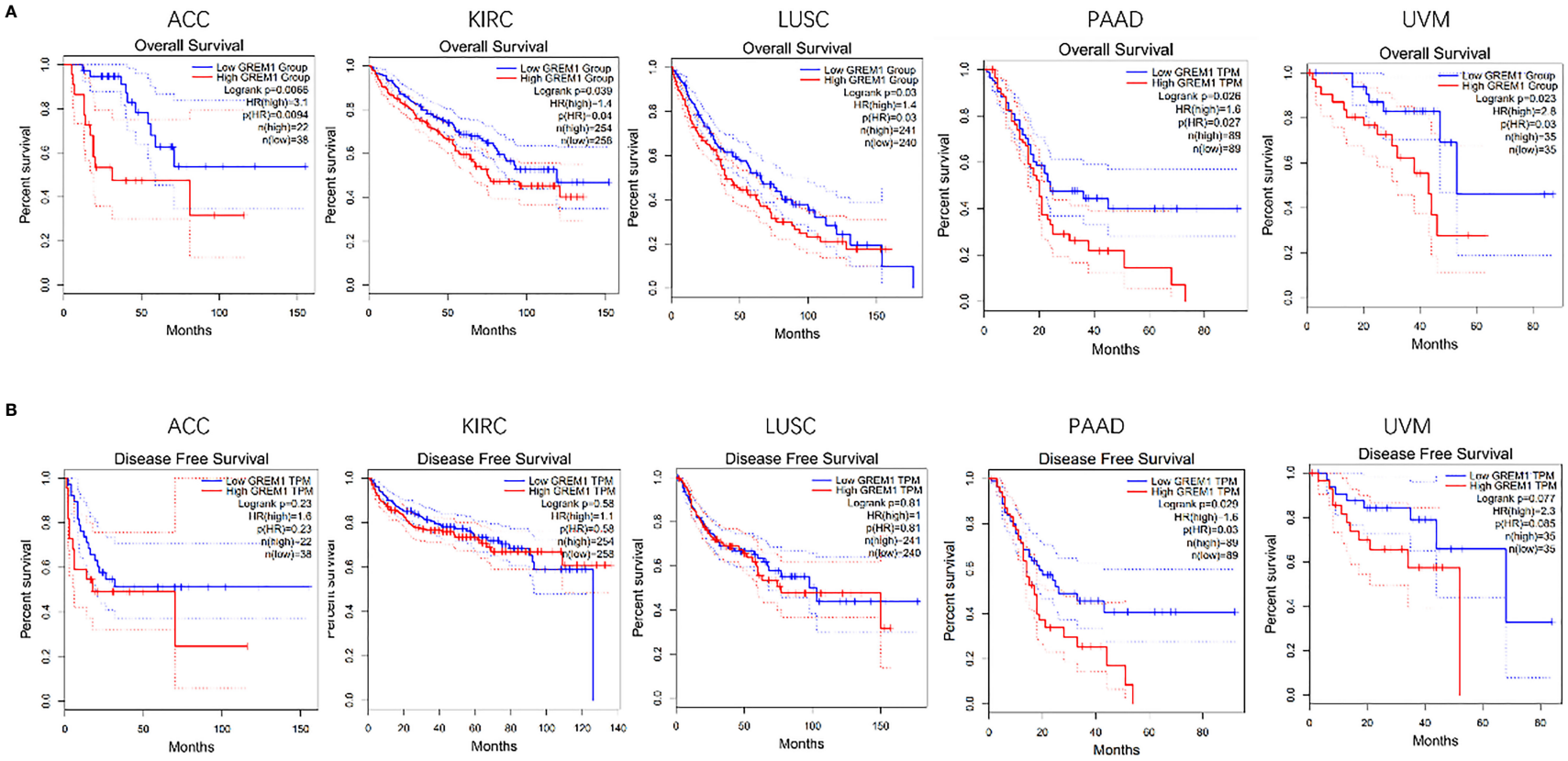


FIGURE 3
Survival analysis of GREM1 in multiple tumors was performed by Kaplan-Meier plotter. **(A)** OS in five tumors including PDAC. **(B)** DFS in five tumors including PDAC. OS, overall survival; PDAC, pancreatic ductal adenocarcinoma; DFS, disease-free survival.

10.3389/fonc.2022.968610

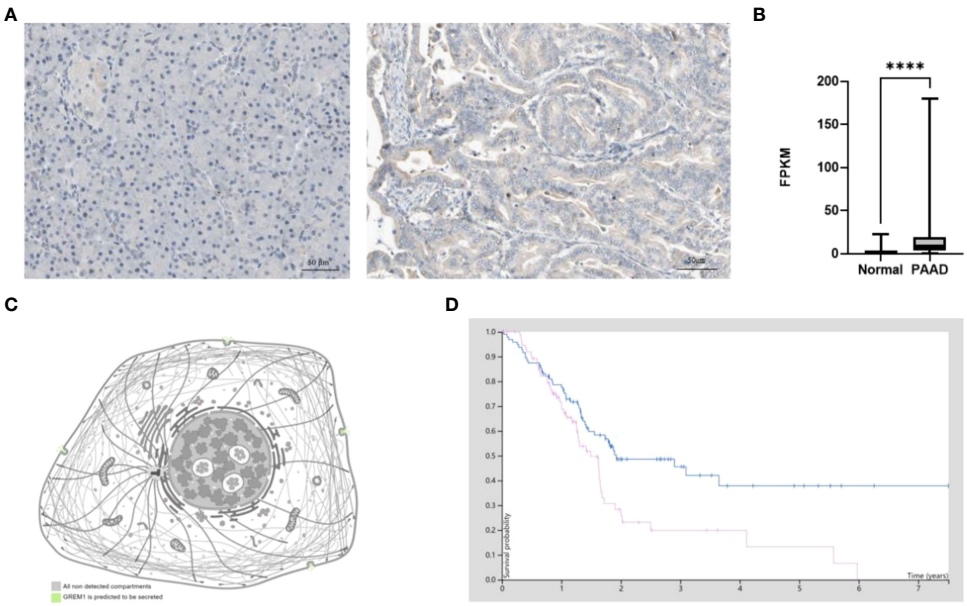


FIGURE 4
Protein level difference of GREM1 in PDAC based on HPA database. **(A)** Immunohistochemical staining of GREM1 in normal pancreas tissues and PDAC. **(B)** The difference in GREM1 mRNA transcription between normal pancreas tissues of 248 patient samples and PDAC of 176 patient samples from TCGA database. **(C)** GREM1 is secreted into the exocellular matrix. **(D)** Survival probability of PDAC patients with GREM1 overexpression (p = 0.011). PDAC, pancreatic ductal adenocarcinoma; HPA, Human Protein Atlas; TCGA, The Cancer Genome Atlas. **** means p-value < 0.0001.

not observe the significant difference between PDAC and other tumors. Accordingly, 128 cases of PDAC serum stepped into further investigation. Serum GREM1 of 260 PDAC patients underwent the ROC analysis, and the results showed its excellent diagnostic value, with an AUC of 0.718 (Figure 9B, $p < 0.001$). Based on the ROC result, the cutoff value was calculated as equal to 945.17 pg/ml. The combinative effect of serum GREM1 and CA199 was also equally evaluated, which presents the higher diagnostic value of CA199 allied with serum GREM1 (Figure 9C, $p < 0.001$).

PDAC patients were divided into the low-GREM1 (n = 72) and high-GREM1 (n = 56) groups by the cutoff value. Subsequently, several associated factors were analyzed by logistic regression (Table 3). Age, sex, smoking, drinking, hypertension, tumor locations, distant metastasis, and tumor stages did not exhibit a significant correlation with the two GREM1 groups. Diabetes was a negatively correlated factor (Table 3, p = 0.034). GREM1 was probably related to tumor growth, resulting from the positive correlation with tumor size (HR = 7.097, p = 0.032) and histopathological grades (HR = 2.898, p = 0.014). However, it was negatively paralleled with lymph node metastasis (HR = 0.149, p = 0.036). It suggested that GREM1 promoting stromal construction may contribute to the blockade of matrix degeneration and indicated its crucial role in tumor growth from another aspect. In addition, a survival analysis of 82 PDAC patients with radical resection surgery by the Kaplan–Meier method was performed to acquire exploration on the relationship between serum GREM1 and postoperative survival (Figure 9D, p = 0.48). The mean survival time of the low-GREM1 group was 877.0 days (831.5, 1,348.3), while that of the high-GREM1 group was 554.0 (30.0, 1,078.0) days (p = 0.48). Additionally, the X-tile program was used to explore the optimal cutoff value of overall survival time. As the cutoff value was equal to 1,117.8 pg/ml, the overall survival of the high-GREM1 group (n = 6) was significantly shorter than that of the low-GREM1 group (n = 76) (Figure 9E, p = 0.0394). Since sample capacity in our cohort was limited, its prognostic efficiency in PDAC patients was restricted. However, it proved the possibility of GREM1 predicting PDAC prognosis on the condition of enlarging patient volumes. Taken together, these results illustrated that serum GREM1 was a risk factor for PDAC and that its level in serum was an excellent potential marker for diagnosis of PDAC and a potential predictor for prognosis.

Discussion

GREM1, a pleiotropic regulator shuttling in fine-tuning BMP, takes charge of tissue development and organ morphology (9, 20). Researchers have focused on bone

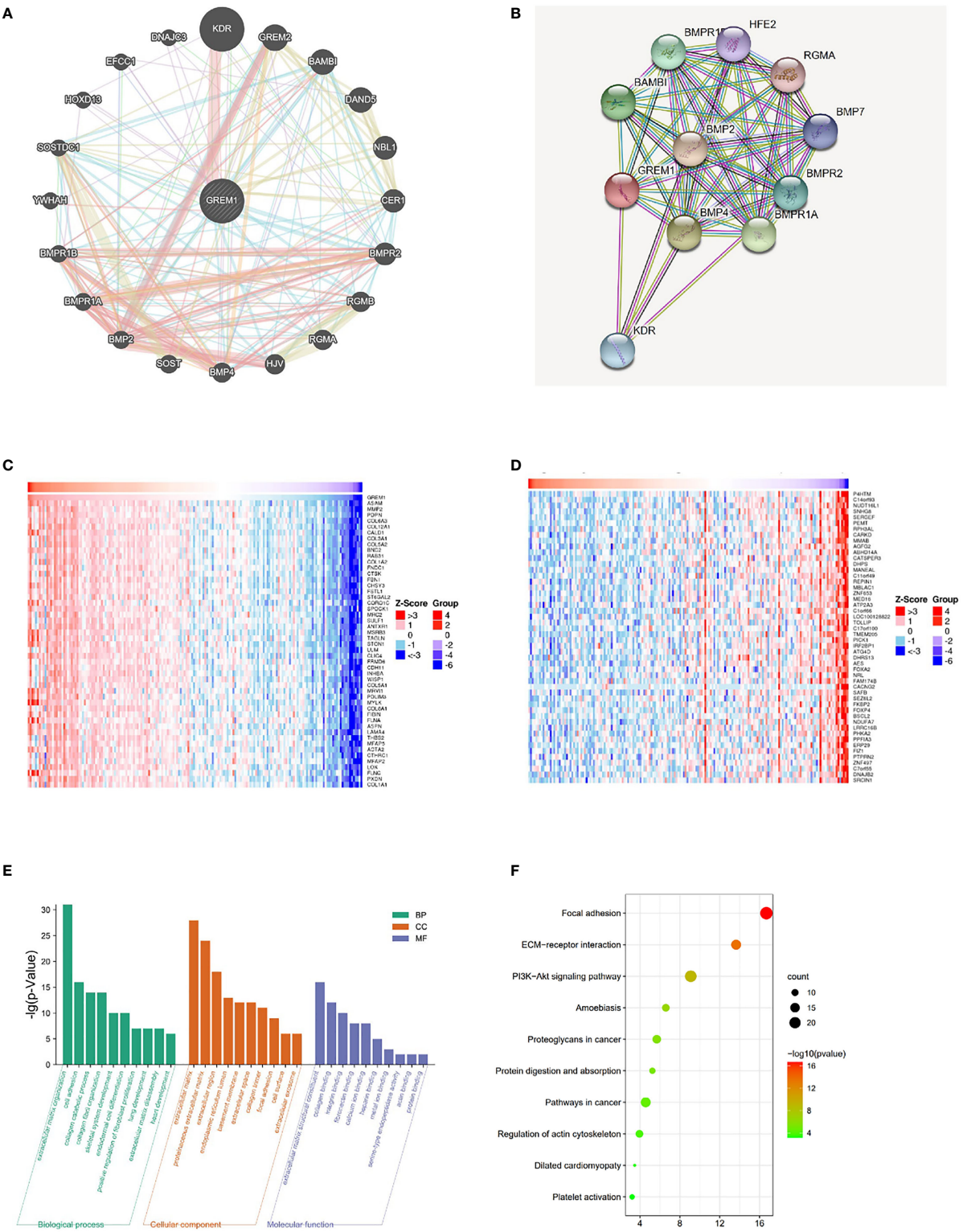


FIGURE 5
Related gene analysis of GREM1 and pathway function prediction in PDAC. **(A)** The gene–gene interaction network of GREM1 was constructed using GeneMANIA. **(B)** The PPI network of GREM1 was generated using STRING. **(C, D)** A heatmap shows the correlations between positively and negatively related significant genes in PDAC by LinkedOmics (Spearman's correlation). **(E)** GO and **(F)** KEGG analyses for GREM1. PDAC, pancreatic ductal adenocarcinoma; PPI, protein–protein interaction; GO, gene ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes.

10.3389/fonc.2022.968610

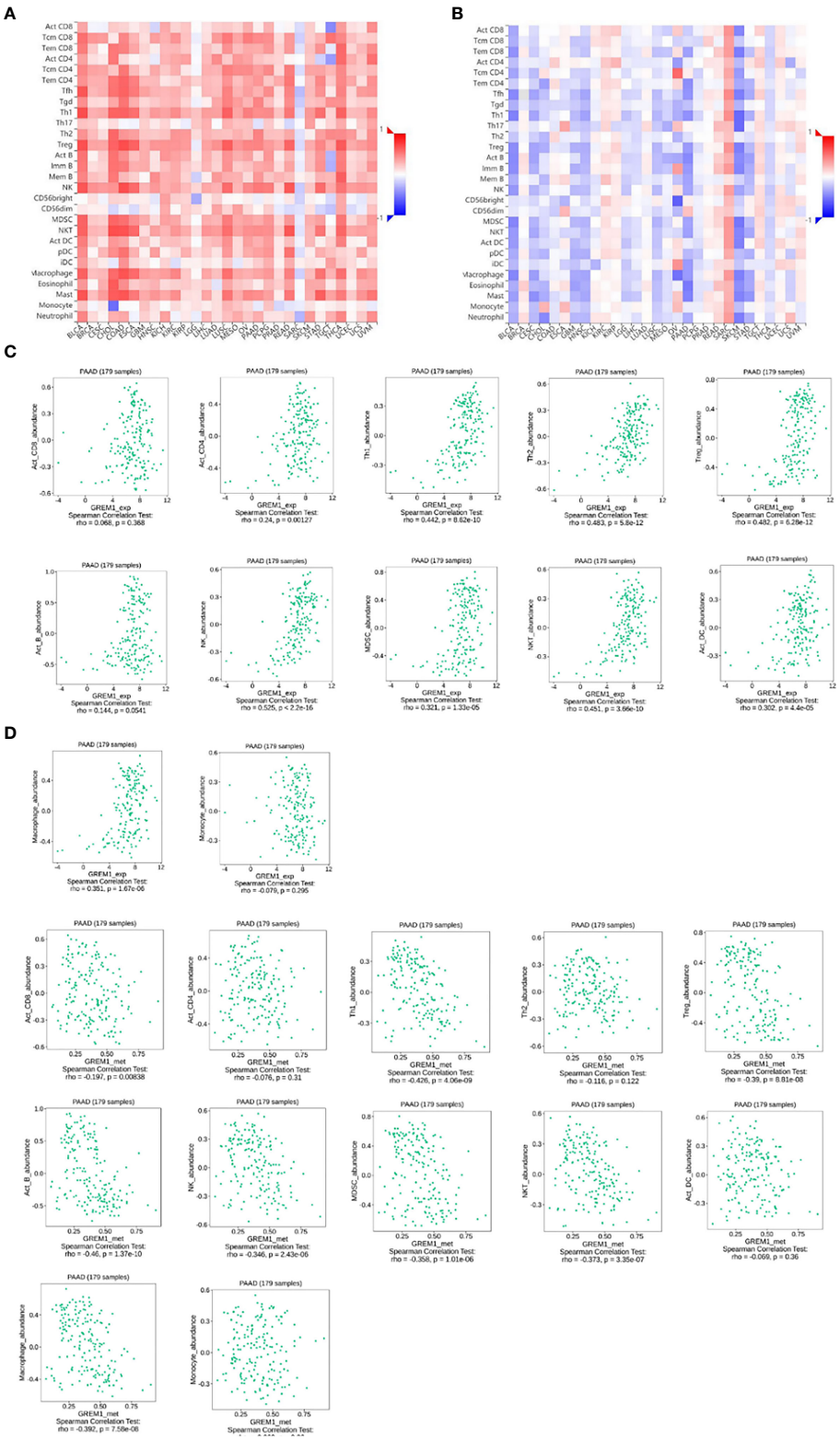


FIGURE 6
GREM1 overexpression regulates immune cell infiltration of PDAC. **(A)** Heatmap of immune cell infiltration in PDAC *via* TISID database. **(B)** Heatmap of immune cell infiltration in PDAC after GREM1 methylation. **(C)** Scatterplots of the correlations between GREM1 expression and immune cell infiltration and **(D)** GREM1 methylation and immune cell infiltration. PDAC, pancreatic ductal adenocarcinoma.

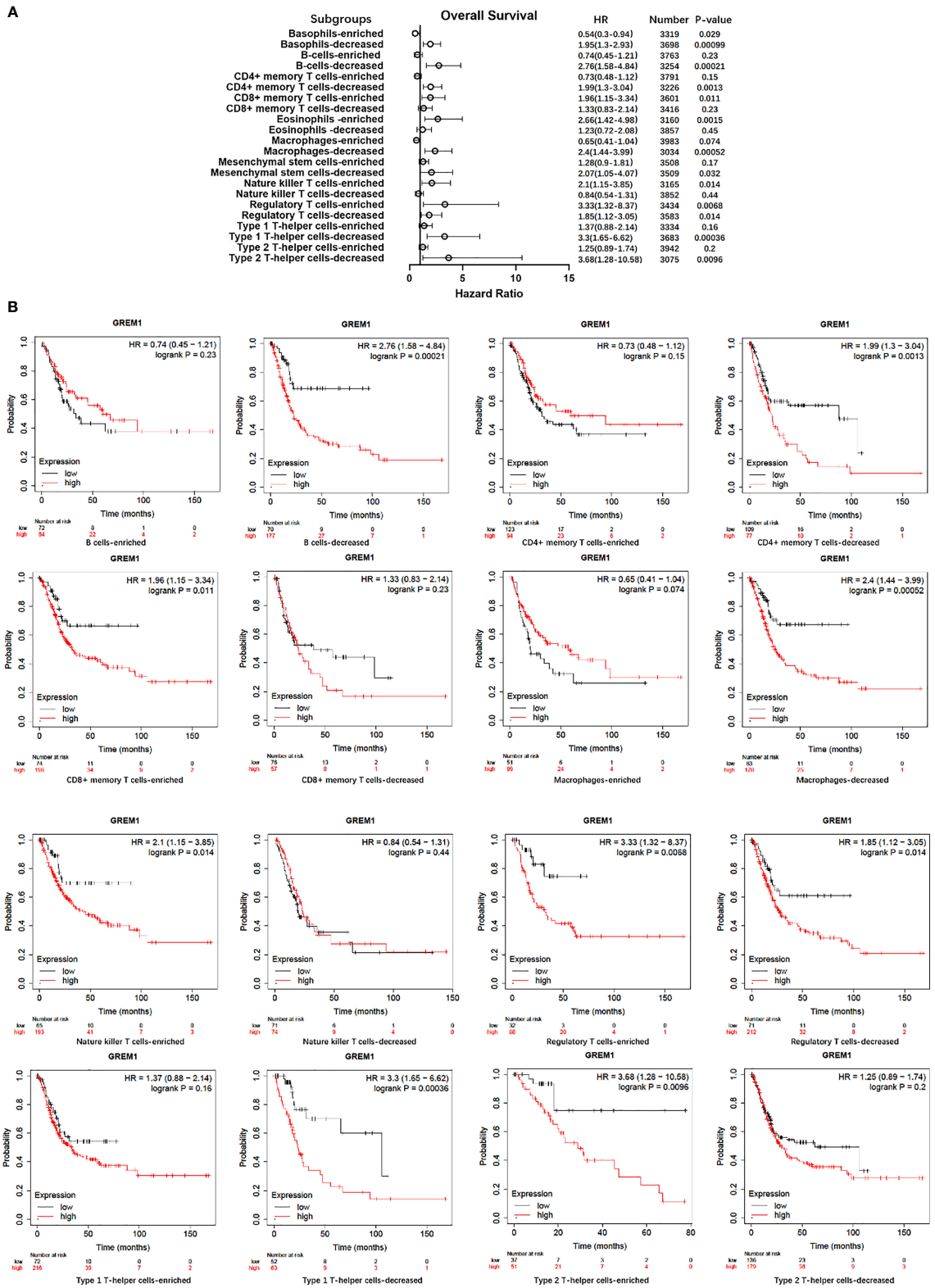


FIGURE 7
Survival analysis of immune cell infiltration in GREM1 high-expressed or low-expressed patients. **(A)** Forest plot for the total analytic data. **(B)** Survival analysis concerning GREM1 expression and immune cell infiltrations.

TABLE 2 Correlation analysis between GREM1 and gene markers of different subgroups of T cells in PDAC by TIMER analysis.

Different groups	Gene markers	None		Tumor purity		Age	
		Cor	*p*	Cor	*p*	Cor	*p*
Th1	TBX21	0.238	**	0.203	**	0.238	**
	STAT4	0.196	**	0.197	**	0.185	*
	STAT1	0.434	***	0.395	***	0.432	***
	TNF	0.260	***	0.229	**	0.254	***
	IFNG	0.330	***	0.301	***	0.336	***
Th1-like	HAVCR2	0.475	***	0.431	***	0.471	***
	IFNG	0.330	***	0.301	***	0.336	***
	CXCR3	0.249	***	0.208	**	0.250	***
	BHLHE40	0.163	*	0.140	0.067	0.157	*
	CD4	0.414	***	0.364	***	0.409	***
Th2	STAT6	0.142	0.057	0.124	0.107	0.138	0.066
	STAT5A	0.337	***	0.305	***	0.335	***
Treg	FOXP3	0.436	***	0.395	***	0.431	***
	CCR8	0.481	***	0.443	***	0.477	***
	TGFB1	0.377	***	0.346	***	0.371	***
Resting Treg	FOXP3	0.436	***	0.395	***	0.431	***
	IL2RA	0.489	***	0.449	***	0.484	***
Effector Treg T cell	FOXP3	0.436	***	0.395	***	0.431	***
	CCR8	0.481	***	0.443	***	0.477	***
	TNFRSF9	0.512	***	0.479	***	0.508	***
Effector T cell	CX3CR1	0.042	0.6	-0.005	0.949	0.036	0.632
	FGFBP2	0.282	***	0.285	***	0.286	***
	FCGR3A	0.535	***	0.506	***	0.536	***
Naive T cell	CCR7	0.249	***	0.202	***	0.241	***
	SELL	0.260	***	0.204	***	0.256	***
Effector memory T cell	DUSP4	0.070	0.353	0.073	0.4	0.070	0.357
	GZMK	0.252	***	0.207	**	0.249	***
	GZMA	0.275	***	0.247	**	0.275	***
Resident memory T cell	CD69	0.356	***	0.314	***	0.350	***
	CXCR6	0.414	***	0.366	***	0.409	***
	MYADM	0.519	***	0.496	***	0.514	***
General memory T cell	CCR7	0.249	***	0.202	***	0.241	***
	SELL	0.260	***	0.204	***	0.256	***
	IL7R	0.503	***	0.466	***	0.498	***
Exhaustion T cell	HAVCR2	0.475	***	0.431	***	0.471	***
	LAG3	0.305	***	0.277	***	0.306	***
	CXCL13	0.276	***	0.230	**	0.273	***
	LAYN	0.735	***	0.717	***	0.733	***

Values are corrected by Tumor purity and Age.
PDAC, pancreatic ductal adenocarcinoma; TIMER, Tumor Immune Estimation Resource.
*p < 0.05, **p < 0.01, ***p < 0.001.

development, in recent years, and have witnessed the miraculous manipulation of tumor progression (13, 23). Consistent with our analytic data, elevations of GREM1 expression were visible in numerous solid tumors, such as lung cancer, kidney cancer, and gastric cancer. PDAC secured the augmentation of GREM1 expression to perform its all-around tumor promotion. As shown in our survival analysis, high expression of GREM1 in some tumors obtained the consequence of shorter OS and DFS. Additionally, we detected serum GREM1 level to uncover its excellent diagnostic and predictive potential for PDAC patients.

Previous studies have reported that GREM1 enhanced the proliferation, invasiveness, and metastasis of tumors through

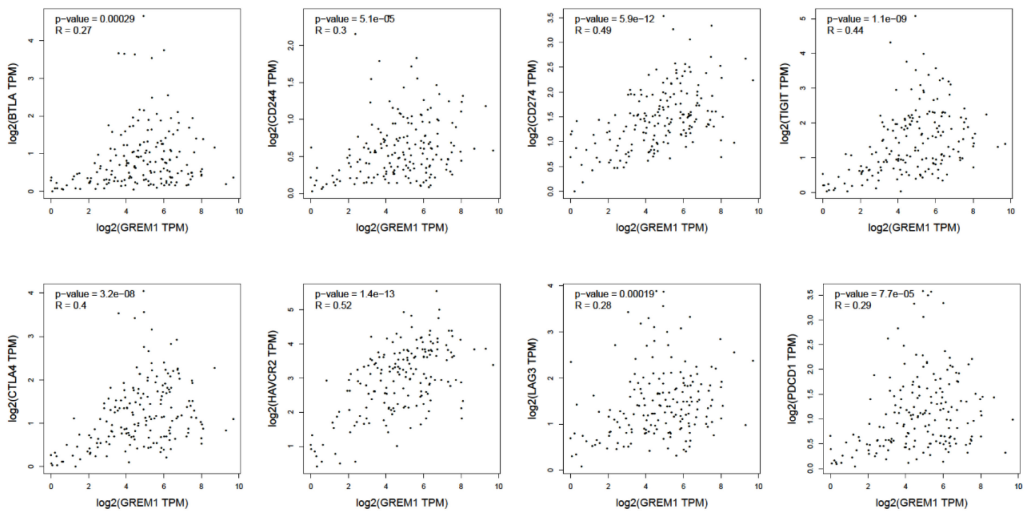


FIGURE 8
The positive relationship between GREM1 expression and several surface markers concerning T-cell exhaustion (BTLA, CD244, CD274, TIGIT, CTLA4, HAVCR2, LAG3, and PDCD1).

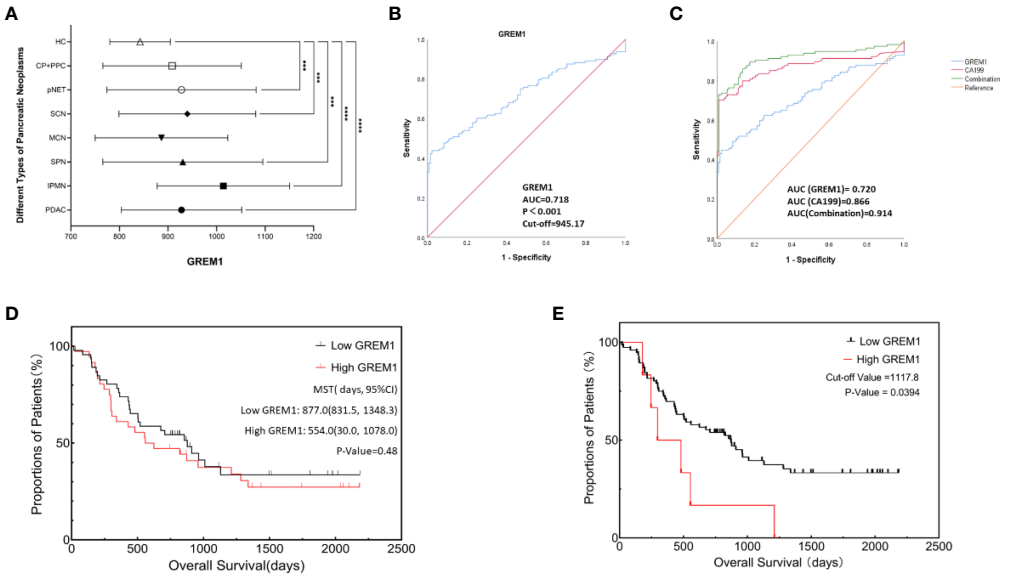


FIGURE 9
Diagnosis and prognosis analysis of serum GREM1 to explore clinical significance. **(A)** GREM1 levels in patient serum with different types of pancreatic neoplasms were detected and were all elevated, compared to the healthy control group (HCs, n = 132); these serum samples included pancreatic ductal adenocarcinoma (PDAC; n = 128), intraductal papillary mucinous neoplasm (IPMN; n = 39), pancreatic solid pseudopapillary neoplasm (SPN; n = 47), pancreatic neuroendocrine tumor (pNET; n = 54), serous cystadenoma (SCN; n = 31), mucinous cystadenoma (MCN; n = 26), chronic pancreatitis (CP; n = 11), pancreatic pseudocyst (PPC; n = 4), and healthy control (HCs; n = 132). **(B)** ROC analysis was performed for GREM1 in 260 cases of PDAC patients (AUC = 0.718, $p < 0.001$), and the cutoff value was calculated to be 945.17. **(C)** ROC analysis for GREM1, CA199, and their combinative diagnostic effect (combination value = GREM1 + CA199 * 0.44/0.09, 0.44, and 0.99 are the coefficient factors from logistic regression equation, AUC = 0.914 $p < 0.001$). **(D)** Survival curves from Kaplan–Meier analysis to compare the low- and high-GREM1 ($p = 0.48$). **(E)** Kaplan–Meier curve for overall survival of PDAC patients based on an optimal cutoff value calculated by X-tile program; OS of high-GREM1 group (n = 6) was significantly poorer than that of the low-GREM1 group (n = 76), $p = 0.0394$. ROC, receiver operating characteristic; AUC, area under the curve; CA199, carbohydrate antigen 199; OS, overall survival.#***p < 0.001, ****p < 0.0001.

TABLE 3 Logistics analysis of GREM1 and correlated factors.

Items	Coefficient	*p*-Value	OR	95%CI	
				Lower limitation	Upper limitation
Age (<62/≥62)	0.127	0.722	1.135	0.565	2.284
Sex (male/female)	−0.193	0.590	0.824	0.408	1.666
Smoking (yes/no)	−0.281	0.542	0.755	0.306	1.861
Drinking (yes/no)	0.138	0.753	1.147	0.488	2.699
Hypertension (yes/no)	0.177	0.639	1.193	0.570	2.498
Diabetes (yes/no)	−0.811	0.034*	0.444	0.210	0.942
Tumor size (T1/T2/T3)	1.960	0.032*	7.097	1.188	42.406
Tumor location (head and neck/body and tail)	0.745	0.058	2.107	0.975	4.552
Histopathological grades (Grades 1–2/Grades 3–4)	1.064	0.014*	2.898	1.236	6.798
Lymph node metastasis (N0/N1/N2)	−1.906	0.036*	0.149	0.025	0.885
Distant metastasis (M0/M1)	−0.017	0.987	0.984	0.129	7.474
Tumor stage (1A-2A/2B-4)	19.871	1	–	–	–

*p < 0.05, means statistical significance.

VEGFR2 and BMP-related pathways (20, 24). In GREM1-silencing cells, p53 phosphorylation and expression of its target gene p21 are enhanced to reduce cell survival *via* programmed death (25). Angiogenesis is regulated by GREM1 to elevate the microvessel density in pancreatic neuroendocrine tumors (24). From the results of HPA, GREM1 protein was predicted to be secreted to the exocellular matrix by the way of exocytosis. The environmental abundance of GREM1 increased in PDAC tissues more than in healthy pancreas tissues, which often predicted a worse prognosis, in agreement with our multiple survival analyses and the confirmation of our follow-up cohort study. Pathway prediction analyses offered the complicated interaction network formed by the downstream signaling pathways targeted. A significant role in extracellular matrix (ECM)–receptor interaction decides the stroma construction in tumor cells. A recent study has proposed the manipulation of MMP generation *via* activating signal transducer and activator of transcription 3 (STAT3) to catalyze the matrix degeneration and cellular disconnection, thus facilitating the metastasis of tumor cells (26). Desmoplastic activation has been linked to the upregulation of GREM1, which determines the rapid progression of tumors (27). Desmoplasia in PDAC is of vital importance, blocking anti-immunity and therapeutic drug delivery, which is regarded as a promoter of malignant progression (28). Combined with our findings, this seems to indicate that GREM1 may promote PDAC progression *via* regulating desmoplasia.

The tumor microenvironment embodies the harmony of tumor cells and the surroundings; the interaction between tumor cells and stroma allows them to reach a state of mutual accommodation. Due to the dense stroma and severe chronic inflammation in PDAC, the constitution of the tumor microenvironment is of vital significance for tumor cells survival; thus, the destruction degree of tumor microenvironment homeostasis determines the success or failure of combating tumor (29). Previous studies have pointed out that PDAC is characterized by the infiltration of immunosuppressive cells and the transformation of antitumor into pro-tumor immunity (30, 31). Distinct from a previous study on GREM1 in pancreatic tumors (24, 32), our analysis has reported characteristic immune cell infiltration featuring distinctive immunosuppressive properties in the pancreatic tumor microenvironment, so that the molecule GREM1 acting outside cell may modulate the immune structure and immune

efficiency distribution to facilitate tumor progression. The previous study has also revealed the adverse prognostic factor in lung cancer, which similarly induces the infiltration of immunosuppressive cells (33). Characteristically, GREM1 induces massive infiltrations of immunosuppressive cells incorporating macrophages, Tregs, and MDSCs, repressing immune cells to identify and eliminate tumor cells (31). Poor prognosis along with GREM1 overexpression correlated with several immune cell infiltration subgroups, indicative of some specific immune structure that can make sense to GREM1 expression in PDAC. Martin *et al.* have proposed that the combination of low budding, low stromal FOXP3 counts, presence of TLTs, and absence of CDKN2A mutations confers a significant survival advantage in patients with PDAC (34). Here, the direct proportion function of GREM1 expression and $FOXP3^+$ Tregs can be observed. Interestingly, the T-cell cluster is investigated in PDAC, presenting a declining tendency in T-cell activation. The upregulation of GREM1 predicts the T-cell exhaustion, a rise in relevant inhibitory surface receptor-like CTLA4, PD-1, PD-L1(CD244), LAG3, TIM3(HAVCR2), BTLA, 2B4(CD244), and TIGIT (35), illuminating the state of T-cell exhaustion. Taken together, GREM1 maintains the immunosuppressive tumor microenvironment, thus promoting PDAC progression.

Furthermore, preliminary verifications were carried out to explore the diagnostic and prognostic values of GREM1 in serum. Concerning the bioinformatics analysis results, serum GREM1 level significantly increased in the PDAC group compared to the HC group, as well as different elevations in assorted pancreatic neoplasms, especially IPMN. Nevertheless, we cannot observe the significant difference among different types of pancreatic diseases due to the limitation of sample quantity. Exactly, serum GREM1 has a good diagnostic value, and its alliance enhances the diagnostic effect of CA199, the most commonly used PDAC diagnostic marker in clinical practice. Interestingly, the increasing level of serum GREM1 occurred in bigger tumor diameters and advanced histopathological grades, as the primary result of GREM1 representing a formidable stromal factor and functioning as stroma modulation. Ultimately, the negative impact on survival of serum GREM1 was visible, despite the disability to statistical significance due to sample restriction, equally identical to multiple survival analyses, which should be further verified by enrolling more PDAC patients.

In summary, GREM1 is significantly upregulated in multiple cancers, including PDAC, which indicates a faster relapse and shorter survival for patients with PDAC. Its pro-tumoral effects in PDAC are pleiotropic, predominantly in promoting stroma formation *via* desmoplasia and inducing immunosuppression in the tumor microenvironment. Our bioinformatics analysis offers a preliminary exploration and discussion on the function of GREM1 in PDAC, and our clinical data further demonstrate the good diagnostic potential of serum GREM1, especially in combination with CA199, which is expected to be a potential candidate for diagnosis and therapy of PDAC.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding authors.

Author contributions

SY, YZ, QFL, and QL conceived this study, analyzed the data, and drafted the manuscript. QFL reviewed and revised the manuscript. MC, MW, JG, and YH collected the data and reviewed the manuscript. QFL and QL were responsible for supervision and project administration. All authors contributed to the article and approved the submitted version.

Funding

This work was supported by the National Natural Science Foundation of China (82172765, 81872501), CAMS Innovation Fund for Medical Sciences (CIFMS,2021-I2M-1-002), Beijing Natural Science Foundation (7172177), and Youth Foundation of Peking Union Medical College Hospital (pumch201911866).

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2022.968610/full#supplementary-material

References

1. Zhu H, Li T, Du Y, Li M. Pancreatic cancer: Challenges and opportunities. *BMC Med* (2018) 16(1):214. doi: 10.1186/s12916-018-1215-3

2. Zhang L, Sanagapalli S, Stoita A. Challenges in diagnosis of pancreatic cancer. *World J Gastroenterol* (2018) 24(19):2047–60. doi: 10.3748/wjg.v24.i19.2047

3. Yin L, Miao Y, Yu J. Advances of pathological complete response after neoadjuvant therapy for pancreatic cancer. *J Pancreatology* (2019) 2(1):11–5. doi: 10.1097/JP9.0000000000000009

4. Tempero MA. NCCN guidelines updates: Pancreatic cancer. *J Natl Compr Canc Netw* (2019) 17(5.5):603–5. doi: 10.6004/jnccn.2019.5007

5. Mizrahi JD, Surana R, Valle JW, Shroff RT. Pancreatic cancer. *Lancet* (2020) 395(10242):2008–20. doi: 10.1016/s0140-6736(20)30974-0

6. Yang S, Liu Q, Liao Q. Tumor-associated macrophages in pancreatic ductal adenocarcinoma: Origin, polarization, function, and reprogramming. *Front Cell Dev Biol* (2020) 8:607209. doi: 10.3389/fcell.2020.607209

7. Wu W, Jin G, Wang C, Miao Y, Wang H, Lou W, et al. The current surgical treatment of pancreatic cancer in China: A national wide cross-sectional study. *J Pancreatology* (2019) 2(1):16–21. doi: 10.1097/JP9.0000000000000012

8. Urist MR. Bone: Formation by autoinduction. *Science* (1965) 150(3698):893–9. doi: 10.1126/science.150.3698.893

9. Lowery JW, Rosen V. The BMP pathway and its inhibitors in the skeleton. *Physiol Rev* (2018) 98(4):2431–52. doi: 10.1152/physrev.00028.2017

10. Katagiri T, Watabe T. Bone morphogenetic proteins. *Cold Spring Harb Perspect Biol* (2016) 8(6):a021899. doi: 10.1101/cshperspect.a021899

11. Kišonaitė M, Wang X, Hyvönen M. Structure of gremlin-1 and analysis of its interaction with BMP-2. *Biochem J* (2016) 473(11):1593–604. doi: 10.1042/bcj20160254

12. Sneddon JB, Zhen HH, Montgomery K, van de Rijn M, Tward AD, West R, et al. Bone morphogenetic protein antagonist gremlin 1 is widely expressed by cancer-associated stromal cells and can promote tumor cell proliferation. *Proc Natl Acad Sci USA* (2006) 103(40):14842–7. doi: 10.1073/pnas.0606857103

13. Ren J, Smid M, Iaria J, Salvatori DCF, van Dam H, Zhu HJ, et al. Cancer-associated fibroblast-derived gremlin 1 promotes breast cancer progression. *Breast Cancer Res* (2019) 21(1):109. doi: 10.1186/s13058-019-1194-0

14. Tang Z, Li C, Kang B, Gao G, Li C, Zhang Z. GEPIA: A web server for cancer and normal gene expression profiling and interactive analyses. *Nucleic Acids Res* (2017) 45(W1):W98–w102. doi: 10.1093/nar/gkx247

15. Rhodes DR, Yu J, Shanker K, Deshpande N, Varambally R, Ghosh D, et al. ONCOMINE: A cancer microarray database and integrated data-mining platform. *Neoplasia* (2004) 6(1):1–6. doi: 10.1016/s1476-5586(04)80047-2

16. Vasaikar SV, Straub P, Wang J, Zhang B. LinkedOmics: analyzing multi-omics data within and across 32 cancer types. *Nucleic Acids Res* (2018) 46(D1): D956–d963. doi: 10.1093/nar/gkx1090

17. Warde-Farley D, Donaldson SL, Comes O, Zuberi K, Badrawi R, Chao P, et al. The GeneMANIA prediction server: Biological network integration for gene prioritization and predicting gene function. *Nucleic Acids Res* (2010) 38(Web Server issue):W214–220. doi: 10.1093/nar/gkq537

18. Ru B, Wong CN, Tong Y, Zhong JY, Zhong SSW, Wu WC, et al. TISIDB: an integrated repository portal for tumor-immune system interactions. *Bioinformatics* (2019) 35(20):4200–2. doi: 10.1093/bioinformatics/btz210

19. Li T, Fan J, Wang B, Traugh N, Chen Q, Liu JS, et al. TIMER: A web server for comprehensive analysis of tumor-infiltrating immune cells. *Cancer Res* (2017) 77(21):e108–10. doi: 10.1158/0008-5472.Can-17-0307

20. Kobayashi H, Gieniec KA, Wright JA, Wang T, Asai N, Mizutani Y, et al. The balance of stromal BMP signaling mediated by GREM1 and ISLR drives colorectal carcinogenesis. *Gastroenterology* (2021) 160(4):1224–39.e1230. doi: 10.1053/j.gastro.2020.11.011

21. Zhu Y, Knolhoff BL, Meyer MA, Nywening TM, West BL, Luo J, et al. CSF1/CSF1R blockade reprograms tumor-infiltrating macrophages and improves response to T-cell checkpoint immunotherapy in pancreatic cancer models. *Cancer Res* (2014) 74(18):5057–69. doi: 10.1158/0008-5472.Can-13-3723

22. Zhu Y, Herndon JM, Sojka DK, Kim KW, Knolhoff BL, Zuo C, et al. Tissue-resident macrophages in pancreatic ductal adenocarcinoma originate from embryonic hematopoiesis and promote tumor progression. *Immunity* (2017) 47 (2):323–38.e326. doi: 10.1016/j.immuni.2017.07.014

23. Gu Q, Luo Y, Chen C, Jiang D, Huang Q, Wang X. GREM1 overexpression inhibits proliferation, migration and angiogenesis of osteosarcoma. *Exp Cell Res* (2019) 384(1):111619. doi: 10.1016/j.yexcr.2019.111619

24. Chen MH, Yeh YC, Shyr YM, Jan YH, Chao Y, Li CP, et al. Expression of gremlin 1 correlates with increased angiogenesis and progression-free survival in patients with pancreatic neuroendocrine tumors. *J Gastroenterol* (2013) 48(1):101–8. doi: 10.1007/s00535-012-0614-z

25. Tamminen JA, Parviainen V, Rönty M, Wohl AP, Murray L, Joenväärä S, et al. Gremlin-1 associates with fibrillin microfibrils *in vivo* and regulates mesothelioma cell survival through transcription factor slug. *Oncogenesis* (2013) 2(8):e66. doi: 10.1038/oncsis.2013.29

26. Sung NJ, Kim NH, Surh YJ, Park SA. Gremlin-1 promotes metastasis of breast cancer cells by activating STAT3-MMP13 signaling pathway. *Int J Mol Sci* (2020) 21(23):9227. doi: 10.3390/ijms21239227

27. Karagiannis GS, Berk A, Dimitromanolakis A, Diamandis EP. Enrichment map profiling of the cancer invasion front suggests regulation of colorectal cancer progression by the bone morphogenetic protein antagonist, gremlin-1. *Mol Oncol* (2013) 7(4):826–39. doi: 10.1016/j.molonc.2013.04.002

28. Ren B, Cui M, Yang G, Wang H, Feng M, You L, et al. Tumor microenvironment participates in metastasis of pancreatic cancer. *Mol Cancer* (2018) 17(1):108. doi: 10.1186/s12943-018-0858-1

29. Jing W, McAllister D, Vonderhaar EP, Palen K, Riese MJ, Gershan J, et al. STING agonist inflames the pancreatic cancer immune microenvironment and reduces tumor burden in mouse models. *J Immunother Cancer* (2019) 7(1):115. doi: 10.1186/s40425-019-0573-5

30. Liu Q, Li Y, Niu Z, Zong Y, Wang M, Yao L, et al. Atorvastatin (Lipitor) attenuates the effects of aspirin on pancreatic cancerogenesis and the chemotherapeutic efficacy of gemcitabine on pancreatic cancer by promoting M2 polarized tumor associated macrophages. *J Exp Clin Cancer Res* (2016) 35:33. doi: 10.1186/s13046-016-0304-4

31. Leinwand J, Miller G. Regulation and modulation of antitumor immunity in pancreatic cancer. *Nat Immunol* (2020) 21(10):1152–9. doi: 10.1038/s41590-020-0761-y

32. Yu Y, Cheng L, Yan B, Zhou C, Qian W, Xiao Y, et al. Overexpression of gremlin 1 by sonic hedgehog signaling promotes pancreatic cancer progression. *Int J Oncol* (2018) 53(6):2445–57. doi: 10.3892/ijo.2018.4573

33. Gentles AJ, Hui AB, Feng W, Azizi A, Nair RV, Bouchard G, et al. A human lung tumor microenvironment interactome identifies clinically relevant cell-type cross-talk. *Genome Biol* (2020) 21(1):107. doi: 10.1186/s13059-020-02019-x

34. Wartenberg M, Cibin S, Zlobec I, Vassella E, Eppenberger-Castori S, Terracciano L, et al. Integrated genomic and immunophenotypic classification of pancreatic cancer reveals three distinct subtypes with Prognostic/Predictive significance. *Clin Cancer Res* (2018) 24(18):4444–54. doi: 10.1158/1078-0432.Ccr-17-3401

35. Wherry EJ, Kurachi M. Molecular and cellular insights into T cell exhaustion. *Nat Rev Immunol* (2015) 15(8):486–99. doi: 10.1038/nri3862

frontiers | Frontiers in Oncology

TYPE Original Research
PUBLISHED 29 September 2022
DOI 10.3389/fonc.2022.993901

OPEN ACCESS

EDITED BY
John Gibbs,
Hackensack Meridian Health,
United States

REVIEWED BY
Quyen Chu,
Orlando Health, United States
Bao-Cai Xing,
Beijing Cancer Hospital, China

*CORRESPONDENCE
Yinmo Yang
YangyinmoSCI@bjmu.edu.cn
Xiaodong Tian
tianxiaodong@pkufh.com

†These authors have contributed equally to this work

SPECIALTY SECTION
This article was submitted to Gastrointestinal Cancers: Hepato Pancreatic Biliary Cancers, a section of the journal Frontiers in Oncology

RECEIVED 14 July 2022
ACCEPTED 15 September 2022
PUBLISHED 29 September 2022

CITATION
Xie X, Chen K, Liu Z, Wang F, Ma Y, Zhang S, Shao Z, Yang Y and Tian X (2022) Safety evaluation of early drain removal following pancreaticoduodenectomy: A single-center retrospective cohort study. *Front. Oncol.* 12:993901. doi: 10.3389/fonc.2022.993901

Safety evaluation of early drain removal following pancreaticoduodenectomy: A single-center retrospective cohort study

Xuehai Xie[1†], Kai Chen[1†], Zonghao Liu[1], Feng Wang[2], Yongsu Ma[1], Shupeng Zhang[3], Zhijiang Shao[3], Yinmo Yang[1*] and Xiaodong Tian[1*]

[1]Department of General Surgery, Peking University First Hospital, Beijing, China, [2]Department of Endoscopy Center, Peking University First Hospital, Beijing, China, [3]Department of General Surgery, Tianjin Fifth Centre Hospital, Tianjin, China

Objectives: The effects of early drain removal (EDR) on postoperative complications after pancreaticoduodenectomy (PD) remains to be investigated. This single-center retrospective cohort study was designed to explore the safety of EDR after PD.

Methods: A total of 112 patients undergoing PD with drain fluid amylase (DFA) on postoperative day (POD) 1 and 3 <= 5000 were divided into EDR and late drain removal (LDR). Propensity Score Matching (PSM) was used. We compared postoperative outcomes between two groups and explore the risk factors of total complications using univariate and multiple logistic regression analyses.

Results: No statistical differences were found in primary outcomes, including Grade B/C postoperative pancreatic fistula (POPF) (Original cohort: 5.71% vs. 3.90%; P = 1.000; PSM cohort: 3.33% vs. 6.67%; P = 1.000), and total complications (Original cohort: 17.14% vs. 32.47%; P = 0.093; PSM cohort: 13.33% vs. 33.33%; P = 0.067). The EDR was associated with shorter in-hospital stay (Original cohort: 11 days vs. 15 days; P < 0.0001; PSM cohort: 11 days vs. 15 days; P < 0.0001).

Conclusions: EDR on POD 3 is safe for patients undergoing PD with low risk of POPF.

KEYWORDS

pancreaticoduodenectomy, early drain removal, postoperative pancreatic fistula, postoperative complications, pancreatic cancer

Introduction

With the rapid development of surgical technique in the last decades, the perioperative mortality of pancreaticoduodenectomy (PD) decreased significantly, whereas the incidences of postoperative complications are still high (1–3). The postoperative pancreatic fistula (POPF) remains one of the most significant postoperative complications after PD, which significantly increases postoperative in-hospital stay and medical burden (4). A growing body of study proposed the predicted models to evaluate the risk of POPF after pancreatic surgery (5, 6). The management of intraperitoneal drainage plays a crucial role in the process of postoperative recovery of patients undergoing PD. The detection of drain fluid around the operative area is perceived as an important indicator for early identifying POPF, postpancreatectomy hemorrhage (PPH) or intra-abdominal infection, therefore prophylactic drainage placement during PD is accepted in most of pancreatic centers (7). However, many studies raised concerns about the placement of intraperitoneal drainage after PD. For example, Conlon et al. (8) performed the first randomized controlled trial (RCT) to demonstrate that the placement of drainage after pancreatic resection failed to reduce postoperative complications, but increased the incidences of intra-abdominal collections and infection. Subsequently, multiple RCTs and meta-analysis proved the safety of omission of drainage after pancreatic resection (9–13). However, one RCT was stopped because of the significantly increased mortality from 3% to 12% for patients undergoing PD without the placement of intraperitoneal drainage (14). Therefore, no consensus was reached with regard to whether to place prophylactic intraperitoneal drainage.

Recently, multiple studies paid more attention to evaluating the feasibility of EDR. Bassi et al. (15) performed the first RCT to explore the safety of EDR, and results showed that EDR significantly decreased complications, in-hospital stay and costs than late drain removal (LDR). Thereafter, Dai et al. (16, 17) performed single and multiple-center RCT to compare EDR and LDR regarding Clavien-Dindo grades 2–4 complications. The strict inclusion criteria were used to select patients with low or middle risk of POPF, which demonstrated EDR is safe in selected patients. The American College of Surgeons' National Surgical Quality Improvement Program (ACS-NSQIP) was also utilized to explore the effects of EDR on postoperative complications for PD. EDR after PD was associated with better outcomes (18). Although the safety of EDR after PD was proved preliminarily, the low risk patient selection criteria and the time-point of EDR remains to be further explored.

Here, we designed single-center retrospective cohort study to the confirm the safety of EDR on POD 3 for PD patients with the low risk of POPF. A total of 112 patients undergoing PD with drain fluid amylase (DFA) on POD 1 and 3 <= 5000 were divided into EDR and LDR groups. Propensity Score Matching (PSM) was used. We found that there were no significant differences in Grade B/C POPF and total complications. In addition, EDR was associated with shorter in-hospital stay.

Methods

Single-center study design

This retrospective cohort study was approved by the Ethical Committee on Peking University First Hospital (Approval No.2021-636) and performed in accordance with the Helsinki Declaration. The consecutive patients undergoing pancreaticoduodenectomy (PD) or pylorus preserving PD (PPPD) from January 2017 and December 2020 in our institution with drain fluid amylase (DFA) on both postoperative day (POD) 1 and 3 ≤ 5000 U/L were enrolled. Specific exclusion criteria consisted in (a) patients underwent distal pancreatectomy (DP) or total pancreatectomy; (b) DFA on POD 1 or 3 > 5000 U/L; (c) patients with age < 18; (d) incomplete records of key postoperative outcomes.

The time-point of early and late drain removal was defined as POD 3 and ≥ POD 5. All the operations were performed by experienced pancreatic surgeons at our institution. Clinicopathological data were collected retrospectively through electronic medical record system.

The primary and secondary outcomes in single-center study

Postoperative complications were evaluated using the Clavien–Dindo classification system (19). The postoperative complications such as postoperative pancreatic fistula (POPF) (20), delayed gastric emptying (DGE) (21), and postpancreatectomy hemorrhage (PPH) (22) were in accordance with the consensus definition of the International Study Group of Pancreatic Fistula (ISGPF). Intra-abdominal collections were defined as collection of fluid measuring at least 3 cm in diameter demonstrated by ultrasound or CT scan. The primary outcomes in this study included Grade B/C POPF and total complications. The secondary outcomes were DGE, PPH, intra-abdominal collections, wound infection, re-operation, re-admission and post-operative in-hospital stay.

Propensity score matching

Propensity Score Matching was used to deal with confounding factors using R package "MatchIt". Matching variables included age, BMI, pancreatitis, diabetes, cardiovascular disease, soft pancreatic texture, operation time, blood loss, diameter of main pancreatic duct, PPPD, vascular

resection, ASA scores, pathology. The nearest neighbor matching method with a tolerate of 0.1 was selected.

Statistical analysis

Data was summarized as mean ± standard deviation or median (interquartile range, IQR) for continuous variables subjected to normal distribution or no normal distribution. The independent-samples t test and Mann-Whitney U test was performed to compare continuous variables between two groups. For categorical variables, data was summarized as frequency (ratio) and the chi-square test, fisher exact test, or rank sum test was used. Study of potential prognostic factors for total complications was carried out using univariate and multiple logistic regression analyses. All statistical analyses were conducted using SPSS version 22.0 software (SPSS22, Chicago, USA). Statistical significance was defined as $p < 0.05$.

Results

Characteristics of patients in single-center study

A total of 112 patients who underwent PD performed at our institution between January 2017 and December 2020 were divided into two groups: EDR (n = 35, drains were removed on POD 3) and LDR (n = 77, drains were removed on or beyond POD 5). Patients with amylase value in drains on POD 1 or 3 > 5000 U/L were excluded. The patients previously enrolled in the multi-center study performed by Peking Union Medical College Hospital were not included in our cohort.

The demographic, surgical, biochemical, and pathological characteristics of patients were summarized in Table 1. There were no significant differences in gender, BMI, pancreatitis, diabetes, cardiovascular disease, smoke, alcohol, intraoperative RBC transfusion, PPPD, vascular resection, preoperative hemoglobin, serum total bilirubin, and pathology between two groups. No significant differences were found as well with particular regard to risk factors of POPF (soft pancreatic texture, blood loss, diameter of main pancreatic duct, DFA on POD 1/3). Only two patients underwent neoadjuvant chemotherapy (2 in EDR, 0 in LDR, P = 0.847). Patients in EDR had lower age (60.23 ± 10.66 vs. 65.32 ± 12.55; P = 0.04), shorter operation time (266 [240 – 300] vs. 301 [251.5 - 418]; P = 0.026), and different ASA scores (Grade I: 17.14% vs. Grade I: 2.60%; P = 0.035) in comparison with LDR. In order to reduce the impact of confounding factors to make two groups more homogeneous, Propensity Score Matching (PSM) was conducted. After PSM, all demographic, surgical, biochemical characteristics, and risk factors of POPF were similar without significant differences between these two groups (Table 1). In addition, the drain placement time was 3 days in EDR group versus 11 days (3 - 15.75) in LDR group (Table 2).

Primary and secondary outcomes in single-center study

Table 2 described postoperative complications of enrolled participants. There were no statistical differences between EDR and LDR group in primary outcomes, including Grade B/C POPF (Original cohort: 5.71% vs. 3.90%; P = 1.000; PSM cohort: 3.33% vs. 6.67%; P = 1.000), and total complications (Original cohort: 17.14% vs. 32.47%; P = 0.093; PSM cohort: 13.33% vs. 33.33%; P = 0.067).

EDR was associated with a decrease of Grade 2-4 complications (Original cohort: 11.43% vs. 28.57%; P = 0.046; PSM cohort: 6.67% vs. 26.67%; P = 0.038), post-operative in-hospital stay (Original cohort: 11 [9 - 14] vs. 15 [12.5 – 22.5]; P < 0.0001; PSM cohort: 11 [9 - 14] vs. 15 [11.75 – 21.5]; P < 0.0001). No significant differences were observed in single abdominal complications, including PPH (Original cohort: 0 vs. 5.19%; P = 0.307; PSM cohort: 0 vs. 6.67%; P = 0.492), intra-abdominal infection (Original cohort: 0 vs. 9.09%; P = 0.096; PSM cohort: 0 vs. 10.00%; P = 0.237), delayed gastric emptying (Original cohort: 8.57 vs. 11.69%; P = 0.869; PSM cohort: 6.67 vs. 6.67%; P = 1.000), and intra-abdominal fluid collections (Original cohort: 8.57 vs. 5.19%; P = 0.792; PSM cohort: 6.67 vs. 6.67%; P = 1.000). The rates of biliary fistula, wound infection, pulmonary complications between two groups were also comparable. The mortality, intervention, re-admission re-operation occurred in 0/0/3/0 patients in EDR group versus 2/3/2/1 patients in LDR group without significant differences. After PSM, the results were the same.

Exploring risk factors of total complications after PD

The correlation analysis of total complications and multiple characteristics were summarized in Table 3. Total complications were related to age, operation time, ASA scores, serum total bilirubin, and pathology. After continuous variables being converted into categorical variables, univariate logistic regression was performed, and the result showed that pathological characteristic was related to total complications. Early drain removal decreased slightly the total complications rate, but it was not significant difference (OR = 0.430; P = 0.098) (Table 4). Finally, the variables (P < 0.1) were included into multivariate logistic regression analysis, which also proved that only pathological characteristic was the independent risk factor associated with the incidence of total complications compared with benign pancreatic diseases (IPMN: OR = 0.087; P = 0.024; duodenal disease: OR = 0.098; P = 0.049) (Table 4).

TABLE 1 Demographic characteristics of enrolled participants in retrospective cohort study.

	Original Cohort				Propensity Score Matching			
	Total (n = 112)	EDR (n = 35)	LDR (n = 77)	P_Value	Total (n = 60)	EDR (n = 30)	LDR (n = 30)	P_Value
Age (year, mean ± SD)	63.73 ± 12.18	60.23 ± 10.66	65.32 ± 12.55	**0.040***	60.78 ± 11.35	60.63 ± 11.32	60.93 ± 11.57	0.919
Gender [female, n (%)]	47 (41.96)	12 (34.26)	35 (45.45)	0.267	23 (38.33)	10 (33.33)	13 (43.33)	0.426
BMI (kg/m2, mean ± SD)	23.60 ± 3.22	23.31 ± 2.82	23.74 ± 3.40	0.517	23.13 ± 2.95	23.02 ± 2.43	23.24 ± 3.43	0.773
Pancreatitis [n (%)]	17 (15.18)	7 (20.00)	10 (12.99)	0.338	11 (18.33)	7 (23.33)	4 (13.33)	0.317
Diabetes [n (%)]	31 (27.68)	13 (37.14)	18 (23.38)	0.131	20 (33.33)	9 (30.00)	11 (36.67)	0.584
Cardiovascular Disease [n (%)]	19 (16.96)	5 (14.29)	14 (18.18)	0.611	10 (16.67)	5 (16.67)	5 (16.67)	1.000
Smoke [n (%)]	36 (32.14)	11 (31.43)	25 (32.47)	0.913	19 (31.67)	8 (26.67)	11 (36.67)	0.405
Alcohol [n (%)]	25 (22.32)	6 (17.14)	19 (24.68)	0.375	14 (23.33)	6 (20.00)	8 (26.67)	0.542
Neoadjuvant Chemotherapy [n (%)]	2 (1.79)	0 (0)	2 (2.60)	0.847	1 (1.67)	0 (0)	1 (3.33)	1.000
Soft Pancreatic Texture [n (%)]	42 (37.50)	13 (37.14)	29 (37.66)	0.958	24 (40.00)	12 (40.00)	12 (40.00)	1.000
Operation Time (min, IQR)	293.5 (244 - 360)	266 (240 - 300)	301 (251.5 - 418)	**0.026***	265.5 (240 - 313)	265.5 (240 - 300)	268 (238.25 - 328.50)	0.684
Blood Loss (ml, IQR)	200 (100 - 300)	200 (100 -400)	200 (100 -300)	0.856	200 (100 - 300)	200 (100 - 400)	180 (100 - 300)	0.560
Intraoperative RBC transfusion [n (%)]	17 (15.18)	5 (14.29)	12 (15.58)	0.859	10 (16.67)	5 (16.67)	5 (16.67)	1.000
Diameter of Main Pancreatic Duct < 3 mm [n (%)]	50 (44.64)	12 (34.29)	38 (49.35)	0.137	20 (33.33)	10 (33.33)	10 (33.33)	1.000
PPPD [n (%)]	73 (65.18)	25 (71.43)	48 (62.34)	0.349	44 (73.33)	23 (76.67)	21 (70.00)	0.559
DFA on POD 1 (U/L, IQR)	470.5 (117.25 - 1722)	256 (128 -1532)	618 (114.5 - 1797.5)	0.292	606.5 (137 - 1786)	256.5 (101.5 - 1579.5)	941.5 (203.75 - 2733)	0.056
DFA on POD 3 (U/L, IQR)	175.5 (30 - 799.75)	115 (20 - 861)	177 (32 - 789.5)	0.778	291.5 (35 - 1023.25)	229.5 (26 - 1100.5)	499.5 (84.25 - 1050.5)	0.255
Vascular Resection [n (%)]	4 (3.57)	3 (8.57)	1 (1.30)	0.170	0 (0)	0 (0)	0 (0)	NA
ASA Score [n (%)]								
Grade I	8 (7.14)	6 (17.14)	2 (2.60)	**0.035***	6 (10.00)	5 (16.70)	1 (3.30)	0.092
Grade II	74 (66.07)	20 (57.14)	54 (70.13)		39 (65.00)	16 (53.3)	23 (76.70)	
Grade III	28 (25.00)	9 (25.71)	19 (24.68)		15 (25.00)	9 (30.00)	6 (20.00)	
Grade IV	2 (1.79)	0 (0)	2 (2.60)		0 (0)	0 (0)	0 (0)	
Preoperative Hemoglobin (g/L, mean ± SD)	123.41 ± 19.31	125.34 ± 23.30	122.53 ± 17.30	0.479	124.43 ± 20.73	125.33 ± 24.07	123.53 ± 17.13	0.740
Serum Total Bilirubin (umol/L, IQR)	52.75 (18.13 - 165.3)	33.2 (19.8 - 158)	56.3 (18.05 - 172.0)	0.925	34.75 (16.60 - 146.9)	32.8 (15.83 - 156.05)	39.30 (17.25 - 110.15)	0.779
Pathology [n (%)]								
Pancreatic Disease	64 (57.14)	21 (60.00)	43 (55.84)	0.622	33 (55.00)	18 (60.00)	15 (50.00)	0.337
Benign	7 (6.25)	3 (8.57)	4 (5.19)		4 (6.67)	3 (10.00)	1 (3.33)	
Neuroendocrine	3 (2.68)	2 (5.71)	1 (1.30)		2 (3.33)	2 (6.67)	0 (0)	
Malignant	46 (41.07)	14 (40.00)	32 (41.56)		21 (35.00)	11 (36.67)	10 (33.33)	
IPMN	8 (7.14)	2 (5.71)	6 (7.79)		6 (10.00)	2 (6.67)	4 (13.33)	
Ampullary Disease	10 (8.93)	2 (5.71)	8 (10.39)		6 (10.00)	1 (3.33)	5 (16.67)	
Biliary Tract Disease	15 (13.39)	3 (8.57)	12 (15.58)		5 (8.33)	2 (6.67)	3 (10.00)	
Duodenal Disease	23 (20.54)	9 (25.71)	14 (18.18)		16 (26.67)	9 (30.00)	7 (23.33)	

IQR, Interquartile range; PPPD, Pylorus preserving pancreaticoduodenectomy; DFA, Drain fluid amylase; ASA, American society of anesthesiologists; IPMN, Intraductal papillary mucinous neoplasm.

* and bold values represent statistical significance ($p < 0.05$).

TABLE 2 Postoperative complications of enrolled participants in retrospective cohort study.

	Original Cohort				Propensity Score Matching			
	Total (n = 112)	EDR (n = 35)	LDR (n = 77)	P_Value	Total (n = 60)	EDR (n = 30)	LDR (n = 30)	P_Value
Drain Placement Time (POD day, IQR)	11 (3 - 15.75)	3	13 (11 - 20)	< **0.000***	4.5 (3 -13.75)	3	13.5 (10.75 - 19.25)	< **0.000***
Grade B/C POPF [n (%)]	5 (4.46)	2 (5.71)	3 (3.90)	1.000	3 (5.00)	1 (3.33)	2 (6.67)	1.000
Total Complications [n (%)]	31 (27.68)	6 (17.14)	25 (32.47)	0.093	14 (23.33)	4 (13.33)	10 (33.33)	0.067
Grade 2-4 Complications [n (%)]	26 (23.21)	4 (11.43)	22 (28.57)	**0.046***	10 (16.67)	2 (6.67)	8 (26.67)	**0.038***
Postpancreatectomy Hemorrhage [n (%)]	4 (3.57)	0 (0)	4 (5.19)	0.307	2 (3.33)	0 (0)	2 (6.67)	0.492
Intra-abdominal Infection [n (%)]	7 (6.25)	0 (0)	7 (9.09)	0.096	3 (5.00)	0 (0)	3 (10.00)	0.237
Post-operative in-hospital Stay (day, IQR)	14 (11 - 21)	11 (9 - 14)	15 (12.5 - 22.5)	< **0.0001***	13 (10 - 16)	11 (9 - 14)	15 (11.75 - 21.5)	< **0.0001***
Delayed Gastric Emptying [n (%)]	12 (10.71)2	3 (8.57)	9 (11.69)	0.869	4 (6.67)	2 (6.67)	2 (6.67)	1.000
Biliary Fistula [n (%)]	6 (5.36)	0 (0)	6 (7.79)	0.174	3 (5.00)	0 (0)	3 (10.00)	0.237
Intra-abdominal Fluid Collections [n (%)]	7 (6.25)	3 (8.57)	4 (5.19)	0.792	4 (6.67)	2 (6.67)	2 (6.67)	1.000
Wound Infection [n (%)]	0 (0)	0 (0)	0 (0)	NA	0 (0)	0 (0)	0 (0)	NA
Pulmonary Complications [n (%)]	1 (0.89)	0 (0)	1 (1.30)	1.000	0 (0)	0 (0)	0 (0)	NA
Mortality [n (%)]	2 (1.79)	0 (0)	2 (2.60)	1.000	1 (1.67)	0 (0)	1 (3.33)	1.000
Intervention [n (%)]	3 (2.68)	0 (0)	3 (3.90)	0.551	1 (1.67)	0 (0)	1 (3.33)	1.000
Re-admission [n (%)]	5 (4.46)	3 (8.57)	2 (2.60)	0.355	2 (3.33)	2 (6.67)	0 (0)	0.492
Re-operation [n (%)]	1 (0.89)	0 (0)	1 (1.30)	1.000	0 (0)	0 (0)	0 (0)	NA

POPF, postoperative pancreatic fistula.
* and bold values represent statistical significance ($p < 0.05$).

Management of postoperative pancreatic fistula

In total, the rate of Grade B/C POPF was 4.46% (5/112), which occurred in 2 patients in EDR and 3 patients in LDR (P = 1.000). Low Grade B/C POPF rate indicated that low risk patient selection strategy (DFA on POD 1 and 3 <= 5000 U/L) works. EDR group had 2 grade B POPF. In contrast, LDR had 2 grade B POPF and 1 grade C POPF. Postoperative course of patients with pancreatic fistula were recorded in (Table 5). The patient had grade C POPF in LDR group even though DFA on POD 1 and 3 < 40 U/L, who underwent PD because of cholangiocarcinoma. Drains were removed on POD 8. Reoperation was conducted to explore for dehiscence of the anastomotic stoma and hemorrhage, which significantly extended in-hospital stay for 77 days, and finally caused mortality. Three of five patients with Grade B/C POPF had positive drain fluid cultures, thus antibiotic therapy was established according to drug sensitivity test. Percutaneous drain insertion or conservative treatment were used for grade B POPF.

Discussion

One of major concerns of EDR is intra-abdominal fluid collection, and caused infection and hemorrhage. This single-center study indicated that EDR could not increase the risk of intra-abdominal fluid collection and hemorrhage. The selection strategy of low risk patients: DFA on POD 1 <= 5000 U/L was utilized by previous studies (15–18, 23), and low Grade B/C POPF rate was observed in these studies. Single and multiple-center RCT performed by Dai and our single-center retrospective study used the stricter selection criteria (DFA on POD 1 and 3 <= 5000 U/L). The Grade B/C POPF rates were 1.75%, 5.13%, and 4.46% respectively. The strict selection strategy guarantees the safety of early drain removal, and aid in surgeon confidence to make a decision of EDR. However, the stricter selection strategy will narrow the clinical application of EDR. Thus, it is very important to balance selection criteria and the range of clinical application of EDR. Nowadays, the selection strategy for low risk of POPF, time-point of EDR, DFA cut-off value remains to be further investigated.

TABLE 3 Correlation analysis of total complications of enrolled participants in retrospective cohort study.

	Original Cohort		
	Total Complications: Yes (n = 31)	**Total Complications: No (n = 81)**	**P_Value**
Age (year, mean ± SD)	67.39 ± 11.08	62.33 ± 12.35	**0.049***
Gender [female, n (%)]	13 (41.94)	34 (41.98)	0.997
BMI (kg/m2, mean ± SD)	24.37 ± 3.53	23.31 ± 3.07	0.122
Pancreatitis [n (%)]	5 (16.13)	12 (14.81)	1.000
Diabetes [n (%)]	10 (32.26)	21 (25.93)	0.503
Cardiovascular Disease [n (%)]	5 (16.13)	14 (17.28)	0.884
Smoke [n (%)]	10 (32.26)	26 (32.10)	0.987
Alcohol [n (%)]	5 (16.13)	20 (24.69)	0.330
Neoadjuvant Chemotherapy [n (%)]	1 (3.22)	1 (1.23)	0.479
Soft Pancreatic Texture [n (%)]	11 (35.48)	31 (38.27)	0.785
Operation Time (min, IQR)	327 (271 - 413)	285 (240 - 349)	**0.019***
Blood Loss (ml, IQR)	300 (150 - 500)	200 (100 - 300)	0.050
Intraoperative RBC transfusion [n (%)]	6 (19.35)	11 (13.58)	0.640
Diameter of Main Pancreatic Duct < 3 mm [n (%)]	13 (41.94)	37 (45.68)	0.721
PPPD [n (%)]	17 (54.84)	56 (69.14)	0.155
DFA on POD 1 (U/L, IQR)	478 (138 - 1532)	452 (107.5 - 1754)	0.642
DFA on POD 3 (U/L, IQR)	181 (43 - 1340)	123 (24 - 700)	0.301
Vascular Resection [n (%)]	2 (6.45)	2 (2.47)	0.655
ASA Score [n (%)]			
Grade I	0 (0)	8 (9.88)	**0.022***
Grade II	19 (61.29)	55 (67.90)	
Grade III	12 (38.71)	16 (19.75)	
Grade IV	0 (0)	2 (2.47)	
Preoperative Hemoglobin (g/L, mean ± SD)	122.81 ± 19.63	123.64 ± 19.31	0.839
Serum Total Bilirubin (umol/L, IQR)	95.5 (24.3 - 239.1)	33.2 (15 - 155.4)	**0.033***
Pathology [n (%)]			
Pancreatic Disease	21 (67.74)	43 (53.09)	**0.048***
Benign	2 (6.45)	5 (6.17)	
Neuroendocrine	1 (3.22)	2 (2.47)	
Malignant	17 (54.84)	29 (35.80)	
IPMN	1 (3.22)	7 (8.64)	
Ampullary Disease	3 (9.68)	7 (8.64)	
Biliary Tract Disease	6 (19.35)	9 (11.11)	
Duodenal Disease	1 (3.22)	22 (27.16)	
Drain Placement Time [EDR, n (%)]	6 (19.35)	29 (35.80)	0.093

* and bold values represent statistical significance ($p < 0.05$).

10.3389/fonc.2022.993901

10.3389/fonc.2022.993901

TABLE 4 Univariate and multivariate logistic regression of total complications of enrolled participants in retrospective cohort study.

	Univariate Analysis		Multivariate Analysis	
	OR (95% CI)	P_Value	OR (95% CI)	P_Value
Age (> 65/<= 65)	0.550 (0.238 - 1.270)	0.161		
Gender (F/M)	1.002 (0.433 - 2.317)	0.997		
BMI (> median/<= median)	0.765 (0.333 - 1.755)	0.527		
Pancreatitis (N/Y)	1.106 (0.355 - 3.446)	0.862		
Diabetes (N/Y)	1.361 (0.552 - 3.354)	0.504		
Cardiovascular Disease (N/Y)	0.920 (0.301 - 2.812)	0.884		
Smoke (N/Y)	1.007 (0.415 - 2.443)	0.987		
Alcohol (N/Y)	0.587 (0.199 - 1.731)	0.334		
Neoadjuvant Chemotherapy (N/Y)	2.667 (0.162 - 44.001)	0.493		
Soft Pancreatic Texture (N/Y)	0.887 (0.375 - 2.099)	0.785		
Operation Time (> median/<= median)	0.531 (0.228 - 1.236)	0.142		
Blood Loss (> 200/<= 200)	0.495 (0.214 - 1.147)	0.101		
Intraoperative RBC transfusion (N/Y)	1.527 (0.511 - 4.563)	0.448		
Diameter of Main Pancreatic Duct < 3 mm (N/Y)	0.859 (0.372 - 1.983)	0.722		
PPPD (N/Y)	0.542 (0.232 - 1.268)	0.158		
DFA on POD 1 (> median/<= median)	0.915 (0.400 - 2.094)	0.833		
DFA on POD 3 (> median/<= median)	0.905 (0.396 - 2.194)	0.826		
Vascular Resection (N/Y)	2.724 (0.367 - 20.242)	0.327		
ASA Score (III - IV/I - II)	0.452 (0.185 - 1.104)	0.082	0.495 (0.186 - 1.321)	0.160
Preoperative Hemoglobin (> median/<= median)	0.728 (0.317 - 1.671)	0.454		
Serum Total Bilirubin (> median/<= median)	0.440 (0.187 - 1.036)	0.060	0.507 (0.178 - 1.443)	0.203
Pathology [n (%)]				
Benign	1		1	
Neuroendocrine	0.114 (0.009 - 1.514)	0.100	0.081 (0.006 – 1.159)	0.064
Malignant	0.091 (0.004 - 2.073)	0.133	0.067 (0.003 - 1.599)	0.095
IPMN	0.078 (0.010 - 0.628)	**0.017***	0.087 (0.010 - 0.720)	**0.024***
Ampullary Disease	0.318 (0.018 - 5.779)	0.439	0.253 (0.013 – 4.908)	0.364
Biliary Tract Disease	0.106 (0.009 - 1.190)	0.069	0.176 (0.015 -2.132)	0.172
Duodenal Disease	0.068 (0.007 - 0.650)	**0.020***	0.098 (0.010 -0.994)	**0.049***
Drain Placement Time (3/>3)	0.430 (0.158 - 1.170)	0.098	0.473 (0.160 - 1.398)	0.176

CI, Confidence interval.
* and bold values represent statistical significance ($p < 0.05$).

The definitions of EDR and LDR varied among previous studies. Bassi et al. (15) chose POD 3 as EDR time-point because the change of drain effluent happens on POD 3 which is also regarded as relatively early time-point, and POD 5 as LDR time-point because POD 5 is standard drain removal time-point in their institution. In our single-center study, we also chose POD 3 as EDR time-point, and >= POD 5 as LDR time-point that is the same as RCTs performed by Dai et al. (16, 17). It is better to select appropriate time-point of EDR and LDR according to local medical conditions. Our single-center study supported that EDR is safe and significantly decrease postoperative in-hospital stay (11 [9 – 14] vs. 15 [12.5 - 22.5]), indicating that faster recovery, lower medical costs for patients, in line with the idea of enhanced recovery after surgery (ERAS). In additions, Dai et al. (17) found late drain removal and laparoscopic procedure were the independent risk factors of major complications using multiple regression analysis. However, our single-center study did not prove the LDR was an independent risk factor of total complications (OR = 0.473, 95% CI: 0.160 - 1.398; P = 0.176), which might be attributed to small sample size.

Our work provides an evidence for the safety of EDR, and help promote the practice of ERAS after PD. However, there are some limitations in this study, such as (a) single-center retrospective cohort study with limited sample size; (b)

TABLE 5 Characteristics of patients with Grade B/C POPF.

ID	Group	Soft PancreaticTexture	Diameter of Main Pancreatic Duct < 3 mm	Blood Loss (ml)	Pathology	DFA on POD 1 (U/L)	DFA on POD 3 (U/L)	Drain Placement Time (POD day)	Grading of POPF	POPF Management	Drain Fluid Culture
1	EDR	Y	Y	100	Moderately differentiated cholangiocarcinoma	3509	861	3	B	Percutaneous drain insertion	Enterococcus fecalis
2	EDR	N	N	100	Benign pancreatic disease	1133	3835	3	B	Percutaneous drain insertion	Enterococcus fecalis, Monilia albican
3	LDR	N	N	300	Moderately differentiated pancreatic ductal adenocarcinoma	463	79	26	B	Percutaneous drain insertion	Negative
4	LDR	Y	N	300	Moderately differentiated ampullary adenocarcinoma	3114	446	33	B	Conservative	Negative
5	LDR	N	N	500	Moderately differentiated cholangiocarcinoma	34	30	8	C	Postoperative hemorrhage, reoperation	Klebsiella pneumoniae

relatively strict low risk patient selection criteria to narrow the clinical application range of EDR; (c) single-center study only focus on PD, the safety of EDR for DP remains to be explored.

In conclusion, our study demonstrates that early drain removal on POD 3 is safe for patients following PD with low risk of POPF.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding authors.

Ethics statement

The studies involving human participants were reviewed and approved by This retrospective cohort study was approved by the Ethical Committee on Peking University First Hospital (Approval No.2021-636). The ethics committee waived the requirement of written informed consent for participation.

Author contributions

Conceptualization, KC, XDT. Literature Search, KC, XHX, ZHL. Data Collection, KC, YSM. Formal Analysis, KC. Validation, KC, XHX, FW, SPZ, ZJS, and XDT. Investigation and Visualization, KC. Methodology, KC and XDT. Writing – original draft, KC. Project administration, YMY. Writing – review & editing XDT. Supervision, XDT and YMY. All authors read and approved the final version of the manuscript.

Funding

This study was supported by The Natural Science Foundation of China (NO.82171722, 81871954) and Beijing Municipal Natural Science Foundation (NO.7212111).

Acknowledgments

We would like to thank all the patients enrolled at Peking University First Hospital, Qi Wang for statistical analysis.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

1. Ryan DP, Hong TS, Bardeesy N. Pancreatic adenocarcinoma. *N Engl J Med* (2014) 371(11):1039–49. doi: 10.1056/NEJMra1404198

2. Vincent A, Herman J, Schulick R, Hruban RH, Goggins M. Pancreatic cancer. *Lancet* (2011) 378(9791):607–20. doi: 10.1016/S0140-6736(10)62307-0

3. Wang M, Peng B, Liu J, Yin X, Tan Z, Liu R, et al. Practice patterns and perioperative outcomes of laparoscopic pancreaticoduodenectomy in China: A retrospective multicenter analysis of 1029 patients. *Ann Surg* (2021) 273(1):145–53. doi: 10.1097/SLA.0000000000003190

4. Pratt WB, Maithel SK, Vanounou T, Huang ZS, Callery MP, Vollmer CMJr. Clinical and economic validation of the international study group of pancreatic fistula (ISGPF) classification scheme. *Ann Surg* (2007) 245(3):443–51. doi: 10.1097/01.sla.0000251708.70219.d2

5. Callery MP, Pratt WB, Kent TS, Chaikof EL, Vollmer CMJr. A prospectively validated clinical risk score accurately predicts pancreatic fistula after pancreatoduodenectomy. *J Am Coll Surg* (2013) 216(1):1–14. doi: 10.1016/j.jamcollsurg.2012.09.002

6. Tang B, Lin Z, Ma Y, Zhang A, Liu W, Zhang J, et al. A modified alternative fistula risk score (a-FRS) obtained from the computed tomography enhancement pattern of the pancreatic parenchyma predicts pancreatic fistula after pancreatoduodenectomy. *HPB (Oxford)* (2021) 23(11):1759–66. doi: 10.1016/j.hpb.2021.04.015

7. Huttner FJ, Probst P, Knebel P, Strobel O, Hackert T, Ulrich A, et al. Meta-analysis of prophylactic abdominal drainage in pancreatic surgery. *Br J Surg* (2017) 104(6):660–8. doi: 10.1002/bjs.10505

8. Conlon KC, Labow D, Leung D, Smith A, Jarnagin W, Coit DG, et al. Prospective randomized clinical trial of the value of intraperitoneal drainage after pancreatic resection. *Ann Surg* (2001) 234(4):487–93. doi: 10.1097/00000658-200110000-00008

9. Liu X, Chen K, Chu X, Liu G, Yang Y, Tian X. Prophylactic intra-peritoneal drainage after pancreatic resection: An updated meta-analysis. *Front Oncol* (2021) 11:658829. doi: 10.3389/fonc.2021.658829

10. Lyu Y, Cheng Y, Wang B, Zhao S, Chen L. Peritoneal drainage or no drainage after pancreaticoduodenectomy and/or distal pancreatectomy: a meta-analysis and systematic review. *Surg Endosc* (2020) 34(11):4991–5005. doi: 10.1007/s00464-019-07293-w

11. McMillan MT, Fisher WE, Van Buren G2nd, McElhany A, Bloomston M, Hughes SJ, et al. The value of drains as a fistula mitigation strategy for pancreatoduodenectomy: something for everyone? results of a randomized prospective multi-institutional study. *J Gastrointest Surg* (2015) 19(1):21–30. doi: 10.1007/s11605-014-2640-z

12. Van Buren G2nd, Bloomston M, Schmidt CR, Behrman SW, Zyromski NJ, Ball CG, et al. A prospective randomized multicenter trial of distal pancreatectomy with and without routine intraperitoneal drainage. *Ann Surg* (2017) 266(3):421–31. doi: 10.1097/SLA.0000000000002375

13. Witzigmann H, Diener MK, Kienkotter S, Rossion I, Bruckner T, Barbel W, et al. No need for routine drainage after pancreatic head resection: The dual-center, randomized, controlled PANDRA trial (ISRCTN04937707). *Ann Surg* (2016) 264(3):528–37. doi: 10.1097/SLA.0000000000001859

14. Van Buren G2nd, Bloomston M, Hughes SJ, Winter J, Behrman SW, Zyromski NJ, et al. A randomized prospective multicenter trial of pancreaticoduodenectomy with and without routine intraperitoneal drainage. *Ann Surg* (2014) 259(4):605–12. doi: 10.1097/SLA.0000000000000460

15. Bassi C, Molinari E, Malleo G, Crippa S, Butturini G, Salvia R, et al. Early versus late drain removal after standard pancreatic resections: results of a prospective randomized trial. *Ann Surg* (2010) 252(2):207–14. doi: 10.1097/SLA.0b013e3181e61e88

16. Dai M, Liu Q, Xing C, Tian X, Cao F, Tang W, et al. Early drain removal is safe in patients with low or intermediate risk of pancreatic fistula after pancreaticoduodenectomy: A multicenter, randomized controlled trial. *Ann Surg* (2022) 275(2):e307–14. doi: 10.1097/SLA.0000000000004992

17. Dai MH, Liu QF, Xing C, Kleeff J, Liao Q, Guo JC, et al. Early drain removal after major pancreatectomy reduces postoperative complications: A prospective, randomized, single-center trial. *J Pancreatol* (2020) 3(2):93–100. doi: 10.1097/JP9.0000000000000049

18. Beane JD, House MG, Ceppa EP, Dolejs SC, Pitt HA. Variation in drain management after pancreatoduodenectomy: Early versus delayed removal. *Ann Surg* (2019) 269(4):718–24. doi: 10.1097/SLA.0000000000002570

19. Bolliger M, Kroehnert JA, Molineus F, Kandioler D, Schindl M, Riss P, et al. Experiences with the standardized classification of surgical complications (Clavien-dindo) in general surgery patients. *Eur Surg* (2018) 50(6):256–61. doi: 10.1007/s10353-018-0551-z

20. Bassi C, Marchegiani G, Dervenis C, Sarr M, Abu Hilal M, Adham M, et al. The 2016 update of the international study group (ISGPS) definition and grading of postoperative pancreatic fistula: 11 years after. *Surgery* (2017) 161(3):584–91. doi: 10.1016/j.surg.2016.11.014

21. Wente MN, Bassi C, Dervenis C, Fingerhut A, Gouma DJ, Izbicki JR, et al. Delayed gastric emptying (DGE) after pancreatic surgery: a suggested definition by the international study group of pancreatic surgery (ISGPS). *Surgery* (2007) 142(5):761–8. doi: 10.1016/j.surg.2007.05.005

22. Wente MN, Veit JA, Bassi C, Dervenis C, Fingerhut A, Gouma DJ, et al. Postpancreatectomy hemorrhage (PPH): an international study group of pancreatic surgery (ISGPS) definition. *Surgery* (2007) 142(1):20–5. doi: 10.1016/j.surg.2007.02.001

23. Villafane-Ferriol N, Baugh KA, McElhany AL, Van Buren G2nd, Fang A, Tashakori EK, et al. Evidence versus practice in early drain removal after pancreatectomy. *J Surg Res* (2019) 236:332–9. doi: 10.1016/j.jss.2018.11.048

frontiers | Frontiers in Oncology

TYPE Original Research
PUBLISHED 13 October 2022
DOI 10.3389/fonc.2022.1012609

OPEN ACCESS

EDITED BY
Xiaodong Tian,
First Hospital, Peking University, China

REVIEWED BY
Jishu Wei,
Nanjing Medical University, China
Yinmo Yang,
First Hospital, Peking University, China

*CORRESPONDENCE
Yudong Qiu
YudongqiuNJ@163.com
Wei He
hewei@wchscu.cn

†These authors have contributed equally to this work

SPECIALTY SECTION
This article was submitted to Gastrointestinal Cancers: Hepato Pancreatic Biliary Cancers, a section of the journal Frontiers in Oncology

RECEIVED 05 August 2022
ACCEPTED 26 September 2022
PUBLISHED 13 October 2022

CITATION
Cheng H, Yang J, Fu X, Mao L, Chu X, Lu C, Li G, Qiu Y and He W (2022) Folate receptor-positive circulating tumor cells predict survival and recurrence patterns in patients undergoing resection for pancreatic cancer. *Front. Oncol.* 12:1012609. doi: 10.3389/fonc.2022.1012609

Folate receptor-positive circulating tumor cells predict survival and recurrence patterns in patients undergoing resection for pancreatic cancer

Hao Cheng[1†], Jun Yang[2†], Xu Fu[1†], Liang Mao[1], Xuehui Chu[1], Chenglin Lu[1], Gang Li[1], Yudong Qiu[1*] and Wei He[3*]

[1]Department of General Surgery, Nanjing Drum Tower Hospital, The Affiliated Hospital of Nanjing University Medical School, Nanjing, China, [2]Department of Pathology, Nanjing Drum Tower Hospital, The Affiliated Hospital of Nanjing University Medical School, Nanjing, China, [3]Institute of Thoracic Oncology, West China Hospital, Sichuan University, Chengdu, China

Objective: To evaluate the prognostic impact of folate receptor (FR)-positive circulating tumor cells (FR^+ CTCs) for patients with pancreatic cancer (PC).

Background: Risk stratification before surgery for PC patients remains challenging as there are no reliable prognostic markers currently. FR^+ CTCs, detected by ligand-targeted polymerase chain reaction (LT-PCR), have shown excellent diagnostic value for PC in our previous study and prognostic value in a variety of cancer types.

Methods: Peripheral blood samples from 44 consecutive patients diagnosed with PC were analyzed for FR^+ CTCs. 25 patients underwent tumor resection and were assigned to the surgical group. 19 patients failed to undergo radical resection because of local advance or distant metastasis and were assigned to the non-surgical group. The impact of CTCs on relapse and survival were explored.

Results: For the prognostic stratification, the optimal cut-off value of CTCs analyzed by receiver operating characteristic (ROC) curve was 14.49 folate units (FU)/3 ml. High CTC levels (> 14.49 FU/3 ml) were detected in 52.0% (13/25) of the patients in the surgical group and 63.2% (12/19) in the non-surgical group. In the surgical group, median disease-free survival (DFS) for patients with high CTC levels versus low CTC levels (< 14.49 FU/3 ml) was 8.0 versus 26.0 months (P = 0.008). In multivariable analysis, CTCs were an independent risk factor for DFS (HR: 4.589, P = 0.012). Concerning the recurrence patterns, patients with high CTC levels showed a significantly frequent rate of distant and early recurrence (P = 0.017 and P = 0.011). CTC levels remained an independent predictor for both distant (OR: 8.375, P = 0.014) and early recurrence (OR: 8.412, P = 0.013) confirmed by multivariable logistic regression. However, CTCs did not predict survival in the non-surgical group (P = 0.220).

Conclusion: FR^+ CTCs in resected PC patients could predict impaired survival and recurrence patterns after surgery. Preoperative CTC levels detected by LT-PCR may help guide treatment strategies and further studies in a larger cohort are warranted.

KEYWORDS

pancreatic cancer, folate receptor, circulating tumor cells, surgical resection, prognosis, recurrence

Introduction

Pancreatic cancer (PC) is the fourth leading cause of cancer mortality in the United States, with an estimated 62,210 new cases diagnosed in 2022 (1). It is currently predicted to become the second leading cause by 2030 (2). Radical resection, in combination with systemic therapy, remains the only hope of cure or meaningful long-time survival with overall 5-year survival rates as high as 30% for patients with PC (3). Due to a combination of late presentation and early metastasis, 80% of the patients are diagnosed in the advanced stage and only 10%-20% of PC patients can get a curative resection at the time of diagnosis. However, due to a substantial rate of under-staging, around 20% of patients already recure within the first 6 months after surgery, resulting the median disease-free survival (DFS) is just over 12 months (4, 5). Therefore for many of these patients, the survival advantage from curative resection is questionable. The main cause of early relapse is likely occult systemic disease below the detection limit of cross-sectional imaging, although there may be other contributing factors such as postoperative morbidity (6, 7). Neoadjuvant therapy is recently recommended to address the issue of occult systemic disease (8). Currently, selection of potentially resectable patients for surgery remains challenging as there are no methods to stratify a patient's risk for metastasis to help guide neoadjuvant and adjuvant therapies (9). One possible strategy is to identify effective prognostic biomarkers to distinguish patients at high risk of early systemic progression who may benefit from systemic treatment first and patients with favourable prognosis who are more likely to benefit from upfront resection (10).

Circulating tumor cells (CTCs), functioning as the "seeds" of metastasis, are tumor cells that originate from primary tumors, survive in circulating and disseminate to colonize distant sites through invading adjacent vasculature (11, 12). Involvement of CTCs in the metastatic process has been identified in the majority of solid tumors (13–15). Accumulating evidence has demonstrated that, as a non-invasive assessment of tumor biology, CTCs are a readily available biomarker for predicting survival in colorectal, breast, and prostate cancers (16–18). In patients with potentially resectable PC, the lack of reliable biomarkers to guide surgical or neoadjuvant treatment decisions also motivated the present studies to investigate the prognostic impact of preoperative CTCs analysis (19–22). Thus far, the CellSearch System remains the first and only CTC detection platform approved by Food and Drug Administration (FDA), although detection, enumeration, and isolation of CTCs have been facilitated by recent technological advancements in a sensitive and reproducible manner for clinical and research applications. It utilizes immunomagnetic separation for isolation of CTCs, and then quantitative evaluation of CTCs is analyzed by capturing epithelial cell adhesion molecule (EpCAM), an epithelial cell marker (23). CellSearch is also the most commonly used method to examine CTCs as a prognostic marker in PC patients in a number of studies, however, across various stages of PC, it has performed with relatively poor detection rates of 7–48% (24–26). In addition, in a study evaluating the efficiency of the CellSearch System, CTC detection rate and counts have been found to be lowest in PC among different types of metastatic cancers (27). Therefore, diverse techniques and technologies have been developed for enrichment, isolation, and identification of CTCs from peripheral blood samples in PC patients in recent years.

Folate receptors (FRs), cysteine-rich cell-surface glycoproteins, are highly expressed in a variety of cancers, including PC (28–31). Our previous study has shown promising clinical value of detecting FR-positive (FR^+) CTCs by a novel ligand-targeted polymerase chain reaction (LT-PCR) method in patients with PC (32). Although FR^+ CTCs have been demonstrated to be useful in the diagnosis of PC, their application in predicting prognosis requires further clarification. Therefore, the purpose of this study was to investigate the prognostic impact of pretreatment FR^+ CTCs analysis in patients with PC.

Patients and methods

Patients, study design, and clinical data collection

Between September 2018 to December 2019, 50 consecutive patients with suspected PC treated at our hospital were enrolled into this observational study. The treatment strategies for all patients were discussed and determined by the multidisciplinary

team of pancreatic disease, independent of the results of CTCs analysis. Exclusion criteria were (1): a history of any other malignancy or any anticancer therapies in the last 10 years (2); failure to adhere to standard surgical procedures in the surgical group (3); other than PC confirmed by final histopathologic observations. Diagnosis was confirmed from the specimens obtained by resection or biopsy. A database of demographic, laboratory, and relevant clinicopathologic variables, including preoperative carbohydrate antigen 19-9 (CA19-9), age, gender, location of primary tumor, tumor differentiation, tumor size, tumor stages et al, was prospectively maintained. Disease stages - tumor, node, and metastasis (TNM) staging, were based on the eighth edition of the American Joint Committee on Cancer (AJCC) manual. A distance from the tumor to the resection margin≥1 mm was defined as R0.

Standard pancreaticoduodenectomy (PD), distal pancreatectomy (DP) or other procedures were performed in patients who were selected for radical surgery in accordance with the tumor location and extension. In addition, patients were followed with a standard postoperative protocol, with routine postoperative clinical status and CA19-9 assessment every 3 months and contrast-enhanced computerized tomography (CT) or magnetic resonance imaging (MRI) scan every 6 months. If necessary, positron emission tomography (PET) was conducted to evaluate recurrence. Early recurrence was defined as within 12 months of surgery, as described in previous studies (5, 33). Locoregional recurrence was defined as recurrent disease along the superior mesenteric artery (SMA)/superior mesenteric vein (SMV), celiac axis, or porta hepatis and in the pancreatic bed, retroperitoneum, or remnant pancreas through radiographic or pathological evidence. Distant recurrence was tumor spread outside of the locoregional area (liver, lungs, peritoneum and extra-regional lymph nodes) (34). All procedures were in accordance with the ethical standards of the Helsinki Declaration. The study was approved by the Ethics Committee of Nanjing Drum Tower Hospital (No. 2020-079-01), and informed written consent was obtained from all subjects before the study.

CTC detection

Before commencing treatment, peripheral venous blood samples (3 ml) from every patient were collected in vacuum tubes containing the anticoagulant ethylenediaminetetraacetic acid for CTCs analysis. For patients with neoadjuvant treatment followed by curative-intent pancreatectomy, CTC detection was performed before surgery. A commercially available CTC detection kit, CytoploRare Kit, invented by Geno Biotech and approved by the China FDA, was used for isolation, enrichment and enumeration of FR^+ CTCs, as described and detailed previously (32). All blood specimens were stored at 4°C and processed within 24 hours of blood withdrawal. The experimental procedure was performed strictly according to the manufacturer's protocol. Briefly, the isolation and enrichment of CTCs was initially achieved by lysing erythrocytes, followed by immunomagnetic depletion of leukocytes from the whole blood. Then, LT-PCR was used for quantitative analysis of the FR^+ CTCs in each blood sample. Finally, the level of FR^+ CTCs in each sample was calculated on the basis of a calibration curve generated with the standard reference materials provided in the kit. FR^+ CTC levels were measured in folate units (FU)/3 ml of blood.

Statistical analysis

Continuous variables were presented as medians (ranges) and categorical variables were summarized as number (percentage). Statistical analysis for these two types of variable was examined using Student's t test (or Mann-Whitney U test) and Fisher's exact test, respectively. Optimal cut-off value of CTCs for recurrence prediction was analyzed by receiver operating characteristic (ROC) curve and calculated with Youden Index. Survival analyses were carried out with the Kaplan-Meier method, using Log-rank test for difference of curve pairs. Overall survival (OS) was defined as the time from date of diagnosis or resection to either death by any cause or censored at last follow-up. DFS was defined as the time interval between date of operation and date of tumor relapse showed by radiological or clinical evidence. To assess the independent influence of CTCs and other covariates on tumor recurrence, univariable and multivariable Cox Proportional Hazard regression model analyses were performed. Multivariable analyses for distant and early recurrence were calculated with a logistic regression model. Variables with a P value < 0.2 in univariable analyses and clinically relevant variables were included in multivariable analyses. Data were analyzed using IBM SPSS, v.25 (IBM Corp., Armonk, NY) and graphs were prepared using PRISM 8 (GraphPad Software, Inc, La Jolla, CA). A P value < 0.05 was considered statistically significant.

Results

Patient characteristics

Of the 50 patients assessed during the study period, 6 patients (12.0%) did not meet the inclusion criteria. The Consort diagram showing the stratification of the 44 eligible cases is presented in Figure 1. Tumor resection with curative intent was performed in 56.8% of the patients (25/44) (surgical group), while 43.2% (19/44) were not resected due to local advance or distant metastasis (non-surgical group) (Table 1). The median age of these enrolled patients was both 64 years in the two groups and 60.0% (15/25) were men in the surgical group, while 57.9% (11/19) in the non-

surgical group. In the surgical group, 60.0% of the tumors were located in the head, and according to the histopathologic type, most were pancreatic ductal adenocarcinoma (PDAC), and only two were malignant intraductal papillary mucinous neoplasm (IPMN). R0 resection was achieved in 56.0% (14/25) of the patients and 80.0% (20/25) received adjuvant treatment. 3 patients received neoadjuvant chemotherapy preoperatively according to the decision taken on pancreatic multidisciplinary and the regimen was gemcitabine with albumin-bound paclitaxel for all patients. In the non-surgical group, 73.7% (14/19) of the patients received systemic treatment.

CTC levels in the patient cohort

For the prognostic stratification, ROC curve analysis showed that the area under the ROC curve (AUROC) was 0.883 ($P = 0.003$), with 14.49 FU/3 ml as the optimal cut-off value of CTCs and the Youden Index was 0.727 (Figure 2). The CTC levels of patients in the surgical group (median 14.92 FU/3 ml, range 5.61 to 26.98 FU/3 ml) were slightly lower than those of patients in the non-surgical group (median 16.76 FU/3 ml, range 5.49 to 41.22 FU/3 ml), although the difference was not significant ($P = 0.147$). In the surgical group, high CTC levels (> 14.49 FU/3 ml) were detected in 52.0% (13/25) of the patients and in the non-surgical group, 12 patients had high CTC levels. We also compared the clinicopathological characteristics of patients with high and low CTC levels, and the results are shown in Table 2. In the surgical group and non-surgical group, there were both no significant difference of clinicopathological characteristics between the two subgroups.

CTC detection and survival

In the surgical group, the median observation time was 20.0 months (range 6.0 to 28.0). Median DFS was 10.0 months for all patients (Figure 3A). Patients with high CTC levels had a significantly shorter DFS (median 8.0 vs. 26.0 months, $P = 0.008$; Figure 3B) compared with patients with low CTC levels. The univariable survival analyses of DFS for CTC levels (HR 3.735; 95% CI: 1.263–11.049; $P = 0.017$) and other risk factors are presented in Table 3. Of those, age also affected the prognosis. After inclusion of these confounding factors as well as other known risk factors (R, tumor stage) in multivariable analysis, CTC levels remained a significant prognostic factor (HR 4.589; 95% CI: 1.404–14.997; $P = 0.012$; Table 3).

In the non-surgical group, the median observation time was 13.0 months (range 2.0 to 21.0). Median OS was 11.0 months for

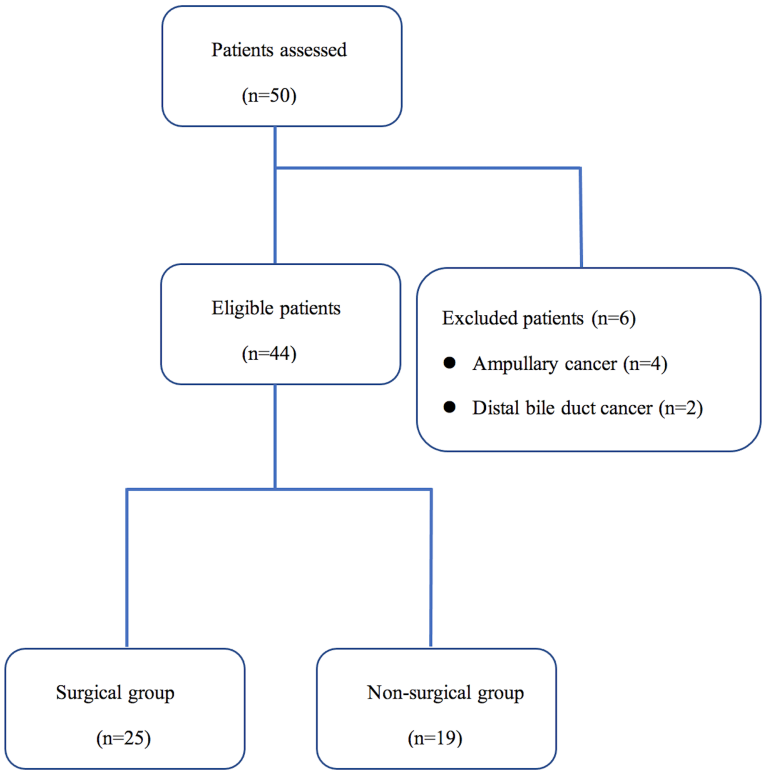


FIGURE 1
Flowchart of study cohort into 2 groups with different treatment strategies.

TABLE 1 Demographics, clinicopathologic, and treatment characteristics of included patients.

Characteristics	Surgical group (n=25)	Non-surgical group (n=19)
Age, years median (range)	64 (45–87)	64 (42-79)
Sex, male n (%)	15 (60.0)	11 (57.9)
Preoperative CA19-9 level U/ml, n (%)		
≥ 200	9 (36.0)	12 (63.2)
< 200	16 (64.0)	7 (36.8)
Operation type, n (%)		
PD	14 (56.0)	BDB 9 (47.4)
DP	10 (40.0)	EUS-FNA 10 (52.6)
TP	1 (4.0)	
Venous resection, n (%)	3 (12.0)	n.a.
Histopathologic type, n (%)		
PDAC	23 (92.0)	19 (100.0)
Malignant IPMN	2 (8.0)	0 (0.0)
Tumor size, mm median (range)	30 (12-95)	n.a.
Tumor location, n (%)		
Head	15 (60.0)	11 (57.9)
Body or tail	10 (40.0)	8 (42.1)
Tumor stage, n (%)		
I	9 (36.0)	0 (0.0)
II	11 (44.0)	0 (0.0)
III	5 (20.0)	11 (57.9)
IV	0 (0.0)	8 (42.1)
Tumor differentiation, n (%)		
Well	4 (16.0)	3 (15.8)
Moderate	10 (40.0)	5 (26.3)
Poor	11 (44.0)	1 (5.3)
Not specified	0 (0.0)	10 (52.6)
Resection margin, n (%)		
R0	14 (56.0)	n.a.
R1	11 (44.0)	n.a.
Adjuvant treatment/Systemic treatment, n (%)		
Yes	20 (80.0)	14 (73.7)
No	5 (20.0)	5 (26.3)
Neoadjuvant treatment, n (%)		
Yes	3 (12.0)	n.a.
No	22 (88.0)	n.a.

BDB, biliodigestive bypass; CA19-9, carbohydrate antigen 19-9; DP, distal pancreatectomy; EUS-FNA, endoscopic ultrasound guided fine needle aspiration; IPMN, intraductal papillary mucinous neoplasm; n.a, not applicable; PD, pancreaticoduodenectomy; PDAC, pancreatic ductal adenocarcinoma; TP, Total pancreatectomy.

all patients (Figure 4A). Median OS for patients with high CTC levels was 8.5 months and 17.0 months for patients with low CTC levels, but the difference was not statistically significant (P = 0.220; Figure 4B).

Patterns of recurrence according to the CTC levels in the surgical group

We compared the presence or absence of recurrence in accordance with CTC levels, and we found that recurrence was significantly higher in the patients with high CTC levels (12/13, 92.3%) compared with patients with low CTC levels (6/12, 50.0%) (P = 0.030). Then the recurrence site and time of emergence were further analyzed (Table 4 and Figure 5). Distant recurrence was significantly higher in the patients with high CTC levels as compared with patients with low CTC levels (76.9% vs. 25.0%, P = 0.017). In the patients with low CTC levels, 5 out of 12 (41.7%) instances of recurrence were within 12 months, but nearly all recurrences (12/13, 92.3%) in the patients with high CTC levels occurred within 12 months (P = 0.011). Finally, we confirmed using multivariable logistic regression analysis that CTCs are an

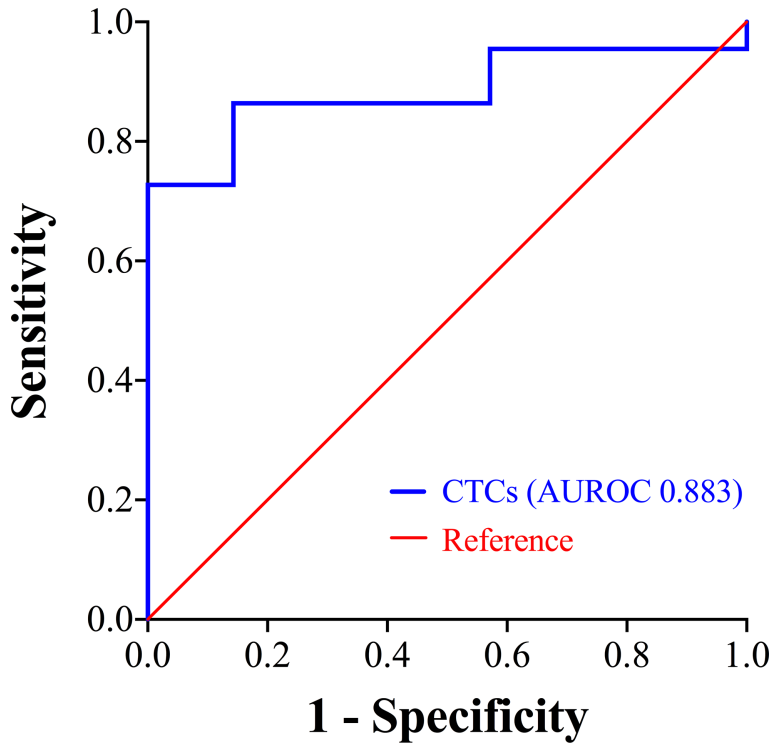


FIGURE 2
ROC curve showing the prognostic stratification performance of CTCs. CTCs, circulating tumor cells; ROC, receiver operating characteristic; AUROC, area under the ROC curve.

independent risk factor for both distant (OR 8.375; 95% CI 1.915–28.222; $P = 0.014$) and early recurrence (OR 8.412; 95% CI 2.342–27.234; $P = 0.013$) (Table 5).

Based on the results, we summarized the relationship between preoperative CTC levels and postoperative patterns of recurrence. If the CTC levels were high (> 14.49 FU/3 ml) before surgery, it tended to show patterns of early and distant recurrence.

Discussion

This is the first report to demonstrate the clinical significance of FR^+ CTCs detected by LT-PCR for predicting the survival in patients with PC. The effect of high CTC levels in the present cohort was detrimental. In patients with resected PC, we demonstrated a strong association between the CTC levels and reduced DFS and in the patterns of recurrence, CTCs were also associated with early and distant recurrence.

As a "liquid biopsy", the utilization of CTCs to assess the tumor biology and guide treatment decisions has developed into an emerging field of study (35). Especially in PC, the current lack of individualized up-front treatment stratification by preoperative risk assessment is a major hindrance for improved treatment results of patients with presumed resectable PC and CTCs have been considered as a very useful biomarker to establish the appropriate therapeutic protocol (22). However, there is a broad heterogeneity in the CTC detection platforms to date and as the "gold standard" technique, the detection rate of the CellSearch system is too low to limit its clinical application (36). In recent years, although the presence of sensitive and reproducible platforms for the isolation, enrichment and detection of CTCs from peripheral blood, methodological standardization for such technologies is still required to have a significant value on clinical care (37). As FRs are also highly expressed in PC, our previous study has shown that FR^+ CTCs have potential as a biomarker for the diagnosis of PC and LT-PCR is feasible and reliable for detecting FR^+ CTCs in patients with PC (32). In addition, FR^+ CTCs have been shown to serve as prognostic markers in several cancer types, including gastric, breast and lung cancers (38–40). In a prospective cohort study including 132 gastric cancer patients, combined model including FR^+ CTC level and other biomarkers (CA19-9, prealbumin and peripheral lymphocyte count) presented high sensitivity (100%) and moderate specificity (59.3%) in predicting peritoneal metastasis, the preoperative FR^+ CTC level could also predict short-term recurrence after surgery (38). In another prospective study to investigate the prognostic and predictive significance of FR^+ CTC in non-small cell lung cancer patients who underwent surgery, patients with lower preoperative CTC level had longer relapse-free

TABLE 2 Comparison of the clinicopathological characteristics of patients with high and low CTC levels in different groups.

Characteristics	Surgical group (n=25)			Non-surgical group (n=19)		
	High CTC levels (n=13)	Low CTC levels (n=12)	*P*	High CTC levels (n=12)	Low CTC levels (n=7)	*P*
Age, years median (range)	65 (55-81)	64 (45-87)	0.970	63.5 (42-79)	63 (58-77)	0.590
Sex, male n (%)	8 (61.5)	7 (58.3)	1.000	6 (50.0)	5 (71.4)	0.633
Preoperative CA19-9 level U/ml, n (%)			0.688			1.000
≥ 200	4 (30.8)	5 (41.7)		8 (66.7)	4 (57.1)	
< 200	9 (69.2)	7 (58.3)		4 (33.3)	3 (42.9)	
Tumor size, mm median (range)	27.0 (12-95)	37.5 (20-61)	0.164	n.a.		
Tumor location, n (%)			0.111			0.960
Head	10 (76.9)	5 (41.7)		7 (58.3)	4 (57.1)	
Body or tail	3 (23.1)	7 (58.3)		5 (41.7)	3 (42.9)	
Tumor stage, n (%)			0.645			
I+II	11 (84.6)	9 (75.0)		0 (0.0)	0 (0.0)	
III+IV	2 (15.4))	3 (25.0)		12 (100.0)	7 (100.0)	
Tumor differentiation, n (%)			0.428			0.342
Well+Moderate	6 (46.2)	8 (66.7)		6 (50.0)	2 (28.6)	
Poor	7 (53.8)	4 (33.3)		1 (8.3)	0 (0.0)	
Not specified	0 (0.0)	0 (0.0)		5 (41.7)	5 (71.4)	
Resection margin, n (%)			1.000			
R0	7 (53.8)	7 (58.3)		n.a.		
R1	6 (46.2)	5 (41.7)		n.a.		
Adjuvant treatment/Systemic treatment, n (%)			0.645			0.603
Yes	11 (84.6)	9 (75.0)		8 (66.7)	6 (85.7)	
No	2 (15.4)	3 (25.0)		4 (33.3)	1 (14.3)	

CA19-9, carbohydrate antigen 19-9; n.a, not applicable.

survival (RFS) and OS, CTC level (HR = 4.10) and pathological stage (HR = 3.16) were independent prognostic factors of RFS (40). Accordingly, in the present analysis, we gave insight into the association between pretreatment CTC levels and patient survival.

The frequency of CTC-detection varies due to the detection methods and the disease status of the patients (19–22). However, in our current study, with 14.49 FU/3 ml as the optimal cut-off value of CTCs, the detection rate of high CTC levels was 52.0% in the patients with resectable PC and 63.2% in the patients with advanced stage cancer. Compared to previous studies, especially the studies in which Cellsearch was used as the detection method, our study showed a higher detection rate in both

TABLE 3 Uni- and multivariable analyses of prognostic factors of DFS for patients in the surgical group.

Variable	Univariable		Multivariable	
	HR (95% CI)	*P*	HR (95% CI)	*P*
Sex, female vs. male	0.530 (0.185-1.514)	0.236		
Age, years	**1.038 (0.992-1.086)**	**0.110**	1.054 (0.999-1.113)	0.054
CA19-9, U/ml	1.001 (0.999-1.002)	0.342		
Tumor site, Body/tail vs. Head	0.814 (0.298-2.223)	0.688		
Neoadjuvant treatment, Yes vs. No	0.389 (0.052-2.940)	0.360		
Adjuvant treatment, Yes vs. No	0.850 (0.276-2.616)	0.777		
R, R1vs. R0	1.074 (0.414-2.787)	0.883	0.733 (0.252-2.128)	0.568
CTC levels, High vs. Low	**3.735 (1.263-11.049)**	**0.017**	**4.589 (1.404-14.997)**	**0.012**
Tumor stage	1.302 (0.652-2.602)	0.454	1.867 (0.922-3.781)	0.083

Age and CA19-9 were calculated as continuous variables. CTC, circulating tumor cell; CA19-9, carbohydrate antigen 19-9; CI, Confidence interval; DFS, disease-free survival; HR, Hazard ratio. Shown in bold are univariable associations (P < 0.2) that were selected for multivariable analysis and significant risk factors (P < 0.05) on multivariable analysis.

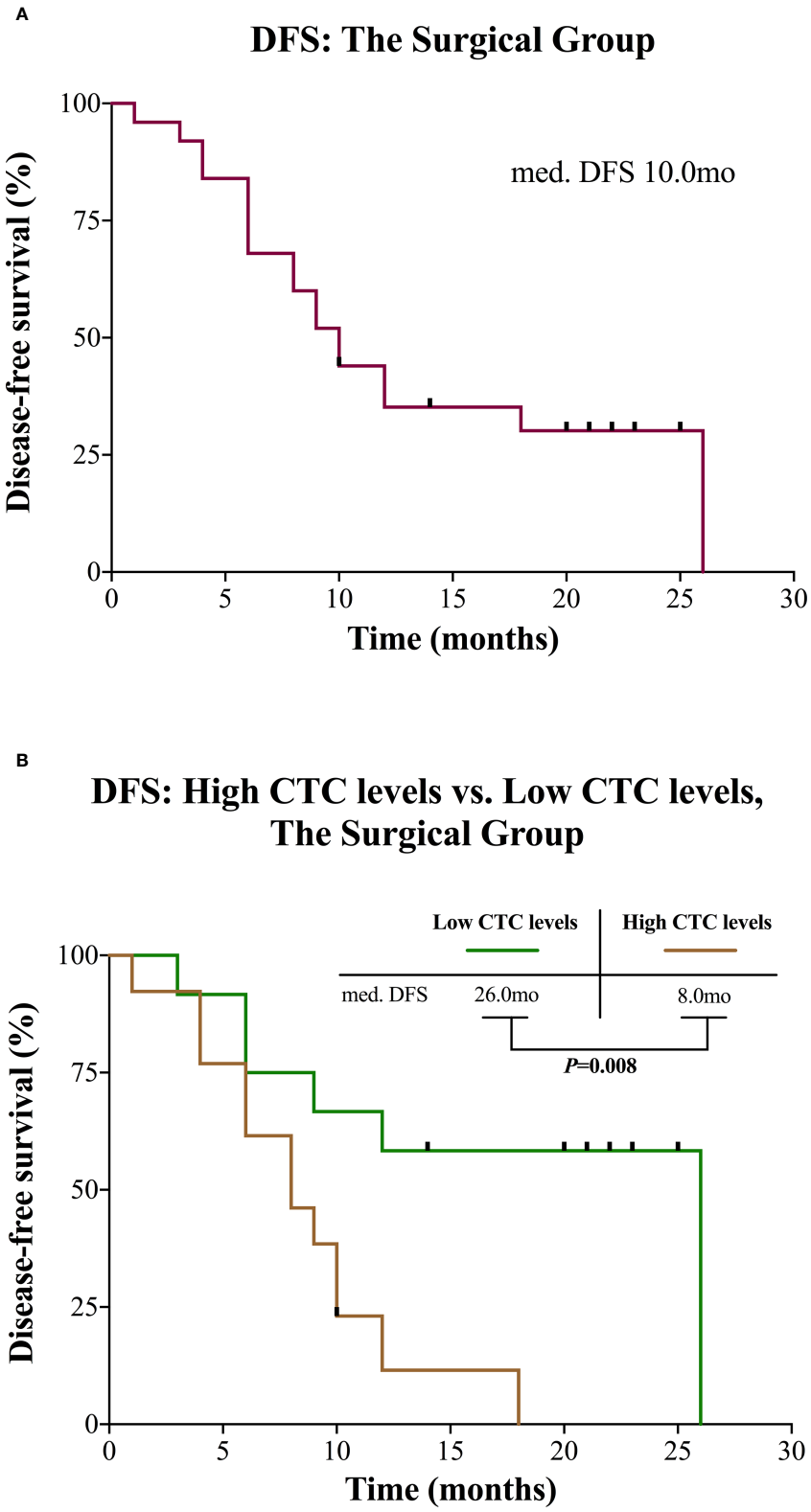


FIGURE 3
Kaplan-Meier curves for patients in the surgical group. **(A)** DFS for the entire group. **(B)** DFS of the surgical group divided into high and low CTC levels. CTC, circulating tumor cell; DFS, disease-free survival; mo, months; med., median.

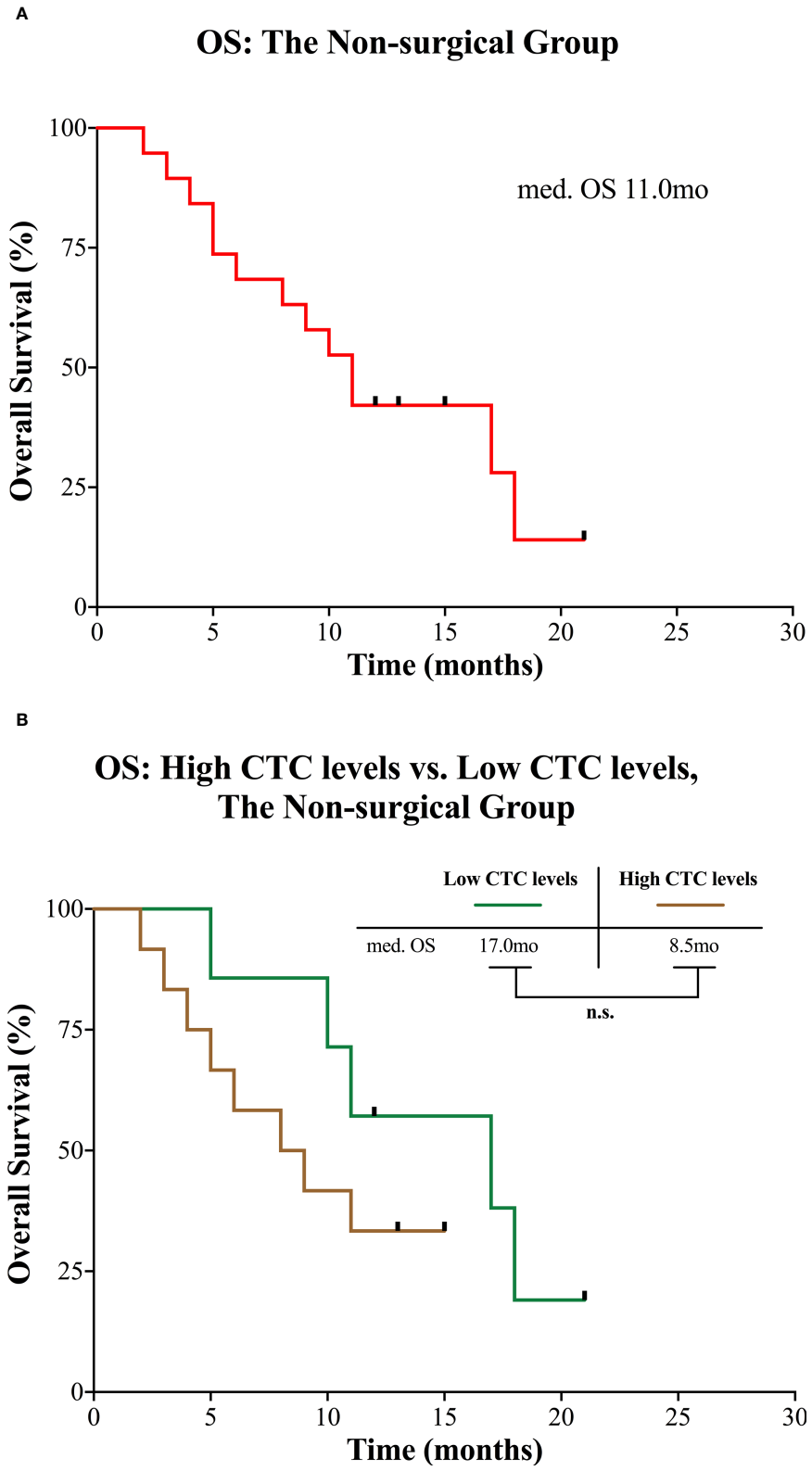


FIGURE 4
Kaplan-Meier curves for patients in the non-surgical group. **(A)** OS for the entire group. **(B)** OS of the non-surgical group divided into high and low CTC levels. CTC, circulating tumor cell; mo, months; med., median; n.s., not significant; OS, overall survival.

TABLE 4 Subanalysis of the recurrence patterns according to the CTC levels.

Variable	Total number (%) or median	High CTC levels (n=13)	Low CTC levels (n=12)	*P*
Recurrence				**0.030**
No	7 (28.0%)	1 (7.7%)	6 (50.0%)	
Yes	18 (72.0%)	12 (92.3%)	6 (50.0%)	
Recurrence site				
Distant	13 (52.0%)	10 (76.9%)	3 (25.0%)	**0.017**
Locoregional	5 (20.0%)	2 (15.4%)	3 (25.0%)	
Recurrence time				
≤ 12 months	17 (68.0%)	12 (92.3%)	5 (41.7%)	**0.011**
> 12 months or recurrence (-)	8 (32.0%)	1 (7.7%)	7 (58.3%)	
Follow-up duration (months)				
Median	20.0	15.0	24.0	0.050
Range	6.0-28.0	10.0-21.0	6.0-28.0	

CTC, circulating tumor cell.
Bold values was used for P values < 0.05.

early and advanced stage cancer. Consequently, these results revealed again that LT-PCR is feasible and reliable for detecting FR^+ CTCs in PC patients.

In our cohort, 43.2% of the patients were assigned to the non-surgical group due to local advance or distant metastasis of tumor. In this group, median OS was 11.0 months and there was no significant difference in OS between the patients with high and low CTC levels. These results are consistent with other reports utilizing the different methods (41, 42). However, in our study, we have secured meaningful results for the difference between the two groups (8.5 months vs. 17.0 months) and larger-scale studies are warranted in the future.

In the current study, 56.8% of the patients were assigned to the surgical group and tumor resection with curative intent was performed. Median DFS was 10.0 months for all patients, which was similar to other studies with larger number of patients (5, 34). More importantly, high CTC levels predicted impaired DFS following potentially curative surgery and patients with low CTC levels could have a DFS as long as 26.0 months, which was an amazing result. However, this may be related to the limitations of our study with small number of cases and short duration for follow-up, and only 50.0% of the patients with low CTC levels during the observation time recured. Moreover, multivariable analysis indicated that CTCs are an independent risk factor for DFS. Concerning OS in the surgical group, also due to the short follow-up, we did not analyze the OS.

Although there are abundant studies on the association between CTCs and survival, we are aware of only few reports

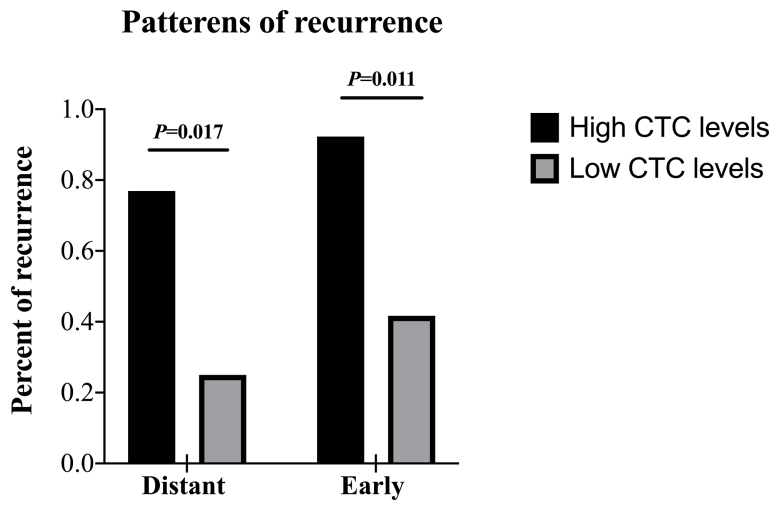


FIGURE 5
Patterns of recurrence of patients in the surgical group according to the CTC levels. CTC, circulating tumor cell.

TABLE 5 Uni- and multivariable logistic regression analysis for risk factors in distant and early recurrence.

Variable	Distant recurrence			
	Univariable		Multivariable	
	OR (95% CI)	*P*	OR (95% CI)	*P*
Sex, female vs. male	0.444 (0.087-2.276)	0.330		
Age, years	0.994 (0.915-1.081)	0.896		
CA19-9, U/ml	1.000 (0.997-1.003)	0.957		
Tumor site, Body/tail vs. Head	0.875 (0.176-4.341)	0.870		
Neoadjuvant treatment, Yes vs. No	0.417 (0.033-5.299)	0.500		
Adjuvant treatment, Yes vs. No	0.667 (0.091-4.889)	0.690		
R, R1vs. R0	**0.317 (0.061-1.644)**	**0.171**	0.121 (0.011-1.370)	0.088
CTC levels, High vs. Low	**5.000 (1.594-26.732)**	**0.014**	**8.375 (1.915-28.222)**	**0.014**
Tumor stage	1.024 (0.350-2.997)	0.965	2.058 (0.445-9.525)	0.356
Variable	**Early recurrence**			
	Univariable		Multivariable	
	OR (95% CI)	*P*	OR (95% CI)	*P*
Sex, female vs. male	0.545 (0.099-3.004)	0.486		
Age, years	**1.141 (0.987-1.319)**	**0.074**	1.224 (0.995-1.504)	0.055
CA199, U/ml	1.001 (0.998-1.004)	0.600		
Tumor site, Body/tail vs. Head	0.545 (0.099-3.004)	0.486		
Neoadjuvant treatment, Yes vs. No	0.933 (0.072-12.015)	0.958		
Adjuvant treatment, Yes vs. No	0.464 (0.043-4.997)	0.527		
R, R1vs. R0	1.481 (0.265-8.267)	0.654	1.681 (0.128-22.061)	0.692
CTC levels, High vs. Low	**6.800 (1.617-24.519)**	**0.018**	**8.412 (2.342-27.234)**	**0.013**
Tumor stage	0.908 (0.288-2.861)	0.870	3.124 (0.361-27.002)	0.301

Age and CA19-9 were calculated as continuous variables. CTC, circulating tumor cell; CA19-9, carbohydrate antigen 19-9; CI, Confidence interval.
Shown in bold are univariable associations (P < 0.2) that were selected for multivariable analysis and significant risk factors (P < 0.05) on multivariable analysis.

of an association between CTCs from peripheral blood and recurrence rate or pattern (22, 43). Therefore, in the present analysis, we particularly analyzed whether CTC levels could be used as an indicator of early recurrence and recurrence patterns among these patients. In this cohort, the recurrence was significantly higher in the patients with high CTC levels compared with patients with low CTC levels. Meanwhile, in patients with high CTC levels, early recurrence (i.e. within 12 months post-operatively) and distant recurrence were significantly frequent. Moreover, multivariable logistic regression analysis indicated that CTCs are a risk factor for both early and distant recurrence.

Noteworthy, in the present study, the CTC levels were the only independent predictor of DFS and recurrence patterns although other clinically relevant factors such as preoperative CA19-9 level, R status, neoadjuvant and adjuvant therapy, tumor stage were included in the analyses. However, in some previous studies, including large-scale and multicenter clinical trials, CA19-9 level, R status, neoadjuvant and adjuvant therapy were all significantly associated with survival and recurrence (44–47). The discrepancy may be mainly explained by the small sample size and the selection bias of patients in our study. Another alternate explanation may be that we did not analyze the surgical margins separately as different surgical margins could have different prognostic roles (48).

Our study showed that with the currently available techniques for CTC-detection and treatment modalities in PC, to support surgical treatment decisions probably would be a promising use of CTC-analysis. Similar to CTCs, circulating tumor DNA (ctDNA) also has the potential to be a preoperative prognostic tool for the stratification of patients with resectable PC (49–51). Although ctDNA was more abundant and easier to detect than CTCs in comparative studies, the prognostic impact of different mutational signatures in ctDNA was not fully resolved (52–54). In a recent meta-analysis, ctDNA was detected in 8.3–68.6% of patients with resectable PDAC preoperatively and was associated with lower RFS and OS (49). Most probably, future improvements in systemic treatment are dependent upon identification of the core molecular characteristics or driver mutations of the cancer cells.

We acknowledge that our study has several important limitations. First, the number of patients is relatively small

and the follow-up time is relatively short, which limit the generalization of the results reported. In addition, FR^+ CTCs can not predict survival in the non-surgical group, indicating that this prognostic biomarker has less utility in patients with advanced disease, which might mainly due to the universally poor prognosis of this patient population. Moreover, the present study design did not include the CTC detection at multiple time points to observe how CTC dynamics predict outcome with treatment, however, our study does provided evidence on the utility of FR^+ CTCs as a biomarker at a time when key treatment decisions are made. Finally, the recurrences of some patients were identified based on radiological evidence, without pathological verification, which may include potential for some provider variability regarding postoperative imaging.

In conclusion, this small-scale, exploratory clinical trial revealed that FR^+ CTCs detected by LT-PCR predict shorter DFS and are associated with early and distant recurrence in patients with resectable PC. Our results indicate that FR^+ CTCs could be a promising tool to individualize treatment planning and to improve outcomes in PC. Further studies to investigate the prognostic value of CTCs detected by LT-PCR in a larger cohort are warranted.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding authors.

Ethics statement

The studies involving human participants were reviewed and approved by Ethics Committee of Nanjing Drum Tower Hospital. The patients/participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

Author contributions

HC, JY, and XF: study design, data acquisition, data analysis, data interpretation; HC: drafting of the manuscript; LM, XC, CL, and GL: data acquisition; YQ and WH: study concept, study design and study supervision; YQ: critical revision of the manuscript for important intellectual content; WH: contribution of CTC detection method and reagents. All authors contributed to the article and approved the submitted version.

Funding

This study was supported by National Natural Science Foundation of China (NSFC) (No. 31971518).

Acknowledgments

The authors thank all members of the multidisciplinary team treating pancreatic tumors at Nanjing Drum Tower Hospital for their guidance in this study. We also appreciate the technical support of Yidu Cloud (Beijing) Technology Co. Ltd., China, in extracting data.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

1. Siegel RL, Miller KD, Fuchs HE, Jemal A. Cancer statistics, 2022. *CA: Cancer J Clin* (2022) 72:7–33. doi: 10.3322/caac.21708

2. Rahib L, Smith BD, Aizenberg R, Rosenzweig AB, Fleshman JM, Matrisian LM. Projecting cancer incidence and deaths to 2030: the unexpected burden of thyroid, liver, and pancreas cancers in the united states. *Cancer Res* (2014) 74:2913–21. doi: 10.1158/0008-5472.Can-14-0155

3. Strobel O, Neoptolemos J, Jäger D, Büchler MW. Optimizing the outcomes of pancreatic cancer surgery. *Nat Rev Clin Oncol* (2019) 16:11–26. doi: 10.1038/s41571-018-0112-1

4. Hugenschmidt H, Labori KJ, Brunborg C, Verbeke CS, Seeberg LT, Schirmer CB, et al. Circulating tumor cells are an independent predictor of shorter survival in patients undergoing resection for pancreatic and periampullary adenocarcinoma. *Ann Surg* (2020) 271:549–58. doi: 10.1097/sla.0000000000003035

5. Groot VP, Gemenetzis G, Blair AB, Rivero-Soto RJ, Yu J, Javed AA, et al. Defining and predicting early recurrence in 957 patients with resected pancreatic ductal adenocarcinoma. *Ann Surg* (2019) 269:1154–62. doi: 10.1097/sla.0000000000002734

6. Court CM, Ankeny JS, Sho S, Winograd P, Hou S, Song M, et al. Circulating tumor cells predict occult metastatic disease and prognosis in pancreatic cancer. *Ann Surg Oncol* (2018) 25:1000–8. doi: 10.1245/s10434-017-6290-8

7. Labori KJ, Katz MH, Tzeng CW, Bjørnbeth BA, Cvancarova M, Edwin B, et al. Impact of early disease progression and surgical complications on adjuvant chemotherapy completion rates and survival in patients undergoing the surgery first approach for resectable pancreatic ductal adenocarcinoma - a population-based cohort study. *Acta Oncol* (2016) 55:265–77. doi: 10.3109/0284186x.2015.1068445

8. Versteijne E, Vogel JA, Besselink MG, Busch ORC, Wilmink JW, Daams JG, et al. Meta-analysis comparing upfront surgery with neoadjuvant treatment in patients with resectable or borderline resectable pancreatic cancer. *Br J Surg* (2018) 105:946–58. doi: 10.1002/bjs.10870

9. Poruk KE, Valero V, Saunders T, Blackford AL, Griffin JF, Poling J, et al. Circulating tumor cell phenotype predicts recurrence and survival in pancreatic adenocarcinoma. *Ann Surg* (2016) 264:1073–81. doi: 10.1097/sla.0000000000001600

10. Hank T, Hinz U, Reiner T, Malleo G, Konig AK, Maggino L, et al. A pretreatment prognostic score to stratify survival in pancreatic cancer. *Ann Surg* (2021). doi: 10.1097/SLA.0000000000004845

11. Chaffer CL, Weinberg RA. A perspective on cancer cell metastasis. *Science* (2011) 331:1559–64. doi: 10.1126/science.1203543

12. Massagué J, Obenauf AC. Metastatic colonization by circulating tumour cells. *Nature* (2016) 529:298–306. doi: 10.1038/nature17038

13. Yu M, Bardia A, Wittner BS, Stott SL, Smas ME, Ting DT, et al. Circulating breast tumor cells exhibit dynamic changes in epithelial and mesenchymal composition. *Science* (2013) 339:580–4. doi: 10.1126/science.1228522

14. Connor AA, McNamara K, Al-Sukhni E, Diskin J, Chan D, Ash C, et al. Central, but not peripheral, circulating tumor cells are prognostic in patients undergoing resection of colorectal cancer liver metastases. *Ann Surg Oncol* (2016) 23:2168–75. doi: 10.1245/s10434-015-5038-6

15. Carter L, Rothwell DG, Mesquita B, Smowton C, Leong HS, Fernandez-Gutierrez F, et al. Molecular analysis of circulating tumor cells identifies distinct copy-number profiles in patients with chemosensitive and chemorefractory small-cell lung cancer. *Nat Med* (2017) 23:114–9. doi: 10.1038/nm.4239

16. Bidard FC, Peeters DJ, Fehm T, Nolé F, Gisbert-Criado R, Mavroudis D, et al. Clinical validity of circulating tumour cells in patients with metastatic breast cancer: a pooled analysis of individual patient data. *Lancet Oncol* (2014) 15:406–14. doi: 10.1016/s1470-2045(14)70069-5

17. Salvianti F, Gelmini S, Mancini I, Pazzagli M, Pillozzi S, Giommoni E, et al. Circulating tumour cells and cell-free DNA as a prognostic factor in metastatic colorectal cancer: the OMITERC prospective study. *Br J Cancer* (2021) 125:94–100. doi: 10.1038/s41416-021-01399-6

18. Antonarakis ES, Lu C, Luber B, Wang H, Chen Y, Zhu Y, et al. Clinical significance of androgen receptor splice variant-7 mRNA detection in circulating tumor cells of men with metastatic castration-resistant prostate cancer treated with first- and second-line abiraterone and enzalutamide. *J Clin Oncol* (2017) 35:2149–56. doi: 10.1200/jco.2016.70.1961

19. Poruk KE, Blackford AL, Weiss MJ, Cameron JL, He J, Goggins M, et al. Circulating tumor cells expressing markers of tumor-initiating cells predict poor survival and cancer recurrence in patients with pancreatic ductal adenocarcinoma. *Clin Cancer Res* (2017) 23:2681–90. doi: 10.1158/1078-0432.Ccr-16-1467

20. Effenberger KE, Schroeder C, Hanssen A, Wolter S, Eulenburg C, Tachezy M, et al. Improved risk stratification by circulating tumor cell counts in pancreatic cancer. *Clin Cancer Res* (2018) 24:2844–50. doi: 10.1158/1078-0432.Ccr-18-0120

21. Song BG, Kwon W, Kim H, Lee EM, Han YM, Kim H, et al. Detection of circulating tumor cells in resectable pancreatic ductal adenocarcinoma: A prospective evaluation as a prognostic marker. *Front Oncol* (2020) 10:616440. doi: 10.3389/fonc.2020.616440

22. Hugenschmidt H, Labori KJ, Borgen E, Brunborg C, Schirmer CB, Seeberg LT, et al. Preoperative CTC-detection by CellSearch is associated with early distant metastasis and impaired survival in resected pancreatic cancer. *Cancers* (2021) 13:485. doi: 10.3390/cancers13030485

23. Yeo D, Bastian A, Strauss H, Saxena P, Grimison P, Rasko JEJ. Exploring the clinical utility of pancreatic cancer circulating tumor cells. *Int J Mol Sci* (2022) 23:1671. doi: 10.3390/ijms23031671

24. Catenacci DV, Chapman CG, Xu P, Koons A, Konda VJ, Siddiqui UD, et al. Acquisition of portal venous circulating tumor cells from patients with pancreaticobiliary cancers by endoscopic ultrasound. *Gastroenterology* (2015) 149:1794–803.e4. doi: 10.1053/j.gastro.2015.08.050

25. Dotan E, Alpaugh RK, Ruth K, Negin BP, Denlinger CS, Hall MJ, et al. Prognostic significance of MUC-1 in circulating tumor cells in patients with metastatic pancreatic adenocarcinoma. *Pancreas* (2016) 45:1131–5. doi: 10.1097/mpa.0000000000000619

26. Buscail E, Alix-Panabières C, Quincy P, Cauvin T, Chauvet A, Degrandi O, et al. High clinical value of liquid biopsy to detect circulating tumor cells and tumor exosomes in pancreatic ductal adenocarcinoma patients eligible for up-front surgery. *Cancers* (2019) 11:1656. doi: 10.3390/cancers11111656

27. Allard WJ, Matera J, Miller MC, Repollet M, Connelly MC, Rao C, et al. Tumor cells circulate in the peripheral blood of all major carcinomas but not in healthy subjects or patients with nonmalignant diseases. *Clin Cancer Res* (2004) 10:6897–904. doi: 10.1158/1078-0432.Ccr-04-0378

28. Chen C, Ke J, Zhou XE, Yi W, Brunzelle JS, Li J, et al. Structural basis for molecular recognition of folic acid by folate receptors. *Nature* (2013) 500:486–9. doi: 10.1038/nature12327

29. Nunez MI, Behrens C, Woods DM, Lin H, Suraokar M, Kadara H, et al. High expression of folate receptor alpha in lung cancer correlates with adenocarcinoma histology and EGFR [corrected] mutation. *J Thorac Oncol* (2012) 7:833–40. doi: 10.1097/JTO.0b013e31824de09c

30. Norton N, Youssef B, Hillman DW, Nassar A, Geiger XJ, Necela BM, et al. Folate receptor alpha expression associates with improved disease-free survival in triple negative breast cancer patients. *NPJ Breast Cancer* (2020) 6:4. doi: 10.1038/s41523-020-0147-1

31. Parker N, Turk MJ, Westrick E, Lewis JD, Low PS, Leamon CP. Folate receptor expression in carcinomas and normal tissues determined by a quantitative radioligand binding assay. *Anal Biochem* (2005) 338:284–93. doi: 10.1016/j.ab.2004.12.026

32. Cheng H, He W, Yang J, Ye Q, Cheng L, Pan Y, et al. Ligand-targeted polymerase chain reaction for the detection of folate receptor-positive circulating tumour cells as a potential diagnostic biomarker for pancreatic cancer. *Cell Prolif* (2020) 53:e12880. doi: 10.1111/cpr.12880

33. Gemenetzis G, Groot VP, Yu J, Ding D, Teinor JA, Javed AA, et al. Circulating tumor cells dynamics in pancreatic adenocarcinoma correlate with disease status: Results of the prospective CLUSTER study. *Ann Surg* (2018) 268:408–20. doi: 10.1097/sla.0000000000002925

34. Honselmann KC, Pergolini I, Castillo CF, Deshpande V, Ting D, Taylor MS, et al. Timing but not patterns of recurrence is different between node-negative and node-positive resected pancreatic cancer. *Ann Surg* (2020) 272:357–65. doi: 10.1097/SLA.0000000000003123

35. Wan L, Pantel K, Kang Y. Tumor metastasis: moving new biological insights into the clinic. *Nat Med* (2013) 19:1450–64. doi: 10.1038/nm.3391

36. Reimers N, Pantel K. Liquid biopsy: novel technologies and clinical applications. *Clin Chem Lab Med* (2019) 57:312–6. doi: 10.1515/cclm-2018-0610

37. Martini V, Timme-Bronsert S, Fichtner-Feigl S, Hoeppner J, Kulemann B. Circulating tumor cells in pancreatic cancer: Current perspectives. *Cancers* (2019) 11:1659. doi: 10.3390/cancers11111659

38. Zeng CDD, Jin CC, Gao C, Xiao AT, Tong YX, Zhang S. Preoperative folate receptor-positive circulating tumor cells are associated with occult peritoneal metastasis and early recurrence in gastric cancer patients: A prospective cohort study. *Front Oncol* (2022) 12:769203. doi: 10.3389/fonc.2022.769203

39. Wu Q, Zheng H, Gu J, Cheng Y, Qiao B, Wang J, et al. Detection of folate receptor-positive circulating tumor cells as a biomarker for diagnosis, prognostication, and therapeutic monitoring in breast cancer. *J Clin Lab Anal* (2022) 36:e24180. doi: 10.1002/jcla.24180

40. Li H, Li B, Pan Y, Zhang Y, Xiang J, Zhang Y, et al. Preoperative folate receptor-positive circulating tumor cell level is a prognostic factor of long term outcome in non-small cell lung cancer patients. *Front Oncol* (2020) 10:621435. doi: 10.3389/fonc.2020.621435

41. Khoja L, Backen A, Sloane R, Menasce L, Ryder D, Krebs M, et al. A pilot study to explore circulating tumour cells in pancreatic cancer as a novel biomarker. *Br J Cancer* (2012) 106:508–16. doi: 10.1038/bjc.2011.545

42. Sergeant G, Roskams T, van Pelt J, Houtmeyers F, Aerts R, Topal B. Perioperative cancer cell dissemination detected with a real-time RT-PCR assay for EpCAM is not associated with worse prognosis in pancreatic ductal adenocarcinoma. *BMC Cancer* (2011) 11:47. doi: 10.1186/1471-2407-11-47

43. Park Y, Jun HR, Choi HW, Hwang DW, Lee JH, Song KB, et al. Circulating tumour cells as an indicator of early and systemic recurrence after surgical resection in pancreatic ductal adenocarcinoma. *Sci Rep* (2021) 11:1644. doi: 10.1038/s41598-020-80383-1

44. Aziz MH, Sideras K, Aziz NA, Mauff K, Haen R, Roos D, et al. The systemic-immune-inflammation index independently predicts survival and recurrence in resectable pancreatic cancer and its prognostic value depends on bilirubin levels: A retrospective multicenter cohort study. *Ann Surg* (2019) 270:139–46. doi: 10.1097/sla.0000000000002660

45. Ghaneh P, Kleeff J, Halloran CM, Raraty M, Jackson R, Melling J, et al. The impact of positive resection margins on survival and recurrence following resection and adjuvant chemotherapy for pancreatic ductal adenocarcinoma. *Ann Surg* (2019) 269:520–9. doi: 10.1097/sla.0000000000002557

46. Macedo FI, Ryon E, Maithel SK, Lee RM, Kooby DA, Fields RC, et al. Survival outcomes associated with clinical and pathological response following neoadjuvant FOLFIRINOX or Gemcitabine/Nab-paclitaxel chemotherapy in resected pancreatic cancer. *Ann Surg* (2019) 270:400–13. doi: 10.1097/sla.0000000000003468

47. Moaven O, Clark CJ, Russell GB, Votanopoulos KI, Howerton R, Levine EA, et al. Optimal adjuvant treatment approach after upfront resection of pancreatic cancer: Revisiting the role of radiation based on pathologic features. *Ann Surg* (2021) 274:1058–66. doi: 10.1097/sla.0000000000003770

48. Crippa S, Giannone F, Schiavo Lena M, Belfiori G, Partelli S, Tamburrino D, et al. R status is a relevant prognostic factor for recurrence and survival after pancreatic head resection for ductal adenocarcinoma. *Ann Surg Oncol* (2021) 28:4602–12. doi: 10.1245/s10434-020-09467-6

49. Guven DC, Sahin TK, Yildirim HC, Aktepe OH, Dizdar O, Yalcin S. A systematic review and meta-analysis of the association between circulating tumor DNA (ctDNA) and prognosis in pancreatic cancer. *Crit Rev Oncol Hematol* (2021) 168:103528. doi: 10.1016/j.critrevonc.2021.103528

50. Woo SM, Kim MK, Park B, Cho EH, Lee TR, Ki CS, et al. Genomic instability of circulating tumor DNA as a prognostic marker for pancreatic cancer survival: A prospective cohort study. *Cancers* (2021) 13:5466. doi: 10.3390/cancers13215466

51. Affolter KE, Hellwig S, Nix DA, Bronner MP, Thomas A, Fuertes CL, et al. Detection of circulating tumor DNA without a tumor-informed search using next-generation sequencing is a prognostic biomarker in pancreatic ductal adenocarcinoma. *Neoplasia* (2021) 23:859–69. doi: 10.1016/j.neo.2021.06.005

52. Earl J, Garcia-Nieto S, Martinez-Avila JC, Montans J, Sanjuanbenito A, Rodríguez-Garrote M, et al. Circulating tumor cells (Ctc) and kras mutant circulating free dna (cfdna) detection in peripheral blood as biomarkers in patients diagnosed with exocrine pancreatic cancer. *BMC Cancer* (2015) 15:797. doi: 10.1186/s12885-015-1779-7

53. Zhu Y, Zhang H, Chen N, Hao J, Jin H, Ma X. Diagnostic value of various liquid biopsy methods for pancreatic cancer: A systematic review and meta-analysis. *Medicine* (2020) 99:e18581. doi: 10.1097/md.0000000000018581

54. Kulemann B, Pitman MB, Liss AS, Valsangkar N, Fernández-Del Castillo C, Lillemoe KD, et al. Circulating tumor cells found in patients with localized and advanced pancreatic cancer. *Pancreas* (2015) 44:547–50. doi: 10.1097/mpa.0000000000000324

frontiers | Frontiers in Oncology

TYPE Original Research
PUBLISHED 21 October 2022
DOI 10.3389/fonc.2022.999002

OPEN ACCESS

EDITED BY
Jorg Kleeff,
University Hospital in Halle, Germany

REVIEWED BY
Matteo De Pastena,
University of Verona, Italy
Pascal Probst,
University of Stuttgart, Germany
Alessandro Esposito,
Verona Integrated University Hospital, Italy

*CORRESPONDENCE
Jun-chao Guo
gjcpumch@163.com

†These authors have contributed equally to this work and share first authorship

SPECIALTY SECTION
This article was submitted to Gastrointestinal Cancers: Hepato Pancreatic Biliary Cancers, a section of the journal Frontiers in Oncology

RECEIVED 20 July 2022
ACCEPTED 06 October 2022
PUBLISHED 21 October 2022

CITATION
Tian F, Luo M-j, Sun M-q, Lu J, Huang B-w and Guo J-c (2022) Staple line lockstitch reinforcement decreases clinically relevant pancreatic fistula following distal pancreatectomy: Results of a propensity score matched retrospective analysis. *Front. Oncol.* 12:999002. doi: 10.3389/fonc.2022.999002

Staple line lockstitch reinforcement decreases clinically relevant pancreatic fistula following distal pancreatectomy: Results of a propensity score matched retrospective analysis

Feng Tian[1†], Ming-jie Luo[2,3†], Meng-qing Sun[1], Jun Lu[2], Bo-wen Huang[2] and Jun-chao Guo[1*]

[1]Department of General Surgery, State Key Laboratory of Complex Severe and Rare Diseases, Peking Union Medical College Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing, China, [2]Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing, China, [3]State Key Laboratory of Ophthalmology, Zhongshan Ophthalmic Center, Sun Yat-sen University, Guangzhou, Guangdong, China

Background: Postoperative pancreatic fistula (POPF) remains the primary complication of distal pancreatectomies. We aimed to review whether staple line reinforcement with continuous lockstitches would lead to decreased grade B and C pancreatic fistula in patients undergoing distal pancreatectomy.

Methods: This retrospective study enrolled consecutive patients scheduled to undergo distal pancreatectomy at a large tertiary hospital. A comparison was conducted between lockstitch reinforcement and non-reinforcement for remnant closure during distal pancreatectomies from August 2016 to February 2021. Propensity score matching was applied to balance the two groups with covariates including abdominal and back pain, diabetes mellitus, and estimated blood loss. The primary outcome was POPF rate.

Results: A total of 153 patients were enrolled in the study (89 lockstitch reinforcements, 64 non-reinforcements), of whom 128 patients (64 per group) were analyzed after propensity score matching (1:1). The total POPF rate was 21.9%. POPF was identified in 12.5% (8/64) of the patients who underwent resection with lockstitch reinforcement and 31.2% (20/64) of the patients without reinforcement (odds ratio 0.314, 95% confidence interval 0.130-0.760, P=0.010). No deaths occurred in either group. Neither the major complication rate nor the length of hospital stay after surgery differed between the groups.

Conclusions: Compared with the use of stapler alone, staple line lockstitch reinforcement for remnant closure during distal pancreatectomy could reduce the POPF rate. Further multicenter randomized clinical trials are required to confirm these results.

KEYWORDS

CR-POPF, distal pancreatectomy, staple line, lockstitch reinforcement, pancreatic fistula

Introduction

Distal pancreatectomy (DP) is the standard surgical procedure for benign, premalignant, or malignant pancreatic tumors located in the body and tail of the pancreas (1). According to the published literature, post-DP morbidity varies from 5–64% in different centers (2–4). Postoperative pancreatic fistula (POPF) remains the major complication after DP and can potentially cause further complications, such as abdominal fluid collection, severe intra-abdominal infection and hemorrhage. Preventing POPF *via* effective pancreatic remnant closure remains challenging, and no consensus on the optimal surgical technique has been established (1, 5–8).

Surgical staples have been widely applied for remnant closure because of their convenience and the mature laparoscopic DP technique used. However, the DISPACT trial demonstrated non-superior results with similar POPF rates in stapler versus scalpel resection followed by hand-sewn closure of the pancreatic remnant (9). Various surgical techniques for staple line reinforcement have been reported to prevent POPF, including reinforced staples, stump coverage with autologous tissue, absorbable or nonabsorbable mesh, and biological glue. However, when compared with stapler or hand-sewn closure, most of the methods showed no convincing benefit in terms of POPF (10–16).

The effective closure of pancreatic remnants of irregular thickness is crucial for fistula prevention. This study reviewed a propensity score matched cohort of patients who underwent DPs with or without splenectomy and compared the efficacy of staple plus lockstitch reinforcement versus non-reinforcement (staples only) on the POPF rate.

Methods

Study design and patient enrolment

This retrospective study included patients scheduled to undergo DPs between August 2016 and February 2021 at the Peking Union Medical College Hospital. Preoperative candidate diagnoses included pancreatic malignancies, pancreatic neuroendocrine tumors, pancreatic cystic neoplasms, chronic pancreatitis, and pancreatic pseudocysts. All patients were identified from a medical record-based database at the authors' institution. A single experienced surgeon, who had performed more than 400 pancreatectomies, performed all the surgeries. This study was approved by the institutional ethics committee (approval number: S-K1937). Written informed consent was obtained from all participants.

Inclusion and exclusion criteria

The inclusion criteria were as follows: patients of both sexes scheduled to undergo DPs with or without splenectomy for either benign or malignant neoplasms; preoperative diagnoses of serous or mucinous cystic adenoma, solid pseudopapillary tumor, neuroendocrine tumor, intraductal papillary mucinous neoplasm, pseudocyst, or distal pancreatic malignancies; use of a stapler when closing the pancreatic remnant; and willingness to provide informed consent. The exclusion criteria were as follows: history of major upper abdominal surgeries; history of splenectomy, gastrectomy, liver resection, or duodenal or pancreatic resection (not including laparoscopic cystectomy); patients with pancreatic trauma; patients who underwent other procedures except DPs, such as pancreaticoduodenectomy, segmental pancreatic resection, enucleation, or exploration; no use of a stapler for remnant closure; and patients with pneumoperitoneum or severe cardiopulmonary contraindications who were unfit for surgery.

Grouping and surgical technique standardization

The included patients were enrolled in two groups according to the closure style of the pancreatic remnant: lockstitch reinforcement of the staple line and no reinforcement (staple only). Initially, lockstitch reinforcement was mainly performed when a staple fire was less than optimal, such as fracture of the pancreatic tissue or remnant bleeding (after 2019, we performed

lockstitch reinforcement in majority of the cases, regardless of staple line performance). The study group in which continuous lockstitches were placed along the staple line after transecting the pancreas (Figure 1) was set as the reinforcement group. Control group, i.e., non-reinforcement group, did not receive additional reinforcement after transecting the pancreas with a stapler.

Regarding the surgical approach, we considered heterogeneous tumor location and its relationship to the left wall of portal vein (PV) and the roots of splenic vessels. When the lesion was located near PV or even invaded the roots of splenic vessels, named "shoulder" pancreatic tumor in our previously published article, we preferred retrograde artery first approach pancreatosplenectomy (17). When the lesion was located far from PV and there was enough space to ligate the splenic vessels, we preferred radical antegrade modular pancreaticosplenectomy (RAMPS) (18). For key surgical steps during minimally invasive RAMPS, the authors' team transected the pancreas before ligating the splenic vessels unless the splenic artery was easy to expose. In that case, the splenic artery was ligated first. After transecting the pancreas, we ligated the splenic vein and then the splenic artery considering the foot-to-head view under laparoscopy. Normally, we resected the pancreas at the neck if the lesion was located at the body and near the PV. If the lesion was far from PV or near the tail of the pancreas, we would transect the pancreas approximately 2 cm right to the lesion to leave normal parenchyma intact as much as possible.

A 60-mm stapler with different heights (Powered Echelon Flex stapler from Johnson & Johnson Medical Company, USA) was used for pancreatic transection. The frequently chosen stapler height was 3.6 mm, whereas a 2.6 mm height was chosen when the targeted parenchyma was particularly thin. In the study group, 5-0 Prolene (Ethicon, Somerville, NJ, USA) was used to perform lockstitches, with a needle gauge of approximately 5 mm, and was pulled tightly according to various thicknesses and firmness of the pancreas.

Two intra-abdominal drainages were routinely placed in all cases (one near the pancreatic remnant and the other in the spleen nest if splenectomy was performed simultaneously). Prophylactic somatostatin analogs, such as octreotide (Merck Serono, Aubonne, Switzerland) were used 1–3 days postoperatively according to the intraoperative performance and amylase levels.

Drain amylase levels were tested on postoperative day(s) 1, 3, 5, 7, and so on. The criteria for drainage removal were strict at the authors' institution. Generally, the drainage was removed when the amylase levels were less than three times the upper normal institutional limit and the patient was asymptomatic. For patients with elevated amylase levels less than 5000 U/L and no intra-abdominal fluid collection, we removed the drainage on postoperative days 5-7. If the amylase levels were higher than 5000 U/L, we initiated the removal process when the drain volume was less than 10 mL per day and lasted for at least 3 days. In detail, we retracted the drainage gradually (3–5 cm at a time) until removal.

The patients met the discharge criteria when they resumed activity and autonomous eating, were afebrile, and did not need fluid transfusions. Whether the drain tube had been removed was not a determinant of discharge. Thus, some patients were discharged with the drainage still in place, which would be removed at the surgical clinic once the patient met the aforementioned criteria.

Outcomes and data collection

The primary outcome measure was POPF, defined and identified according to the 2016 version of the International Study Group on Pancreatic Surgery (ISGPS) classification and grading of POPF (19). POPF is defined as the drain output of any

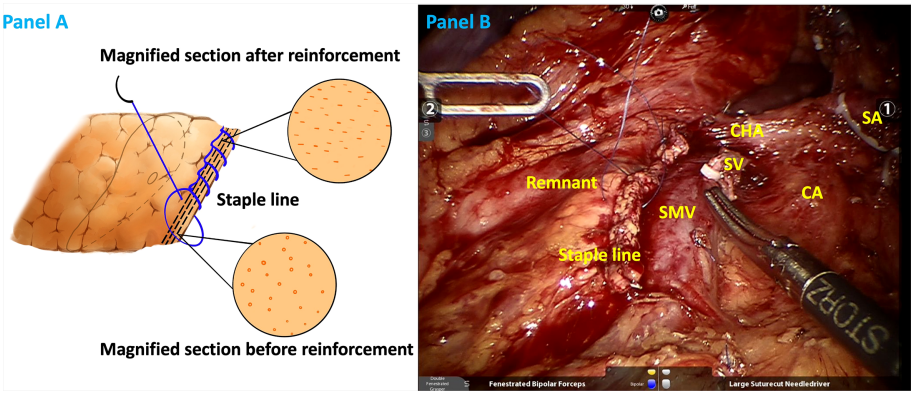


FIGURE 1
Illustrations of staple line reinforcement with continuous lock stitches in schematic **(A)** and realistic drawings **(B)**. SA splenic artery (ligated), CA celiac axis, CHA common hepatic artery, SV splenic vein (ligated), SMV superior mesentery vein.

measurable fluid volume with amylase levels greater than three times the upper limit of the institutional normal serum amylase level (115 U/L at the authors' institution) associated with one or more clinical conditions related directly to the POPF. For the classification of POPF, different physicians' interpretations may deviate from the ISGPS definition, causing potential bias. Therefore, two investigators independently performed POPF classification (TF and LMJ). If an inconsistency occurred, a senior professor reviewed this and made a judgment (GJC).

The second outcome included surgical variables (parenchymal firmness, operative time, lockstitch reinforcement time, estimated blood loss [EBL], blood transfusion rate, and conversion rate], short-term postoperative complication rate within 90 days, and pathological results [final pathologic diagnosis, margin status, and the number of harvested lymph nodes]). The postoperative length of stay (LOS) was also recorded. Due to the retrospective nature of the study, we did not collect data on the duration of performing lockstitch reinforcements; however, data were collected from several random samples of surgical videos. The R0 resection rate was defined as a tumor within 1 mm of the specimen margin (20). Definitions of postoperative complications, such as delayed gastric emptying (DGE) (21), post-pancreatectomy hemorrhage (22), and abdominal infection (23, 24), have been reported previously. The Clavien–Dindo classification was adopted to describe the severity of postoperative complications (25), with grade III or higher considered as a major complications.

In addition to the above variables, demographic data including age, sex, body mass index (BMI), American Society of Anesthesiologists (ASA) status, symptoms, medical history, carbohydrate antigen 19-9 level, and maximum tumor size measured on preoperative computed tomography (CT) scans were also collected using a standardized form.

Postoperative follow-up

The first follow-up was arranged 30 days postoperatively and the second 90 days postoperatively at the outpatient clinic or by phone if the patient could not attend the clinic. Medical history, physical examination, and laboratory tests were performed routinely. Non-enhanced or contrast-enhanced CT scan was performed accordingly. For patients with the surgical drainage, the surgical drains were removed in the clinic when the drain volume was less than 10 mL per day and lasted for 3 days.

Propensity-score matching

Mann–Whitney U tests and χ^2 tests were conducted for patient demographics and clinicopathological characteristics. Significant between-group differences in the symptoms, rates of diabetes mellitus, and intraoperative blood loss were identified, which might potentially affect the risk of POPF. Characteristics which possibly contribute to fistula such as pancreatic firmness, BMI, or sex did not differ between the groups (Supplementary Tables). Subsequently, we conducted propensity score matching (PSM) (staple plus reinforcement vs. non-reinforcement in a 1:1 match) to balance the two groups. The covariates included abdominal and back pain, diabetes mellitus, and EBL. The pancreatic remnant closure technique was used as the dependent variable for the PSM.

Statistical analysis

PSM and statistical analyses were performed using R (R Core Team, 2018) and SPSS® version 20.0 (IBM, Armonk, New York, USA). Continuous variables are described as medians (range) after testing for normality. Categorical variables are presented as frequencies and percentages. Student's t-test or Mann–Whitney U test was used for continuous variables, and χ^2 and Fisher's exact tests were applied for categorical variables. P-values were considered significant at $P < 0.05$.

Results

A total of 261 patients with distal pancreatic lesions were eligible for enrolment between the study intervals. Of these, 53 patients had to be excluded because procedures other than DP were performed (n=30), or the required data were incomplete (n=23). Thus, the intention-to-treat population consisted of 208 patients, of whom 55 did not use a stapler for remnant closure were excluded. A total of 153 patients were enrolled according to the inclusion criteria before matching, of whom 89 adopted staplers plus lockstitch reinforcement and 64 used only staplers without reinforcement. After PSM, a balanced cohort was created with 64 patients in each of the study and control groups (Figure 2).

The top five pathologies among the 208 patients who underwent DP were pancreatic adenocarcinomas (32.7%), solid pseudopapillary tumors (17.8%), serous cystic adenomas (11.5%), neuroendocrine tumors (10.1%), and mucinous cystic adenomas (10.1%). There were also a few rare pathological types, including adenosquamous carcinoma (n=2), acinar cell carcinoma (n=1), metastatic lesions from breast cancer (n=1), liposarcoma (n=2), leiomyoma (n=1), spindle cell sarcoma (n=1), tubular villous adenoma (n=1), and hemangioma (n=1; Table 1)

The study population comprised 48 men (37.5%) and 80 women (62.5%). There were no differences in the baseline data (age, sex, or BMI), clinical features (symptoms, accompanying medical histories, ASA status, tumor marker deviation), or radiological variables (tumor size, the relationship between the

tumor and major vessels such as the portal vein-superior mesentery vein [PV-SMV] axis, and roots of splenic vessels) (Table 2).

A total of 92.2% (118/128) of the DPs were completed *via* minimally invasive approaches, of which 6.3% (8/128) were converted to open surgeries for severe adhesion or uncontrollable hemorrhage. The minimally invasive surgery and conversion rates did not differ significantly between the two groups. Based on five random samples of surgical videos, the mean duration of performing lockstitch reinforcements was 521 ± 146.1 s (493, 614, 463, 327, 708 s, respectively). As shown in Table 3, the overall POPF rate was 21.9% (28/128), with rates of 12.5 and 31.2% in the reinforcement and non-reinforcement groups, respectively (P=0.010). Among the 28 patients with grade B POPF, 27 needed persistent drainage>21days (delayed removal of the surgical drainage), whereas no surgical, endoscopic, or radiological intervention were required. Only one patient needed percutaneous puncture due to intra-abdominal fluid and fever. No grade C fistula was observed.

The 90-day all-cause mortality rate was zero in both groups. The rates of spleen preservation and concomitant PV-SMV wall resection did not show any differences between the groups. Both groups were similar regarding parenchymal firmness, intraoperative median EBL, transfusion rate, and operative time. The duration of drainage tended to be shorter in the reinforcement group than in the non-reinforcement group (8 vs. 10 days, respectively; P=0.066). Major postoperative complications and LOS were similar between the two groups. There were two grade IIIa complications in the non-reinforcement group, including one case of DGE requiring a gastric tube reinsertion and one case of peripancreatic fluid

TABLE 1 Pathological array of 208 distal pancreatectomies.

Pathology	n(%)
Pancreatic ductal adenocarcinoma	68 (32.7)
Solid pseudopapillary tumor	37 (17.8)
Serous cystic adenoma	24 (11.5)
Neuroendocrine tumor	21 (10.1)
Mucinous cystic adenoma	21 (10.1)
Intraductal papillary mucinous neoplasm	14 (6.7)
Chronic pancreatitis	13 (6.2)
Other rare pathologic types*	10 (4.8)

*Including: adenosquamous carcinoma (n=2); alveolar cell carcinoma (n=1); metastatic cancer from breast cancer (n=1); liposarcoma (n=2); tubular villous adenoma (n=1); liomyoma (n=1); spindle cell sarcoma (n=1); hemangioma (n=1).

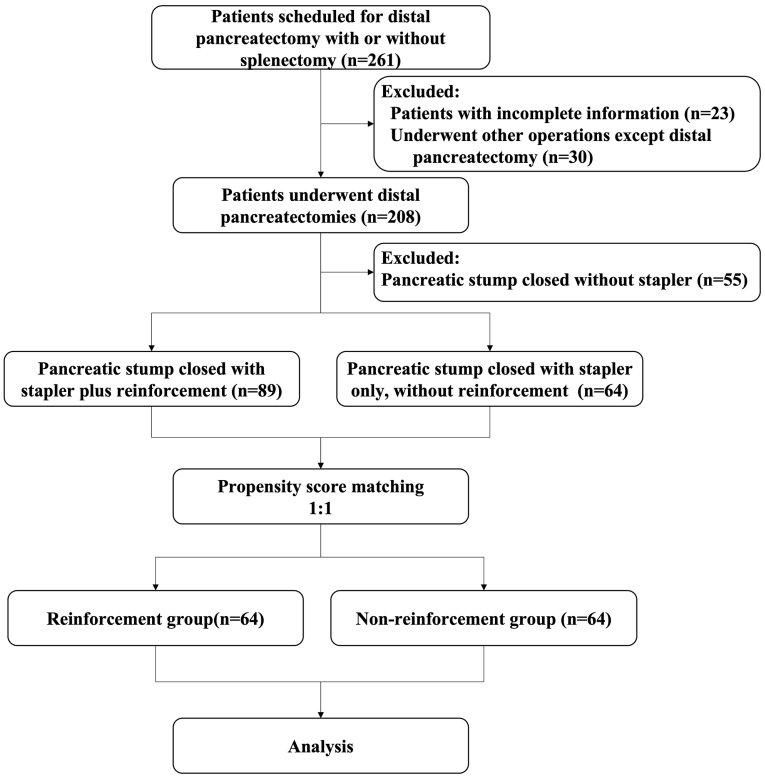


FIGURE 2
Study profile.

accumulation with fever requiring reintervention, although pathogen cultures were all negative. In addition, there were nine grade II complications in both groups: one abdominal infection (*Enterococcus faecalis*), one blood infection (*Brucella*), and two intestinal infections (one *Candida albicans* and one *Clostridium difficile*), which were treated with antibiotics; three chylous leakages treated *via* fasting; and two patients with transient hemoglobin decline treated conservatively (Table 4).

Discussion

The major finding of this study was that reinforcement of the staple line with continuous lockstitches resulted in a significantly decreased POPF rate compared with its nonreinforcement counterpart for DP (12.5 vs. 31.2%, P=0.010). Meanwhile, lockstitch reinforcement did not lead to differences in the major postoperative complication rate or patient recovery. Both remnant closure strategies were equally safe for DP.

TABLE 3 Comparison of rate of postoperative pancreatic fistula in staple line reinforcement and non-reinforcement groups .

	Non-reinforcement group (n=64)	Reinforcement group (n=64)	*P* value§
No leakage	7 (10.9)	10 (15.6)	0.601
Biochemical leak	37 (57.8)	46 (71.9)	0.138
POPF			0.010*
Grade B	20 (31.2)	8 (12.5)	
Grade C	0 (0)	0 (0)	

Values in parentheses are percentages. POPF, postoperative pancreatic fistula. §χ^2 test, except *Fisher's exact test.

This study presented a total POPF rate of 21.9% (28/128), which is similar to the 23% benchmark POPF rate reported in a study involving 3,016 patients from 24 randomized controlled trials undergoing DP (6). Risk scores for predicting POPF would promote preventive and mitigation strategies. Several studies have identified risk factors related to POPF occurrence after DP.

TABLE 2 Comparison of baseline and clinicopathological characteristics of patients between the groups.

	Non-reinforcement group (n=64)	Reinforcement group (n=64)	*P* value§
Age, years *	54.5 (13-81)	55.5 (17-79)	0.618#
Sex			0.273
Male	21 (32.8)	27 (42.2)	
Female	43 (67.2)	37 (57.8)	
Body mass index (kg/m^2) *	23.6 (15.6-32.7)	23.2 (15.4-33.6)	0.941#
Abdominal/back pain	31 (48.4)	26 (40.6)	0.374
Weight loss	17 (26.6)	25 (39.1)	0.132
Hypertension in medical history	14 (21.9)	18 (28.1)	0.414
Diabetes mellitus	8 (12.5)	7 (10.9)	0.783
Coronary heart disease	2 (3.1)	4 (6.2)	0.680¶
Cerebrovascular disease	2 (3.1)	3 (4.7)	1.000¶
Hyperlipemia	10 (15.6)	9 (14.1)	0.804
Maximum tumor size (cm) *	5 (1.2-12)	4.8 (1-23)	0.960#
Preoperative pancreatic portal hypertension	11 (17.2)	13 (20.3)	0.651
PV/SMV axis invasion on imaging	10 (15.6)	15 (23.4)	0.265
Splenic artery invasion on imaging	13 (20.3)	12 (18.8)	0.824
Splenic vein invasion on imaging	9 (14.1)	12 (18.8)	0.474
Elevated CA19-9	18 (28.1)	20 (31.2)	0.699
Elevated CEA	11 (17.2)	11 (17.2)	1.000
Preoperative albumin (g/L) *	43 (36-54)	44 (30-51)	0.521
Preoperative hemoglobin (g/dL) *	133 (83-169)	136.5 (85-178)	0.333#
ASA Classification			0.571
Grade I or II	58 (90.6)	56 (87.5)	
Grade ≥III	6 (9.4)	8 (12.5)	
Pathology			0.461
PDAC	21 (32.8)	25 (39.1)	
Non-PDAC	43 (67.2)	39 (60.9)	

Values in parentheses are percentages unless indicated otherwise; *values are median (range). ASA, American Society of Anesthesiologists. PDAC, pancreatic ductal adenocarcinoma. §χ^2 test, except ¶ Fisher's exact test and #Mann–Whitney U test.

TABLE 4 Comparison of safety and efficiency-related outcomes between the two groups.

	Non-reinforcement group (n=64)	Reinforcement group (n=64)	*P* value[§]
Surgical approach			0.510[¶]
Open	4 (6.2)	6 (9.4)	
Laparoscopic or robotic	60 (93.8)	58 (90.6)	
Conversion to open surgery	4 (6.3)	4 (6.3)	1.000[¶]
Parenchyma firmness			0.466
Soft	56 (87.5)	52 (81.3)	
Hard	8 (12.5)	12 (18.7)	
Operative time (min) *	200 (100-460)	200 (110-440)	0.834[#]
Spleen preservation	12 (18.8)	12 (18.8)	1
Concomitant PV/SMV wall resection	8 (12.5)	8 (12.5)	1
Estimated blood loss (ml) *	100 (20-1000)	125 (20-1000)	0.712[#]
Transfusion	6 (9.4)	8 (12.5)	0.571
Duration of drainage (days) *	10 (6-60)	8 (3-60)	0.066[#]
Postoperative LOS (days) *	9 (6-25)	10 (6-26)	0.378[#]
Clavien-Dindo classification			0.528[¶]
Grade I or II	46 (71.9)	28 (43.8)	
Grade IIIa[†]	2 (3.1)	0 (0)	
Grade IIIb	0 (0)	0 (0)	
Grade IV or V	0 (0)	0 (0)	
90-day mortality	0 (0)	0 (0)	1

Values in parentheses are percentages unless indicated otherwise; *values are median (range). PV, portal vein. SMV, superior mesentery vein. LOS, length of stay. † Including one delayed gastric emptying and one peri-pancreatic fluid accumulation with fever needing reintervention. § $\chi 2$ test, except ¶ Fisher's exact test and # Mann–Whitney U test.

Based on a retrospective study involving 2026 patients, Ecker et al. reported that age <60 years, obesity, hypoalbuminemia, absence of epidural anesthesia, nonmalignant pathology, concomitant splenectomy, and vascular resection were independent risk factors of POPF. Unfortunately, most of the factors were not modifiable and the prediction model showed unsatisfactory discrimination (1). Recently, Bonsdorff et al. and Pastena et al. developed and validated new risk scores for POPF after DP, introducing crucial risk factors, including the pancreatic thickness at the transection, the diameter of the pancreatic duct, diabetes, and the level of transection (neck or body-tail) (7, 8).

Effective closure of pancreatic remnants of irregular thickness is crucial for fistula prevention. The pancreatic parenchyma, particularly the soft and thick parenchyma, may be too fragile to retain the staples. The stapler may only tear the pancreatic tissue, potentially increasing the risk of leakage. Moreover, mismatch between the irregular remnant thickness and the stapler's height might cause invisible minor leaks (Figure 1). Zimmitti et al. reported pancreatic capsule disruption and staple line bleeding at a high occurrence rate of 39% and 50%, respectively, during DPs. Moreover, they concluded that pancreatic capsule disruption and staple line bleeding were factors associated with higher POPF rate (26). The thicker the pancreas at the pancreatic transection site, the higher the possibility of disruption. Initially, for heterogeneous remnants, we only used electrocoagulation and single stitch for pancreatic capsule disruption and staple line bleeding. Obviously, no improvement of POPF was observed. Around 2019, we applied continuous lockstitch reinforcement along the staple line and found it might decrease POPF. From then on, we added reinforcement as a routine step during DP and applied it in majority of the cases, regardless of the occurrence of disruption. The logic of the staple line lockstitch reinforcement technique is that the fine lockstitches could close tiny pancreatic ducts according to different gland characteristics and tighten the remnant to the largest extent, thus preventing potential leakage from the remnant. It systemically, not focally, enhances the staple line and decreases POPF rate as demonstrated in our results, which is a reverse proof of the effectiveness of the lockstitch reinforcement technique. Therefore, the inconsistency between results of Zimmitti et al.'s study and our study lie in that they described the situation, and we put forward an alternative solution for this situation.

In the past decade, several studies have shown a significant reduction in POPF using reinforced stapler for closure of the remnant (10, 27, 28). However, a recent randomized trial reported no difference in terms of POPF or overall postoperative complications after DP comparing reinforced stapler versus standard stapler (14). Therefore, the potential superiority of reinforced stapler has not been confirmed and controversy remains (5). Moreover, the expenses could have limited the wide use of reinforced stapler. In the present study, the POPF rate in the reinforcement group is similar to the rate in

reinforced stapler group (12%) reported by Wennerblom et al., even though they did not consider reintervention and the rate of POPF was possibly underestimated (14).

The reduced POPF rate should have shortened the postoperative LOS and the drainage duration in the reinforcement group, but there was no difference in the LOS (median 9 vs. 10 days in the reinforcement group; P=0.378) or duration of drainage (median 10 vs. 8 days in the reinforcement group; P=0.066) between the two groups. This may be related to our conservative strategies for postoperative management, especially the aspect dealing with surgical drains. We believe that around postoperative day 7, there is a high-risk period of pancreatic fistula due to tissue edema, necrosis, and increased secretion of pancreatic juice following oral intake. Therefore, we were accustomed to retaining the surgical drain until around postoperative day 7, unless the amylase levels were very low. This perhaps narrowed the difference of LOS and drainage duration between the two groups.

Surgical drainages are commonly used to mitigate POPF. Likewise, no-drain strategy in selected cases after DP was reported to not be associated with increased POPF rate when compared with routine prophylactic abdominal drainage (29, 30). However, the selection bias limits the conclusion of studies and controversy still exists (31). Future evidence is required for identifying which subset of patients is suitable for no-drain strategy. Prophylactic abdominal drainage has been reported to be associated with a greater fistula rate but reduced POPF severity (1). Strict criteria for drainage removal may increase inconvenience for patients after discharge. However, longer drainage may lower the possibility of intra-abdominal fluid collection and reduce the need for punctures. Meanwhile, drainage in place keeps an existing and easier pathway for a possible percutaneous drain. Wennerblom et al. (14) and Diener et al. (9), both reported a remarkably high rate of patients with intra-abdominal fluid and abscess (17–19%), majority of whom needed subsequent radiological or surgical reintervention. In this study, only one patient with fluid accumulation required percutaneous reintervention after removal of the drainage, leading to a very low Clavien–Dindo grade III or higher complication rate in both groups, which benefits patients. Concern might be raised that delayed drain removal was related to an increased incidence of bacterial contamination. However, in the present study, rare retrograde infection was detected. The low rate of Clavien–Dindo grade III or higher complication (3.1% vs. 0 in the reinforcement group; P=0.528) might also affect the detection of differences between the two groups. Of those with grade B fistula, most patients had prolonged intra-abdominal drainage (over 21 days) due to high drain amylase levels but no clinical symptoms.

This study had several limitations. First, although we applied PSM to decrease selection bias, inherent bias still existed in this retrospective study. For example, neither remnant characteristics, such as parenchymal thickness, duct diameter at the transection site nor the staple height were recorded, which was a potential source of bias. Second, this study described experience from a single surgeon, and repeatability of the reinforcement technique might be an issue if widely adopted.

In conclusion, compared with staplers only, stapler line reinforcement with lockstitches for remnant closure during DP could reduce the POPF rate. Randomized controlled trials are needed to validate the results of our study before generalizing the reinforcement technique. The quality of the reinforcement lockstitches, transection level, and pancreatic duct and parenchyma thickness at the transection site should be considered in future randomized controlled trials.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving human participants were reviewed and approved by PUMCH Institutional Review Board. The patients/participants provided their written informed consent to participate in this study.

Author contributions

FT: study design; acquisition of data; analysis and interpretation of data; drafting the article and revising it critically for important intellectual content. M-JL: acquisition and disposal data; data analysis and interpretation of data; and manuscript revision. M-QS: picture drawing; make contributions to conception and design; reviewed and revised the manuscript. JL: acquisition of data; make contributions to conception and design; reviewed and revised the manuscript. B-WH: acquisition of data; make contributions to conception and design; reviewed and revised the manuscript. J-CG: operated all the cases; make contributions to conception and design; manuscript revision; give final approval of the version to be published. FT and M-JL contributed equally to this work and share first authorship. All authors contributed to the article and approved the submitted version.

Funding

This work was funded by the National Natural Science Foundation of China (grant number: 81972324), CAMS

Innovation Fund for Medical Sciences (grant number: 2021-I2M-1-002), Bethune Charitable Foundation (grant number: FW-HXKT2019013000198), and National High Level Hospital Clinical Research Funding. The funding bodies played no roles in the design or conduction of this study, had no access to the data or role in data collection, management, analysis, or interpretation, and had no role in preparation, review, or approval of the manuscript.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2022.999002/full#supplementary-material

References

1. Ecker BL, McMillan MT, Allegrini V, Bassi C, Beane JD, Beckman RM, et al. Risk factors and mitigation strategies for pancreatic fistula after distal pancreatectomy: Analysis of 2026 resections from the international, multi-institutional distal pancreatectomy study group. *Ann Surg* (2019) 269:143–9. doi: 10.1097/SLA.0000000000002491

2. Knaebel HP, Diener MK, Wente MN, Buchler MW, Seiler CM. Systematic review and meta-analysis of technique for closure of the pancreatic remnant after distal pancreatectomy. *Br J Surg* (2005) 92:539–46. doi: 10.1002/bjs.5000

3. Hackert T, Werner J, Buchler MW. Postoperative pancreatic fistula. *Surgeon* (2011) 9:211–7. doi: 10.1016/j.surge.2010.10.011

4. Reeh M, Nentwich MF, Bogoevski D, Koenig AM, Gebauer F, Tachezy M, et al. High surgical morbidity following distal pancreatectomy: still an unsolved problem. *World J Surg* (2011) 35:1110–7. doi: 10.1007/s00268-011-1022-x

5. Miao Y, Lu Z, Yeo CJ, Vollmer CMJr., Fernandez-Del Castillo C, Ghaneh P, et al. Management of the pancreatic transection plane after left (distal) pancreatectomy: Expert consensus guidelines by the international study group of pancreatic surgery (ISGPS). *Surgery* (2020) 168:72–84. doi: 10.1016/j.surg.2020.02.018

6. Probst P, Huttner FJ, Meydan O, Abu Hilal M, Adham M, Barreto SG, et al. Evidence map of pancreatic surgery-a living systematic review with meta-analyses by the international study group of pancreatic surgery (ISGPS). *Surgery* (2021) 170:1517–24. doi: 10.1016/j.surg.2021.04.023

7. De Pastena M, van Bodegraven EA, Mungroop TH, Vissers FL, Jones LR, Marchegiani G, et al. Distal pancreatectomy fistula risk score (D-FRS): Development and international validation. *Ann Surg* (2022) 7. doi: 10.1097/SLA.0000000000005497

8. Bonsdorff A, Ghorbani P, Helantera I, Tarvainen T, Kontio T, Belfrage H, et al. Development and external validation of DISPAIR fistula risk score for clinically relevant postoperative pancreatic fistula risk after distal pancreatectomy. *Br J Surg* (2022) 19:znac266. doi: 10.1093/bjs/znac266

9. Diener MK, Seiler CM, Rossion I, Kleeff J, Glanemann M, Butturini G, et al. Efficacy of stapler versus hand-sewn closure after distal pancreatectomy (DISPACT): a randomised, controlled multicentre trial. *Lancet* (2011) 377:1514–22. doi: 10.1016/S0140-6736(11)60237-7

10. Hamilton NA, Porembka MR, Johnston FM, Gao F, Strasberg SM, Linehan DC, et al. Mesh reinforcement of pancreatic transection decreases incidence of pancreatic occlusion failure for left pancreatectomy: a single-blinded, randomized controlled trial. *Ann Surg* (2012) 255:1037–42. doi: 10.1097/SLA.0b013e31825659ef

11. Jensen EH, Portschy PR, Chowaniec J, Teng M. Meta-analysis of bioabsorbable staple line reinforcement and risk of fistula following pancreatic resection. *J Gastrointest Surg* (2013) 17:267–72. doi: 10.1007/s11605-012-2016-1

12. Jimenez RE, Mavanur A, Macaulay WP. Staple line reinforcement reduces postoperative pancreatic stump leak after distal pancreatectomy. *J Gastrointest Surg* (2007) 11:345–9. doi: 10.1007/s11605-006-0034-6

13. Montorsi M, Zerbi A, Bassi C, Capussotti L, Coppola R, Sacchi M, et al. Efficacy of an absorbable fibrin sealant patch (TachoSil) after distal pancreatectomy: a multicenter, randomized, controlled trial. *Ann Surg* (2012) 256:853–859; discussion 859-860. doi: 10.1097/SLA.0b013e318272dec0

14. Wennerblom J, Ateeb Z, Jönsson C, Björnsson B, Tingstedt B, Williamsson C, et al. Reinforced versus standard stapler transection on postoperative pancreatic fistula in distal pancreatectomy: multicentre randomized clinical trial. *Br J Surg* (2021) 108:265–70. doi: 10.1093/bjs/znaa113

15. Probst P, Hüttner FJ, Klaiber U, Knebel P, Ulrich A, Büchler MW, et al. Stapler versus scalpel resection followed by hand-sewn closure of the pancreatic remnant for distal pancreatectomy. *Cochrane Database Syst Rev* (2015) 6(11): Cd008688. doi: 10.1002/14651858.CD008688.pub2

16. Landoni L, De Pastena M, Fontana M, Malleo G, Esposito A, Casetti L, et al. A randomized controlled trial of stapled versus ultrasonic transection in distal pancreatectomy. *Surg Endosc* (2022) 36:4033–41. doi: 10.1007/s00464-021-08724-3

17. Tian F, Sun MQ, Lu J, Guo JC. Retrograde artery first approach for "shoulder" pancreatic cancers in minimally invasive distal pancreatectomy. *Surg Endosc* (2021) 35:74–80. doi: 10.1007/s00464-020-07908-7

18. Strasberg SM, Drebin JA, Linehan D. Radical antegrade modular pancreatosplenectomy. *Surgery* (2003) 133:521–7. doi: 10.1067/msy.2003.146

19. Bassi C, Marchegiani G, Dervenis C, Sarr M, Abu Hilal M, Adham M, et al. The 2016 update of the international study group (ISGPS) definition and grading of postoperative pancreatic fistula: 11 years after. *Surgery* (2017) 161:584–91. doi: 10.1016/j.surg.2016.11.014

20. Schlitter AM, Esposito I. Definition of microscopic tumor clearance (r0) in pancreatic cancer resections. *Cancers (Basel)* (2010) 2:2001–10. doi: 10.3390/cancers2042001

21. Healy JM, Kunstman JW, Salem RR. Proposal and critical appraisal of exclusion criteria to the international study group for pancreatic surgery definition of delayed gastric emptying. *J Am Coll Surg* (2015) 220:1036–1043 e1031. doi: 10.1016/j.jamcollsurg.2014.12.017

22. Correa-Gallego C, Brennan MF, D'Angelica MI, DeMatteo RP, Fong Y, Kingham TP, et al. Contemporary experience with postpancreatectomy hemorrhage: results of 1,122 patients resected between 2006 and 2011. *J Am Coll Surg* (2012) 215:616–21. doi: 10.1016/j.jamcollsurg.2012.07.010

23. Dellinger RP, Levy MM, Rhodes A, Annane D, Gerlach H, Opal SM, et al. Surviving sepsis campaign guidelines committee including the pediatric s Surviving sepsis campaign: international guidelines for management of severe sepsis and septic shock, 2012. *Intensive Care Med* (2013) 39:165–228. doi: 10.1007/s00134-012-2769-8

24. Sawyer RG, Claridge JA, Nathens AB, Rotstein OD, Duane TM, Evans HL, et al. Trial of short-course antimicrobial therapy for intraabdominal infection. *N Engl J Med* (2015) 372:1996–2005. doi: 10.1056/NEJMoa1411162

25. Dindo D, Demartines N, Clavien PA. Classification of surgical complications: a new proposal with evaluation in a cohort of 6336 patients and results of a survey. *Ann Surg* (2004) 240:205–13. doi: 10.1097/01.sla.0000133083.54934.ae

26. Zimmitti G, La Mendola R, Manzoni A, Sega V, Malerba V, Treppiedi E, et al. Investigation of intraoperative factors associated with postoperative pancreatic fistula following laparoscopic left pancreatectomy with stapled

closure: a video review-based analysis : Video-review for predictors of pancreatic leak. *Surg Endosc* (2021) 35:941–54. doi: 10.1007/s00464-020-07912-x

27. Jang JY, Shin YC, Han Y, Park JS, Han HS, Hwang HK, et al. Effect of polyglycolic acid mesh for prevention of pancreatic fistula following distal pancreatectomy: A randomized clinical trial. *JAMA Surg* (2017) 152:150–5. doi: 10.1001/jamasurg.2016.3644

28. Pulvirenti A, Landoni L, Borin A, De Pastena M, Fontana M, Pea A, et al. Reinforced stapler versus ultrasonic dissector for pancreatic transection and stump closure for distal pancreatectomy: A propensity matched analysis. *Surgery* (2019) 166:271–6. doi: 10.1016/j.surg.2019.02.016

29. van Bodegraven EA, De Pastena M, Vissers FL, Balduzzi A, Stauffer J, Esposito A, et al. Routine prophylactic abdominal drainage versus no-drain strategy after distal pancreatectomy: A multicenter propensity score matched analysis. *Pancreatology* (2022) 22(6):797-802. doi: 10.1016/j.hpb.2021.08.122

30. Van Buren G2nd, Bloomston M, Schmidt CR, Behrman SW, Zyromski NJ, Ball CG, et al. A prospective randomized multicenter trial of distal pancreatectomy with and without routine intraperitoneal drainage. *Ann Surg* (2017) 266:421–31. doi: 10.1097/SLA.0000000000002375

31. Zhang JW, Yang X. Distal pancreatectomy with and without routine intraperitoneal drainage. *Pancreatology* (2022) 22(6):826-7. doi: 10.1016/j.pan.2022.06.256

frontiers | Frontiers in Oncology

TYPE Original Research
PUBLISHED 31 October 2022
DOI 10.3389/fonc.2022.1059581

OPEN ACCESS

EDITED BY
Xiaodong Tian,
First Hospital, Peking University, China

REVIEWED BY
Dalong Yin,
University of Science and Technology of China, China
Wei Guo,
Department of HPB Surgery, Capital Medical University, China

*CORRESPONDENCE
Zhi-Yu Chen
chenzhiyu_umn@163.com
Shi-Quan Deng
dengshiquantmmu@163.com

†These authors have contributed equally to this work

SPECIALTY SECTION
This article was submitted to Gastrointestinal Cancers: Hepato Pancreatic Biliary Cancers, a section of the journal Frontiers in Oncology

RECEIVED 01 October 2022
ACCEPTED 17 October 2022
PUBLISHED 31 October 2022

CITATION
Liu Z-P, Cheng Z-J, Dai H-S, Zhong S-Y, Zhao D-C, Gong Y, Zuo J-H, Che X-Y, Chen W-Y, Wang Z-R, Yu T, Cheng J-J, Liu X-C, Bai J, Jiang Y, Zhang Y-Q, Lau WY, Deng S-Q and Chen Z-Y (2022) Impact of perioperative blood transfusion on long-term survival in patients with different stages of perihilar cholangiocarcinoma treated with curative resection: *A multicentre propensity score matching study*. *Front. Oncol.* 12:1059581. doi: 10.3389/fonc.2022.1059581

Impact of perioperative blood transfusion on long-term survival in patients with different stages of perihilar cholangiocarcinoma treated with curative resection: *A multicentre propensity score matching study*

Zhi-Peng Liu[1†], Zheng-Jun Cheng[2†], Hai-Su Dai[1†], Shi-Yun Zhong[1†], Dong-Chu Zhao[1†], Yi Gong[1], Jing-Hua Zuo[1], Xiao-Yu Che[1], Wei-Yue Chen[1,3], Zi-Ran Wang[1,4], Ting Yu[1], Jun-Jie Cheng[1], Xing-Chao Liu[5], Jie Bai[1], Yan Jiang[1], Yan-Qi Zhang[6], Wan Yee Lau[1,7], Shi-Quan Deng[8*] and Zhi-Yu Chen[1*]

[1]Department of Hepatobiliary Surgery, Southwest Hospital, Third Military Medical University (Army Medical University), Chongqing, China, [2]Department of Hepatobiliary Surgery, Jiulongpo District Second People's Hospital, Chongqing, China, [3]Clinical Research Center of Oncology, Lishui Hospital of Zhejiang University, Lishui, China, [4]Department of General Surgery, 903rd Hospital of People's Liberation Army, Hangzhou, China, [5]Department of Hepatobiliary Surgery, Sichuan Provincial People's Hospital, Chengdu, China, [6]Department of Health Statistics, College of Military Preventive Medicine, Third Military Medical University (Army Medical University), Chongqing, China, [7]Faculty of Medicine, The Chinese University of Hong Kong, Hong Kong, Hong Kong SAR, China, [8]Department of Hepatobiliary Surgery, Chongqing Jiulongpo District Integrated Traditional Chinese and Western Medicine Hospital, Chongqing, Hong Kong SAR, China

Background & aim: The association of perioperative blood transfusion (PBT) with long-term survival in perihilar cholangiocarcinoma (pCCA) patients after surgical resection with curative intent is controversial and may differ among different stages of the disease. This study aimed to investigate the impact of PBT on long-term survival of patients with different stages of pCCA.

Methods: Consecutive pCCA patients from three hospitals treated with curative resection from 2012 to 2019 were enrolled and divided into the PBT and non-PBT groups. Propensity score matching (PSM) was used to balance differences in baseline characteristics between the PBT and non-PBT groups. Kaplan–Meier curves and log-rank test were used to compare overall survival (OS) and recurrence-free survival (RFS) between patients with all tumor stages, early stage (8th AJCC stage I), and non-early stage (8th AJCC stage II-IV) pCCA in the PBT and non-PBT groups. Cox regression analysis was used to determine the impact of PBT on OS and RFS of these patients.

Results: 302 pCCA patients treated with curative resection were enrolled into this study. Before PSM, 68 patients (22 patients in the PBT group) were in the early stage and 234 patients (108 patients in the PBT group) were in the non-early stage. Patients with early stage pCCA in the PBT group had significantly lower OS and RFS rates than those in the non-PBT group. However, there were with no significant differences between the 2 groups with all tumor stages and non-early stage pCCA. After PSM, there were 18 matched pairs of patients with early stage and 72 matched pairs of patients with non-early stage. Similar results were obtained in the pre- and post-PSM cohorts: patients with early stage pCCA in the PBT group showed significantly lower OS and RFS rates than those in the non-PBT group, but there were no significant differences between the 2 groups for patients with all tumor stages and non-early stage pCCA. Cox regression analysis demonstrated that PBT was independently associated with worse OS and RFS for patients with early stage pCCA.

Conclusions: PBT had a negative impact on long-term survival in patients with early stage pCCA after curative resection, but not in patients with non-early stage pCCA.

KEYWORDS

perihilar cholangiocarcinoma, perioperative blood transfusion, resection, survival, recurrence

Introduction

Cholangiocarcinoma accounts for 3% of all gastrointestinal tumors and represents 10~25% of all primary hepatic malignancies globally (1, 2). Perihilar cholangiocarcinoma (pCCA) is the most common type of cholangiocarcinoma, accounting for approximately 60% of these cases (3). The only treatment that can result in long-term survival for patients with pCCA is curative resection (4, 5). However, the complicated nature of the surgical procedure which includes bile duct resection and reconstruction, hepatectomy, perihilar dissection, vascular resection and reconstruction if necessary, as well as coagulopathy due to preoperative jaundice, make the possibility of intraoperative bleeding and perioperative blood transfusion extremely likely (6).

Perioperative blood transfusion (PBT) plays an essential role in perioperative safety of pCCA patients. However, the impact of PBT on long-term survival in pCCA patients treated with curative resection has been controversial. Müller et al. indicated that allogeneic blood transfusion did not affect long-term survival after curative resection for advanced cholangiocarcinoma (7). However, Kimura et al. indicated that PBT was a poor prognostic factor for hilar cholangiocarcinoma treated with curative resection (8). Both these two studies focused on long-term survival in cholangiocarcinoma patients following curative resection, they reached completely different conclusions. In fact, allogeneic blood transfusion has been demonstrated to have immunosuppressive effects, which are associated with a higher chance of tumor recurrence and a poor long-term prognosis in patients with malignancies (9, 10). There are two possible explanations for the different results obtained in the above two mentioned studies. First, both these studies were single-centre studies with small sample sizes, and the results were of low-level of medical evidence. Second, the conclusions drawn based on the total cohort did not apply to an individual, as the patients had tumors of different stages. Previous studies on hepatocellular carcinoma showed PBT to have different impact on long-term survival in different tumor stages (11, 12). However, the impact of PBT on long-term survival has not been studied in patients with different stages of pCCA.

Abbreviations: AJCC, American Joint Committee on Cancer; ALB, albumin; ALT, alanine aminotransferase; ASA, American Society of Anesthesiologists; AST, aspartate transaminase; CA 19-9, carbohydrate antigen 19-9; CI, confidence interval; HGB, haemoglobin; HR, hazard ratio; INR, international normalized ratio; IQRs, interquartile ranges; LN, lymph node; PBT, perioperative blood transfusion; pCCA, perihilar cholangiocarcinoma; PRBCs, packed red blood cells; PSM, propensity score matching; OR, odds ratio; OS, overall survival; RFS, recurrence-free survival; SDs, standard deviations.

Ethical reasons do not allow clinical researchers to conduct a randomized controlled trial on PBT. To improve the level of medical evidence, 302 patients from 3 institutions were identified from a multicentre database to be included to conduct this first study by using propensity score matching (PSM) analysis to study the impact of PBT on long-term survival in patients with different stages of pCCA treated with curative resection.

Methods

Patients

From February 2012 to February 2019, consecutive pCCA patients treated with curative resection at three hospitals (Southwest Hospital, Sichuan Provincial People's Hospital, Jiulongpo District Second People's Hospital) were enrolled in this study. Tumors originating from common hepatic duct, junction of common hepatic duct, and left/right first-order hepatic ducts were all grouped as pCCA. All diagnoses were confirmed by postoperative histopathology. The exclusion criteria were patients with (1): recurrent pCCA; (2) loss to follow-up; (3) lack of data for essential variables; and (4) death within 30 days after curative resection. This study complied with the Declaration of Helsinki and was approved by the Ethics Committees of the 3 participating hospitals. Due to its retrospective nature and because all data were deidentified, informed consent was exempted.

Surgical procedure

Curative resection was defined as resection resulting in microscopically clear margins. Curative resection included bile duct resection, biliary reconstruction, hepatectomy, lymph node dissection, and vascular reconstruction for vascular invasion as previously reported (13–15). Curative resection was performed by experienced surgeons in hepatobiliary surgery in the 3 institutions.

Data collection

Data was prospectively collected into a database used by the 3 participating hospitals and the study was conducted retrospectively. The data collected on patient demographic, preoperative laboratory, postoperative histopathological and surgical variables included gender, age, comorbidity, preoperative jaundice, preoperative hepatolithiasis, chronic hepatitis, American Society of Anesthesiologists (ASA) score, alanine aminotransferase (ALT), aspartate transaminase (AST), international normalized ratio (INR), albumin (ALB), hemoglobin (HGB), carbohydrate antigen 19-9 (CA 19-9), tumor size, degree of tumor differentiation, macrovascular invasion, microvascular invasion, lymph node (LN) involvement, nerve invasion, cirrhosis, 8th American Joint Committee on Cancer (AJCC) staging (16), extent of hepatectomy, PBT, perioperative blood loss and operation time.

Patients were divided into two groups according to the upper or lower limits of normal of each preoperative laboratory variable. Specifically, the following thresholds were employed: ALT and AST: 40 U/L, INR: 1.15, ALB: 35 g/L, HGB: 120 g/L, and CA 19-9: 37 U/L (13, 14, 17). All postoperative histopathological variables were confirmed by postoperative histopathological examination of tumor or nontumor tissues. Preoperative jaundice was defined as a preoperative total bilirubin higher than 37 μmol/L. Extent of hepatectomy was divided into major hepatectomy (three or more resected Couinaud liver segments) and minor hepatectomy (two or less resected Couinaud liver segments). In previous studies, pCCA patients with a tumor size > 3 cm showed poor long-term survival (13, 14). As a consequence, 3 cm was used to divide patients into 2 groups. Both portal vein invasion and hepatic artery invasion were considered as macrovascular invasion.

Perioperative blood transfusion

PBT was defined as transfusion of whole blood and/or packed red blood cells (PRBCs) either during surgery or within 7 days of surgery as determined from the surgical and postoperative medical records. PBT excluded autologous blood, allogeneic platelets, fresh frozen plasma, and cryoprecipitate. The need for intraoperative blood transfusions was determined by excessive intraoperative blood loss and/or hemodynamic instability. Postoperative blood transfusions were administered if the patient's hemoglobin level was below 70 g/L or the patient was hemodynamically unstable. Two units were the standard for transfusion (one unit of PRBCs refers to the red blood cells isolated from 200 ml of whole blood).

Survival outcomes and follow-up

The main outcomes were overall survival (OS) and recurrence-free survival (RFS). OS was defined as the interval from curative resection to death or the last follow-up. The definition of RFS for patients with recurrence was the interval from curative resection to recurrence, and for patients with no recurrence as the interval from curative resection to death or last follow-up. This study was censored on February 28, 2022. After discharged from hospital, patients were followed-up once every 1-2 months for 2 years after curative resection, once every 3-4 months for 3-5 years and then once every 6 months for 5 years.

Contrast-enhanced ultrasonography, contrast-enhanced computed tomography, and/or magnetic resonance cholangiopancreatography were performed at each follow-up. Conservative therapy, systemic chemotherapy, or repeat surgical resection were performed if patients were confirmed to have relapsed.

Statistical analysis

Continuous variables with normal distributions were presented as means and standard deviations (SDs) and were compared using the Student's t test, whereas continuous variables with non-normal distributions weare presented as medians with interquartile ranges (IQRs) and were compared using the Mann–Whitney U test. Categorical variables were presented as frequencies and percentages and were compared using the Pearson's chi-square test. All patients were divided into two groups according to whether PBT was given. All the baseline characteristics of the two groups were compared. To overcome the influence of selection bias, PSM was used to balance the differences in the baseline characteristics between the PBT and non-PBT groups. Tendency scoring system was used for PSM to integrate all observed variable information, in order to balance variable and reduce the bias. Potential variables which might affect PBT were included into the propensity model, including preoperative jaundice, ASA grade, INR, ALB, HGB, tumor size, cirrhosis, and extent of hepatectomy. Propensity scores for pCCA patients who received PBT or not were created using logistic regression estimation. A one-to-one match between the two groups was then performed using the nearest-neighbor matching method with a caliper width equal to 0.2 of the standard deviation of the logit of the propensity score. Kaplan–Meier curves were used to calculate the OS and RFS rates of patients, and the log-rank test was used for comparisons. Variables with a significance level of $P < 0.1$ in univariate analysis were included in multivariate analysis using the Cox regression model to determine independent predictors of OS and RFS. In addition, using the 8th AJCC staging system, all patients were divided into the early stage (AJCC stage I) group and the non-early stage (AJCC stage II-IV) group. Subgroup analysis was used to investigate the impact of PBT on OS and RFS for patients with different tumor stagings. SPSS® version 26.0 (IBM, Armonk, New York, United States) was used for all statistical analyses. A P value (two-sided) < 0.05 was considered statistically significant.

Results

Characteristics of all pCCA patients

Of 364 pCCA patients treated with curative resection during the study period, 62 patients were excluded according to the exclusion criteria, resulting in 302 pCCA patients being included in this study (Supplement Figure 1). There were 198 (65.6%) males, and 125 (41.4%) patients were more than 60 years old. The median follow-up time was 22.5 months. The PBT group had 130 patients (43.0%), and the non-PBT group had 172 patients (57.0%). Before PSM, baseline characteristics showed the PBT group to have significantly more patients with preoperative jaundice, ASA grade > II, INR > 1.15, ALB < 35 g/L, HGB < 120 g/L, tumor size > 3 cm, 8th AJCC stage II-IV disease, major hepatectomy, blood loss > 500 mL and operation time > 360 min than the non-PBT group. After PSM with 90 matched pairs of patients were analyzed, baseline characteristics of the PBT group still showed significantly more patients with the 8th AJCC stage II-IV disease than the non-PBT group (Table 1).

Long-term survival of all pCCA patients

On follow-up, before PSM, the 5-year OS rates for all pCCA patients treated with curative resection were 18.9% in the PBT group and 29.4% in the non-PBT group, respectively, while the 5-year RFS rates were 10.6% in the PBT group and 19.5% in the non-PBT group, respectively. After PSM, the 5-year OS rates for all pCCA patients treated with curative resection were 22.7% in the PBT group and 27.8% in the non-PBT group, respectively, while the 5-year RFS rates were 11.4% in the PBT group and 18.0% in the non-PBT group, respectively (Supplement Table 1). Both before and after PSM, Kaplan–Meier curves revealed that there were no significant differences between the PBT and non-PBT groups in OS and RFS (Figure 1).

Characteristics of patients with early stage pCCA

68 patients with early stage (AJCC stage I) pCCA were treated with curative resection. Among these patients, 22 patients (32.4%) were in the PBT group, and 46 patients (67.6%) were in the non-PBT group. Before PSM, baseline characteristics showed the PBT group to have significantly more patients with an ALB < 35 g/L, HGB < 120 g/L and blood loss > 500 mL than the non-PBT group. After PSM with 18 matched pairs of patients being analyzed, there were no significant differences in baseline characteristics between the PBT group and the non-PBT group (Table 2).

Long-term survival of patients with early stage pCCA

On follow-up, before PSM, the 5-year OS rates of patients with early stage pCCA treated with curative resection were 32.6% in the PBT group and 62.2% in the non-PBT group,

TABLE 1 Clinicopathologic characteristics of the PBT and non-PBT groups among all pCCA patients treated with curative resection.

Variables	Before PSM			After PSM		
	PBT (n=130)	Non-PBT (n=172)	*P* value [a]	PBT (n=90)	Non-PBT (n=90)	*P* value [a]
Male	90 (69.2)	108 (62.8)	0.224	62 (68.2)	62 (68.2)	1.000
Age > 60 years	56 (43.1)	69 (40.1)	0.605	40 (44.4)	46 (51.1)	0.371
Comorbidity	33 (25.4)	39 (22.7)	0.584	27 (30.0)	21 (23.3)	0.312
Preoperative jaundice	108 (83.1)	102 (59.3)	< 0.001	70 (77.8)	70 (77.8)	1.000
Preoperative hepatolithiasis	11 (8.5)	14 (8.1)	0.920	8 (8.9)	7 (7.8)	0.787
Chronic hepatitis	16 (12.3)	11 (6.4)	0.075	10 (11.1)	9 (10.0)	0.808
ASA grade > II	19 (14.6)	12 (7.0)	0.030	9 (10.0)	10 (11.1)	0.808
ALT > 40 U/L	110 (84.6)	140 (81.4)	0.463	76 (84.4)	76 (84.4)	1.000
AST > 40 U/L	109 (83.8)	135 (78.5)	0.242	76 (84.4)	75 (83.3)	0.839
INR > 1.15	17 (13.1)	10 (5.8)	0.029	5 (5.6)	8 (8.9)	0.388
ALB < 35 g/L	59 (45.4)	51 (29.7)	0.005	40 (44.4)	34 (37.8)	0.363
HGB < 120 g/L	40 (30.8)	33 (19.2)	0.020	27 (30.0)	22 (24.4)	0.402
CA 19-9 > 37 U/L	98 (75.4)	126 (73.3)	0.676	66 (73.3)	65 (72.2)	0.867
Tumor size > 3 cm	59 (45.4)	56 (32.6)	0.023	39 (43.3)	40 (44.4)	0.881
Poor differentiation	22 (16.9)	20 (11.6)	0.188	16 (17.8)	13 (14.4)	0.543
Macrovascular invasion	39 (30.0)	43 (25.0)	0.333	20 (22.2)	12 (13.3)	0.119
Microvascular invasion	15 (11.5)	19 (11.0)	0.893	8 (8.9)	11 (12.2)	0.467
LN involvement	55 (42.3)	61 (35.5)	0.226	35 (38.9)	33 (36.7)	0.758
Peripheral nerve invasion	46 (35.4)	53 (30.8)	0.402	34 (37.8)	29 (32.2)	0.435
Cirrhosis	15 (11.5)	10 (5.8)	0.074	6 (6.7)	9 (10.0)	0.418
8th AJCC stage II-IV	108 (83.1)	126 (73.3)	0.043	75 (83.3)	56 (62.2)	0.001
Major hepatectomy	94 (72.3)	102 (59.3)	0.019	62 (68.9)	59 (65.6)	0.634
Blood loss > 500 mL	98 (75.4)	99 (57.6)	0.001	63 (70.0)	51 (56.7)	0.063
Operation time > 360 min	76 (58.5)	75 (43.6)	0.011	45 (50.0)	41 (45.6)	0.551

[a]The calibration formula of chi-square test was used.
ALB, albumin; ALT, alanine aminotransferase; AJCC, American Joint Committee on Cancer; ASA, American Society of Anesthesiologists; AST, aspartate transaminase; CA 19-9, carbohydrate antigen 19-9; HGB, hemoglobin; INR, international normalized ratio; LN, lymph node; pCCA, perihilar cholangiocarcinoma; PBT, perioperative blood transfusion; PSM, propensity score matching.

respectively, while the 5-year RFS rates were 13.2% in the PBT group and 47.9% in the non-PBT group, respectively. After PSM, the 5-year OS rates of patients with early stage pCCA treated with curative resection were 20.6% in the PBT group and 72.6% in the non-PBT group, respectively, while the 5-year RFS rates were 23.0% in the PBT group and 60.7% in the non-PBT group, respectively (Supplement Table 2). Both before and after PSM, Kaplan–Meier curves revealed that in patients with early stage pCCA, the OS and RFS rates in the PBT group were significantly lower than those in the non-PBT group (Figure 2). After PSM, multivariable analyses revealed that for patients with early stage pCCA, PBT and tumor size >3 cm to be independently associated with worse OS (Table 3) and RFS (Table 4).

Characteristics of patients with non-early stage pCCA

234 patients with non-early stage (AJCC stage II-IV) pCCA were treated with curative resection. Of which, 108 patients with AJCC stage II, pCCA 103 patients with AJCC stage III pCCA, 23 patients with AJCC stage IV pCCA were treated with curative resection. The PBT group had 108 patients (46.2%), and the non-PBT group had 126 patients (53.8%). Before PSM, baseline characteristics showed the PBT group to have significantly more patients with preoperative jaundice, chronic hepatitis, ASA > II grade, INR > 1.15, ALB < 35 g/L, blood loss > 500 ml, and operation time > 360 min than the non-PBT group. After PSM, with 72 matched pairs of patients were analyzed, there were no significant differences in baseline characteristics between the PBT group and non-PBT group (Table 5).

Long-term survival for patients with non-early stage pCCA

On follow-up, before PSM, the 5-year OS rates for patients with non-early stage pCCA treated with curative resection were 15.6% in the PBT group and 17.0% in the non-PBT group,

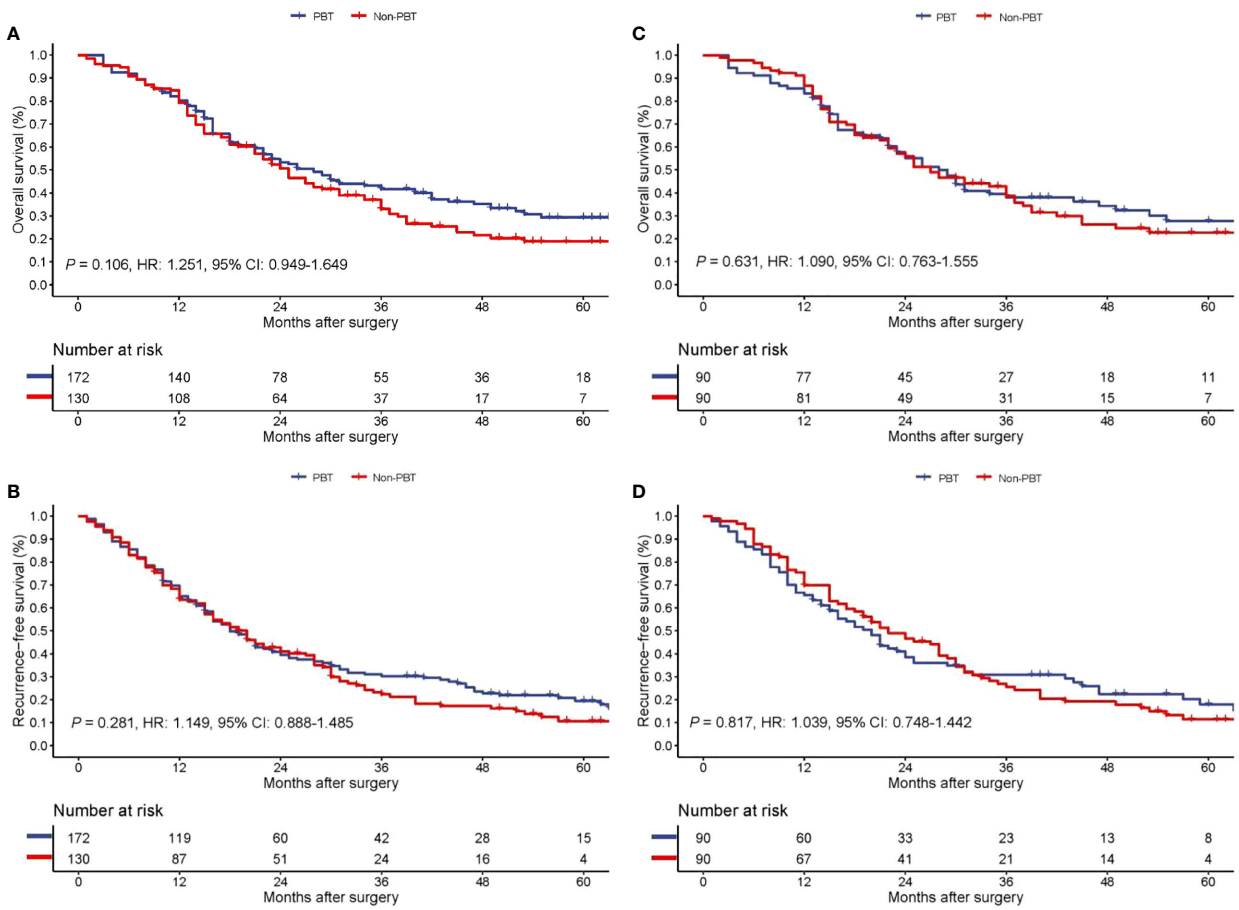


FIGURE 1
Kaplan–Meier curves of overall survival **(A)** and recurrence-free survival **(B)** between the PBT and non-PBT groups among all pCCA patients treated with curative resection before PSM. Kaplan–Meier curves of overall survival **(C)** and recurrence-free survival **(D)** between the PBT and non-PBT groups among all pCCA patients treated with curative resection after PSM. CI, confidence interval; HR, hazard ratio; PBT, perioperative blood transfusion; PSM, propensity score matching.

respectively, while the 5-year RFS rates were 10.6% in the PBT group and 8.5% in the non-PBT group, respectively. After PSM, the 5-year OS rates for patients with non-early stage pCCA treated with curative resection were 17.7% in the PBT group and 13.3% in the non-PBT group, respectively, while the 5-year RFS rates were 12.1% in the PBT group and 3.0% in the non-PBT group, respectively (Supplement Table 3). Both before and after PSM, Kaplan–Meier curves revealed in patients with non-early stage pCCA, there were no significant differences between the PBT and non-PBT groups in OS and RFS (Figure 3).

Discussion

PBT has been shown to be associate with perioperative safety of patients with hepatobiliary diseases. However, in some hepatobiliary diseases, such as hepatocellular carcinoma and colorectal liver metastases, immunomodulation brought on by PBT has been shown to associate with cancer recurrence (18–23). There have been very few studies reported on the association of PBT with long-term survival in pCCA patients. Radical resection of pCCA requires bile duct resection and reconstruction, hepatectomy, perihilar dissection, vascular resection and reconstruction if necessary, and it is a more complex, demanding, and high risk operation than resection for hepatocellular carcinoma or colorectal liver metastases. As a consequence, radical resection of pCCA has a greater need for PBT.

The association between PBT and long-term survival following resection for pCCA, to our knowledge, has only been studied in three previously published studies (8, 24, 25). Liu et al. observed a significant association between PBT and poor survival in 40 patients who underwent surgical resection for pCCA. However, blood transfusion could not be identified as

TABLE 2 Clinicopathologic characteristics of the PBT and non-PBT groups among patients with early stage (8th AJCC stage I) pCCA treated with curative resection.

Variables	Before PSM			After PSM		
	PBT (n = 22)	Non-PBT (n = 46)	*P* value [a]	PBT (n = 18)	Non-PBT (n = 18)	*P* value
Male	16 (72.7)	35 (76.1)	0.765	15 (83.3)	12 (66.7)	0.248
Age > 60 years	15 (68.2)	28 (60.9)	0.559	13 (72.3)	13 (72.3)	1.000
Comorbidity	8 (36.4)	9 (19.6)	0.134	6 (33.3)	6 (33.3)	1.000
Preoperative jaundice	18 (81.8)	27 (58.7)	0.059	14 (77.8)	15 (83.3)	0.674
Preoperative hepatolithiasis	1 (4.5)	4 (8.7)	0.540	1 (5.6)	1 (5.6)	1.000
Chronic hepatitis	3 (13.6)	5 (10.9)	0.740	3 (16.7)	2 (11.1)	0.630
ASA grade > II	3 (13.6)	6 (13.0)	0.946	3 (16.7)	3 (16.7)	1.000
ALT > 40 U/L	19 (86.4)	37 (80.4)	0.549	17 (94.4)	15 (83.3)	0.289
AST > 40 U/L	17 (77.3)	36 (78.3)	0.927	15 (83.3)	15 (83.3)	1.000
INR > 1.15	4 (18.2)	4 (8.7)	0.256	3 (16.7)	1 (5.6)	0.289
ALB < 35 g/L	11 (50.0)	11 (23.9)	0.031	7 (38.9)	7 (38.9)	1.000
HGB < 120 g/L	11 (50.0)	8 (17.4)	0.005	7 (38.9)	6 (33.3)	0.729
CA 19-9 > 37 U/L	14 (63.6)	23 (50.0)	0.291	11 (61.1)	10 (55.6)	0.735
Tumor size > 3 cm	7 (31.8)	11 (23.9)	0.489	7 (38.9)	5 (27.8)	0.480
Poor differentiation	2 (9.1)	3 (6.5)	0.704	2 (11.1)	2 (11.1)	1.000
Cirrhosis	4 (18.2)	4 (8.7)	0.256	4 (22.2)	2 (11.1)	0.371
Major hepatectomy	13 (59.1)	18 (39.1)	0.122	12 (66.7)	7 (38.9)	0.095
Blood loss > 500 mL	17 (77.3)	23 (50.0)	0.003	13 (72.2)	9 (50.0)	0.171
Operation time > 360 min	9 (40.9)	12 (26.1)	0.216	7 (38.9)	4 (22.2)	0.278

[a]The calibration formula of chi-square test was used.
ALB, albumin; ALT, alanine aminotransferase; AJCC, American Joint Committee on Cancer; ASA, American Society of Anesthesiologists; AST, aspartate transaminase; CA 19-9, carbohydrate antigen 19-9; HGB, hemoglobin; INR, international normalized ratio; pCCA, perihilar cholangiocarcinoma; PBT, perioperative blood transfusion; PSM, propensity score matching.

an independent predictor in multivariate analysis of this study (24). In contrast, Young et al. demonstrated through multivariate analysis that PBT was a significant independent predictor of poor survival following surgery in a study of 83 patients with pCCA (25). Similarly, Kimura et al. retrospectively analysed the clinical data of 66 patients with pCCA who underwent surgical resection and found PBT to be an independent risk factor for poor OS and disease-free survival (8). The controversial results of the above three studies may well be due to differences in patient baseline characteristics, timings of the studies, tumor stagings and surgery types. However, in our opinion, the differences may be associated more with small sample sizes and selection biases of the studies. First, all these three studies had sample sizes of less than 100 patients coming from a single institution. The validity of the results of these studies could be improved by expanding the sample size and enrolling patients from multicenters. Second, as conducting a randomized controlled trial for PBT is not feasible due to ethical issues, PSM analysis can be used to minimize selection bias when randomized controlled studies cannot be carried out (26) in the same way as studies investigating the association between PBT and long-term survival of hepatocellular carcinoma patients using PSM analyses (11, 12).

To our knowledge, our study is the first study using PSM analysis and a multicenter database to investigate the impact of PBT on OS and RFS in patients with different stages of pCCA treated with curative resection. Of the 302 pCCA patients from three institutions included in this study, univariable analysis indicated that PBT did not adversely affect long-term survival of pCCA patients treated with curative resection. Two commonly used tumour staging systems or classifications were evaluated at the outset of this study, including the 8th AJCC staging system and the Bismuth classification to divide these patients into an early stage group and a non-early stage group to study the long-term survival of patients with different stagings of pCCA. The Bismuth classification was more relevant for choosing surgical procedures rather than classifying the degrees of tumor invasion. To better reflect the extent and location of tumor invasion, this study chose the 8th AJCC staging to group these patients. After grouping, the PBT rate of patients in the early stage group was significantly lower than that in the non-early stage group (32.4% vs. 46.2%). On long-term survival analysis, multivariable Cox

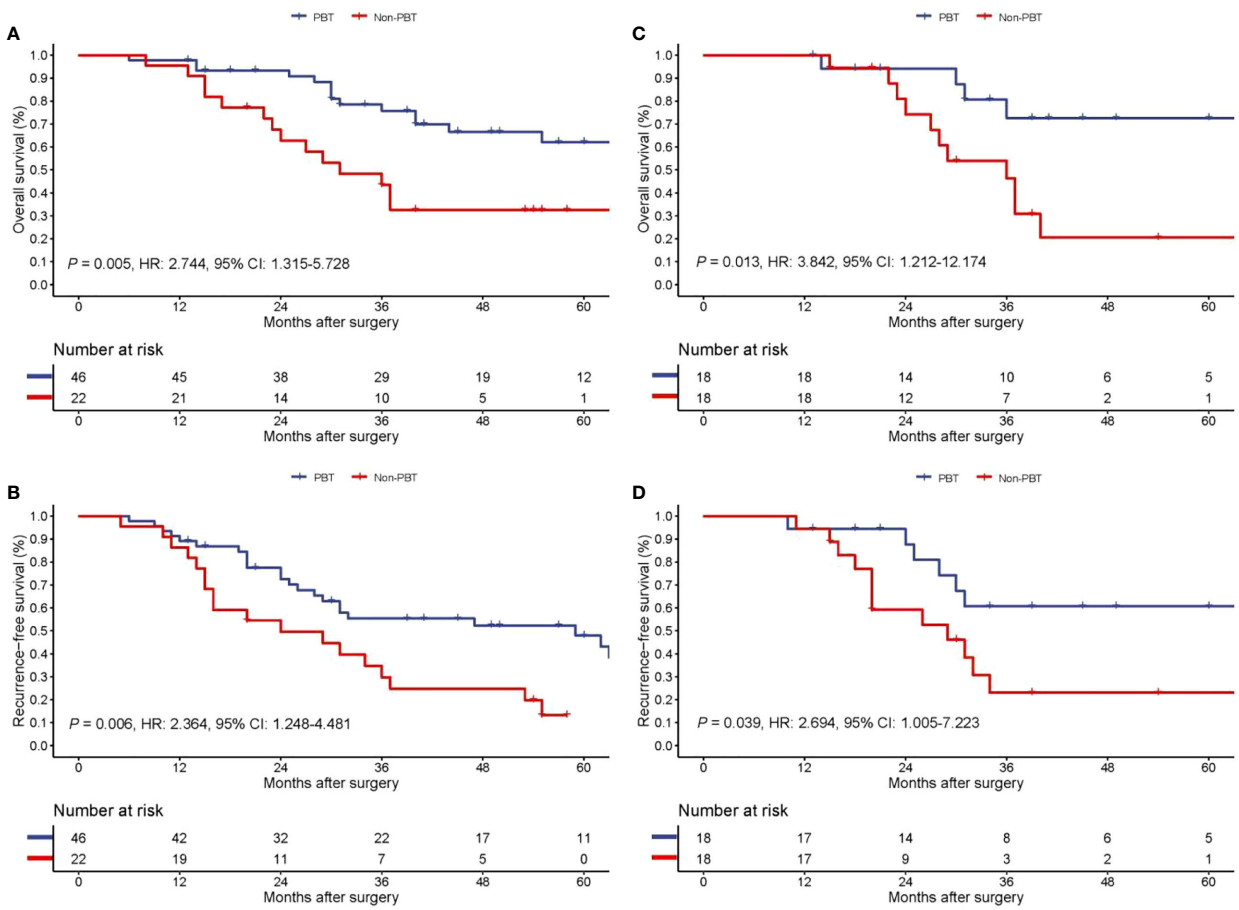


FIGURE 2
Kaplan–Meier curves of overall survival **(A)** and recurrence-free survival **(B)** between the PBT and non-PBT group among patients with early stage (8th AJCC stage I) pCCA treated with curative resection before PSM. Kaplan–Meier curves of overall survival **(C)** and recurrence-free survival **(D)** between the PBT and non-PBT group among patients with early stage (8th AJCC stage I) pCCA treated with curative resection after PSM. CI, confidence interval; HR, hazard ratio; PBT, perioperative blood transfusion; PSM, propensity score matching.

regression analysis showed that PBT was independently associated with decreased OS and RFS rates in pCCA patients in the early-stage group treated with curative resection. However, in patients with non-early stage pCCA treated with curative resection, univariable analysis suggested that PBT had no significant effect on OS and RFS.

These exciting and interesting results can be explained by the conclusions drawn from the following reported studies. Blood transfusion has been well documented to increase immunosuppression in the host to promote cancer recurrence and metastasis. Blood transfusion in basic and clinical studie ahs been shown to decrease host immunity by reducing natural killer cell activity and cytotoxic T-cell function, increase suppressor T-cell activity, and decrease helper/suppressor (T4/T8) lymphocyte ratios (27, 28). In addition, normal physiological ageing and metabolic processes result in leaching of biologically active substances from cells into stored blood products. These leached bioactive substances have immunomodulatory effects that promote cell growth and angiogenesis and may therefore have a direct effect on tumor growth (29). The immunosuppressive impact of blood transfusion may therefore have a significant influence on recurrence of malignant tumors. A recent study by Goeppert et al. indicated that presence of both intratumoral T and B cells to be associated with prolonged survival in patients with cholangiocarcinoma and that prognosis was associated with inflammation (30). These findings provide a strong foundation in understanding the biological significance of inflammatory infiltrates in cholangiocarcinoma, as well as for further functional and clinical investigations on regulation of inflammatory responses in cholangiocarcinoma patients (30). Although immunosuppression may influence recurrence and survival in cholangiocarcinoma patients, the deleterious consequences of blood transfusion on host immunity remain unknown.

TABLE 3 Univariable and multivariable analyses of independent risk factors for overall survival among patients with early stage (8th AJCC stage I) pCCA treated with curative resection after propensity score matching.

Variables	Comparison	Univariable analyses		Multivariable analyses*	
		P value	HR (95% CI)	*P* value	HR (95% CI)
PBT	Yes *vs.* No	0.022	3.842 (1.212-12.174)	0.024	3.772 (1.187-11.983)
Sex	Male *vs.* Female	0.863	0.904 (0.286-2.852)		
Age, years	> 60 vs. ≤ 60	0.981	1.014 (0.322-3.195)		
Comorbidity	Yes vs. No	0.445	1.497 (0.532-4.212)		
Preoperative jaundice	Yes vs. No	0.561	1.556 (0.350-6.918)		
Preoperative hepatolithiasis	Yes vs. No	0.560	1.841 (0.237-14.302)		
Chronic hepatitis	Yes vs. No	0.631	1.443 (0.323-6.438)		
ASA grade > II	Yes vs. No	0.358	2.032 (0.448-9.211)		
ALT, U/L	> 40 *vs.* ≤ 40	0.320	2.807 (0.367-21.453)		
AST, U/L	> 40 vs. ≤ 40	0.124	4.932 (0.645-37.704)		
INR	> 1.15 vs. ≤ 1.15	0.210	2.265 (0.630-8.146)		
ALB, g/L	< 35 vs. ≥ 35	0.572	1.349 (0.478-3.806)		
HGB, g/L	< 110 vs. ≥ 100	0.176	2.017 (0.731-5.569)		
CA 19-9, U/L	> 37 vs. ≤ 37	0.065	2.959 (0.937-9.350)	0.147	NA
Tumor size, cm	> 3 vs. ≤ 3	0.013	3.707 (1.317-10.432)	0.015	3.683 (1.287-10.279)
Poor differentiation	Yes vs. No	0.531	1.612 (0.362-7.178)		
Cirrhosis	Yes vs. No	0.483	1.574 (0.442-5.601)		
Major hepatectomy	Yes vs. No	0.146	2.346 (0.743-7.415)		

*Those variables that had a P <.10 in the univariable analyses were entered into the multivariable analyses.
ALB, albumin; ALT, alanine aminotransferase; AJCC, American Joint Committee on Cancer; ASA, American Society of Anesthesiologists; AST, aspartate transaminase; CA 19-9, carbohydrate antigen 19-9; HGB, hemoglobin; INR, international normalized ratio; NA, not available; pCCA, perihilar cholangiocarcinoma; PBT, perioperative blood transfusion.

TABLE 4 Univariable and multivariable analyses of independent risk factors for recurrence-free survival among patients with early stage (8th AJCC stage I) pCCA treated with curative resection after propensity score matching.

Variables	Comparison	Univariable analyses		Multivariable analyses*	
		P value	HR (95% CI)	*P* value	HR (95% CI)
PBT	Yes vs. No	0.049	2.694 (1.005-7.223)	0.015	3.709 (1.289-10.674)
Sex	Male vs. Female	0.724	0.829 (0.294-2.337)		
Age, years	> 60 vs. ≤ 60	0.568	1.383 (0.454-4.216)		
Comorbidity	Yes vs. No	0.323	1.615 (0.624-4.181)		
Preoperative jaundice	Yes vs. No	0.738	1.263 (0.358-4.274)		
Preoperative hepatolithiasis	Yes vs. No	0.855	1.207 (0.160-9.135)		
Chronic hepatitis	Yes vs. No	0.963	1.035 (0.237-4.519)		
ASA grade > II	Yes vs. No	0.646	1.419 (0.320-6.296)		
ALT, U/L	> 40 vs. ≤ 40	0.235	3.399 (0.451-25.635)		
AST, U/L	> 40 vs. ≤ 40	0.082	6.034 (0.798-45.615)	0.060	NA
INR	> 1.15 vs. ≤ 1.15	0.367	1.775 (0.511-6.167)		
ALB, g/L	< 35 vs. ≥ 35	0.727	1.184 (0.458-3.062)		
HGB, g/L	< 110 vs. ≥ 100	0.367	1.539 (0.603-3.933)		
CA 19-9, U/L	> 37 vs. ≤ 37	0.126	2.160 (0.805-5.794)		
Tumor size, cm	> 3 vs. ≤ 3	0.023	2.954 (1.160-7.524)	0.006	4.108 (1.485-11.362)
Poor differentiation	Yes vs. No	0.228	2.160 (0.617-7.558)		
Cirrhosis	Yes vs. No	0.885	1.096 (0.316-3.794)		
Major hepatectomy	Yes vs. No	0.573	1.314 (0.509-3.393)		

*Those variables that had a P <.10 in the univariable analyses were entered into the multivariable analyses.
ALB, albumin; ALT, alanine aminotransferase; AJCC, American Joint Committee on Cancer; ASA, American Society of Anesthesiologists; AST, aspartate transaminase; CA 19-9, carbohydrate antigen 19-9; HGB, hemoglobin; INR, international normalized ratio; NA, not available; pCCA, perihilar cholangiocarcinoma; PBT, perioperative blood transfusion.

TABLE 5 Clinicopathologic characteristics of the PBT and non-PBT groups among patients with non-early stage (8th AJCC stage II-IV) pCCA treated with curative resection.

Variables	Before PSM			After PSM		
	PBT (n = 108)	Non-PBT (n = 126)	*P* value [a]	PBT (n = 72)	Non-PBT (n = 72)	*P* value [a]
Male	74 (68.5)	73 (57.9)	0.096	48 (66.7)	41 (56.9)	0.230
Age > 60 years	41 (38.0)	41 (32.5)	0.387	27 (37.5)	25 (34.7)	0.729
Comorbidity	25 (23.1)	30 (23.8)	0.906	19 (26.4)	15 (20.8)	0.433
Preoperative jaundice	90 (83.3)	77 (61.1)	< 0.001	56 (77.8)	58 (80.6)	0.682
Preoperative hepatolithiasis	10 (9.3)	10 (7.9)	0.719	8 (11.1)	5 (6.9)	0.383
Chronic hepatitis	13 (12.0)	6 (4.8)	0.043	6 (8.3)	5 (6.9)	0.754
ASA grade > II	16 (14.8)	6 (4.8)	0.009	7 (9.7)	4 (5.6)	0.347
ALT > 40 U/L	91 (84.3)	103 (81.7)	0.611	64 (88.9)	59 (81.9)	0.238
AST > 40 U/L	92 (85.2)	99 (78.6)	0.194	62 (86.1)	56 (77.8)	0.194
INR > 1.15	13 (12.0)	6 (4.8)	0.043	4 (5.6)	4 (5.6)	1.000
ALB < 35 g/L	48 (44.4)	40 (31.7)	0.046	30 (41.7)	27 (37.5)	0.609
HGB < 120 g/L	29 (26.9)	25 (19.8)	0.205	20 (27.8)	11 (15.3)	0.068
CA 19-9 > 37 U/L	84 (77.8)	103 (81.7)	0.451	56 (77.8)	57 (79.2)	0.839
Tumor size > 3 cm	52 (48.1)	45 (35.7)	0.055	27 (37.5)	33 (45.8)	0.310
Poor differentiation	20 (18.5)	17 (13.5)	0.294	12 (16.7)	12 (16.7)	1.000
Macrovascular invasion	39 (36.1)	43 (34.1)	0.751	28 (38.9)	18 (25.0)	0.074
Microvascular invasion	15 (12.0)	19 (15.1)	0.797	6 (8.3)	12 (16.7)	0.131
LN involvement	55 (50.9)	61 (48.4)	0.701	30 (41.7)	38 (52.8)	0.182
Peripheral nerve invasion	46 (42.6)	53 (42.1)	0.935	26 (36.1)	24 (33.3)	0.726
Cirrhosis	39 (36.1)	43 (34.1)	0.752	7 (9.7)	2 (2.8)	0.085
Major hepatectomy	15 (13.9)	19 (15.1)	0.797	51 (70.8)	51 (70.8)	1.000
Blood loss > 500 mL	55 (50.9)	61 (48.4)	0.702	51 (70.8)	43 (59.7)	0.161
Operation time > 360 min	40 (37.0)	40 (31.7)	0.396	39 (54.2)	37 (51.4)	0.738

[a]The calibration formula of chi-square test was used.
ALB, albumin; ALT, alanine aminotransferase; AJCC, American Joint Committee on Cancer; ASA, American Society of Anesthesiologists; AST, aspartate transaminase; CA 19-9, carbohydrate antigen 19-9; HGB, hemoglobin; INR, international normalized ratio; pCCA, perihilar cholangiocarcinoma; PBT, perioperative blood transfusion; PSM, propensity score matching.

In our study, all patients were staged using the 8th AJCC staging system, and patients with early stage disease had tumors confined to the bile ducts. On the other hand, for individuals with non-early stage disease, their tumors had exhibited at least one of the following characteristics: invasion into surrounding adipose tissues, invasion into adjacent liver, invasion into one (or more) portal vein branches hepatic artery/common hepatic artery, lymph node invasion, and distant metastases. We hypothesize that the difference between the impact of PBT on prognosis of patients with early stage and non-early stage pCCA are the results of the difference in biological behaviors of the tumors in the 2 groups. PBT had detrimental effects on prognosis of patients with early stage disease, but its impact on prognosis of patients with more advanced diseases was obscured by the invasive and/or metastatic behavior of the tumors.

For pCCA patients who received PBT, the effects of postoperative adjuvant therapy remain to be further studied, as such a treatment way improve long-term survival. At present, immune checkpoint inhibitors have achieved remarkable results in biliary tract cancer, and some immune checkpoint inhibitors have achieved breakthroughs in clinical studies (clinical trial information: NCT03875235 and NCT03875235) (31). Since PBT could lead to immunosuppression in tumor patients who underwent radical surgery, it is worth studying whether such patients should receive adjuvant immunotherapy after surgery.

This study has several limitations. First, this retrospective study has its inherent defects, PSM analysis was used in this study to minimize selection bias. Second, there was only a small sample size of patients with early stage pCCA. However, as pCCA is a highly malignant tumor and it has no specific symptoms in the early stages, most patients in this study were already in the non-early stage at diagnosis. Patients enrolled in this study were much higher than those in other studies which investigated the association between long-term survival of pCCA patients with PBT. Third, patient selection and surgical procedures were not standardized among the three institutions in this study. For a multicenter study, such a bias cannot be completely be avoided. Despite this, the surgery

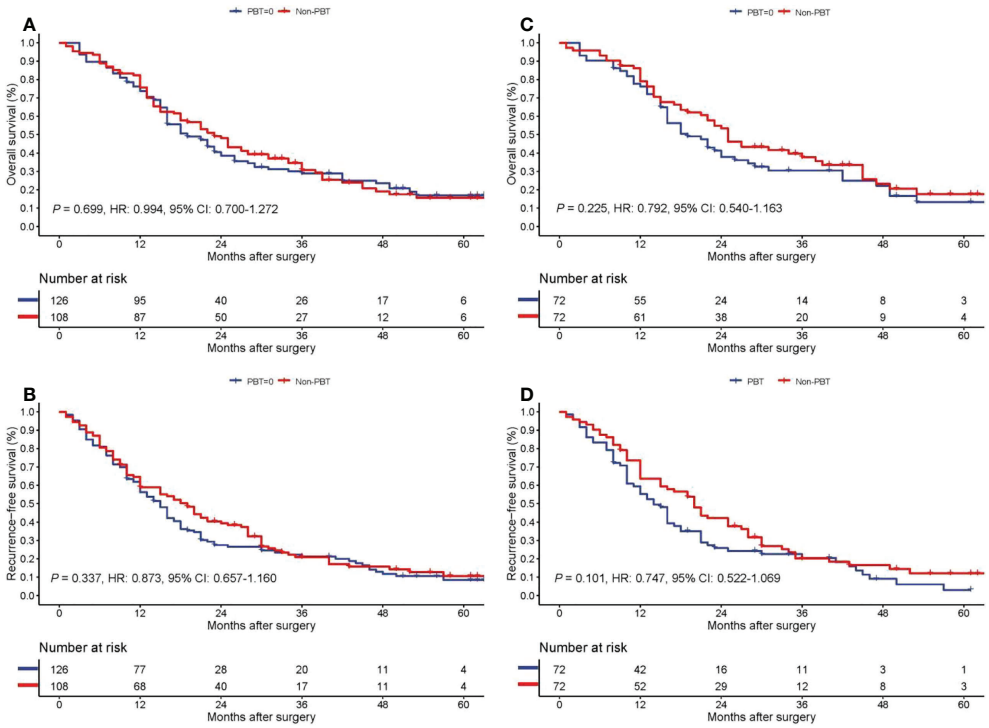


FIGURE 3
Kaplan–Meier curves of overall survival **(A)** and recurrence-free survival **(B)** for the PBT and non-PBT groups among patients with non-early stage (8th AJCC stage II-IV) pCCA treated with curative resection before PSM. Kaplan–Meier curves of overall survival **(C)** and recurrence-free survival **(D)** for the PBT and non-PBT groups among patients with non-early stage (8th AJCC stage II-IV) pCCA treated with curative resection after PSM. CI, confidence interval; HR, hazard ratio; PBT, perioperative blood transfusion; PSM, propensity score matching.

was all performed by surgeons with rich experience in hepatobiliary surgery.

In conclusion, PBT was demonstrated in this study to be independently associated with worse long-term survival in patients with early stage pCCA treated with curative resection, but not in patients with non-early stage diseases. To improve the long-term survival of pCCA patients treated with curative resection, particularly those with early stage disease, PBT should be avoided if technically possible.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

This study complied with the Declaration of Helsinki and was approved by the Ethics Committees of the 3 participating hospitals. The patients/participants provided their written informed consent to participate in this study.

Author contributions

Conception, Z-YC, Z-PL; Study design, Z-PL, Z-JC, H-SD, S-YZ, D-CZ, Z-YC, S-QD; Administrative support, Z-YC, S-QD; Data collection and acquisition, S-YZ, J-HZ, X-YC, TY, X-CL, J-JC, JB, YJ; Data analysis, Z-PL, W-YC, Z-RW, Y-QZ; Manuscript preparation, Z-PL, Z-JC, H-SD; Critical revision, Z-YC, S-QD, WL; Final approval of manuscript, All authors.

Funding

This work was supported in part by the National Natural Science Foundation of China (No. 81874211) and Chongqing Technology Innovation and Application Development Special Key Project (No. CSTC2021jscx-gksb-N0009).

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2022.1059581/full#supplementary-material

References

1. Razumilava N, Gores GJ. Cholangiocarcinoma. *Lancet* (2014) 383 (9935):2168–79. doi: 10.1016/S0140-6736(13)61903-0

2. Rizvi S, Khan SA, Hallemeier CL, Kelley RK, Gores GJ. Cholangiocarcinoma - evolving concepts and therapeutic strategies. *Nat Rev Clin Oncol* (2018) 15(2):95–111. doi: 10.1038/nrclinonc.2017.157

3. Nath MC, Torbenson MS, Erickson LA. Perihilar cholangiocarcinoma. *Mayo Clin Proc* (2018) 93(3):397–8. doi: 10.1016/j.mayocp.2018.01.017

4. Balci D, McCormack L. Perihilar cholangiocarcinoma: A difficult surgery in a difficult patient where experience matters most. *Surgery* (2021) 170(2):644–5. doi: 10.1016/j.surg.2021.02.049

5. Cillo U, Fondevila C, Donadon M, Gringeri E, Schlitt HS, Ijzermans JNM, et al. Surgery for cholangiocarcinoma. *Liver Int* (2019) 39 Suppl 1(Suppl Suppl 1):143–55. doi: 10.1111/liv.14089

6. Li Z, Sun YM, Wu FX, Yang LQ, Lu ZJ, Yu WF. Controlled low central venous pressure reduces blood loss and transfusion requirements in hepatectomy. *World J Gastroenterol* (2014) 20(1):303–9. doi: 10.3748/wjg.v20.i1.303

7. Müller SA, Mehrabi A, Rahbari NN, Warschkow R, Elbers H, Leowardi C, et al. Allogeneic blood transfusion does not affect outcome after curative resection for advanced cholangiocarcinoma. *Ann Surg Oncol* (2014) 21(1):155–64. doi: 10.1245/s10434-013-3226-9

8. Kimura N, Toyoki Y, Ishido K, Kudo D, Yakoshi Y, Tsutsumi S, et al. Perioperative blood transfusion as a poor prognostic factor after aggressive surgical resection for hilar cholangiocarcinoma. *J Gastrointest Surg* (2015) 19(5):866–79. doi: 10.1007/s11605-014-2741-8

9. Blajchman MA. Immunomodulation and blood transfusion. *Am J Ther* (2002) 9(5):389–95. doi: 10.1097/00045391-200209000-00005

10. Bordin JO, Blajchman MA. Immunosuppressive effects of allogeneic blood transfusions: implications for the patient with a malignancy. *Hematol Oncol Clin North Am* (1995) 9(1):205–18. doi: 10.1016/S0889-8588(18)30117-5

11. Peng T, Wang L, Cui H, Li X, Liu M, Yu J, et al. Impact of perioperative allogeneic blood transfusion on the long-term prognosis of patients with different stage tumors after radical resection for hepatocellular carcinoma. *Eur J Surg Oncol* (2021) 47:620–7. doi: 10.1016/j.ejso.2020.09.021

12. Chen GX, Qi CY, Hu WJ, Wang XH, Hua YP, Kuang M, et al. Perioperative blood transfusion has distinct postsurgical oncologic impact on patients with different stage of hepatocellular carcinoma. *BMC Cancer* (2020) 20:487. doi: 10.1186/s12885-020-06980-5

13. Liu ZP, Chen WY, Wang ZR, Liu XC, Fan HN, Xu L, et al. Development and validation of a prognostic model to predict recurrence-free survival after curative resection for perihilar cholangiocarcinoma: A multicenter study. *Front Oncol* (2022) 12:849053. doi: 10.3389/fonc.2022.849053

14. Liu ZP, Chen WY, Zhang YQ, Jiang Y, Bai J, Pan Y, et al. Postoperative morbidity adversely impacts oncological prognosis after curative resection for hilar cholangiocarcinoma. *World J Gastroenterol* (2022) 28(9):948–60. doi: 10.3748/wjg.v28.i9.948

15. Liu ZP, Zhang QY, Chen WY, Huang YY, Zhang YQ, Gong Y, et al. Evaluation of four lymph node classifications for the prediction of survival in hilar cholangiocarcinoma. *J Gastrointest Surg* (2022) 26(5):1030–40. doi: 10.1007/s11605-021-05211-x

16. Chun YS, Pawlik TM, Vauthey JN. 8th edition of the AJCC cancer staging manual: Pancreas and hepatobiliary cancers. *Ann Surg Oncol* (2018) 25(4):845–7. doi: 10.1245/s10434-017-6025-x

17. Liu ZP, Yao LQ, Diao YK, Chen ZX, Feng ZH, Gu WM, et al. Association of preoperative body mass index with surgical textbook outcomes following hepatectomy for hepatocellular carcinoma: A multicenter study of 1206 patients. *Ann Surg Oncol* (2022) 29:4278–86. doi: 10.1245/s10434-022-11721-y

18. Gozzetti G, Mazziotti A, Grazi GL, Jovine E, Gallucci A, Gruttadauria S, et al. Liver resection without blood transfusion. *Br J Surg* (1995) 82(8):1105–10. doi: 10.1002/bjs.1800820833

19. Yamamoto J, Kosuge T, Takayama T, Yamamoto J, Shimada K, Inoue K, et al. Perioperative blood transfusion promotes recurrence of hepatocellular carcinoma after hepatectomy. *Surgery* (1994) 115(3):303–9.

20. Shiba H, Ishida Y, Wakiyama S, Iida T, Matsumoto M, Sakamoto S, et al. Negative impact of blood transfusion on recurrence and prognosis of hepatocellular carcinoma after hepatic resection. *J Gastrointest Surg* (2009) 13 (9):1636–42. doi: 10.1007/s11605-009-0963-y

21. Wang CC, Iyer SG, Low JK, Lin CY, Wang SH, Lu SN, et al. Perioperative factors affecting long-term outcomes of 473 consecutive patients undergoing hepatectomy for hepatocellular carcinoma. *Ann Surg Oncol* (2009) 16(7):1832–42. doi: 10.1245/s10434-009-0448-y

22. Stephenson KR, Steinberg SM, Hughes KS, Vetto JT, Sugarbaker PH, Chang AE. Perioperative blood transfusions are associated with decreased time to recurrence and decreased survival after resection of colorectal liver metastases. *Ann Surg* (1988) 208(6):679–87. doi: 10.1097/00000658-198812000-00002

23. Kooby DA, Stockman J, Ben-Porat L, Gonen M, Jarnagin WR, Dematteo RP, et al. Influence of transfusions on perioperative and long-term outcome in patients following hepatic resection for colorectal metastases. *Ann Surg* (2003) 237 (6):860–9. doi: 10.1097/01.SLA.0000072371.95588.DA

24. Liu CL, Fan ST, Lo CM, Tso WK, Lam CM, Wong J. Improved operative and survival outcomes of surgical treatment for hilar cholangiocarcinoma. *Br J Surg* (2006) 93(12):1488–94. doi: 10.1002/bjs.5482

25. Young AL, Igami T, Senda Y, Adair R, Farid S, Toogood GS, et al. Evolution of the surgical management of perihilar cholangiocarcinoma in a Western centre demonstrates improved survival with endoscopic biliary drainage and reduced use of blood transfusion. *HPB (Oxford)* (2011) 13(7):483–93. doi: 10.1111/j.1477-2574.2011.00328.x

26. Staffa SJ, Zurakowski D. Five steps to successfully implement and evaluate propensity score matching in clinical research studies. *Anesth Analg* (2018) 127:1066–73. doi: 10.1213/ANE.0000000000002787

27. Gascón P, Zoumbos NC, Young NS. Immunologic abnormalities in patients receiving multiple blood transfusions. *Ann Intern Med* (1984) 100(2):173–7. doi: 10.7326/0003-4819-100-2-173

28. Kaplan J, Sarnaik S, Gitlin J, Lusher J. Diminished helper/suppressor lymphocyte ratios and natural killer activity in recipients of repeated blood transfusions. *Blood* (1984) 64(1):308–10. doi: 10.1182/blood.V64.1.308.308

29. Upile T, Jerjes W, Mahil J, Upile N, Sudhoff H, Wright A, et al. An explanation for the worsened prognosis in some cancer patients of perioperative transfusion: the time-dependent release of biologically active growth factors from stored blood products. *Eur Arch Otorhinolaryngol* (2011) 268(12):1789–94. doi: 10.1007/s00405-011-1525-y

30. Goeppert B, Frauenschuh L, Zucknick M, Stenzinger A, Andrulis M, Klauschen F, et al. Prognostic impact of tumour-infiltrating immune cells on biliary tract cancer. *Br J Cancer* (2013) 109:2665–74. doi: 10.1038/bjc.2013.610

31. Collingridge D. ASCO annual meeting. *Lancet Oncol* (2022) 23(7):844.

frontiers | Frontiers in Oncology

TYPE Original Research
PUBLISHED 10 November 2022
DOI 10.3389/fonc.2022.1040508

OPEN ACCESS

EDITED BY
Xiaodong Tian,
First Hospital, Peking University, China

REVIEWED BY
Tullio Piardi,
Centre Hospitalier Universitaire de Reims, France
Tian Yang,
Eastern Hepatobiliary Surgery Hospital, China
Xu-an Wang,
Shanghai Jiao Tong University, China

*CORRESPONDENCE
Pimsiri Sripongpun
spimsiri@medicine.psu.ac.th

SPECIALTY SECTION
This article was submitted to Gastrointestinal Cancers: Hepato Pancreatic Biliary Cancers, a section of the journal Frontiers in Oncology

RECEIVED 09 September 2022
ACCEPTED 24 October 2022
PUBLISHED 10 November 2022

CITATION
Pattarapuntakul T, Charoenrit T, Netinatsunton N, Yaowmaneerat T, Pitakteerabundit T, Ovartlarnporn B, Attasaranya S, Wong T, Chamroonkul N and Sripongpun P (2022) Postoperative outcomes of resectable periampullary cancer accompanied by obstructive jaundice with and without preoperative endoscopic biliary drainage. *Front. Oncol.* 12:1040508. doi: 10.3389/fonc.2022.1040508

Postoperative outcomes of resectable periampullary cancer accompanied by obstructive jaundice with and without preoperative endoscopic biliary drainage

Tanawat Pattarapuntakul[1], Tummarong Charoenrit[1], Nisa Netinatsunton[2], Thanapon Yaowmaneerat[2], Thakerng Pitakteerabundit[3], Bancha Ovartlarnporn[2], Siriboon Attasaranya[2], Thanawin Wong[1], Naichaya Chamroonkul[1] and Pimsiri Sripongpun[1]*

[1]Gastroenterology and Hepatology Unit, Division of Internal Medicine, Faculty of Medicine, Prince of Songkla University, Hat Yai, Songkhla, Thailand, [2]Nanthana-Kriangkrai Chotiwattanaphan (NKC) institute of Gastroenterology and Hepatology, Faculty of Medicine, Prince of Songkla University, Hat Yai, Songkhla, Thailand, [3]HepatoPancreatoBiliary surgery unit, Department of Surgery, Faculty of Medicine, Prince of Songkla University, Hat Yai, Songkhla, Thailand

Background: Preoperative biliary drainage (PBD) is useful in resectable periampullary cancer with obstructive jaundice. Whether it is better than direct surgery (DS) in terms of postoperative complications and mortality is controversial.

Methods: All cases of successful pancreaticoduodenectomy (PD) in patients with periampullary cancer with obstructive jaundice performed between January 2016 and January 2021 were retrospectively reviewed. Endoscopic PBD was performed; data pertaining to serum bilirubin level, procedural technique, and duration before surgery were obtained. The incidence of postoperative complications and survival rate were compared between the PBD and DS group.

Results: A total of 104 patients (PBD, n = 58; DS, n = 46) underwent curative PD. The mean age was 63.8 ± 10 years and 53 (51%) were male. Age, body mass index (BMI), sex, Eastern Cooperative Oncology Group status, presence of comorbid disease, initial laboratory results, and pathological diagnoses were not significantly different between the two groups. The incidence of postoperative complications was 58.6% in the PBD group while 73.9% in the DS group (relative risk [RR] 1.26, 95% confidence interval [CI] 0.92, 1.73, p=0.155) and the difference was not significant except in bile leakage (RR 8.83, 95% CI 1.26, 61.79, p = 0.021) and intraoperative bleeding (RR 3.97, 95% CI 0.88, 17.85, p = 0.049) which were higher in the DS group. The one-year survival rate was slightly less in the DS group but the difference was not

statistically significant. The independent predictors for death within 1-year were intraoperative bleeding and preoperative total bilirubin > 14.6 mg/dL.

Conclusions: PBD in resectable malignant distal biliary obstruction showed no benefit in terms of 1-year survival over DS approach. But it demonstrated the benefit of lower risks of intraoperative bleeding, and bile leakage. Additionally, the level of pre-operative bilirubin level of over 14.6 mg/dL and having intraoperative bleeding were associated with a lower 1-year survival in such patients. Overall, PBD may be not necessary for all resectable periampullary cancer patients, but there might be a role in those with severely jaundice (>14.6 mg/dL), as it helps lower risk of intraoperative bleeding, and might lead to a better survival outcome.

KEYWORDS

preoperative biliary drainage, direct surgery, resectable periampullary cancer, obstructive jaundice, postoperative outcomes

Background

Obstructive jaundice is the one of common presentations of periampullary cancers, and surgical resection is the curative treatment but could be applied only in the early-stage patients (1). Presence of obstructive jaundice is also a significant risk factor for postoperative complications attributed to impaired immune response, coagulopathy, kidney dysfunction, and impaired healing of anastomosis secondary to surgical site infection (2, 3). Therefore, preoperative biliary drainage (PBD) is potentially beneficial in lowering serum bilirubin level and reducing the incidence of subsequent complications, thereby preventing hepatobiliary dysfunction and improving the quality of life of patients (4). Previous meta-analyses showed comparable postoperative outcomes such as infection and mortality between those who underwent PBD and direct surgery (DS) (5, 6). However, conflicting outcomes of PBD including increasing risk of preoperative bacterial contamination of bile, cholangitis, and postoperative complications have also been reported (7–9). While the benefit of endoscopic biliary drainage in unresectable periampullary cancers with malignant obstruction is evidently concrete (10, 11), there are no current recommendations regarding the decision between PBD and DS for potentially resectable periampullary cancer patients.

Several factors might affect the outcomes of PBD. Theoretically, patients with a higher degree of jaundice may be benefit from PBD. A prior randomized controlled trial published in 2010 showed that PBD showed no benefit over early surgery in those with serum bilirubin level <14.6 mg/dL (1). nonetheless, whether PBD in patients with deeper jaundice will be beneficial over direct surgery is still unknown (1, 12). Moreover, the waiting duration for surgery, from PBD to surgery, has varied among studies, and has been reported as 2 weeks, 2-4 weeks, and > 4 weeks. A longer waiting duration seemed to increase the incidence of biliary complications and poor operative outcomes, especially infectious complications, over time (1).

The advantages of PBD in patients with periampullary cancer who present with obstructive jaundice at the resectable stage remain unclear. Most studies have been conducted in the United State of America and Europe, which have good healthcare referring systems, high socioeconomic statuses, and short waiting duration for surgery.

In this study, we aimed to evaluate postoperative outcomes in terms of postoperative complications, length of hospital stays, and mortality in patients with periampullary cancers who underwent curative surgical resection with or without PBD.

Abbreviations: CI, Confidence interval; CT, Computed tomography; ECOG, Eastern Cooperative oncology Group; ERC, Endoscopic retrograde cholangiography; ESGE, European Society of Gastrointestinal Endoscopy; HPB, Hepato-pancreato-biliary; MRI, Magnetic resonance imaging; OR, Odd ratios; PBD, Preoperative biliary drainage; PD, Pancreaticoduodenectomy; PPPD, Pyloric-preserving pancreaticoduodenectomy; 5FU, fluorouracil; LV, leucovorin.

Methods

We conducted a retrospective single center cohort study, which included patients with periampullary cancer who had undergone curative surgical resection (Whipple's operation or pyloric preserving pancreaticoduodenectomy [PPPD]) with or

without endoscopic PBD at our center between January 2016 and January 2021. Specifically, all endoscopic procedures were performed at Songklanagarind hospital, the largest university hospital in Southern Thailand. The inclusion criteria were as follows: 1) patients with periampullary tumor diagnosed by computed tomography (CT), magnetic resonance imaging (MRI) and considered to be resectable after an evaluation by hepato-pancreato-biliary (HPB) surgeons, 2) age of at least 18 years, 3) total bilirubin level at the time of diagnosis > 3 mg/dL, and 4) with complete follow-up data. Patients with disease progression to the unresectable stage or locally advanced stage during the waiting duration for surgery were excluded. Eligible patients were identified from our endoscopic center and HPB registration center's database. All patients' profiles and procedural data were extracted and collected from the hospital's electronic database.

The study protocol was approved by the Institutional Review Board of Faculty of Medicine, Prince of Songkla University (REC 64-473-21-1). The need for informed consent from the participants was waived owing to the retrospective nature of the study.

Resectability determination of periampullary cancer

Periampullary cancer was identified through CT, MRI and reviewed by experienced body radiologists. The tumor considered to be resectable if none of the following criteria were met: infiltration of peripancreatic fat planes, the hepatoduodenal ligament and the mesentery; > 180 ° encasement of the portal or superior mesenteric vein or of the hepatic or superior mesenteric artery; or distant metastasis (13).

At our institute, the decision to perform direct surgery (DS) or PBD in patients with resectable periampullary cancers presenting with obstructive jaundice is made by the attending physician. Generally, PBD is performed in patients with obstructive jaundice, ascending cholangitis, or prolonged waiting duration for surgery (2), but there were no pre-defined criteria for PBD in our institute, to drain or not drain prior to the surgery is as per the attending physician's decision. According to the current practice in hepato-pacreato-biliary(HPB) surgery, definite tissue diagnosis is not mandatory before curative resection.

Endoscopic biliary drainage procedure

The general protocol for endoscopic retrograde cholangiography (ERC) for PBD in our center is as follow: before the procedure, cross-sectional abdominal imaging is reviewed, and the location of the biliary stricture is identified. ERC is performed using a duodenoscope (TJF 160VR and TJF Q180V: Olympus Optical Co.,Ltd., Tokyo, Japan). Biliary cannulation is achieved using a sphincterotome or cannula catheter and guidewires. Biliary sphincterotomy, biliary brushing cytology, and intraductal biopsy are performed before biliary stent deployment under fluoroscopy guidance. The biliary stent(s) is placed above the stricture, and the position is confirmed by fluoroscopy. Either the 7-10 Fr straight plastic (Boston scientific, COOK and Olympus) or 10-mm covered self-expandable metal stent (Teawong [Korea], Hanaro [Korea] and Boston Scientific [USA]) is selected at the discretion of the endoscopist.

The procedure was performed under conscious sedation by five experienced endoscopists (TP, NN, JS, TY and BO). Antibiotic prophylaxis was given to all patients.

All patients were followed up as out-patient setting at 2-week after endoscopic PBD for monitoring the clinical condition, serum bilirubin level and other procedure-related complications before pancreaticoduodenectomy, this additional follow up after PBD may affect the timing for schedule the operation.

Surgical resection procedure

The eligibility for surgery was evaluated by experienced HPB surgeons and radiologists. All patients received preoperative antibiotics for prophylaxis. The choice of curative resection, classic Whipple's operation or PPPD, was as the surgeon's discretion.

- PPPD was performed for tumors located around the ampulla with no evidence of invasion of the duodenum or stomach and includes the removal of all lymph nodes on the right side of the portal vein and mesenteric artery (14).

- The classic Whipple's operation was performed if the tumor had metastasized to the proximal duodenum or pylorus and includes the resection of the distal stomach (14).

Patients were transferred to a critical care or intermediate postoperative care unit after the operation. Routine postoperative biochemical blood tests were performed. Oral intake was generally initiated after the gastric content output was ensured to be less than 500 mL/day and presence of bowel movements.

Data collection

Data pertaining to demographic and clinical characteristics such as age, sex, tumor location, imaging results, laboratory investigations, Eastern Cooperative Oncology Group (ECOG) status, details of the endoscopic procedure (stent type, duration of the endoscopic procedure, technical details, and complications), details of the operative procedure (procedure type, blood loss, complications bleeding, bile leakage and

internal organ injury), incidence of postoperative complications (intraoperative bleeding, surgical site infection, intra-abdominal collection, bile leakage, pancreatic leakage and anastomosis leakage), and date and cause of death were collected and recorded. Intraoperative bleeding was categorized according to the extent of blood loss: < 500 mL was considered normal operative bleeding, 500-1000 mL was considered mild, >/= 1000 mL was moderate and >/= 1000 mL with need for early resuscitation was considered severe.

Statistical analysis

Patients were categorized into the PBD and DS groups. Continuous variables were compared between the two groups using Wilcoxon's test for non-normally distributed data and student's *t*-test for normally distributed data, whereas categorical data were compared using chi-square test or Fisher's exact test. A p-value < 0.05 was considered statistically significant. Survival probability data were demonstrated using Kaplan-Meier survival curve and log-rank test was used for the comparison. The potential factors associated with 1-year mortality were analyzed by univariable and multivariable methods using logistic regression analyses and expressed as odds ratios (ORs) with 95% confidence intervals (CIs). All statistical analyses were performed using the R program version 4.1.0 (R foundation for statistical computing, Vienna, Austria).

Results

During the study period, 181 patients diagnosed periampullary cancer underwent Whipple's operation or PPPD. Of those, a total of 104 patients (58 in the PBD group and 46 in the DS group) fulfilled our eligibility criteria.

Table 1 summarizes baseline characteristics of the patients. Age, body mass index (BMI), sex, ECOG status, presence of comorbid diseases, initial laboratory data and definite pathological diagnoses were not significantly different between the two groups. Most patients in both groups were over 60 years old but exhibited good performance status of ECOG class 1-2. The results of baseline laboratory data at diagnosis were not significantly different except for the higher median total bilirubin level, lower platelet counts, and longer prothrombin time were observed in the patients in the DS group compared with those in the PBD group. Pancreatic adenocarcinoma, ampullary adenocarcinoma, and cholangiocarcinoma were accounted for >80% of the entire cohort. All patients underwent curative resection with either Whipple's operation or PPPD. As expected, the PBD group had a significantly longer waiting time for surgery than the DS group by approximately 30 days (p < 0.001), and the mean preoperative serum bilirubin level was respectively lower (1.8 vs 16.8 mg/dL, p = 0.001). None of the patients in the study received neoadjuvant chemotherapy, but 22.1% of them (24.1% in the PBD group, and 19.6% in the DS group) received adjuvant chemotherapy after surgical resection are shown in Table 1.

Postoperative complications and outcomes between the two groups are shown in Table 2. Although the time in intensive care unit, the length of hospital stays, the proportion of patients who survived less than one-year, and overall immediate complications were comparable between the two groups, interestingly, the patients in the DS group experienced a significantly higher rate of severe intraoperative bleeding (2% vs 0%) and bile leakage (15.2% vs 1.7%), respectively, and the relative risks are shown in the table.

The overall survival of the patients in both groups are presented in Figure 1. At one-year after surgery, 20.6% of the patients in the PBD group and 34.7% of the patients in the DS group deceased. However, the difference in overall survival between the two groups was not statistically significant.

In the univariable analyses exploring the factors associated with 1-year mortality (Figure 2), PBD showed a trend towards a lower 1-year mortality rate with an odds ratio (OR) of 0.42 (95% CI: 0.16-1.08, p=0.073). Other factors associated with increased risk of 1-year mortality by univariate analyses were increasing age (OR 1.07; 95%CI 1.01-1.13, p=0.013 per year), preoperative bilirubin level of higher than 14.6 mg/dL (OR 4.11; 95%CI 1.55-10.91, p=0.005), and presence of intraoperative bleeding (OR 5.44; 95%CI 2.03-14.62, p<0.001). Having normal creatinine clearance (>/=90 ml/min/1.73m^2) at the time of diagnosis and receiving adjuvant chemotherapy were additional factors that showed trends towards a lower risk of death within 1 year (p<0.1). Sex, diabetes, degree of jaundice at the time of diagnosis, tumor size, surgery waiting time, and other laboratory data were not associated with one-year mortality.

All variables with p<0.1 from the univariable analyses were then entered to the multivariable analysis and the results are shown in Figure 3. From the multivariable analysis, only intraoperative bleeding, and preoperative bilirubin level of >14.6 mg/dL were independent predictors for death within 1-year with an adjusted OR of 8.60 (95%CI: 2.45-30.46, p<0.001) and 6.39 (95%CI: 1.39-37.55, p<0.001), respectively. While age, normal creatinine clearance, PBD, and adjuvant chemotherapy recipients were not independently associated with 1-year mortality.

Discussion

Pancreaticoduodenectomy is a high-risk surgery, associated with high morbidity in patients with periampullary cancer, but also is an only curative treatment option (15). Adequate preoperative preparation of patients undergoing PD is crucial to minimize adverse outcomes. The frequency of complications is higher in patients with severe obstructive jaundice, malnutrition, and cholangitis; therefore, PBD is theoretically

TABLE 1 Baseline characteristics of the patients in the study.

Variables	Preoperative biliary drainage (n = 58)	Direct surgery (n = 46)	p-value
Sex (male) #	34 (58.6)	19 (41.3)	0.119
Age (years) *	62.1± 11	65.8 ± 8.3	0.066
Body mass index (kg/m2)+	20.8 (18.9,23.7)	21.5 (19.3, 23.7)	0.101
ECOG status #			0.249
Class 1	46 (79.3)	31 (67.4)	
Class 2	12 (20.7)	15 (32.6)	
Comorbid disease #			
Cardiovascular disease	3(5.2)	1(2.2)	0.653
Chronic lung disease	2(3.4)	3(6.5)	0.653
Chronic liver disease	2(3.4)	1(2.2)	1
Neurological disease	1(1.7)	0	1
Hypertension	13(22.4)	15(32.6_	0.346
Diabetic mellitus	17(29.3)	6(13)	0.081
Hyperlipidaemia	9(15.5)	13(28.3)	0.181
Chronic kidney disease	0	3(6.5)	0.083
Laboratory finding at diagnosis			
Total bilirubin (mg/dL)+	12.7 (7, 18.3)	16.2 (9.1, 22.1)	0.049
Alanine transaminase (U/L) +	96 (51.2, 165.2)	97 (47.5,210.8)	0.751
Alkaline phosphatase (IU/L) +	451 (346.8, 674.2)	372 (275, 477)	0.029
Albumin (g/dL) *	3.6 ± 0.5	3.6 ± 0.5	0.974
Creatinine (mg/dL) +	0.8 (0.6, 0.9)	0.8 (0.6, 0.9)	0.945
Platelet count (X 10^3) *	388.8 ± 114	336.7 ± 106	0.019
Haematocrit (%) *	33.6 ± 4.8	32.7 ± 4.2	0.297
Prothrombin time +	12.6 (12, 13.9)	14.3 (12.2, 15.8)	0.012
International normalized ratio +	1.1 (1, 1.2)	1.3 (1.1,1.5)	0.019
Type of operation #			0.446
-Whipple's operation	12 (20.7)	6 (13)	
-PPPD	46 (79.3)	40 (87)	
Waiting duration for surgery (days)+	49 (28.2, 64.2)	19 (9.2, 29)	< 0.001
Total procedure duration (min) +	480 (420, 540)	480 (380, 540)	0.815
Preoperative serum bilirubin (mg/dL) +			
-Total bilirubin (TB), mg/dL	1.8 (0.8, 3)	16.8 (9, 22.1)	< 0.001
-Direct bilirubin (DB), mg/dL	1.4 (0.4, 2.6)	14.8 (8, 21.2)	< 0.001
Pathological diagnosis #			0.575
-Pancreatic adenocarcinoma	16 (27.6)	19 (41.3)	
-Adenocarcinoma of ampulla of Vater	27 (46.6)	19 (41.3)	
-Cholangiocarcinoma	7 (12.1)	5 (10.9)	
-Adenocarcinoma of duodenum	2 (3.4)	–	
-Neuroendocrine tumor of pancreas	1 (1.7)	–	
-Mass forming chronic pancreatitis	2 (3.4)	–	
-Cystic tumor of pancreas	3 (5.2)	3 (6.5)	
Receiving adjuvant chemotherapy	14 (24.1)	9 (19.6)	0.577

*Data are expressed as mean± SD, + Data are expressed as median (Interquartile range), # Data are expressed as n (%).

useful in such patients (16, 17). Two approaches for PBD, endoscopic and percutaneous, are generally used for biliary decompression in patients with periampullary cancer presenting with obstructive jaundice in clinical practice (1, 4). Nonetheless, whether patients with obstructive jaundice should go for direct surgery or PBD first is still debatable.

Our study demonstrates that, in resectable periampullary cancer patients, PBD did not show a survival benefit in comparison to DS. However, those who underwent PBD had a lower rate of immediate postoperative complications namely bile leakage, and degree of intraoperative bleeding. And having intraoperative bleeding, and preoperative bilirubin over 14.6

TABLE 2 Postoperative outcomes.

Variables	Preoperative biliary drainage (n = 58)	Direct surgery (n = 46)	Relative risk (95% confidence interval)
Postoperative complications #			
- Overall complications	34 (58.6)	34 (73.9)	1.26 (0.92, 1.73)
- Intra-abdominal bleeding	17 (29.3)	15 (32.6)	1.11 (0.28, 4.42)
- Severity of bleeding (mL)	41 (75.9)	36 (78.3)	–
• < 500	13 (24.1)	8 (17.3)	reference
* 500-1000	0	2 (4.3)	0.86 (0.47, 1.56)
* >/=1000	13 (22.4)	17 (37)	2.25 (1.76, 2.87)
- Intra-abdominal collection	16 (27.6)	9 (19.6)	1.65 (0.82, 3.31)
- Surgical site infection	1 (1.7)	7 (15.2)	0.71 (0.28, 1.8)
- Bile leakage	10 (17.2)	13 (28.3)	8.83 (1.26, 61.79)
- Pancreatic leakage	2 (3.4)	2 (4.3)	1.64 (0.68, 3.94)
- Small bowel injury -			
Need for re-operation #	7 (12.1)	3 (6.5)	0.54 (0.08, 3.81)
Length of intensive care unit (days) *	6.4 (4.4)	5.7 (4.1)	–
Length of hospital stay (days)+	11.5 (10,17.8)	13.5 (10, 23.2)	–
Death within one year #	12 (20.6)	16 (34.7)	1.45 (0.86, 2.45)

* Data are expressed as mean ± standard deviation, + Data are expressed as median (interquartile range), # Data are expressed as n (%).

mg/dL were independent predictors associated with an increased risk of death within one year.

The baseline characteristics of the PBD and DS group were generally similar in this study. The median total bilirubin level at the diagnosis and INR were slightly higher, and the platelet count was lower in the DS group compared to the PBD group. Nonetheless, these 3 variables were not significantly associated with 1-year mortality. Of note, serum bilirubin level at the diagnosis in our study (mean 14.3 ± 7.8 mg/dL) was higher than that reported in other studies (1, 18), which may represent the late presentation or the longer time to diagnosis of patients in this study. Moreover, the waiting time for surgery in the present

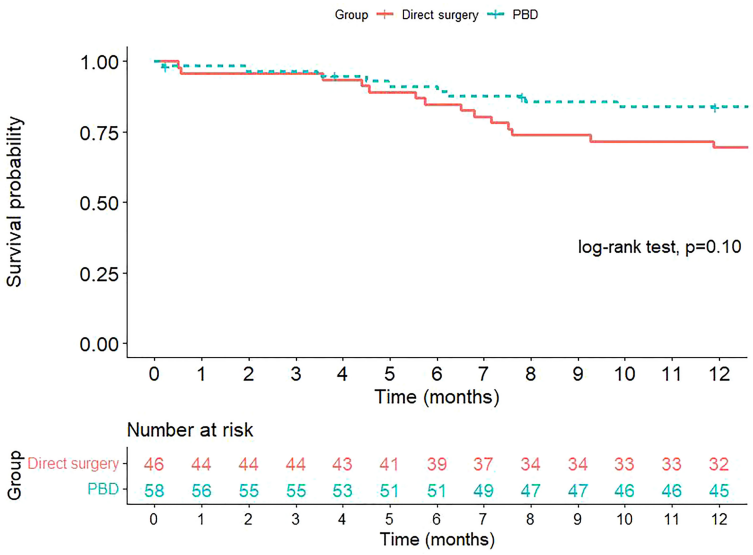


FIGURE 1
Kaplan-Meier survival curve for overall survival after pancreaticoduodenectomy.

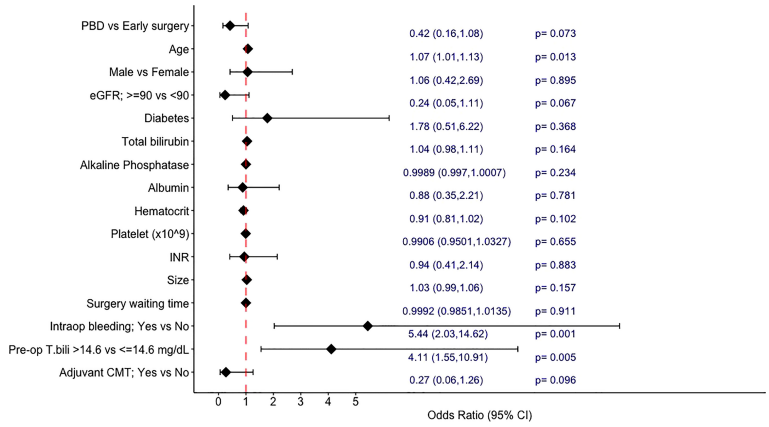


FIGURE 2
Univariable analysis for factors associated with mortality.

study (median 30 days; IQR 15-53.5 days) was quite longer than that reported in previous studies (1-2 weeks) even none of the patients received neoadjuvant chemotherapy (1, 19), which reflects the situation in developing countries where the healthcare system is usually overwhelmed.

The mean preoperative bilirubin level sharply declined from 12.7 to 1.8 mg/dL within 2 weeks after endoscopic biliary drainage in the PBD group, indicates the adequacy of biliary decompression, according to the European Society of Gastrointestinal Endoscopy (ESGE) guidelines (20). Adequate PBD in patients with periampullary cancer has been reported benefit in reducing the occurrence of major morbidities (38.9% vs 61.1%) postoperatively compared to inadequate drainage (18). This might support our results PBD advantages in terms of the lower rates of intraoperative bleeding and bile leakage compared with the DS group. And it is also in concordant with the result from a previous meta-analysis that patients with resectable malignant distal biliary obstruction who had undergone internal PBD had significantly lower incidence of major postoperative complications than those who had undergone DS (9), and the recent retrospective study in patients with severe jaundice that the fewer cases of post-pancreatectomy hemorrhage was observed in the PBD group (21). In addition, endoscopic approach is less invasive than percutaneous

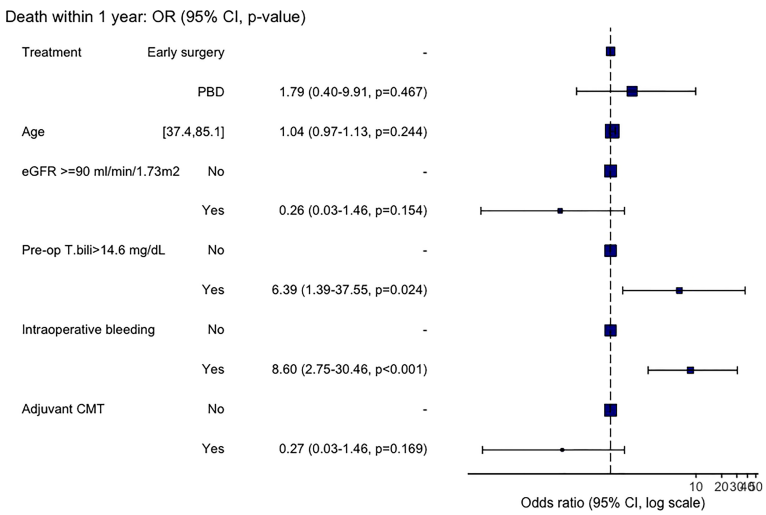


FIGURE 3
Multivariate analysis for factor associated with mortality.

approach for PBD (22). The benefit of significantly lower intraoperative bleeding and postoperative bile leakage in the PBD group than in the DS group may be explained by the regained coagulation function, as effective biliary drainage leads to an improvement of vitamin K absorption; and biliary decompression could downsize the dilated bile duct and might result in a lower chance of bile duct injury during surgical procedures.

The choice of biliary drainage stent type was controversy, previous reports showed self-expanding metal stents (SEMs) were associated with lower risks of post procedural cholangitis (4.1% VS 9.7%, p=0.043) and fewer postoperative pancreatic fistula (9.8% vs 18.5%, p = 0.004) than plastic stents (23). However, a recent meta-analysis showed comparable postoperative outcomes between metal and plastic stents (24). In this cohort, plastic stents were used in most patients (>95%) according to the national reimbursement policy, making the further analysis regarding stent subtype was not possible in our study.

On the contrary, studies have reported the negative impact of PBD on postoperative outcomes in terms of increased incidence of infectious complications and bleeding (at a relative risk [RR] of 1.66; 95% CI, 1.28-2.16; p = 0.0002) (1, 6, 25). Garcea et al, showed that PBD was associated with a significantly increased probability of wound infection (OR, 1.827; p < 0.005) (8). Nonetheless, these negative outcomes were not observed in our cohort. The routine prophylactic antibiotic prior to the operation in our study might play a role in this finding.

In the present study, the one-year overall mortality was 20.6% in the PBD group and 34.7% in the DS group, while the probability of postoperative death within one-year was slightly higher in the DS group than in the PBD group, the difference was not statistically significant (p = 0.107). The lack of benefit of PBD in lowering mortality has also been shown in previous reports (1, 6, 9).

We also evaluated the factors associated with 1-year mortality after curative surgical resection. As mentioned earlier, PBD itself was not associated with a better 1-year survival. After adjustment with many potential factors, we found that only pre-operative serum bilirubin level of >14.6 mg/dL, not the bilirubin level at the time of diagnosis, was significantly associated with a higher risk of death within one-year with an adjusted OR of 6.39 (95%CI: 1.39-37.55, p<0.001). And the other independent factor showed an increased risk of 1-year mortality was having intraoperative bleeding (an adjusted OR of 8.60 (95%CI: 2.45-30.46, p<0.001)), whereas the development of pancreatic fistula and bile leak were not significantly associated with the poorer survival outcome. These are interesting findings that may highlight the potential role of PBD in a subgroup of patients. As prior studies that showed no beneficial effect (or negative impact) of PBD on postoperative outcomes were mainly studied in patients with a lower level of bilirubin; for instance, the RCT by van der Gaag, et al. (1) included only patients with baseline bilirubin level of lower than 250 µmol per liter (<14.6 mg/dL), and the large retrospective study by de Pastena, et al. most of the patients had bilirubin level of less than 10.2 mg/dL (26).. Moreover, the recent article studied for the overall mortality also demonstrated that the higher level of total bilirubin before surgery (over 150 µmol per liter – about 8.77 mg/dL) was associated with a lower risk of overall survival (27).

Adjuvant chemotherapy is another interesting factor, receiving postoperative chemotherapy was associated with a better outcome in the univariate analysis, but not in the multivariate analysis in our study, yet the towards a better 1-year survival was still observed (adjusted OR 0.26, p=0.169). The data regarding the benefit of adjuvant chemotherapy in these patients are still controversial, a prospective study (ESPAC-3) showed the survival advantage of the adjuvant chemotherapy (a combination of fluorouracil and folinic acid or gemcitabine) with a hazard ratio of 0.75 (95% CI 0.57-0.98, p = 0.03) (28). while in a retrospective study of patients with resectable pancreatic adenocarcinoma, the use of adjuvant chemotherapy (FU-based or gemcitabine-based) was not associated with improved long-term survival (p=0.69) (29). A study with a larger sample size may demonstrate the benefit of adjuvant chemotherapy more clearly.

This study represents a real-world situation of resectable periampullary cancer patients in developing countries, in which the presentation of the patients is usually late as severe jaundice was commonly observed, and the waiting time before surgery was quite long. We found no deleterious effect of PBD compared to DS, and some beneficial postoperative outcomes were also observed. The limitations of our study are noted. As it is retrospective in nature, some differences in baseline characteristics of the patients in the PBD and the DS groups existed, however, those differences in baseline laboratory data were not associated with the outcomes in our study. In addition, a significant proportion of the patients were referred back to their local hospital after surgical resection and being followed-up for over a year, this makes it is difficult to evaluate the disease-free survival and long-term (e.g., 3-year, or 5-year) survival of the patients in our study.

Conclusions

PBD in resectable malignant distal biliary obstruction showed no benefit in terms of 1-year survival over DS approach. But it demonstrated the benefit of lower risks of intraoperative bleeding, and bile leakage. Additionally, the level of pre-operative bilirubin level of over 14.6 mg/dL and

having intraoperative bleeding were associated with a lower 1-year survival in such patients. Overall, PBD may be not necessary for all resectable periampullary cancer patients, but there might be a role in those with severely jaundice (>14.6 mg/dL), as it helps lower risk of intraoperative bleeding, and might lead to a better survival outcome.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding author.

Author contributions

TPat has made substantial contribution to the conception and design of the study, data collection as well as manuscript writing. TC and PS have made contributions to the design of the study, data analysis and manuscript writing. NN, BO, TY, TP and SA performed and completely reported endoscopic and surgical data. PS, NC and SA have made contributions to manuscript writing and English language approval. TPat is the first author. PS is the corresponding author, and responsible for ensuring that all listed authors have approved the manuscript before submission. All authors contributed to the article and approved the submitted version.

Funding

The study was supported by Faculty of Medicine, Prince of Songkla University.

Acknowledgments

The authors thank Faculty of Medicine, Prince of Songkla University, for providing resource and aiding data collection in the study. We also thank all patients and hope this work will be beneficial for the clinical practice in the future.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

1. van der Gaag NA, Rauws EA, van Eijck CH, Bruno MJ, van der Harst E, Kubben FJ, et al. Preoperative biliary drainage for cancer of the head of the pancreas. *N Engl J Med* (2010) 362(2):129–37. doi: 10.1056/NEJMMoa093230

2. Lai EC, Lau SH, Lau WY. The current status of preoperative biliary drainage for patients who receive pancreaticoduodenectomy for periampullary carcinoma: a comprehensive review. *Surgeon* (2014) 12(5):290–6. doi: 10.1016/j.surg.2014.02.004

3. Bakens M, van Rijssen B, van Woerden V, Besselink M, Boerma D, Busch O, et al. Evaluation of preoperative biliary drainage in patients undergoing pancraetoduodenectomy for suspected pancreatic or periampullary cancer. *J Pancreas* (2018) 19(1):24–8.

4. van der Gaag NA, Kloek JJ, de Castro SM, Busch OR, van Gulik TM, Gouma DJ. Preoperative biliary drainage in patients with obstructive jaundice: history and current status. *J Gastrointest Surg* (2009) 13(4):814–20. doi: 10.1007/s11605-008-0618-4

5. Lee PJ, Podugu A, Wu D, Lee AC, Stevens T, Windsor JA. Preoperative biliary drainage in resectable pancreatic cancer: a systematic review and network meta-analysis. *HPB (Oxford)* (2018) 20(6):477–86. doi: 10.1016/j.hpb.2017.12.007

6. Fang Y, Gurusamy KS, Wang Q, Davidson BR, Lin H, Xie X, et al. Pre-operative biliary drainage for obstructive jaundice. *Cochrane Database Syst Rev* (2012) 9(9):CD005444. doi: 10.1002/14651858.CD005444.pub3

7. Herzog T, Belyaev O, Muller CA, Mittelkotter U, Seelig MH, Weyhe D, et al. Bacteribilia after preoperative bile duct stenting: a prospective study. *J Clin Gastroenterol* (2009) 43(5):457–62. doi: 10.1097/MCG.0b.13e318186b19b

8. Garcea G, Chee W, Ong SL, Maddern GJ. Preoperative biliary drainage for distal obstruction: the case against revisited. *Pancreas* (2010) 39(2):119–26. doi: 10.1097/MPA.0b013e3181bd65de

9. Moole H, Bechtold M, Puli SR. Efficacy of preoperative biliary drainage in malignant obstructive jaundice: a meta-analysis and systematic review. *World J Surg Oncol* (2016) 14(1):182. doi: 10.1189/s12957-016-0933-2

10. Sripongpun P, Attasaranya S, Chamroonkul N, Sookpaisal T, Khow-Ean U, Siripun A. Simple clinical score to predict 24-week survival times in patients with inoperable malignant distal biliary obstruction as a tool for selecting palliative metallic or plastic stents. *J Gastrointest Cancer* (2018) 49(2):138–43. doi: 10.1007/s12029-017-9918-9

11. Pattarapuntakul T, Netinatsuton N, Sottisuporn J, Witeerungrot T, Ovartlarnporn B. Impact of palliative biliary drainage between metal stents and plastic stents on survival rate in unresectable distal biliary stricture in songklanagarind hospital. *J Med Assoc Thai* (2018) 101(4):38. doi: 10.26226/morressier.59a6b342d462b80290b53f62

12. Ducreux M, Cuhna A, Caramella C, Hollebecque A, Burtin P, Goere D, et al. Cancer of the pancreas: ESMO clinical practice guidelines for diagnosis, treatment and follow-up. *Ann Oncol* (2015) 26(5):56–68. doi: 10.1093/annonc/mdv295

13. Faisal M, Fathy H, Abu-Elela STB, Shams ME. Prediction of resectability and surgical outcomes of periampullary tumors. *Clin Surg* (2018) 3:1969.

14. Gouma DJ, Nieveen van Dijkum EJ, Obertop H. The standard diagnostic work-up and surgical treatment of pancreatic head tumours. *Eur J Surg Oncol* (1999) 25(2):113–23. doi: 10.1053/ejso.1998.0612

15. Sriussadaporn S, Sriussadaporn S, Pak-Art R, Kritayakirana K, Prichayudh S, Samorn P, et al. Outcomes of pancreaticoduodenectomy in patients with obstructive jaundice with and without preoperative biliary drainage: a retrospective observational study. *Asian BioMed* (2018) 12(5):237–41. doi: 10.1515/abm-2019-0025

16. Dumonceau JM, Tringali A, Papanikolaou IS, Blero D, Mangiavillano B, Schmidt A, et al. Endoscopic biliary stenting: indications, choice of stents, and results: European society of gastrointestinal endoscopy (ESGE) clinical guideline - updated October 2017. *Endoscopy*. (2018) 50(9):910–30. doi: 10.1055/a-0659-9864

17. Adams MA, Anderson MA, Myles JD, Khalatbari S, Scheiman JM. Self-expanding metal stents (SEMS) provide superior outcomes compared to plastic stents for pancreatic cancer patients undergoing neoadjuvant therapy. *J Gastrointest Oncol* (2012) 3(4):309–13. doi: 10.3978/j.issn.2078-6891.2011.050

18. Rungsakulkij N, Thongchai V, Suragul W, Vassanasiri W, Tangtawee P, Muangkaew P, et al. Association of the rate of bilirubin decrease with major morbidity in patients undergoing preoperative biliary drainage before pancreaticoduodenectomy. *SAGE Open Med* (2021) 9:20503121211039667. doi: 10.1177/20503121211039667

19. Costa Santos M, Cunha C, Velho S, Ferreira AO, Costa F, Ferreira R, et al. Preoperative biliary drainage in patients performing pancreaticoduodenectomy: guidelines and real-life practice. *Acta Gastroenterol Belg* (2019) 82(3):389–95.

20. Dumonceau JM, Tringali A, Blero D, Devière J, Laugiers R, Heresbach D, et al. Biliary stenting: indications, choice of stents and results: European society of gastrointestinal endoscopy (ESGE) clinical guideline. *Endoscopy* (2012) 44:277–92. doi: 10.1055/s-0031-1291633

21. Shen Z, Zhang J, Zhao S, Zhou Y, Wang W, Shen B. Preoperative biliary drainage of severely obstructive jaundiced patients decreases overall post-operative complications after a retrospective and propensity score-matched analysis. *Pancreatology* (2020) 20(3):529–36. doi: 10.1016/j.pan.2020.02.002

22. Speer AG, Cotton PB, Russell RC, Mason RR, Hatfield AR, Leung JW, et al. Randomised trial of endoscopic versus percutaneous stent insertion in malignant obstructive jaundice. *Lancet* (1987) 2:57–62. doi: 10.1016/S0140-6736(87)92733-4

23. Latenstein AEJ, Mackay TM, van Huijgevoort NCM, Bonsing BA, Bosscha KB, Hol L, et al. Nationwide practice and outcomes of endoscopic biliary drainage in resectable pancreatic head and periampullary cancer. *HPB (Oxford)* (2021) 23 (2):270–78. doi: 10.1016/j.hpb.2020.06.009

24. Du J, Gao X, Zhang H, Wan Z, Yu H and Wang D. Stent selection in preoperative biliary drainage for patients with operable pancreatic cancer receiving neoadjuvant therapy: A meta-analysis and systematic review. *Front Surg* (2022) 30:875504(9). doi: 10.3389/fsurg.2022.875504

25. Pisters PW, Hudec WA, Hess KR, Lee JE, Vauthey JN, Lahoti S, et al. Effect of preoperative biliary decompression on pancreaticoduodenectomy-associated morbidity in 300 consecutive patients. *Ann Surg* (2001) 234:47–55. doi: 10.1097/00000658-200107000-00008

26. Pastena MD, Marchegiani G, Paiella S, Malleo G, Ciprani D, Gasparini C, et al. Impact of preoperative biliary drainage on postoperative outcome after pancreaticoduodenectomy: An analysis of 1500 consecutive cases. *Dig Endosc* (2018) 30(6):777–84. doi: 10.1111/den.13221

27. Shen Z, Zhang J, Chen H, Wang W, Xu W, Lu X, et al. Does pre-operative biliary drainage influence long-term survival in patients with obstructive jaundice with resectable pancreatic head cancer? *Front Oncol* (2020) 16:575316(10). doi: 10.3389/fonc.2020.575316

28. Neoptolemos JP, Moore MJ, Cox TF, Valle JW, Palmer DH, McDonald AC, et al. Effect of adjuvant chemotherapy with fluorouracil plus folinic acid or gemcitabine vs observation on survival in patients with resected periampullary adenocarcinoma: the ESPAC-3 periampullary cancer randomized trial. *JAMA* (2012) 308(2):147–56. doi: 10.1001/jama.2012.7352

29. Ecker BL, Vollmer CM, Behrman SW, Allegrini V, Aversa J, Ball CG, et al. Role of adjuvant multimodality therapy after curative-intent resection of ampullary carcinoma. *JAMA Surg* (2019) 154(8):706–14. doi: 10.1001/jamasurg.2019.1170

frontiers | Frontiers in Oncology

TYPE Original Research
PUBLISHED 21 November 2022
DOI 10.3389/fonc.2022.1040411

OPEN ACCESS

EDITED BY
Menghua Dai,
Peking Union Medical College Hospital (CAMS), China

REVIEWED BY
Charlotte Maulat,
Université Toulouse III Paul Sabatier, France
George Gemenetzis,
University of Edinburgh, United Kingdom

*CORRESPONDENCE
Shuqi Mao
mmmaoshuqi@163.com
Shengdong Wu
arsdrell@hotmail.com
Caide Lu
lucaide@nbu.edu.cn

SPECIALTY SECTION
This article was submitted to Gastrointestinal Cancers: Hepato Pancreatic Biliary Cancers, a section of the journal Frontiers in Oncology

RECEIVED 09 September 2022
ACCEPTED 24 October 2022
PUBLISHED 21 November 2022

CITATION
Tong J, Jiang W, Mao S, Wu S and Lu C (2022) Development and validation of a nomogram to predict liver metastasis for pancreatic ductal adenocarcinoma after radical resection.
Front. Oncol. 12:1040411.
doi: 10.3389/fonc.2022.1040411

Development and validation of a nomogram to predict liver metastasis for pancreatic ductal adenocarcinoma after radical resection

Jingshu Tong, Wei Jiang, Shuqi Mao*, Shengdong Wu* and Caide Lu*

Department of Hepatopancreatobiliary Surgery, Ningbo Medical Centre Lihuili Hospital, Ningbo University, Ningbo, China

Objectives: This study aimed to develop and externally validate a nomogram for predicting liver metastasis after radical resection in patients with pancreatic ductal adenocarcinoma (PDAC).

Methods: A total of 247 PDAC patients who underwent radical resection were retrospectively reviewed from January 2015 to March 2022 at Ningbo Medical Centre Lihuili Hospital Eastern Section, and used as a training cohort to develop the nomogram. 83 PDAC patients from the Ningbo Medical Centre Lihuili Hospital Xingning Section were enrolled as the validation cohort. The postoperative liver metastasis was recorded during the follow-up, and the liver metastasis-free survival was defined as the time from operation to the date of liver metastasis diagnosis or death. The nomogram was established based on independent prognostic factors selected by LASSO and multivariate Cox regression model. The performance was assessed using the concordance index (C-index) and calibration curves. The receiver operating characteristic (ROC) curve and decision curve analysis (DCA) were used to determine the clinical utility of the nomogram model.

Results: From the training cohort of 247 patients, a total of 132 patients developed liver metastasis during the follow-up, the 1-, 2- and 3- year liver metastasis-free survival were 52.4%, 43.5% and 40% respectively. The LASSO and multivariate Cox regression analysis indicated that postoperative CA125 (hazard ratio [HR] = 1.007, p <0.001), tumor differentiation (HR = 1.640, p = 0.010), tumor size (HR = 1.520, p = 0.029), lymph node ratio (HR = 1.897, p = 0.002) and portal/superior mesenteric/splenic vein invasion degree (PV/SMV/SV) (HR = 2.829, p <0.001) were the independent factors of liver metastasis. A nomogram with independent factors was developed and the C-index was 0.760 (95% confidence interval [CI], 0.720-0.799) and 0.739 (95% CI, 0.669-0.810) in the training and validation cohorts, respectively. The areas under curve (AUC) of the nomogram at 1-, 2- and 3-year were 0.815, 0.803 and 0.773 in the training cohort, and 0.765, 0.879 and 0.908 in the validation cohort, respectively, higher than those in TNM stage.

Decision curve analysis (DCA) analysis revealed that the nomogram model provided superior net benefit in clinical utility. Liver metastasis-free survival curves showed a significant discriminatory ability for liver metastasis risk based on the nomogram (p <0.001).

Conclusions: The nomogram showed high accuracy in predicting liver metastasis for PDAC after radical resection, and may serve as a clinical support tool to guide personalized and prescient intervention.

KEYWORDS

pancreatic ductal adenocarcinoma, nomogram, liver metastasis, recurrence, radical resection

Introduction

Pancreatic ductal adenocarcinoma (PDAC) is the 12th most common malignancy and the 7th leading cause of cancer mortality, as one of the most intractable malignant neoplasms worldwide (1). Due to its extremely aggressive nature, radical resection is the only chance for long-term survival for patients with PDAC. However, even after radical resection, most patients still have tumor recurrence or metastasis, resulting in 5-year survival of only 12% to 27%, negatively affecting the curative nature of the operation and the prognosis of PDAC patients (2, 3).

Liver metastasis has the worst prognosis among all recurrence patterns, the median OS is significantly shorter than that of other recurrence patterns (15.4 months vs 17.7-39.6 months) (4). Meanwhile, liver metastasis accounts for the largest proportion of all recurrence patterns, up to 35%-40% of patients (5). Postoperative liver metastasis in patients with PDAC may present a unique biologic characteristic and always indicates a poor prognosis, constituting a key cohort worthy of further study (6). Several stage systems have been used to estimate the overall survival or recurrence-free survival (7, 8), however considering the absence of a prognostic model specifically for liver metastasis after radical resection, it was necessary to develop a predictive model for liver metastasis with an unfavorable prognosis.

In the present study, we developed and externally validated a nomogram to predict the liver metastasis for PDAC after radical resection, which has not been reported in previous studies, aimed to explore the patients with a high risk of liver metastasis after radical resection and potentially assist in clinical.

Materials and methods

Patients

The retrospective study consisted of 247 patients who underwent radical pancreatic cancer resection between January 2015 and March 2022 at Ningbo Medical Centre Lihuili Hospital Eastern Section, Ningbo University. The inclusion criteria were as follows: (1) pathology confirmed PDAC, (2) integrated intraoperative and clinical data, (3) enhanced CT/MR performed within 1 month before the operation, and (4) negative final margins with no residual tumor based on pathology. The exclusion criteria were as follows: (1) death within 30 days after the operation, (2) complications with other malignancies, and (3) failure to evaluate the vascular invasion degree from the preoperative imagines or during the operation. To examine the generalizability of the model, the external validation cohort consisted of 83 PDAC patients who underwent radical resection and met the above criteria at Ningbo Medical Centre Lihuili Hospital Xingning Section between January 2016 and August 2021. The study was approved by the ethics committee of Ningbo Medical Center Lihuili Hospital (Approval number: KY2021PJ263). All research procedures complied with the relevant guidelines and regulations. Informed consent was obtained from all patients before inclusion. We confirmed that this study was conducted following the Declaration of Helsinki.

Assessment of the vascular invasion degree

To assess portal vein/superior mesenteric vein (PV/SMV) and splenic vein (SV) invasion, we recorded the PV/SMV/SV invasion condition in each patient during the operation, evaluated by the chief surgeon. We also review the PV/SMV/SV invasion on preoperative images, evaluated by two radiologists (Supplementary Figure S1). The degree of PV/SMV/SV invasion was assessed as follows (9): (1) PV/SMV/SV without tumor abutment or invasion, (2) PV/SMV/SV invasion <180°, (3) PV/SMV/SV invasion >180°.

For most patients, the intraoperative evaluation of vascular invasion was usually consistent with preoperative CT imaging

evaluation, if there was a difference, the intraoperative evaluation was prevail.

Liver metastasis and follow up

Liver metastasis-free survival was defined as the time from operation to the date of liver metastasis diagnosis, death or the last follow-up. The liver metastasis is essentially a particular pattern of tumor recurrence, so the liver metastasis-free survival is a bit like the term recurrence-free survival (RFS), and we concentrated on liver metastasis in this study. The diagnosis of liver metastasis and other recurrence patterns was based on imaging studies, and rarely tissue confirmation. Information regarding liver metastasis was obtained at regular follow-up.

Patients were followed up until September 2022, and all patients were followed up for more than 6 months unless they died. The median follow-up time of patients from the Ningbo Medical Centre Lihuili Hospital Eastern Section and the Xingning Section were 15.0 (range 3-78) months and 19.0 (range 3-77) months, respectively. In general, patients had at least 1 follow-up by imaging study (CT, MRI or PET/CT) and tumor biomarkers every 3 months for the first year after the operation and then every 3-6 months after the first year. Follow-up was performed in the outpatient clinic or *via* phone call.

Study variables and operation

The following clinicopathological variables were analyzed: demographic data, biochemical tests, tumor markers, pathological features, vascular invasion degree, operative and adjuvant treatment characteristics. The preoperative biochemical and tumor markers test were performed within 7 days before the radical resection, and postoperative tumor markers were measured at the first follow-up. The lymph node ratio was defined as the proportion of positive lymph nodes in the total examined lymph node. The disease stage was evaluated according to the American Joint Committee on Cancer (AJCC) 8th edition and the 7th edition Japanese Pancreas Society (JPS) derived from tumor-node-metastasis (TNM) staging system (10, 11). Adjuvant chemotherapy was routinely recommended and started within 3 months after the operation if conditions permit.

Resectability evaluation and synchronous liver metastasis exclusion were performed by a multidisciplinary team, based on CT and MRI. Surgical methods included pancreaticoduodenectomy and distal pancreatectomy, resected tissues were pathologically examined in frozen and final sections to confirm negative surgical margins. According to preoperative imaging studies and intraoperative exploration, if the tumor invaded, PV/SMV resection and reconstruction were performed in pancreaticoduodenectomy, invaded SV along with the pancreatic body/tail and spleen resection was performed in distal pancreatectomy.

Statistical analysis

Continuous variables were presented as mean with standard deviation or median with range, categorical variables were presented as frequencies with percentages. Survival curves were calculated using the Kaplan-Meier method and the Log-rank test. Optimal features were selected using the least absolute shrinkage and selection operator (LASSO) regression, and factors with nonzero coefficients were identified and selected. Independent prognostic factors of liver metastasis were identified by univariate and multivariate Cox proportional hazards regression. Subsequently, a nomogram was developed to predict the probability of 1-, 2-, and 3-year liver metastasis-free survival rates after the operation. The performance was evaluated based on the discriminating ability (discrimination) and accuracy of point estimates of the survival function (calibration) with 1000 time bootstraps, and to calculate a relatively corrected concordance index (C-index). The area under curves of the receiver operating characteristic (ROC) curves were calculated and compared with TNM stage, to validate the nomogram model performance. The clinical utility of the nomogram was investigated using the decision curve analysis (DCA), by quantifying the net benefits along with the increase in threshold probabilities. Each patient had a total risk score for risk stratification of liver metastasis according to the nomogram model. Patients were divided into different risk groups (low-; moderate-; high-) with the cut-off points automatically calculated using X-tile software (version 3.6.1; Yale University, New Haven, CT, USA) (12), and further applied to the validation cohort, and the respective Kaplan-Meier curves were constructed.

All statistical analyses were conducted using SPSS software version 24.0 (IBM Corporation, 2020, USA) and R software version 3.6.2 (http://www.r-project.org/). $p < 0.05$ was considered statistically significant.

Results

Patients characteristics in the training and validation cohorts

The training cohort consisted of 247 patients who underwent pancreatic cancer resection and had histologically confirmed PDAC at Ningbo Medical Centre Lihuili Hospital Eastern Section, Ningbo University between January 2015 and March 2022. A total of 132 patients developed liver metastasis during the follow-up, and the 1-, 2- and 3- year liver metastasis-free survival were 52.4%, 43.5% and 40% respectively. The validation cohort consisted of 83 eligible patients who underwent radical resection at the Ningbo Medical Centre Lihuili Hospital Xingning Section between January 2016 and August 2021, a total of 46 patients developed liver metastasis, the

1-, 2- and 3- year liver metastasis-free survival were 56.6%, 45.0% and 43.5%, respectively. All clinicopathological characteristics of patients in the training and validation cohorts were summarized (Table 1). The patients with liver metastasis may be accompanied by other patterns of recurrence, the specific recurrence patterns of postoperative liver metastasis were summarized (Table 2). There was no difference in overall survival between the patients with only-liver metastasis (14.0 months, 95%CI, 11.323-16.677) and the patients with other multiple recurrence (12.0 months, 95%CI, 1.653-22.347, p=0.871).

Prognostic factors selection with LASSO analysis in the training cohort

LASSO regression was performed for all 34 clinicopathological characteristics to select the prognostic factors of liver metastasis (Figures 1A, B). The neoadjuvant chemotherapy was not an independent prognostic factor of liver metastasis after the operation (HR=1.468, 95%CI, 0.881-2.447, p=0.141). The analysis indicated that postoperative CA125, total examined lymph node number, tumor differentiation, lymphovascular invasion, capsule invasion, tumor size, lymph node ratio and PV/SMV/SV invasion degree were associated with liver metastasis after the operation. All significant factors selected from the LASSO regression were further included in the multivariable Cox analysis, and showed that postoperative CA125 (hazard ratio [HR] = 1.007, p <0.001), tumor differentiation (HR = 1.640, p = 0.010), tumor size (HR = 1.520, p = 0.029), lymph node ratio (HR = 1.897, p = 0.002) and PV/SMV/SV invasion degree (HR = 2.829, p <0.001) were the independent factors for liver metastasis (Table 3).

Construction and validation of nomogram for liver metastasis-free survival prediction

As shown in Figure 2, the nomogram was established based on the independent factors of liver metastasis. PV/SMV/SV invasion degree and postoperative CA125 level were the largest contributions to liver metastasis prediction, followed by tumor differentiation and lymph node ratio. The calibration curves showed high agreement between predicted and actual liver

TABLE 1 Clinicopathological and treatment characteristics of PDAC patients in the training and validation cohorts.

Characteristic	Training cohort (n=247)	Validation cohort (n=83)
Age, years, mean ± SD	67.2 ± 9.5	64.5 ± 10.2
Sex, (%)		
Male	137 (55.5)	44 (53.0)
Female	110 (44.5)	39 (47.0)
BMI, kg/m^2, mean ± SD	22.4 ± 2.8	22.8 ± 2.7
TBIL, umol/L, median (rang)	14.3 (3.6-434.0)	15.6 (1.7-420.0)
DBIL, umol/L, median (rang)	5.2 (0.8-334.8)	7.0 (1.0-380.0)
ALB, U/L, median (rang)	39.3 (27.3-59.0)	39.0 (26.4-54.8)
ALT, U/L, median (rang)	30 (4-723)	30 (10-575)
AST, U/L, median (rang)	26 (9-993)	35 (14-306)
CA199, IU/ml, median (rang)	147.2 (1.2-18722)	21.5 (3.8-4904)
CA125, IU/ml, median (rang)	11.9 (2.0-524.3)	28.4 (1.1-367.0)
CEA, ug/L, median (rang)	2.1 (0.1-66.9)	1.8 (0.1-21.3)
Postoperative CA199, IU/ml, median (rang)	30.1 (1.2-9760)	21.8 (0.7-4708)
Postoperative CA125, IU/ml, median (rang)	24.0 (1.1-198.3)	61.1 (1.4-168.1)
Postoperative CEA, ug/L, median (rang)	1.8 (0.1-55.2)	2.4 (0.1-21.6)
Neoadjuvant chemotherapy, (%)		
Yes	27 (10.9)	8 (9.6)
No	220 (89.1)	75 (90.4)
Tumor location, (%)		
Head/Neck	149 (60.3)	54 (65.1)
Body/Tail	98 (39.7)	29 (34.9)
Surgical path, (%)		
Open	192 (77.7)	66 (79.5)
Laparoscopic	55 (22.3)	17 (20.5)

(Continued)

TABLE 1 Continued

Characteristic	Training cohort (n=247)	Validation cohort (n=83)
Tumor size, cm (%)		
>4	91 (36.8)	29 (34.9)
≤4	156 (63.2)	54 (65.1)
Lymphnodes metastasis, (%)		
Yes	120 (48.6)	41 (49.4)
No	127 (51.4)	42 (50.6)
Lymph node ratio, (%)		
≥0.2	54 (21.9)	25 (30.1)
<0.2	193 (78.1)	58 (69.9)
Tumor differentiation, (%)		
Poor	119 (48.2)	49 (59.0)
Well-moderate	128 (51.8)	34 (41.0)
Lymphovascular invasion, (%)		
Present	143 (57.9)	43 (51.8)
Absent	104 (42.1)	40 (48.2)
Perineural invasion, (%)		
Present	213 (86.2)	57 (68.7)
Absent	34 (13.8)	26 (31.3)
Frozen resection margin, (%)		
Positive	33 (13.4)	17 (20.5)
Negative	214 (86.6)	66 (79.5)
Capsule invasion, (%)		
Present	107 (43.3)	39 (47.0)
Absent	140 (56.7)	44 (53.0)
PV/SMV/SV invasion degree, (%)		
Absent	145 (58.7)	47 (56.6)
<180°	53 (21.5)	19 (22.9)
>180°	49 (19.8)	17 (20.5)
Artery reconstruction, (%)		
Yes	4 (1.6)	0 (0)
No	243 (98.4)	83 (100)
Adjuvant chemotherapy, (%)		
Yes	159 (64.4)	67 (80.7)
No	88 (35.6)	16 (19.3)
Morbidity, (%)		
Clavien-Dindo grade 0-II	229 (92.7)	76 (91.6)
Clavien-Dindo grade III-IV	18 (17.3)	7 (8.4)
TNM stage, (%)		
I-IIA	119 (48.2)	37 (44.6)
IIB-IV	128 (51.8)	46 (55.4)

metastasis-free survival in both training and validation cohorts (Figure 3). The C-indexes of nomogram based on the training and validation cohorts were 0.760 (95% confidence interval [CI], 0.720-0.799) and 0.739 (95% CI, 0.669-0.810), respectively. The AUC of the nomogram at 1-, 2- and 3-year was 0.815, 0.803 and 0.773 in the training cohort, and 0.765, 0.879 and 0.908 in the validation cohort, respectively, all of which were higher than AJCC and JPS of TNM stage system (Figure 4 and Table 4).

Clinical utility of the nomogram

DCA analysis revealed that the nomogram model could provide superior net benefits and exhibited a wider range of threshold probabilities than the AJCC and JPS stage system in both training and validation cohorts (Figure 5). Patients were divided into three different risk groups based on the total risk scores calculated by the nomogram models, to validate the

10.3389/fonc.2022.1040411

TABLE 2 Recurrence patterns of patients with liver metastasis after the operation.

Liver metastasis patterns (at the date of liver metastasis diagnosis)	Training cohort (n=247)	Validation cohort (n=83)
Liver metastasis only	116 (47.0%)	39 (47.0%)
Multiple recurrences		
Liver+Retroperitoneum	8 (3.2%)	5 (6.0%)
Liver+Locoregional	4 (1.6%)	1 (1.2%)
Liver+Lung	2 (0.8%)	0 (0%)
Liver+Retroperitoneum+Lung	1 (0.4%)	0 (0%)
Liver+Retroperitoneum+Peritoneal+Spleen	1 (0.4%)	0 (0%)
Liver+Bone	0 (0%)	1 (1.2%)
Sum up	132 (53.4%)	46 (55.4%)

predictive abilities of the nomogram for liver metastasis after the operation. The optimal cut-off points were auto-calculated by X-tile software. The risk scores calculated divide patients into the low-risk group (<99.6), moderate-risk group (99.6-160.1) and high-risk group (>160.1). The liver metastasis-free survival rates were calculated in three groups, the results showed a significant discriminatory ability for liver metastasis risk based on the nomogram risk scores (Figure 6).

Discussion

In the present study, we developed and externally validated a nomogram model based on clinicopathological and vascular invasion characteristics, which could be used to predict liver metastasis in patients with PDAC after radical resection. The nomogram model showed superior performance in predicting liver metastasis, with C-indexes of 0.760 (95% CI, 0.720-0.799) and 0.739 (95% CI, 0.669-0.810) in the training and validation cohorts, respectively. As the prognosis of PDAC patients with liver metastasis after radical resection is significantly poor, and currently there is no specifical model for predicting liver metastasis, the present nomogram provided an intuitive and utility tool for guiding the personalized and rational choice of prescient intervention, which is of increased clinical significance.

Liver metastasis is an important feature of PDAC after radical resection, which accounts for the largest proportion and the poorest prognosis among all recurrence patterns, resulting in an increase in mortality (5, 13). Previous study demonstrated that specific patterns of PDAC recurrence result in different survival outcomes, the post progression survival of patients with liver metastasis (4.7months) or multiple-site recurrence (7.2months) had significantly worse when compared to patients with local recurrence (9.7months) or lung metastasis (15.4 months, p<0.001) (4). Hishinuma et al. (14) reported that local recurrence is rarely the direct cause of death, instead most patients died of liver metastasis, based on 27 patient autopsies. Previous reports have shown that more than 40% of PDAC patients develop liver metastasis after radical resection (4, 15), similar to the results of this study, but we further focused on liver metastasis throughout the follow-up period, to obtain accurate liver metastasis-free survival in each patient, for developing a more precise and prognostic nomogram model. So, we introduced the term of liver metastasis-free survival, which is a bit like the term recurrence-free survival (RFS), since the liver metastasis is essentially a particular pattern of tumor recurrence, and we

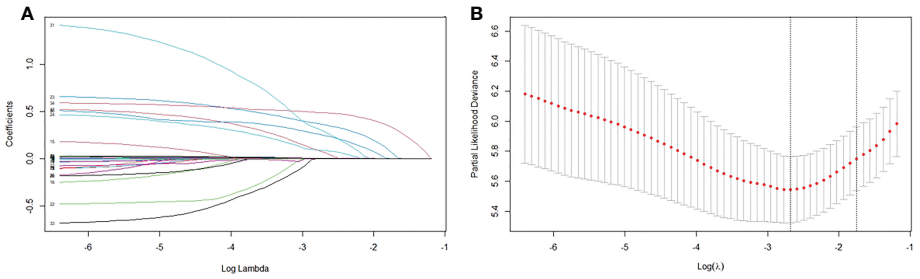


FIGURE 1
Factors associated with liver metastasis. **(A)** LASSO coefficient profiles of the 34 variables. **(B)** Optimum parameter (Lambda) selection in the LASSO model performed ten-fold cross-validation *via* minimum criteria.

10.3389/fonc.2022.1040411

TABLE 3 Univariate and multivariate analysis of predictive factors for liver metastasis in the training cohort.

Variable	Univariate Cox analysis		Multivariate analysis	
	Hazard ratio (95%CI)	*p*-value	Hazard ratio (95%CI)	*p*-value
Postoperative CA125, IU/ml	1.007 (1.003-1.010)	<0.001	1.007 (1.003-1.011)	0.001
Total examined lymph nodes number	1.028 (1.009-1.048)	0.004		
Tumor differentiation				
Poor	Reference		Reference	
Well-moderate	2.168 (1.531-3.070)	<0.001	1.640 (1.126-2.388)	0.010
Lymphovascular invasion				
Present	Reference			
Absent	1.748 (1.219-2.505)	0.002		
Capsule invasion				
Present	Reference			
Absent	1.463 (1.037-2.064)	0.030		
Tumor size, cm				
>4	Reference		Reference	
≤4	2.178 (1.547-3.065)	<0.001	1.520 (1.045-2.210)	0.029
Lymph node ratio				
≥0.2	Reference		Reference	
<0.2	1.844 (1.249-2.722)	0.002	1.897 (1.256-2.866)	0.002
PV/SMV/SV invasion degree				
None	Reference		Reference	
<180°	2.754 (1.806-4.197)	<0.001	2.572 (1.664-3.977)	<0.001
>180°	3.991 (2.641-6.030)	<0.001	2.829 (1.817-4.404)	<0.001

only concentrate on liver metastasis during follow-up, for the nomogram development. Morever, the patients with postoperative liver metastasis may also be accompanied by other patterns of recurrence, and we found that there was no significant difference in overall survival between the patients with only-liver metastasis and patients with multiple recurrence, highlighting the malignancy of liver metastasis and the importance of this nomogram.

In the process of developing our nomogram, PV/SMV/SV invasion degree is an important factor, which is not easily

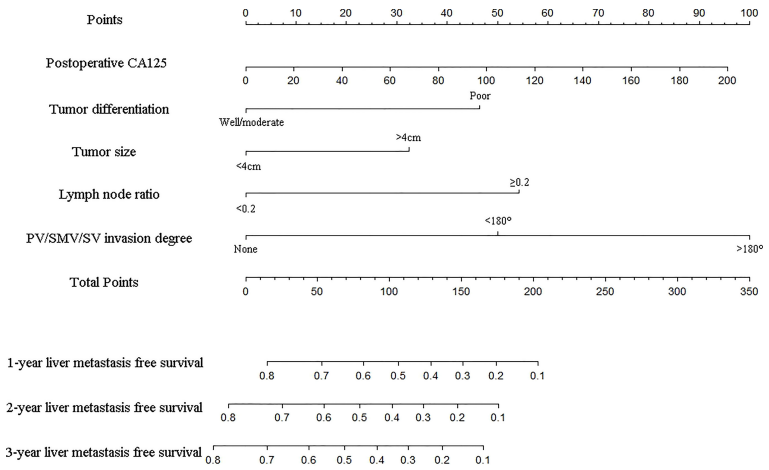


FIGURE 2
Nomogram for predicting the 1-, 2- and 3-year liver metastasis-free survival in PDAC patients after the operation. The nomogram was established in the training group, with postoperative CA125, tumor differentiation, tumor size, lymph node ratio and PV/SMV/SV invasion degree.

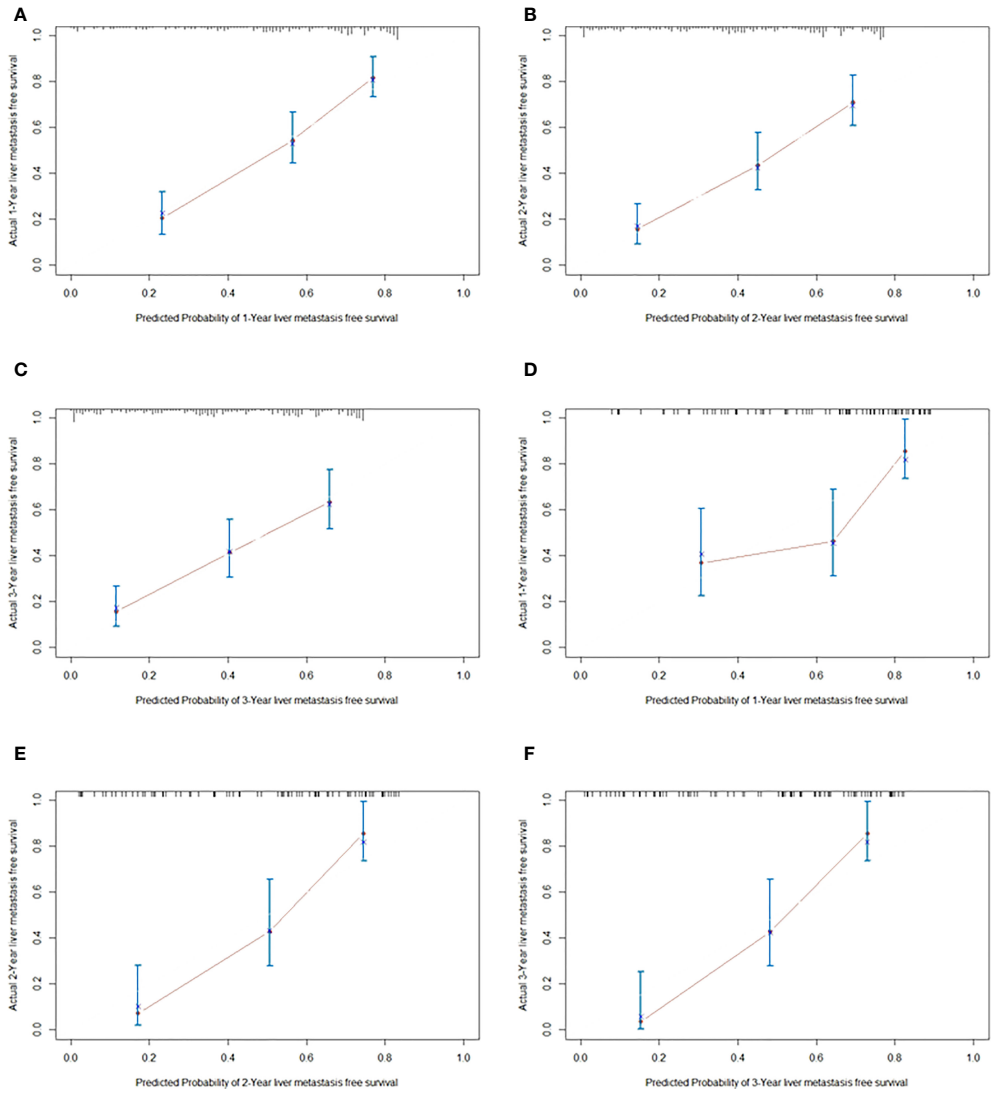


FIGURE 3
The calibration curves for predicting liver metastasis-free survival at 1 year **(A)**, 2 years **(B)** and 3 years **(C)** in the training cohort, and those at 1 year **(D)**, 2 years **(E)** and 3 years **(F)** in validation cohorts, respectively.

measurable as other clinicopathological variables, needed an intuitive and standard classification to define the different invasion degrees. Nakao et al. (16) based on the narrowing of vascular invaded by the tumor, suggested four types of vascular invasion degree: normal, unilateral narrowing, bilateral narrowing and complete obstruction. However this classification has limited capacity in predicting prognosis. Shen et al. (17) reported four types to indicate the relationship between vein and tumor: type 1 (absent), type 2 (mild deformity), type 3 (tethering or stenosis >1/2) and type 4 (obstruction or embolus), this classification can accurately predict the prognosis and similiar to ours. According to the degree of the tumor abutment or invasion, we classified into PV/SMV/SV without invasion, invasion <180°, and invasion >180°, considering both the SMV and SV belong to the portal vein circulatory system, this classification could combine the pancreatic head and body/tail cancer, evaluating the invasion degree in a simple and duplicatable way. As the close adjacent anatomical relationship between the pancreas and PV/SMV/SV, these veins are a common site of direct tumor involvement, but the impact on the prognosis is not clear (18–20). In the present study, PV/SMV/SV invasion was a significant independent risk factor for liver metastasis, 83.7% of patients with vascular invasion >180° developed liver metastasis after radical resection. The "circulating tumor cell (CTC)" hypothesis may explain: that the tumor cells invading the PV/SMV/SV were

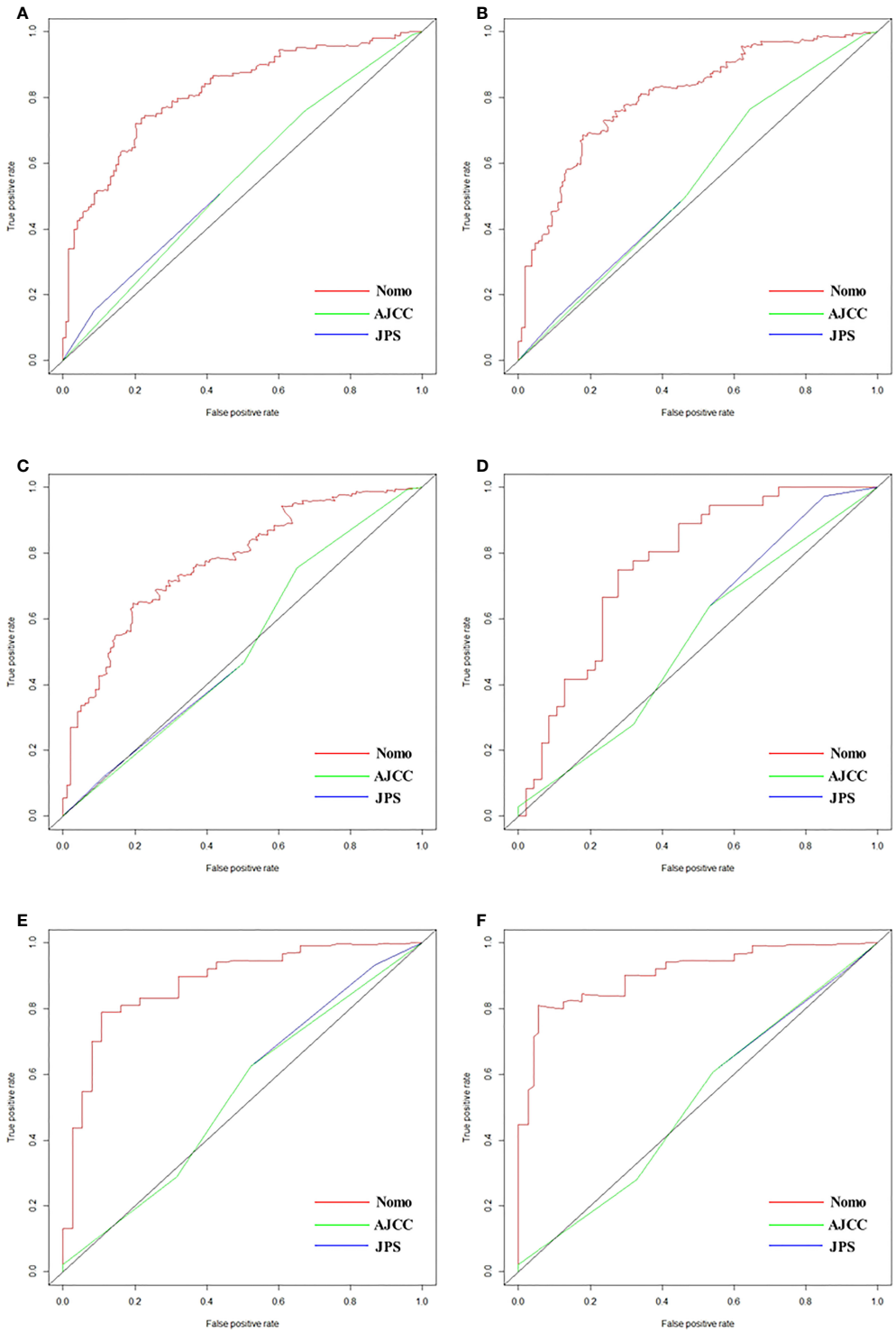


FIGURE 4
ROCs of nomogram, AJCC and JPS for predicting liver metastasis-free survival at 1 year **(A)**, 2 years **(B)** and 3 years **(C)** in the training cohort, and those at 1 year **(D)**, 2 years **(E)** and 3 years **(F)** in validation cohorts, respectively.

TABLE 4 Prognostic performance of different models for predicting liver metastasis after radical resection.

	AUC		
	1 year	2 years	3 years
Training cohort			
Nomogram	0.815	0.803	0.773
AJCC	0.561	0.545	0.522
JPS	0.549	0.549	0.518
Validation cohort			
Nomogram	0.765	0.879	0.908
AJCC	0.530	0.531	0.513
JPS	0.550	0.539	0.511

likely to enter portal vein circulation and metastasize to liver (21, 22). Tien et al. (23) detected the CTCs in portal vein blood obtained during the operation, and found that patients with positive CTCs tended to develop liver metastasis after the operation, supporting the above hypothesis.

Postoperative CA125 level is another independent risk factor of liver metastasis, increased CA125 level after radical resection was an important feature of high PDAC tumor burden and distant metastasis tendency, which indicated the poor curative effect of the operation. Previous study suggested that serum CA125 levels were the most strongly associated with early distant metastasis after pancreatectomy, when compared with other tumor markers such as CA199, CEA, CA242 and CA724. High CA125 levels was consistent with the expression of a "drive" metastasis associated gene signature, which may be the reason for CA125 highly sensitive to liver metastasis (24). Xu et al. (25, 26) also reported that postoperative CA125 level can better predict the prognosis when compared with preoperative tumor markers. Moreover, poor tumor differentiation was associated with liver metastasis as well, in this study, the probabilities of liver metastasis were 35.3%, 50% and 59.5% in the high, moderate and poor tumor differentiation, respectively. A previous large sample study supported our result, indicating that poor differentiation of tumor could promote infiltration and invasion, and contribute to liver metastasis (5). The "intriguing hypothesis" may explain: that poorly differentiated tumors highly expressed epidermal growth factor and E-cadherin, enhanced the ability of liver metastasis (27). Apart from the above risk factors, the nomogram model also covered several risk factors including lymph node ratio and tumor size. Compared with positive lymph node number, the lymph node ratio is a more valuable prognostic indicator, also associated with liver metastasis after radical resection (28, 29). Furthermore, we found that preoperative neoadjuvant chemotherapy was not associated with liver metastasis, which is a regrettable result. We believe that selective bias is the cause: the patients in cohort of neoadjuvant chemotherapy tend to have bigger tumor size and worse vascular invasion degrees, these undesirable tumor characteristic may lead to postoperative liver metastasis, leading to negative result of neoadjuvant chemotherapy.

Compared with the previous traditional nomograms for survival and recurrence prediction, our model can predict liver metastasis after radical resection more specifically and accurately, for early intervention of this unfavorable

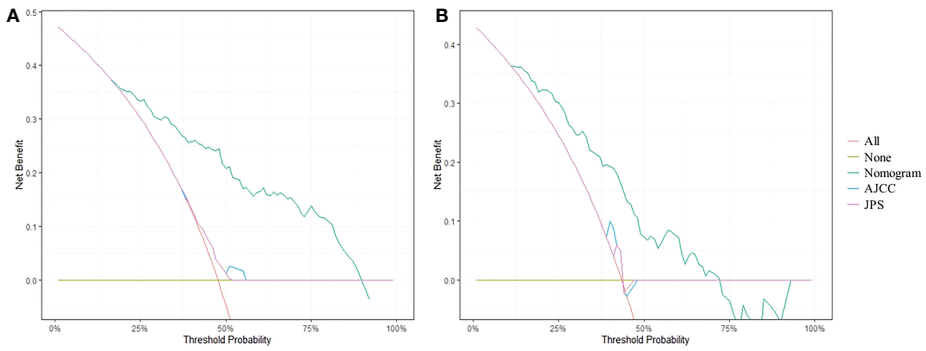


FIGURE 5
DCA curves for predicting 1-year liver metastasis-free survival based on nomogram as compared with 8th AJCC and 7th JPS stage system in the training cohort **(A)** and the validation cohort **(B)**.

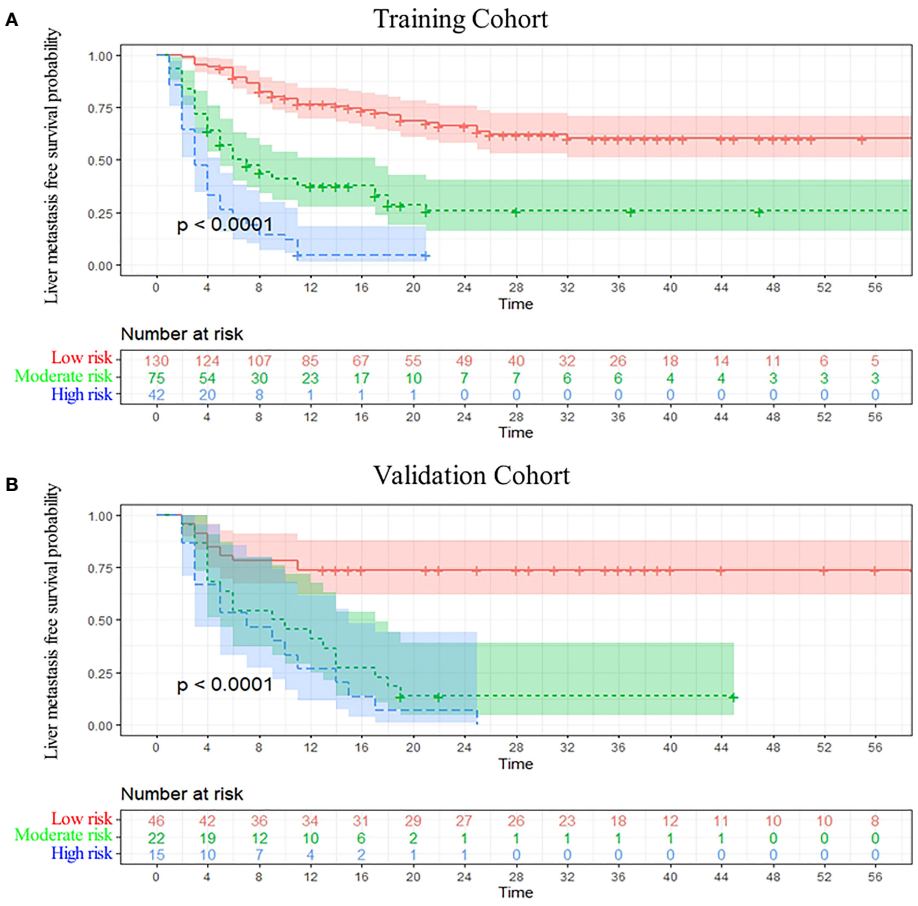


FIGURE 6
Kaplan-Meier curve analysis. Liver metastasis-free survival curves were stratified by the model risk score in the training cohort **(A)** and the validation cohort **(B)**.

metastasis. The nomogram achieved a C-index of 0.760 and 0.739 in the training and external validation cohorts, respectively, and the calibration curve indicated the precisely predictive ability of the nomogram in prediction. The present nomogram showed higher AUC and better performance in predicting liver metastasis, when compared with the TNM stage system of 8th AJCC and 7th JPS (10, 11). In addition, DCA analysis indicated that the nomogram could augment net benefits and expose a wider range of threshold probabilities by risk stratification in the prediction of liver metastasis. Furthermore, we calculated the nomogram risk score and compared the liver metastasis-free survival rates, the results showed a significant discriminatory ability for liver metastasis risk based on the nomogram. Liver metastasis possibly represents a unique biological subtype of PDAC (6), personalized follow-up and intervention was needed for the patients with a high nomogram risk score. Randomized clinical trials confirmed that several gemcitabine-based chemotherapies were effective in preventing postoperative liver metastasis and prolonging survival (30). Masayuki et al. (31) reported that hepatic artery infusion chemotherapy can observably increase intrahepatic drug concentration and eliminate tumor metastatic lesions. Additionally, hepatectomy for PDAC patients with postoperative liver metastasis has been proven successful in improving survival (32).

The present study had several limitations. First, liver metastasis was generally based on imaging studies, the tiny hepatic nodules were difficult to identify as metastasis or cyst, limiting the accuracy of the liver metastasis dignosis date. Second, the specific adjuvant chemotherapy regimen after the operation were not included in the variable, making the cohorts relatively heterogenous. In future, a study especially for the patients with/without systemic adjuvant treatment will be established, to explore the effect of systemic adjuvant treatment, as an upgrade to the present nomogram. Third, some differences exist between the training and validation cohorts, but in general, the two cohorts are basically balanced, and the C-index were 0.760 and 0.739, indicating the nomogram has good consistency. Furthermore, a

large sample of prospective cohorts is still needed, to further confirm the predictive value.

In conclusion, we developed and externally validated a nomogram to predict liver metastasis after radical resection in patients with PDAC. The nomogram based on clinicopathological characteristics showed great accuracy in predictive performance, and provided an intuitive and utility tool to guide personalized and prescient intervention for patients with a potential risk of liver metastasis.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: 1174608081@qq.com.

Ethics statement

The study was approved by the ethics committee of Ningbo Medical Center Lihuili Hospital (Approval number: KY2021PJ263). All research procedures complied with the relevant guidelines and regulations. Informed consent was obtained from all patients before inclusion. We confirmed that this study was conducted following the Declaration of Helsinki.

Author contributions

JT and CL proposed and designed the study. SW, W J and SM collected the data. JT and SM analyzed the data, interpreted the results, and drafted the article. All authors contributed to the article and approved the submitted version.

Funding

Funded by Ningbo medical and health brand discipline (PPXK2018-03).

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2022.1040411/full#supplementary-material

References

1. Huang J, Lok V, Ngai CH, Zhang L, Yuan J, Lao XQ, et al. Worldwide burden of, risk factors for, and trends in pancreatic cancer. *Gastroenterology* (2021) 160:3. doi: 10.1053/j.gastro.2020.10.007

2. Ferrone CR, Pieretti-Vanmarcke R, Bloom JP, Zheng H, Szymonifka J, Wargo JA, et al. Pancreatic ductal adenocarcinoma: Long-term survival does not equal cure. *Surgery* (2012) 152:S43–9. doi: 10.1016/j.surg.2012.05.020

3. Luu AM, Braumann C, Belyaev O, Janot-Matuschek M, Rudolf H, Praktiknjo M, et al. Long-term survival after pancreaticoduodenectomy in patients with ductal adenocarcinoma of the pancreatic head. *Hepatobiliary Pancreat Dis Int* (2021) 20:271–78. doi: 10.1016/j.hbpd.2020.12.006

4. Groot VP, Gemenetzis G, Blair AB, Ding D, Javed AA, Burkhart RA, et al. Implications of the pattern of disease recurrence on survival following pancreatectomy for pancreatic ductal adenocarcinoma. *Ann Surg Oncol* (2018) 25:2475–83. doi: 10.1245/s10434-018-6558-7

5. Groot VP, Rezaee N, Wu W, Cameron JL, Fishman EK, Hruban RH, et al. Patterns, timing, and predictors of recurrence following pancreatectomy for pancreatic ductal adenocarcinoma. *Ann Surg* (2018) 267:936–45. doi: 10.1097/SLA.0000000000002234

6. Tong J, Wu S, Lu C, Yang Y, Mao S, Lu C. Risk factors of early liver metastasis for pancreatic ductal adenocarcinoma after radical resection. *Gastroenterol Res Pract* (2022) 2022:8061879. doi: 10.1155/2022/8061879

7. Li H, Zhou F, Cao Z, Tang Y, Huang Y, Li Y, et al. Development and validation of a nomogram based on nutritional indicators and tumor markers for prognosis prediction of pancreatic ductal adenocarcinoma. *Front Oncol* (2021) 11:682969. doi: 10.3389/fonc.2021.682969

8. Peng F, Qin T, Wang M, Wang H, Dang C, Wu CH, et al. Development and validation of a nomogram to predict survival in pancreatic head ductal adenocarcinoma after pancreaticoduodenectomy. *Front Oncol* (2021) 11:734673. doi: 10.3389/fonc.2021.734673

9. Tempero MA, Arnoletti JP, Behrman SW, Ben-Josef E, Benson AR, Casper ES, et al. Pancreatic adenocarcinoma, version 2.2012: Featured updates to the NCCN guidelines. *J Natl Compr Canc Netw* (2012) 10:703–13. doi: 10.6004/jnccn.2012.0073

10. van Roessel S, Kasumova GG, Verheij J, Najarian RM, Maggino L, de Pastena M, et al. International validation of the eighth edition of the American joint committee on cancer (AJCC) TNM staging system in patients with resected pancreatic cancer. *JAMA Surg* (2018) 153:e183617. doi: 10.1001/jamasurg.2018.3617

11. Yamaguchi K, Okusaka T, Shimizu K, Furuse J, Ito Y, Hanada K, et al. Clinical practice guidelines for pancreatic cancer 2016 from the Japan pancreas society: A synopsis. *Pancreas* (2017) 46:595–604. doi: 10.1097/MPA.0000000000000816

12. Hauschka PV, Harrington WF. Collagen structure in solution. IV. conformational properties of refolded cross-linked chains. *Biochemistry-Us* (1970) 9:3745–54. doi: 10.1021/bi00821a013

13. Zheng B, Ohuchida K, Yan Z, Okumura T, Ohtsuka T, Nakamura M. Primary recurrence in the lung is related to favorable prognosis in patients with pancreatic cancer and postoperative recurrence. *World J Surg* (2017) 41:2858–66. doi: 10.1007/s00268-017-4068-6

14. Hishinuma S, Ogata Y, Tomikawa M, Ozawa I, Hirabayashi K, Igarashi S. Patterns of recurrence after curative resection of pancreatic cancer, based on autopsy findings. *J Gastrointest Surg* (2006) 10:511–18. doi: 10.1016/j.gassur.2005.09.016

15. Suenaga M, Fujii T, Kanda M, Takami H, Okumura N, Inokawa Y, et al. Pattern of first recurrent lesions in pancreatic cancer: Hepatic relapse is associated with dismal prognosis and portal vein invasion. *Hepatogastroenterology* (2014) 61:1756–61.

16. Nakao A, Kanzaki A, Fujii T, Kodera Y, Yamada S, Sugimoto H, et al. Correlation between radiographic classification and pathological grade of portal vein wall invasion in pancreatic head cancer. *Ann Surg* (2012) 255:103–8. doi: 10.1097/SLA.0b013e318237872e

17. Shen YN, Bai XL, Jin G, Zhang Q, Lu JH, Qin RY, et al. A preoperative nomogram predicts prognosis of up front resectable patients with pancreatic head cancer and suspected venous invasion. *HPB (Oxford)* (2018) 20:1034–43. doi: 10.1016/j.hpb.2018.04.010

18. Kaneoka Y, Yamaguchi A, Isogai M. Portal or superior mesenteric vein resection for pancreatic head adenocarcinoma: Prognostic value of the length of venous resection. *Surgery* (2009) 145:417–25. doi: 10.1016/j.surg.2008.12.009

19. Muller SA, Hartel M, Mehrabi A, Welsch T, Martin DJ, Hinz U, et al. Vascular resection in pancreatic cancer surgery: Survival determinants. *J Gastrointest Surg* (2009) 13:784–92. doi: 10.1007/s11605-008-0791-5

20. Addeo P, Velten M, Averous G, Faitot F, Nguimpi-Tambou M, Nappo G, et al. Prognostic value of venous invasion in resected T3 pancreatic adenocarcinoma: Depth of invasion matters. *Surgery* (2017) 162:264–74. doi: 10.1016/j.surg.2017.03.008

21. Liu X, Li C, Li J, Yu T, Zhou G, Cheng J, et al. Detection of CTCs in portal vein was associated with intrahepatic metastases and prognosis in patients with advanced pancreatic cancer. *J Cancer* (2018) 9:2038–45. doi: 10.7150/jca.23989

22. Wang Y, Yu X, Hartmann D, Zhou J. Circulating tumor cells in peripheral blood of pancreatic cancer patients and their prognostic role: A systematic review and meta-analysis. *HPB (Oxford)* (2020) 22:660–69. doi: 10.1016/j.hpb.2019.11.003

23. Tien YW, Kuo HC, Ho BI, Chang MC, Chang YT, Cheng MF, et al. A high circulating tumor cell count in portal vein predicts liver metastasis from periampullary or pancreatic cancer: A high portal venous CTC count predicts liver metastases. *Med (Baltimore)* (2016) 95:e3407. doi: 10.1097/MD.0000000000003407

24. Liu L, Xu HX, Wang WQ, Wu CT, Xiang JF, Liu C, et al. Serum CA125 is a novel predictive marker for pancreatic cancer metastasis and correlates with the metastasis-associated burden. *Oncotarget* (2016) 7:5943–56. doi: 10.18632/oncotarget.6819

25. Xu HX, Li S, Wu CT, Qi ZH, Wang WQ, Jin W, et al. Postoperative serum CA19-9, CEA and CA125 predicts the response to adjuvant chemoradiotherapy following radical resection in pancreatic adenocarcinoma. *Pancreatology* (2018) 18:671–77. doi: 10.1016/j.pan.2018.05.479

26. Xu HX, Liu L, Xiang JF, Wang WQ, Qi ZH, Wu CT, et al. Postoperative serum CEA and CA125 levels are supplementary to perioperative CA19-9 levels in predicting operative outcomes of pancreatic ductal adenocarcinoma. *Surgery* (2017) 161:373–84. doi: 10.1016/j.surg.2016.08.005

27. Sugawara T, Ban D, Nishino J, Watanabe S, Maekawa A, Ishikawa Y, et al. Prediction of early recurrence of pancreatic ductal adenocarcinoma after resection. *PLoS One* (2021) 16:e249885. doi: 10.1371/journal.pone.0249885

28. Groot VP, Gemenetzis G, Blair AB, Rivero-Soto RJ, Yu J, Javed AA, et al. Defining and predicting early recurrence in 957 patients with resected pancreatic ductal adenocarcinoma. *Ann Surg* (2019) 269:1154–62. doi: 10.1097/SLA.0000000000002734

29. You MS, Lee SH, Choi YH, Shin BS, Paik WH, Ryu JK, et al. Lymph node ratio as valuable predictor in pancreatic cancer treated with R0 resection and adjuvant treatment. *BMC Cancer* (2019) 19:952. doi: 10.1186/s12885-019-6193-0

30. Oettle H, Neuhaus P, Hochhaus A, Hartmann JT, Gellert K, Ridwelski K, et al. Adjuvant chemotherapy with gemcitabine and long-term outcomes among patients with resected pancreatic cancer: The CONKO-001 randomized trial. *JAMA* (2013) 310:1473–81. doi: 10.1001/jama.2013.279201

31. Sho M, Tanaka T, Yamada T, Nomi T, Akahori T, Doh J, et al. Novel postoperative adjuvant strategy prevents early hepatic recurrence after resection of pancreatic cancer. *J Hepatobiliary Pancreat Sci* (2011) 18:235–39. doi: 10.1007/s00534-010-0336-7

32. Kleeff J, Reiser C, Hinz U, Bachmann J, Debus J, Jaeger D, et al. Surgery for recurrent pancreatic ductal adenocarcinoma. *Ann Surg* (2007) 245:566–72. doi: 10.1097/01.sla.0000245845.06772.7d

frontiers | Frontiers in Oncology

TYPE Original Research
PUBLISHED 07 December 2022
DOI 10.3389/fonc.2022.1006131

OPEN ACCESS

EDITED BY
Xiaodong Tian,
First Hospital, Peking University, China

REVIEWED BY
Hanxiang Zhan,
Qilu Hospital, Shandong University, China
Zipeng Lu,
Nanjing Medical University, China

*CORRESPONDENCE
Benno Traub
Benno.Traub@uniklinik-ulm.de

SPECIALTY SECTION
This article was submitted to Gastrointestinal Cancers: Hepato Pancreatic Biliary Cancers, a section of the journal Frontiers in Oncology

RECEIVED 29 July 2022
ACCEPTED 21 November 2022
PUBLISHED 07 December 2022

CITATION
Shi J, Yang X, Kang Q, Lu J, Denzinger M, Kornmann M and Traub B (2022) JNK inhibitor IX restrains pancreatic cancer through p53 and p21.
Front. Oncol. 12:1006131.
doi: 10.3389/fonc.2022.1006131

JNK inhibitor IX restrains pancreatic cancer through p53 and p21

Jingwei Shi[1,2], Xing Yang[2], Qi Kang[2], Jian Lu[2], Maximilian Denzinger[2], Marko Kornmann[2] and Benno Traub[2*]

[1]Department of Cardiothoracic Surgery, Nanjing Drum Tower Hospital, The Affiliated Hospital of Nanjing University Medical School, Nanjing, China, [2]Department of General and Visceral Surgery, Ulm University Hospital, Ulm, Germany

Novel treatment options for pancreatic cancer are desperately needed. De-regulated kinases can be regularly detected in pancreatic cancer. Multiple pathway inhibitors were developed to exploit these features, among them selective inhibitors of the c-Jun N-terminal kinase isoforms 1 and 2 (JNK1 and 2). We evaluated the effectiveness of four different JNK inhibitors on pancreatic cancer cell lines. Cell mobility and migration were evaluated in scratch assay and Boyden chamber assay. Mechanism of cell death was analyzed *via* apoptosis assays in FACS and immunoblotting as well as cell cycle analysis *via* FACS, and qPCR. JNK2 knockout cells were generated using siRNA transfection. Among the inhibitors, JNK inhibitor IX (JNK-in-IX), designed as specific inhibitor against JNK2 was proven highly effective in inhibiting cell growth, mobility and migration. We were able to show that JNK-in-IX caused DNA damage resulting in G2 arrest mediated through p53 and p21. Interestingly, JNK-in-IX acted independently of its primary target JNK2. In summary, JNK-in-IX was shown highly effective in pancreatic cancer. This study underlines the need for modeling systems in testing therapeutic options as JNK2 was previously not indicated as a potential target.

KEYWORDS

pancreatic cancer, c-Jun N-terminal kinase, JNK inhibitor IX, cell cycle arrest, G2 arrest

Introduction

The devastating numbers of pancreatic cancer (PC) are well known around researchers and clinicians: fourth leading cause of cancer-related mortality, estimated to rise to second position within next decade (1, 2); no improvement in mortality rates over the last decade, unlike other malignancies (1); failure of early detection and severe side-effects in treatment (3).

In search for alternative treatment options, researchers have focused on signaling pathways that were found altered in PC. While mutant KRAS is the key driver of PC, it has yet been mostly undruggable (4). Addressing signaling pathways downstream of KRAS has thus become of interest, but the combined inhibition of major downstream pathways, the PI-3-/Akt-kinase and the MAPK pathways has failed in patients (5). Thus, a more thorough understanding of these pathways is needed.

The c-Jun N-terminal kinases 1, 2, and 3 (JNK1/2/3) form a major subgroup among MAP kinases, and, together with the p38 kinase are grouped as stress activated kinases (6). This is due to their physiologic role in the cellular response towards stressors like proliferation and metabolic stress as well as the response towards exogenic triggers like chemotherapeutic drugs and irradiation (7). These variable stimuli are transduced into a cellular response carried out by transcription factors, mainly c-Jun (8). Thereby, the central role of JNKs becomes especially evident in PC, as RAS-induced oncogenesis is dependent on Jun phosphorylation (9, 10).

Still, it is yet to determine if the JNK isoforms act as tumor promotors or suppressors. Multiple well-designed studies evaluated the role of JNK1 and 2 in cellular transformation and have offered evidence for both malignant transformation (11–15) and tumor suppression (15–17). In PC we recently showed a tumor promoting phenotype after JNK2 knockdown, while JNK1 knockdown seemed to reduce the cellular malignant potential (18).

Studies in pharmacological targeting of JNK can be biased by inhibiting isoforms nonspecifically as well as off-target effects and compensatory mechanisms, e.g in the p38 pathway (6). However, isoform specific JNK inhibitors were developed recently and the inactivation of JNK1 sensitized PC cells towards FOLFOX treatment (19).

This study aimed to determine value and mechanism of JNK inhibition in PC. We used novel isoform specific inhibitors of JNK to test their efficacy in PC cell lines. Contrary to previous findings we showed the highest efficacy for JNK inhibitor IX (JNK-in-IX), designed as specific inhibitor of JNK2. JNK-in-IX caused a G2 arrest through upregulation of p21 and p53 phosphorylation and markedly reduced cell migration, but surprisingly seemed to act independently of JNK2.

Abbreviations: ATCC, American Type Culture Collection; BAX, Bcl-2-associated X protein; Bcl2, B-cell lymphoma 2; CCNB1, G2/mitotic-specific cyclin-B1; CDK1, Cyclin-dependent kinase 1; CDKN1A(p21), Cyclin-dependent kinase inhibitor 1; CHEK1, Checkpoint Kinase 1; CTG, CellTiter-Glo; DMEM, Dulbecco's Modified Eagle's Medium; DMSO, Dimethyl sulfoxide; FOLFIRINOX, FOL-folinic acid, F-fluorouracil, IRIN-irinotecan, OX-oxaliplatin; GAPDH, Glycerinaldehyd-3-phosphat-Dehydrogenase; IC50, The half maximal inhibitory concentration; ICC, Immunocytochemistry; JNK, c-Jun N-terminal kinase; JNK-in-IX, JNK inhibitor IX; KRAS, Kirsten rat sarcoma; MAP, Mitogen-activated protein; MAPK, Mitogen-activated protein kinase; MFI, Mean fluorescence intensity; PC, Pancreatic cancer; PI, Propidium Iodide; PLK1, Polo-like Kinase 1; qPCR, Quantitative real-time polymerase chain reaction; RAS, Rat sarcoma virus; RPMI, Roswell Park Memorial Institute medium; Z-VAD-FMK, carbobenzoxy-valyl-alanyl-aspartyl-[O-methyl]- fluoromethylketone.

Materials and methods

Cell lines and cell culture

Human pancreatic cancer cell lines AsPC-1 (RRID : CVCL_0152), BxPC-3 (RRID : CVCL_0186), MIA PaCa-2 (RRID : CVCL_0428), and PANC-1 (RRID : CVCL_0480) were purchased from the American Type Culture Collection (ATCC, Manassas, USA). AsPC-1 was cultured in RPMI (Roswell Park Memorial Institute medium). BxPC-3 was cultured in 50% RPMI and 50% DMEM (Dulbecco's Modified Eagle's Medium). MIA PaCa-2 and PANC-1 were cultured in DMEM. All mediums were supplemented with 10% fetal calf serum (FCS), 1% Penicillin (10,000 U/ml)/Streptomycin (10,000 µg/ml). Cells were maintained in 100 mm cell culture dishes and in a monolayer culture at 37°C in humidified air with 5% CO2. Regular mycoplasma testing was carried out.

Human pancreatic cancer organoids

Human pancreatic cancer organoids were generated and maintained as described before (20). Organoid work was performed in cooperation with the Core Facility Organoids of the University Hospital of Ulm. Organoid generation and analysis were approved by the independent ethics committee of the University of Ulm (approval number 72/19) and written informed consent was obtained from patients before collecting the samples.

Dose response of pancreatic cancer cell lines to JNK inhibitors and chemotherapeutic drugs

JNK inhibitors SP600125, AS602801, JNK-in-IX and Licochalcone, supplied by Selleck Chemicals GmbH (Planegg, Germany), were dissolved and aliquoted according to manufacturer's instructions. The combination regimen of FOLFIRINOX is composed of 5-FU (Sigma Aldrich, Taufkirchen, Germany), Oxaliplatin (Sigma Aldrich), and SN-38 (active compound of Iriontecan, Sigma Aldrich) in ratios of 5-FU: Oxaliplatin: SN-38 = 80.95: 0.80: 1 and Gemcitabine (Sigma Aldrich) – Paclitaxel (Sigma Aldrich) (Gem-Pac) in ratios of Gemcitabine: Paclitaxel = 1: 0.04. PC cells were seeded at a

density of 500 cells/well in 384-well plates. Cells were allowed to adhere for 24 h. Then, drugs were dispensed using the Tecan D300e (Tecan Deutschland GmbH, Crailsheim, Germany) with titration concentrations ranging from 0.0001 μM to 50μM for chemotherapeutic drugs and from 0.01 μM to 50 μM for inhibitors, while DMSO was normalized to the highest concentration (0.5%, v/v). Synergy mode supplied by the D300e was used to dispense the combination of chemotherapies and JNK inhibitors. 5 days after treatment, cell viability was assessed by CellTiter-Glo® Luminescent Cell Viability Assay (Promega GmbH, Walldorf, Germany) following the manufacturer's instructions.

Human pancreatic cancer organoids were dissociated into single cells and 500 cells per well were seeded in triplicate in 1μl Matrigel domes in 384-well plates and 25μl of organoid growth medium was added. After 24 h, 25μl of organoid growth medium containing the drugs was added. 10 concentrations per drug were used. Cell viability was assessed using CellTiter-Glo® 3D Cell Viability Assay (Promega GmbH) after 5 days of treatment.

Data were analyzed using GraphPad Prism 8.0.1 (GraphPad Software, San Diego, USA). Synergetic effects of chemotherapies and JNK inhibitors was evaluated by the SynergyFinder 2.0 platform (21).

Effect of V-ZAD-FMK (50 μM, Selleck Chemicals) on cell growth with or without JNK-in-IX treatment was also determined following the above procedures.

Cell migration assay

Wound healing assay was performed to examine cell movement and Boyden chamber assay was used to determine cell migration as described before (22). In brief, the gaps of wounds scratched by sterile 200 μl tips on confluent cells in a monolayer were measured. Gap distances were quantitatively evaluated by ImageJ 1.8.0 (National Institutes of Health, Bethesda, USA). In the Boyden chamber assay, migratory cells were stained with DAPI (Sigma Aldrich) and counted by ImageJ 1.8.0. In both assays, the intervention group was treated with JNK-in-IX for 48 h using the previously determined cell line specific concentrations matching the IC50: 0.409 μM for AsPC-1, 0.220 μM for BxPC-3, 0.071 μM for MIA PaCa-2 and 0.066 μM for PANC-1. Wound healing rate and number of migratory cells were analyzed and compared between the treated and untreated groups using GraphPad Prism 8.0.1.

Giemsa stain assay

Giemsa staining was performed as described before (22). PC cells stained by Giemsa stain (Sigma Aldrich) were photographed under a light microscope at 20x and 40x magnification. Afterwards, cell morphology was observed.

Western blot analysis

Western blot was performed as described before (22). Primary antibodies were used as follows: Bcl2 (1:200, Cell Signalling Technology (Frankfurt am Main, Germany)), BAX (1:1000, Cell Signalling Technology), c-Jun(1:100, Santa Cruz Biotechnology (Dallas, USA)), p-c-Jun(1:100, Santa Cruz Biotechnology), Lamin B1 (0.1 μg/ml, Abcam). β-actin(1:5000, Sigma Aldrich) and GAPDH (1:5000, Sigma Aldrich) act as the internal control. Images were acquired by FUSION FX (Vilber Lourmat Deutschland GmbH, Weinheim, Germany).

Flow cytometry

Apoptosis was analyzed in the Annexin V assay using the Annexin V-FITC Kit (Miltenyi Biotec, Bergisch Gladbach, Germany) following the manufacturer's instructions. 10^6 of PC cells with or without JNK-in-IX incubation for 48 h were harvested, mixed with 10 μl of Annexin V-FITC and incubated for 15 min at room temperature. After washing, the cell pellet was resuspended in 500 μl of binding buffer. 5 μl of PI (Propidium Iodide) solution was added immediately prior to flow cytometry using MACSQuant® X Flow Cytometer (Miltenyi Biotec).

Cell cycle was analyzed by performing PI staining (Sigma Aldrich). 10^6 PC cells were harvested and fixed in cold 70% ethanol. After fixation for 2 h, the cell pellet was rinsed and resuspended in staining solution containing 50 μg/ml of PI. Data were acquired by flow cytometry.

For flow cytometric expression of JNK1 and JNK2 protein levels, 10^6 PC cells were harvested and blocked in FC-block solution (Miltenyi Biotec, Bergisch Gladbach, Germany) for 20 min. 100 ul of single cell suspension containing 10^6 cells of interest were aliquoted and incubated in 300 μl of 2.7% paraformaldehyde for 30 min. After being rinsed, cells were resuspended in 500 of μl ice-cold permeabilizing solution and centrifuged at 350 x g at 4°C for 8 min and the supernatant was discarded. Then, cells were stained by AF647-conjugated antibodies (anti-JNK1 antibody and anti-JNK2 Antibody, 1:50, Santa Cruz Biotechnology (Dallas, USA)). Cells resuspended in 300 μl of FACS buffer were subjected to flow cytometry.

Data were analyzed by FlowJo_v10.6.1 (FlowJo LLC, Ashland, USA).

Immunocytochemistry/ Immunofluorescence (ICC/IF)

Cells were cultured on glass coverslips until 50-80% confluence. A control and drug-treated group were set up, and cells were treated with or without JNK-in-IX for 48 h at beforementioned concentrations. After rinsing with PBS, cells

were fixed with 4% paraformaldehyde for 20 min and permeabilized by incubation with 0.2% Triton for 10 min at room temperature. Cells were incubated with 5% BSA to block nonspecific binding and then incubated with phospho-histone H2A.X (1:400, Cell Signaling Technology) overnight at 4°C. After rinsing, cells were incubated with Alexa Fluor Plus 594-labeled anti-rabbit secondary antibody (10 µg/ml, Invitrogen) for 1 h at room temperature. Nuclear staining was performed with DAPI (2µg/ml, Sigma-Aldrich). Representative fluorescence photographs were taken using Axio Observer 7 (Zeiss) at 40x magnification.

Quantitative real-time PCR

Quantitative real-time PCR (qPCR) was used to determine the target gene expression on mRNA level. Total RNA in cell lysates was extracted using Monarch® Total RNA Miniprep Kit (New England BioLabs, Ipswich, USA). The concentrations of purified total RNA were detected using the QIAxpert (QIAGEN, Hilden, Germany). cDNA was synthesized using the AffinityScript Multiple Temperature cDNA Synthesis Kit (Agilent Technologies, Santa Clara, USA). The expression of target genes (JNK1, JNK2, CDK1, CCNB1, CDC25C1, PLK1, CHEK1, CDKN1A, p53, and Wee1) was verified by qPCR using SYBR-Green Master Mix (New England BioLabs) with LightCyter®480 II (Roche Life Science, Mannheim, Germany). Experiments were performed following the manufacturers' protocols. Primers were supplied by QIAGEN and are listed in the Supplementary Table S1.

siRNA transfection

Each cell line was seeded into 2 ml of complete medium in triplicate in a 6-well plate and cultured until cells were at 50-70% confluence at the time of transfection. For transfected cells, 25 pmol of MAPK-9 RNAi (Silencer Select siRNA, ID: S11159, Catalog 4390824 (Thermo Fisher Scientific, Waltham, MA, USA)) or 25 pmol of Silencer Select Negative Control No.1 siRNA (Catalog 4390843, Thermo Fisher Scientific) was dissolved in 150 µl of Opti-MEM® Medium (Catalog 31985070, Thermo Fisher Scientific). Diluted Lipofec-tamine™ RNAiMAX (Catalog 13778100, Thermo Fisher Scientific) as transfection reagents were separately added. Lipofectamine and siRNA solutions were mixed gently and incubated for 5 min at room temperature prior to adding into each well. Cells were passaged 24 h after transfection, and transfection efficiency was verified by qPCR.

In order to determine cell growth, 1000 of wildtype, JNK-2 knockdown and control transfected pancreatic cancer cells were seeded into each well of a 384-well plate containing 50 µl of medium. JNK-in-IX was added after cell adherence with the concentrations previously mentioned. 48 h and 96 h after JNK-in-IX treatment, viability of wildtype, JNK-2 knockdown or control transfected cells in four cell lines was detected as described above.

Human Phospho-Kinase array (proteome profiler)

To analyze the involved pathways after JNK-in-IX treatment, phosphorylation of relevant kinases and transcription factors were detected by using the Human Phospho-Kinase Array Kit (ARY003C, R&D Systems, Inc, Minneapolis, USA) following manufacturer's instructions. Briefly, PC cells with or without JNK-in-IX incubation for 48 h were lysed and subjected to the array membranes. Images were acquired by FUSION FX. Mean pixel density was analyzed by ImageJ 1.8.0 and GraphPad Prism 8.0.1.

Statistics

GraphPad Prism 8.0.1 was used for statistical analysis. To evaluate the significance of differences among groups, statistical methods including t test, paired t test, Tukey's multiple comparisons test and uncorrected Fisher's LSD were used. P values less than 0.05 are taken as significant and are shown as follows: ns $p > 0.05$, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ and **** $p < 0.0001$.

Results

We have previously demonstrated the role of the JNK isoforms (JNK1 and JNK2) in PC. A potential therapeutic role has been outlined by others, even more since the development of isoform-specific inhibitors. These results have prompted us to investigate the potential use of pharmacological targeting of JNK in PC.

Pancreatic cancer cell survival is reduced by JNK inhibitors, most significantly by the JNK2-specific inhibitor JNK inhibitor IX

In order to test the response of human PC cell lines to JNK inhibition, the efficacy of two pan-JNK inhibitors (SP600125 and AS602801) as well as the JNK1-specific inhibitor Licochalcone A and the JNK2-specific inhibitor JNK inhibitor IX (JNK-in-IX) were tested. The dose-response curves demonstrated in Figure 1A clearly show that no difference between JNK1 specific inhibition and pan-JNK inhibition can be observed. However, JNK-in-IX was proven highly efficient with IC50

values of about 0.1 μM across all cell lines (Figure 1A, Supplementary Table S2).

We next examined the combination therapy of JNK-in-IX and the standard chemotherapeutic regimen for human pancreatic cancer cell lines, FOLFIRINOX and Gemcitabine-Paclitaxel (Gem-Pac). The dosages used for combination treatment matched the *in vivo* situation with concentrations of drugs at the ratios of Gemcitabine: Paclitaxel = 1: 0.04 and 5-FU: Oxaliplatin: SN-38 = 80.95: 0.80: 1 (23). Both Gem-Pac and FOLFIRINOX were effective in all tested cell lines (Figure 1B). We evaluated the combination treatment by using SynergyFinder 2.0 in order to discriminate between additive, synergistic or antagonistic effects (Figures 1C, D). Data was evaluated using the ZIP (Zero Interaction Potency) model (Supplementary Table S3) where drugs are assumed to be non-interacting and differences in the dose-response curve can be evaluated (21). Thereby, we were able to demonstrate that no synergistic effect can be observed in the combination treatment of Gem-Pac or FOLFIRINOX with JNK-in-IX. For AsPC-1, the combination of FOLFIRINOX and JNK-in-IX even seemed antagonistic (Supplementary Table 2).

We also tested our findings in 2 organoid lines derived from PC specimen. The effects were less pronounced, but JNK-in-IX was still effective in both organoid lines (Figure 2).

JNK-in-IX reduces pancreatic cancer cell migration

After JNK-in-IX was shown to be highly effective in suppressing cell survival of PC cells, its effect on cell behavior was examined next. First, it became obvious, that JNK-in-IX treatment leads to morphological changes with enlarged cells including larger nuclei as demonstrated *via* Giemsa staining (Supplementary Figure S1). Therefore, we evaluated cell mobility and migration next: AsPC-1, BxPC-3, MIA PaCa-2 and PANC-1 were treated with 0.409 μM, 0.220 μM, 0.071 μM and 0.066 μM of JNK-in-IX, corresponding to the previously determined IC50 for each cell line. In previous findings from our group, the reduced expression of JNK2 led to enhanced cell migration (18).

In contrast to these findings, by treatment with JNK-in-IX, pancreatic cancer cell migration was profoundly reduced. The scratch assay showed prolonged wound closure rates of about 20% across all cell lines (Figures 3A, B). Even more strikingly, the cellular migration ability was nearly completely abolished in the modified Boyden chamber assay (Figures 3C, D). These findings were replicable in all 4 biological replicates.

JNK-in-IX induced cell death

These results indicate that JNK-in-IX can strongly reduce oncogenic hallmarks of PC cells and we next sought to examine the mechanism of action.

PC cells were treated with JNK-in-IX using the same concentrations as above (corresponding to IC50) for 48 h. We first evaluated apoptosis-related cell death. By using Annexin V/Propidium Iodide (PI)-staining, we were able to demonstrate, that JNK-in-IX only slightly increased the proportion of apoptotic Annexin V+/PI- cells. Proportions of dead cells (Annexin V+/PI-) increased significantly in BxPC-3 and MIA PaCa-2. However as detached dead cells are mostly lost during cell harvest, these results need to be interpreted cautiously (Figure 4A).

After JNK-in-IX treatment, expression of pro-apoptotic Bax was decreased in all cell lines. The anti-apoptotic Bcl-2 was only found highly expressed in MIA PaCa-2 and was also decreased after JNK-in-IX treatment (Figure 4B). These results indicate, that JNK-in-IX only slightly increases apoptosis in PC cells and the observed effect of reduced cell survival appears to be mostly apoptosis independent. We were able to verify these results by using the pan-caspase inhibitor Z-VAD FMK which can prevent apoptosis-related cell death. Corresponding to previous results, caspase-inhibition through Z-VAD FMK was not able to reverse JNK-in-IX induced cell death (Figure 4C).

Cell-cycle progression was evaluated next. Again, cells were treated for 48 h with JNK-in-IX in the before mentioned concentrations. There, we were able to detect a strongly increased proportion of cells in the G2 phase, suggesting a G2 arrest through JNK-in-IX (Figure 5A).

Expression of Lamin B1, as a nuclear envelope marker was reduced after JNK-in-IX treatment, also indicating that cells fail to successfully undergo mitosis (Supplemental Figure S2)

In order to elucidate the mechanism of G2 arrest after JNK-in-IX treatment, we analyzed the expression of key regulators of cell cycle progression. We included pro-mitotic regulators (Cyclin Dependent Kinase 1 (CDK1), Cyclin B1 (CCNB1), CDC25C1, and Polo-like Kinase 1 (PLK1)) as well as restrictors of cell cycle progression (Checkpoint Kinase 1 (CHEK1), p21 (CDKN1A), p53, and Wee1). After treatment with JNK-in-IX, p21 (CDKN1A) was consistently upregulated in all cell lines (Figure 5B). Treatment with JNK-in-IX thus leads to a G2 arrest mediated by p21.

Target effectors of JNK-in-IX

We next sought to evaluate the effect of JNK-in-IX on the expression of JNK1 and 2. On RNA level, there was a trend towards increased expression of both kinases (Figure 6A). We also evaluated protein expression levels using the mean fluorescence intensity in flow cytometry. There, we were only able to observe a slight trend towards increased kinase expression in AsPC-1 and MIA PaCa-2, while expression levels in BxPC-3 and PANC-1 were unaltered (Figure 6B). Surprisingly, after evaluation of c-Jun expression and phosphorylation (Figure 6C), we did not detect any differences in expression levels of c-Jun and interestingly, also did not detect differences of its phosphorylation.

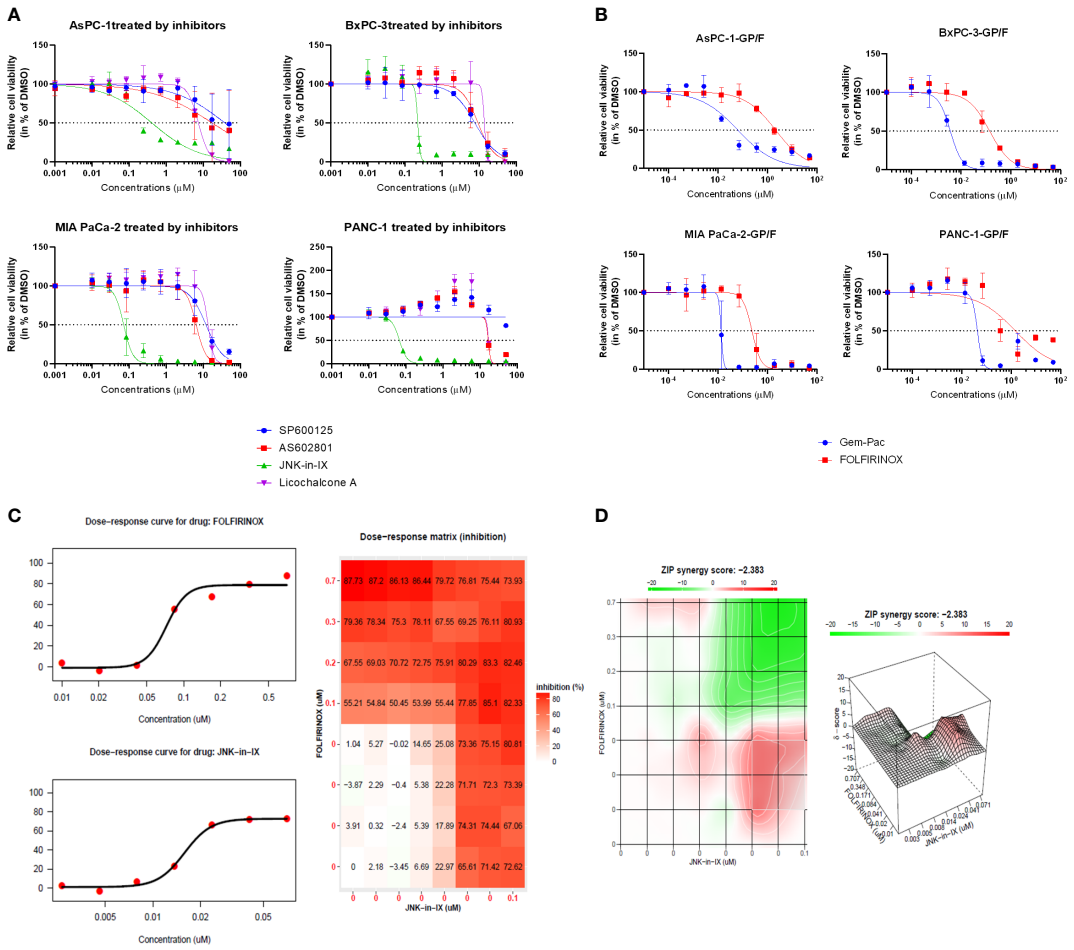


FIGURE 1
Targeting pancreatic cancer cells. **(A, B)** 500 cells per well were seeded in 384-well plates containing 50 µl of complete medium. 24 h after seeding, increasing concentrations of JNK inhibitors **(A)** and chemotherapeutics **(B)** dissolved in DMSO were added in triplicate using the Tecan D300e Dispenser. DMSO was normalized to the highest concentration (0.5%, v/v). Cell viability was evaluated 5 days after treatment using CellTiter-Glo. Results shown are means of 3 independent experiments. **(C, D)** Combination treatment with JNK-in-IX and FOLFIRINOX in MIA PaCa-2. Data were calculated and visualized using SynergyFinder 2.0. Results are means of 3 independent experiments. With a ZIP score of less than -10, the interaction between two drugs is likely to be antagonistic; from -10 to 10: the interaction is likely to be additive, and for scores larger than 10, the interaction is likely to be synergistic. GP: Gemcitabine-Paclitaxel (Gem-Pac): the combination of Gemcitabine and Paclitaxel with the ratio of 1:0.04 (c/c); F FOLFIRINOX: the combination of SN-38, Oxaliplatin and 5-FU with the ratio of 1:0.8:80.95 (c/c/c).

These results suggested that the effects of JNK-in-IX may be independent of JNK2 activity and c-Jun phosphorylation. Therefore, we used a human phospho-kinase assay to analyze related pathways in MIA PaCa-2 and PANC-1, as those were the most sensitive towards JNK-in-IX (Figures 6D, E). Again, we could not detect a consistent difference in c-Jun phosphorylation. However, we could observe a consistent phosphorylation of p53 at serine 15, 46 and 392 in both cell lines.

To verify the hypothesis, that JNK-in-IX acts independently of its designed target JNK2, we used siRNA knockouts of JNK2 in all four cell lines. qPCR confirmed a successful knockdown (Figure 7A). We then evaluated cell growth of wildtype cells, control transfected cells and knockdown cells with or without JNK-in-IX treatment using the concentrations previously determined. Again, JNK-in-IX successfully suppressed cell growth in wildtype and control-transfected cells. However, it was equally effective in cells with reduced expression of JNK2 (Figures 7B, C). This further strengthened our findings that JNK-in-IX acts independently of its primary target JNK2 in PC.

The increased expression of p21 taken together with increased phosphorylation of p53 was indicative for DNA. We therefore analyzed phosphorylation of the histone variant H2A.X as a marker for DNA double stand breaks. In all cell lines, especially in BxPC-3 and MIA PaCa-2, increased phosphorylation was detected in immunocytochemistry (Figure 8).

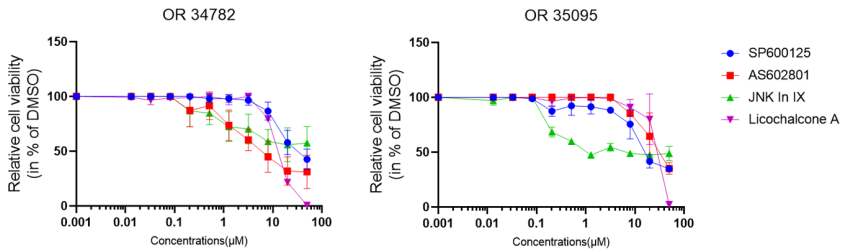


FIGURE 2
Human PC organoid treatment. Human organoids OR34782 and OR35095 were generated from PC specimen. 500 cells per well were plated in 1μl of Matrigel and cultured in 25μl Organoid growth medium. After 24 h, another 25 μl of Organoid medium was added including the indicated inhibitors. Results are shown as means of 3 (OR34782) and 2 independent replicates (OR35095).

Taken together, these results indicate that JNK-in-IX is effective in PC through reduced cell survival and restrained cell migration. These effects are mediated through DNA damage resulting in a G2 arrest and incapability to enter mitosis through activation of p53 and p21 independent of JNK2.

Discussion

PC is continuously proving itself as one of the most challenging malignancies to detect and treat. Recent efforts in systemic treatment options had two main goals: large,

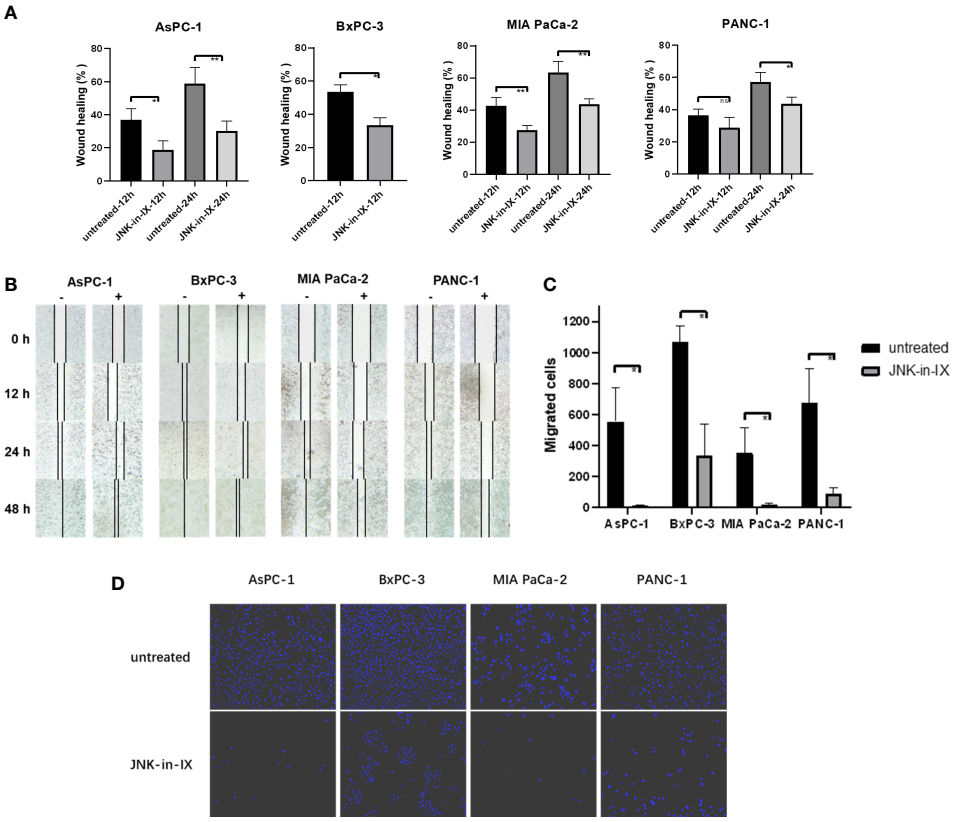


FIGURE 3
Effect of JNK-in-IX on pancreatic cancer cell mobility and migration. **(A)** Cell movement in the wound healing assay. Results are shown as wound healing distance (in % of 0 h) after 12 h and 24 h and are means of 5 separate experiments. The wound healing rate of BxPC-3 at 24 h is not shown as the wound margins were highly irregular. **(B)** Representative areas of wound gaps at 0 h, 12 h, 24 h and 48 h at 2.5× magnification. **(C, D)** Modified Boyden chamber assay. Results are shown as number of the migrated cells within 24 h and are means of 5 separate experiments **(C)** with representative areas of migrated cells at 10× magnification **(D)**. ns p>0.05, * p< 0.05, ** p<0.01.

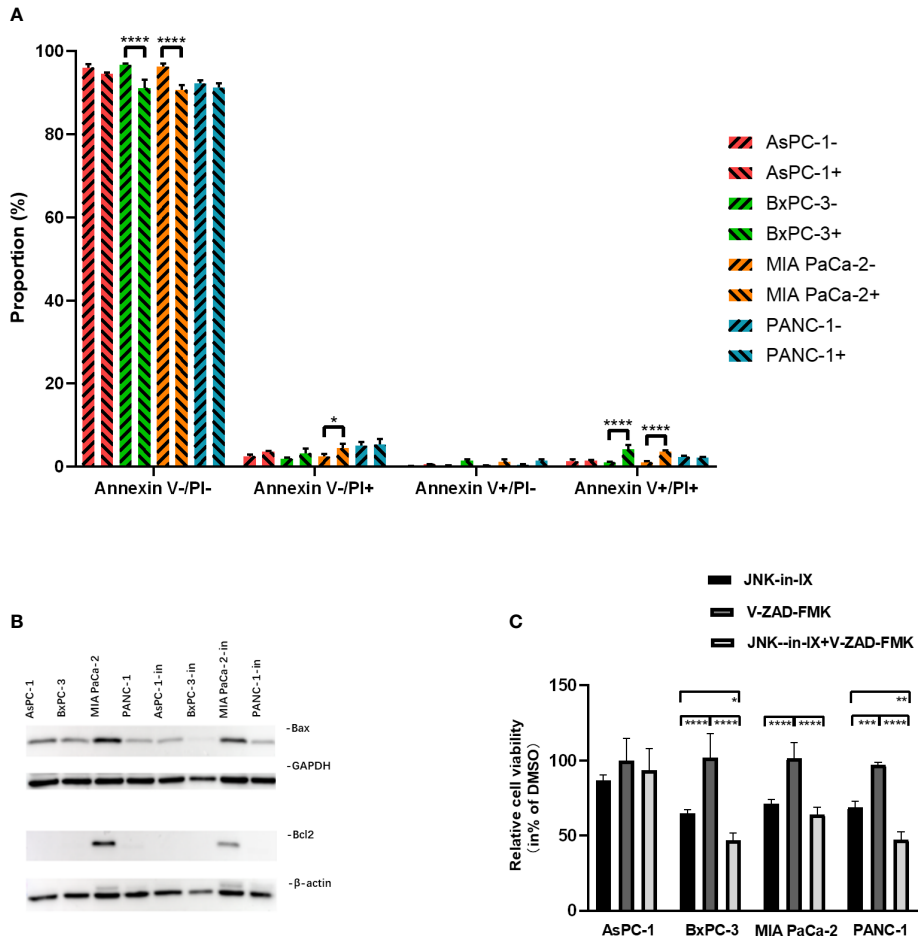


FIGURE 4
Effect of JNK-in-IX on apoptosis. **(A)** After incubation with JNK-in-IX (AsPC-1: 0.409 μM, BxPC-3: 0.220 μM, MIA PaCa-2: 0.071 μM, and PANC-1: 0.066 μM) for 48 h, 10^6 cells were harvested and stained by Annexin V and PI. Data were analyzed by flow cytometry. Viable cells are stained Annexin V- / PI-, apoptotic cells are stained Annexin V+ / PI-, and dead cells are stained Annexin V+ / PI+. Only a slight increase in apoptotic cells was observed. Results are shown of 3 independent experiments. **(B)** Western blot analysis of Bax and Bcl2 after JNK-in-IX treatment. GAPDH and β-actin were used as internal control. **(C)** Pancreatic cancer cell lines treated by JNK-in-IX and V-ZAD-FMK. Percentage of viable cells compared to untreated control are shown. Data are expressed as mean ± SEM. of at least four independent experiments. *p < 0.05, **p < 0.01, ***p <; 0.001, ****p < 0.0001.

multicenter studies aimed at determining the optimal chemotherapies for whole patient cohorts (24, 25). On the other hand, efforts were made in personalizing treatment options using novel modeling systems like organoids (26). One of the major obstacles in drugging PC is the fact that its major driver, mutant KRAS, has presented itself undruggable (4). Furthermore, it has been shown that mutant KRAS causes alterations in associated pathways, thereby potentiating its deadly potential (27). However, we believe that targeting these associated pathways may be an attractive treatment option.

The c-Jun N-terminal kinases (JNK)1, 2, and 3, together with p38 kinases, are also called stress activated kinases as they are involved in the cellular response towards exogenous and endogenous stressors like metabolic stress or cytokine stimulation, as well as UV radiation or cytotoxic drugs (6). Due to their central role in the cellular signaling cascade they merge signals from multiple membrane receptors and intracellular kinases including RAS. Multiple substrates of JNK have been identified and include transcription factors like c-Jun and p53, apoptosis regulating proteins but also cytoskeleton elements (6).Previous studies have implicated a potential role of JNKs in PC (11, 13).

In determining the roles of JNK in PC, several factors need to be considered: isoform specific roles of JNK1 and 2 need to be taken into account, while JNK3 is only expressed in brain, heart and testis. Additionally, as demonstrated by Sato and colleagues (11), the overall role of JNK in PC also includes the kinase action in cells of the tumor microenvironment. By silencing JNK1 and 2

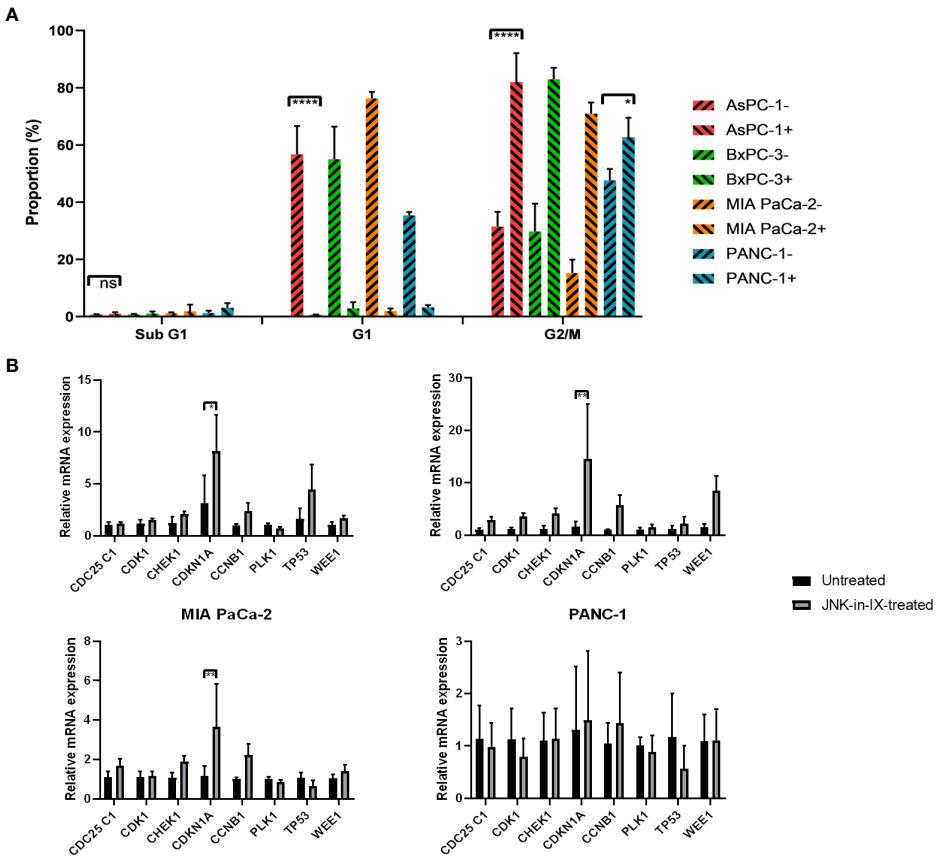


FIGURE 5
(A) Cell cycle analysis. After treatment with JNK-in-IX in the previously determined concentrations for 48 h, 10^6 PC cells were harvested, fixed, and stained with PI staining buffer. Afterwards, data were acquired using a flow cytometer. Cell cycles were normalized to the untreated control of which proportions are presented. All cell lines show a significant decrease in the G1 phase and a corresponding increase of cells in G2/M phase ($p < 0.0001$ for all cells except PANC-1, $p < 0.05$ for PANC-1). **(B)** mRNA expression of cell cycle regulators. Cyclin Dependent Kinase 1 (CDK1), Cyclin B1 (CCNB1), CDC25C1, Polo-like Kinase 1 (PLK1), Checkpoint Kinase 1 (CHEK1), p21 (CDKN1A), p53, and Wee1 expression in pancreatic cancer cells treated by JNK-in-IX was tested by qPCR. Data are shown as fold changes with respect to the untreated group ($2^{-\Delta\Delta Ct}$, mean ± SEM, n = 3). GADPH was used as the internal control. *p < 0.05, **p < 0.01, ****p < 0.0001.

separately, we recently demonstrated a tumor restraining function of JNK2 and a tumor promoting role of JNK1 (18). These results prompted us to investigate the value of isoform specific JNK inhibitors for the treatment of PC.

We used four pancreatic cancer cell lines with different genetic backgrounds, including KRAS-wildtype BxPC-3 (28). All cell lines were treated with JNK inhibitors addressing different backgrounds: SP600125 is a commonly used reversible ATP-competitive pan-JNK inhibitor (29) which effectiveness in PC was shown by us and others (18, 30). AS602801 (Bentamapimod) also acts as an ATP competitive inhibitor with similar IC50 values for JNK1 and 2. Interestingly it was shown effective against cancer stem cells including cell survival, as well as self-renewal and tumor-initiating capacity in PC (31). Contrary to those, Licochalcone A is not ATP-competitive but competes with the scaffolding protein JIP1 in its binding with JNK and thereby inhibits specifically JNK1 activity (32). Lastly, we used the JNK2 specific, ATP competitive JNK inhibitor IX (JNK-in-IX) (33). We can now demonstrate that both pan-JNK inhibitors as well as the JNK1 specific inhibitor exert similar growth restraining effects across most cell lines. However, JNK-in-IX has proven itself as the most effective by far of all inhibitors and was even effective in PANC-1 which consistently shows resistance against JNK inhibition (18, 34). Although less pronounced, JNK-in-IX was also effective in primary PC organoids, underlining the potential inhibitor use. These interesting findings were opposing to our previous demonstration of a growth suppressive function of JNK2 and prompted further studies.

Being stress-activated kinases, JNKs are involved in the cellular response towards endogenous and exogenous stressors. Therapy-induced cell stress through radiation or cytotoxic stress is a fundamental part of successful cancer treatment. By inhibiting the cellular coping mechanisms to therapy induced

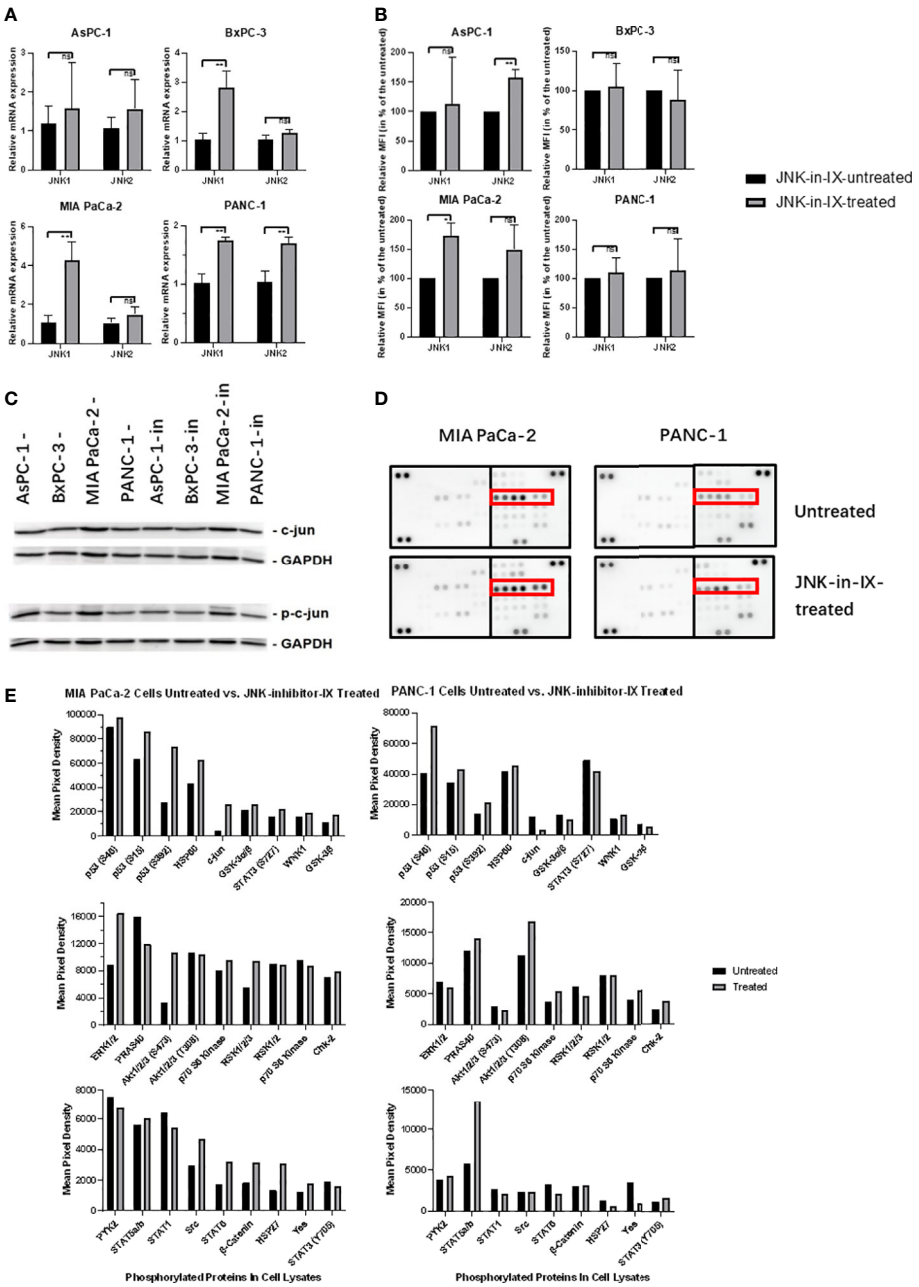


FIGURE 6
Target effectors of JNK-in-IX. **(A, B)** Relative mRNA expression in qPCR **(A)** and Mean Fluorescence Intensity (MFI) detected by flow cytometry **(B)** of JNK1 and JNK2 with or without JNK-in-IX treatment. Data shown are the means (±SEM) from 3 **(A)** and 4 **(B)** independent experiments. **(C)** Immunoblot analysis of total expression and phosphorylation of c-jun with or without JNK-in-IX treatment. GAPDH acts as internal control. **(D, E)** Human Phospho-Kinase Array. Pictures shown are the blots of respective protein phosphorylation with or without JNK-in-IX treatment, p53 phosphorylation is marked in red **(D)**. **(E)** Quantitative expression of phosphorylated kinases. Data are shown as mean pixel density. ns $p>0.05$, *$p<0.05$, **$p<0.01$.

stress, therapy resistance might be overcome. We therefore decided to evaluate the combination treatment of the most effective JNK-in-IX and the standard cytotoxic treatments for PC, Gemcitabine-Paclitaxel (Gem-Pac) and FOLFIRINOX. Contrary to Lipner and colleagues who showed sensitization of PC cells towards 5-FU/FOLFOX treatment after JNK1 inhibition (19), JNK-in-IX did not show treatment synergy with FOLFIRINOX or Gemcitabine-Paclitaxel.

As the invasion ability is a hallmark of cancer cells, we next examined the effectiveness of JNK-in-IX on reducing cell

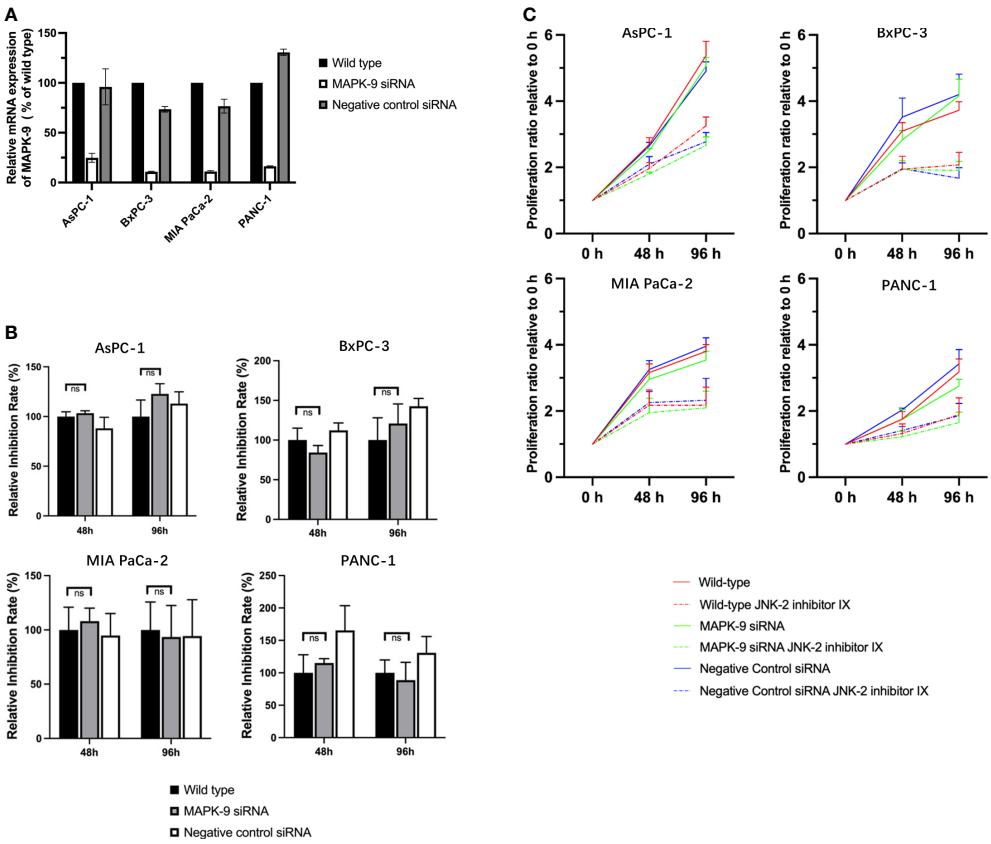


FIGURE 7
siRNA mediated knockdown of JNK2 does not impact the effect of JNK-in-IX in pancreatic cancer. **(A)** qPCR analysis revealed that siRNA targeting MAPK-9/JNK2 effectively reduces the expression of MAPK-9. Results are shown as means of two replicates in qPCR. **(B)** Quantitative inhibition rate. 2x10^4/ml of wildtype, control transfected and knockdown pancreatic cancer cells were seeded in quintuplicate into each well of a 384-well plate containing 50 µl of complete medium. JNK-in-IX was added after 24 h with the before mentioned concentrations. Cell viability was measured by CTG after 48 h and 96 h after treatment. The results presented demonstrate the effect of JNK-in-IX on wildtype cells, JNK2 knockdown cells and negative control cells respectively. 100% equals the inhibition rate of wildtype cells at each timepoint. This demonstrates that JNK-in-IX is equally effective irrespective of JNK2 expression. **(C)** Cell growth with or without JNK-in-IX. JNK-in-IX suppressed cell growth after 48 h and 96 h after treatment. Results are shown as proliferation ratio relative to 0 h after JNK-in-IX was added. ns p >0.05.

migration *in vitro*. Again, wound closure of all cell lines was significantly inhibited. More importantly, the seen effect was even more pronounced in cell migration. Recently, Jemaà and colleagues used JNK inhibition in order to reduce colon cancer cell migration. Interestingly, they were able to reduce migration of human RKO cancer cell line *in vitro* by pan-JNK inhibition and specific JNK1 inhibition. However, JNK-in-IX was ineffective (35). On the other hand, the group of van Berg demonstrated the value of JNK2 for breast cancer cell migration (36) and pharmacological JNK2 inhibition reduced cell migration (37).

In our study, the reduced cell proliferation and ultimately cell death was apoptosis independent. Furthermore, we were able to show that the seen effects are caused by a G2/M arrest. G2/M progression is dependent on the active complex of CDK1 and Cyclin B1. CDK1/Cyclin B1 phosphorylation inhibits the complex pro-mitotic activity and is mediated through Wee1. In contrast, the phosphatase CDC25C activates CDK1/Cyclin B1 through dephosphorylation. Other regulators of cell cycle progression act through activation (PLK1) or deactivation (CHK1) of CDC25C (38). Finally, p53 can repress CDK1 and CCNB1 expression and additionally inhibit CDK1 directly through activation of p21 which binds and inhibits CDK1 directly (39).

In the present study, we demonstrate that JNK-in-IX leads to a G2 arrest in pancreatic cancer cells *via* increased expression of p21 (CDKN1A). Together with the increased phosphorylation of p53, as demonstrated in the proteome profiling, this suggested that the inhibitor treatment may result in DNA damage. We were able to confirm increased DNA double stand breaks through ICC labeling of Histone H2A.X phosphorylation.

Similar findings have been demonstrated for JNK-in-IX in Jurkat T-cells (40). Although in this lymphoid cell line, cell death

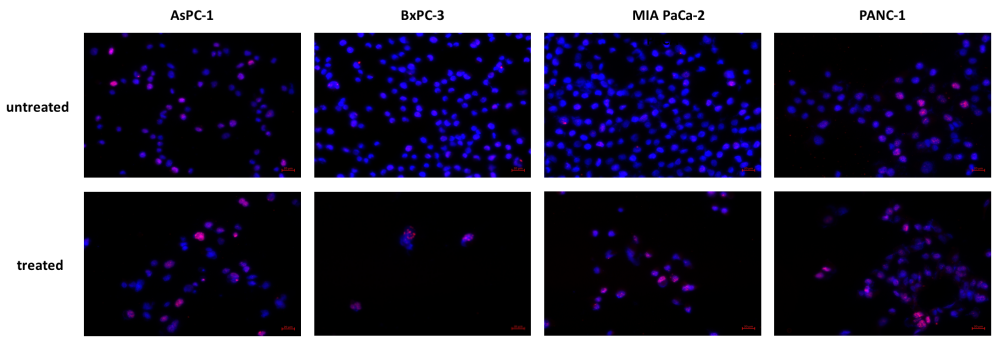


FIGURE 8
Histone H2A.X phosphorylation. Increased phosphorylation of Histone H2A.X was detected after JNK-in-IX treatment. Representative pictures are shown at 40x magnification.

was due to apoptosis, cells also underwent a G2 arrest. Furthermore, JNK-in-IX treatment resulted in a defective mitotic spindle defect (40). This mechanism could serve as an explanation for the morphological differences observed in our treatment group.

The findings of our present study indicate JNK inhibitor IX as a promising therapeutic in PC. However, our present findings were in contrast to previous studies from our group: Stable knockout of JNK2 increased proliferation and migration of PC cells (18). Thus, we examined the effectiveness of JNK-in-IX in inhibiting the JNK2 activity. Interestingly, c-Jun phosphorylation as major downstream target of Jun kinases was unaltered. Furthermore, siRNA-based knockdown of JNK2 did not impair the effectiveness of JNK-in-IX. These findings indicate that JNK-in-IX may act independently of JNK2, at least in the studied cell lines.

Overall, our study underlines the current trend in personalizing therapies. JNK2 as the primary target of the compound used in our study did not seem to be promising based on genetic findings but the way of action of JNK-in-IX was proven effective. Novel model systems like tumor organoids (41) can test these compounds and thus be a powerful tool in prioritizing treatment options and verify or disprove treatment options based on genetic testing.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding author.

Ethics statement

The studies involving human participants were reviewed and approved by Independent Ethics Committee of the University of Ulm. The patients/participants provided their written informed consent to participate in this study.

Author contributions

Conceptualization, MK and BT; Literature Search, XY, QK, MD, MK, and BT; Experiment and Data Analysis, JS, XY, QK, JL, and MD; Writing – JS, MK, and BT; Supervision, BT. All authors read and approved the final version of the manuscript.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

The handling editor XT declared a past co-authorship with the authors BT, MK, and JS.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2022.1006131/full#supplementary-material

References

1. Siegel RL, Miller KD, Fuchs HE, Jemal A. Cancer statistics, 2021. *CA Cancer J Clin* (2021) 71:7–33. doi: 10.3322/caac.21654

2. Rahib L, Smith BD, Aizenberg R, Rosenzweig AB, Fleshman JM, Matrisian LM. Projecting cancer incidence and deaths to 2030: the unexpected burden of thyroid, liver, and pancreas cancers in the united states. *Cancer Res* (2014) 74:2913–21. doi: 10.1158/0008-5472.CAN-14-0155

3. Traub B, Link KH, Kornmann M. Curing pancreatic cancer. *Semin Cancer Biol* (2021) 76:232–46. doi: 10.1016/j.semcancer.2021.05.030

4. Marín-Ramos NI, Ortega-Gutiérrez S, López-Rodríguez ML. Blocking ras inhibition as an antitumor strategy. *Semin Cancer Biol* (2019) 54:91–100. doi: 10.1016/j.semcancer.2018.01.017

5. Chung V, McDonough S, Philip PA, Cardin D, Wang-Gillam A, Hui L, et al. Effect of selumetinib and MK-2206 vs oxaliplatin and fluorouracil in patients with metastatic pancreatic cancer after prior therapy: SWOG S1115 study randomized clinical trial. *JAMA Oncol* (2017) 3:516–22. doi: 10.1001/jamaoncol.2016.5383

6. Traub B, Roth A, Kornmann M, Knippschild U, Bischof J. Stress-activated kinases as therapeutic targets in pancreatic cancer. *World J Gastroenterol* (2021) 27:4963–84. doi: 10.3748/wjg.v27.i30.4963

7. Johnson GL, Nakamura K. The c-jun kinase/stress-activated pathway: regulation, function and role in human disease. *Biochim Biophys Acta* (2007) 1773:1341–8. doi: 10.1016/j.bbamcr.2006.12.009

8. Zeke A, Misheva M, Reményi A, Bogoyevitch MA. JNK signaling: Regulation and functions based on complex protein-protein partnerships. *Microbiol Mol Biol Rev* (2016) 80:793–835. doi: 10.1128/MMBR.00043-14

9. Johnson R, Spiegelman B, Hanahan D, Wisdom R. Cellular transformation and malignancy induced by ras require c-jun. *Mol Cell Biol* (1996) 16:4504–11. doi: 10.1128/MCB.16.8.4504

10. Ruiz EJ, Lan L, Diefenbacher ME, Riising EM, Da Costa C, Chakraborty A, et al. JunD, not c-jun, is the AP-1 transcription factor required for ras-induced lung cancer. *JCI Insight* (2021) 6. doi: 10.1172/jci.insight.124985

11. Sato T, Shibata W, Hikiba Y, Kaneta Y, Suzuki N, Ihara S, et al. C-jun n-terminal kinase in pancreatic tumor stroma augments tumor development in mice. *Cancer Sci* (2017) 108:2156–65. doi: 10.1111/cas.13382

12. Okada M, Shibuya K, Sato A, Seino S, Suzuki S, Seino M, et al. Targeting the K-Ras–JNK axis eliminates cancer stem-like cells and prevents pancreatic tumor formation. *Oncotarget* (2014) 5:5100–12. doi: 10.18632/oncotarget.2087

13. Takahashi R, Hirata Y, Sakitani K, Nakata W, Kinoshita H, Hayakawa Y, et al. Therapeutic effect of c-jun n-terminal kinase inhibition on pancreatic cancer. *Cancer Sci* (2013) 104:337–44. doi: 10.1111/cas.12080

14. Cellurale C, Sabio G, Kennedy NJ, Das M, Barlow M, Sandy P, et al. Requirement of c-jun NH(2)-terminal kinase for ras-initiated tumor formation. *Mol Cell Biol* (2011) 31:1565–76. doi: 10.1128/MCB.01122-10

15. Sabapathy K, Hochedlinger K, Nam SY, Bauer A, Karin M, Wagner EF. Distinct roles for JNK1 and JNK2 in regulating JNK activity and c-jun-dependent cell proliferation. *Mol Cell* (2004) 15:713–25. doi: 10.1016/j.molcel.2004.08.028

16. Davies CC, Harvey E, McMahon RF, Finegan KG, Connor F, Davis RJ, et al. Impaired JNK signaling cooperates with KrasG12D expression to accelerate pancreatic ductal adenocarcinoma. *Cancer Res* (2014) 74:3344–56. doi: 10.1158/0008-5472.CAN-13-2941

17. She QB, Chen N, Bode AM, Flavell RA, Dong Z. Deficiency of c-Jun-NH(2)-terminal kinase-1 in mice enhances skin tumor development by 12-O-tetradecanoylphorbol-13-acetate. *Cancer Res* (2002) 62:1343–8.

18. Tian X, Traub B, Shi J, Huber N, Schreiner S, Chen G, et al. C-jun n-terminal kinase 2 suppresses pancreatic cancer growth and invasion and is opposed by c-jun n-terminal kinase 1. *Cancer Gene Ther* (2022) 29:73–86. doi: 10.1038/s41417-020-00290-5

19. Lipner MB, Peng XL, Jin C, Xu Y, Gao Y, East MP, et al. Irreversible JNK1-JUN inhibition by JNK-IN-8 sensitizes pancreatic cancer to 5-FU/FOLFOX chemotherapy. *JCI Insight* (2020) 5. doi: 10.1172/jci.insight.129905

20. Beutel AK, Schütte L, Scheible J, Roger E, Müller M, Perkhofer L, et al. A prospective feasibility trial to challenge patient–derived pancreatic cancer organoids in predicting treatment response. *Cancers* (2021) 13:2539. doi: 10.3390/cancers13112539

21. Ianevski A, Giri KA, Aittokallio T. SynergyFinder 2.0: visual analytics of multi-drug combination synergies. *Nucleic Acids Res* (2020) 48(W1): W488-W493, gkaa216. doi: 10.1093/nar/gkaa216

22. Shi J, Shen X, Kang Q, Yang X, Denzinger M, Kornmann M, et al. Loss of interleukin-13-Receptor-Alpha-1 induces apoptosis and promotes EMT in pancreatic cancer. *Int J Mol Sci* (2022) 23. doi: 10.3390/ijms23073659

23. Begg SKS, Birnbaum DJ, Clark JW, Mino-Kenudson M, Wellner UF, Schilling O, et al. FOLFIRINOX versus gemcitabine-based therapy for pancreatic ductal adenocarcinoma: Lessons from patient-derived cell lines. *Anticancer Res* (2020) 40:3659–67. doi: 10.21873/anticanres.14355

24. Conroy T, Desseigne F, Ychou M, Bouché O, Guimbaud R, Bécouarn Y, et al. FOLFIRINOX versus gemcitabine for metastatic pancreatic cancer. *N Engl J Med* (2011) 364:1817–25. doi: 10.1056/NEJMoa1011923

25. Von Hoff DD, Ervin T, Arena FP, Chiorean EG, Infante J, Moore M, et al. Increased survival in pancreatic cancer with nab-paclitaxel plus gemcitabine. *N Engl J Med* (2013) 369:1691–703. doi: 10.1056/NEJMoa1304369

26. Frappart PO, Walter K, Gout J, Beutel AK, Morawe M, Arnold F, et al. Pancreatic cancer-derived organoids - a disease modeling tool to predict drug response. *United Eur Gastroenterol J* (2020) 8:594–606. doi: 10.1177/2050640620905183

27. Dey P, Li J, Zhang J, Chaurasiya S, Strom A, Wang H, et al. Oncogenic KRAS-driven metabolic reprogramming in pancreatic cancer cells utilizes cytokines from the tumor microenvironment. *Cancer Discovery* (2020) 10:608–25. doi: 10.1158/2159-8290.CD-19-0297

28. Deer EL, González-Hernández J, Coursen JD, Shea JE, Ngatia J, Scaife CL, et al. Phenotype and genotype of pancreatic cancer cell lines. *Pancreas* (2010) 39:425–35. doi: 10.1097/MPA.0b013e3181c15963

29. Bennett BL, Sasaki DT, Murray BW, O'Leary EC, Sakata ST, Xu W, et al. SP600125, an anthrapyrazolone inhibitor of jun n-terminal kinase. *Proc Natl Acad Sci U.S.A.* (2001) 98:13681–6. doi: 10.1073/pnas.251194298

30. Konno T, Ninomiya T, Kohno T, Kikuchi S, Sawada N, Kojima T. C-jun n-terminal kinase inhibitor SP600125 enhances barrier function and elongation of human pancreatic cancer cell line HPAC in a Ca-switch model. *Histochem Cell Biol* (2015) 143:471–9. doi: 10.1007/s00418-014-1300-4

31. Okada M, Kuramoto K, Takeda H, Watarai H, Sakaki H, Seino S, et al. The novel JNK inhibitor AS602801 inhibits cancer stem cells *in vitro* and in vivo. *Oncotarget* (2016) 7:27021–32. doi: 10.18632/oncotarget.8395

32. Yao K, Chen H, Lee MH, Li H, Ma W, Peng C, et al. A natural inhibitor of c-jun n-terminal kinase 1. *Cancer Prev Res (Phila)* (2014) 7:139–49. doi: 10.1158/1940-6207.CAPR-13-0117

33. Angell RM, Atkinson FL, Brown MJ, Chuang TT, Christopher JA, Cichy-Knight M, et al. N-(3-Cyano-4,5,6,7-tetrahydro-1-benzothien-2-yl)amides as potent, selective, inhibitors of JNK2 and JNK3. *Bioorg Med Chem Lett* (2007) 17:1296–301. doi: 10.1016/j.bmcl.2006.12.003

34. Recio-Boiles A, Ilmer M, Rhea PR, Kettlun C, Heinemann ML, Ruetering J, et al. JNK pathway inhibition selectively primes pancreatic cancer stem cells to TRAIL-induced apoptosis without affecting the physiology of normal tissue resident stem cells. *Oncotarget* (2016) 7:9890–906. doi: 10.18632/oncotarget.7066

35. Jemaà M, Abassi Y, Kifagi C, Fezai M, Daams R, Lang F, et al. Reversine inhibits colon carcinoma cell migration by targeting JNK1. *Sci Rep* (2018) 8:11821. doi: 10.1038/s41598-018-30251-w

36. Mitra S, Lee JS, Cantrell M, Van den Berg CL. C-jun n-terminal kinase 2 (JNK2) enhances cell migration through epidermal growth factor substrate 8 (EPS8). *J Biol Chem* (2011) 286:15287–97. doi: 10.1074/jbc.M109.094441

37. Kaoud TS, Mitra S, Lee S, Taliaferro J, Cantrell M, Linse KD, et al. Development of JNK2-selective peptide inhibitors that inhibit breast cancer cell migration. *ACS Chem Biol* (2011) 6:658–66. doi: 10.1021/cb200017n

38. Ventura E, Giordano A. Cell cycle. *Reference Module Life Sci* (2019). doi: 10.1016/B978-0-12-809633-8.90189-4

39. Taylor WR, Stark GR. Regulation of the G2/M transition by p53. *Oncogene* (2001) 20:1803–15. doi: 10.1038/sj.onc.1204252

40. Jang WY, Lee JY, Lee ST, Jun do Y, Kim YH. Inhibition of JNK2 and JNK3 by JNK inhibitor IX induces prometaphase arrest-dependent apoptotic cell death in human jurkat T cells. *Biochem Biophys Res Commun* (2014) 452:845–51. doi: 10.1016/j.bbrc.2014.09.015

41. Tiriac H, Belleau P, Engle DD, Plenker D, Deschênes A, Somerville TDD, et al. Organoid profiling identifies common responders to chemotherapy in pancreatic cancer. *Cancer Discovery* (2018) 8:1112–29. doi: 10.1158/2159-8290.CD-18-0349

frontiers | Frontiers in Oncology

TYPE Original Research
PUBLISHED 25 January 2023
DOI 10.3389/fonc.2023.1053375

OPEN ACCESS

EDITED BY
Xiaodong Tian,
First Hospital, Peking University, China

REVIEWED BY
Junmeng Li,
Henan Provincial People's Hospital, China
Marco Massani,
ULSS2 Marca Trevigiana, Italy

*CORRESPONDENCE
Bai Ji
ji_bai@jlu.edu.cn

†These authors have contributed equally to this work

SPECIALTY SECTION
This article was submitted to Gastrointestinal Cancers: Hepato Pancreatic Biliary Cancers, a section of the journal Frontiers in Oncology

RECEIVED 25 September 2022
ACCEPTED 09 January 2023
PUBLISHED 25 January 2023

CITATION
Guo X, Song X, Long X, Liu Y, Xie Y, Xie C and Ji B (2023) New nomogram for predicting lymph node positivity in pancreatic head cancer.
Front. Oncol. 13:1053375.
doi: 10.3389/fonc.2023.1053375

New nomogram for predicting lymph node positivity in pancreatic head cancer

Xingren Guo†, Xiangyang Song†, Xiaoyin Long, Yahui Liu, Yixin Xie, Cheng Xie and Bai Ji*

The Department of General Surgery Center-Hepatobiliary and Pancreatic Surgery, The First Affiliated Hospital of Jilin University, Changchun, China

Background: Pancreatic cancer is one of the most malignant cancers worldwide, and it mostly occurs in the head of the pancreas. Existing laparoscopic pancreaticoduodenectomy (LPD) surgical techniques have has undergone a learning curve, a wide variety of approaches for the treatment of pancreatic cancer have been proposed, and the operation has matured. At present, pancreatic head cancer has been gradually changing from "surgeons' evaluation of anatomical resection" to "biologically inappropriate resection". In this study, the risk of lymph node metastasis in pancreatic head cancer was predicted using common preoperative clinical indicators.

Methods: The preoperative clinical data of 191 patients with pancreatic head cancer who received LPD in the First Affiliated Hospital of Jilin University from May 2016 to December 2021 were obtained. A univariate regression analysis study was conducted, and the indicators with a significance level of $P<0.05$ were included in the univariate logistic regression analysis into multivariate. Lastly, a nomogram was built based on age, tumor size, leucocyte,albumin(ALB), and lymphocytes/monocytes(LMR). The model with the highest resolution was selected by obtaining the area under a curve. The clinical net benefit of the prediction model was examined using decision curve analyses.Risk stratification was performed by combining preoperative CT scan with existing models.

Results: Multivariate logistic regression analysis found age, tumor size, WBC, ALB, and LMR as five independent factors. A nomogram model was constructed based on the above indicators. The model was calibrated by validating the calibration curve within 1000 bootstrap resamples. The ROC curve achieved an AUC of 0.745 (confidence interval of 95%: 0.673-0.816), thus indicating that the model had excellent discriminative skills. DCA suggested that the predictive model achieved a high net benefit in the nearly entire threshold probability range.

Conclusions: This study has been the first to investigate a nomogram for preoperative prediction of lymphatic metastasis in pancreatic head cancer. The result suggests that age, ALB, tumor size, WBC, and LMR are independent risk factors for lymph node metastasis in pancreatic head cancer. This study may provide a novel perspective for the selection of appropriate continuous treatment regimens, the increase of the survival rate of patients with pancreatic head cancer, and the selection of appropriate neoadjuvant therapy patients.

KEYWORDS

LNM, nomogram, pancreatic head cancer, clinical indicators, age

Introduction

Pancreatic cancer is one of the most malignant tumors worldwide, the five-year survival rate is less than 5%, and 75% occurs in the pancreatic head (1). When patients are diagnosed with pancreatic cancer, most have lost the opportunity for surgery. Feasible pancreaticoduodenectomy without distant metastasis.

Pancreaticoduodenectomy has been confirmed as one of the largest operations in general surgery. LPD has experienced a learning curve in many large tertiary hospitals with shorter postoperative recovery times and fewer complications over the past few years. However, a considerable amount of research has suggested that the survival rate of postoperative patients remains not ideal (2, 3). On the one hand, it is dependent on the malignant biological characteristics of pancreatic cancer. On the other hand, numerous patients have lymph node metastasis before surgery (4), resulting in poor surgical results. Existing research has shown that preoperative lymph node metastasis is an independent risk factor for the postoperative survival rate of patients. The guidelines also recommend preoperative neoadjuvant therapy for patients with positive large regional lymph nodes (5).

Accordingly, preoperative prediction of lymph node metastasis takes on a critical significance to neoadjuvant therapy. At present, it is still difficult to predict lymph node metastasis by preoperative imaging indicators (6). Currently, a number of imaging modalities, such as endoscopic ultrasonography (EUS), computed tomography (CT), magnetic resonance imagin (MRI), and Positron emission tomography (PET), have been used to identify lymph node metastases (LNM). Radiologists often judge LNM by the size of the lymph node, the smooth edge of the lymph node and the homogeneous density or signal on CT or MRI images. Positron emission tomography/computed tomography (PET/CT), in addition to offering anatomical information, can provide an intuitive picture of the metabolic status of the lesion through semi-quantitative parameters such as standard uptake values and total glycolysis of the lesion. For instance, if the lymph node is present in a high uptake state on a PET image, it is highly suspected to be malignant. However, its accuracy in predicting lymph node metastasis in patients with pancreatic cancer is not very high (7–10). In recent years, Serum markers MMP7, MUC1, and MUC2 have been used to detect the preoperative status of PDAC lymph nodes and the rise of radiology, but their clinical application has been limited due to technical restriction and low accuracy (11, 12). Because, we use the advantage of nomogram, combined with preoperative clinical common indicators to predict the probability of lymph node cancer of the head of the pancreas.

In this study, there were 129 patients with positive lymph nodes, and only 34 patients had lymph node metastasis confirmed by preoperative imaging, and the predictive rate only reached 26%. This study aimed to investigate the correlation between common preoperative clinical indicators and lymph node metastasis(LNM) of pancreatic head cancer and to construct a corresponding nomogram to better identify patients with positive lymph nodes, which has potential significance for individualized comprehensive treatment.

Materials and procedures

Patients

Evaluation of the therapeutic information of patients with pancreatic head cancer who had LPD between May 2016 to December 2021 at the First Affiliated Hospital of Jilin University. This study was done in line with the Helsinki Declaration, with the agreement of the Research Ethics Committee of the First Affiliated Hospital of Jilin University, and with the informed consent of all patients.

The inclusion criteria are presented as follows:

(1) The pathological results were pancreatic carcinoma; (2) The lesion was located in the head of the pancreas; (3) Thin-layer CECT was performed in 191 patients within 1 month before operation and (4) There was a minimum number of LNs (eln) of 12 examinations.

The exclusion criteria are presented as follows:

(1) distant metastases (liver metastases or peritoneal carcinomatosis) on surgical exploration (2) preoperative anticancer therapy (chemotherapy, radiotherapy, or both) (3) incomplete clinicopathological data.

Establishment of cutoff values for variables and pathological characteristics

We recorded the LNM indices of Pancreatic head cancer based on the postoperative pathological report after analyzing the routine and preoperative blood biochemical test findings. The ideal cutoff values for the variables in this study were established using receiver operating characteristic curves and the maximum Youden index. The definition of LSR was ALT (U/L)/AST (U/L). LMR was determined as lymphocytes (109/L)/monocytes (109/L). Their cutoff levels were set based on the receiver operating characteristic curve and the highest Youden index. At P less than 0.05, differences achieved statistical significance. Lastly, 191 patients were included, of which 129 patients had lymph node metastases and 62 patients had no lymph node metastases. The information regarding patients is listed in Table 1.

Statistical analyses

Cutoffs were determined by transforming continuous information into categorical variables based on the ROC's maximum Youden index (sensitivity plus specificity minus 1). Categorical variables are described as numbers (percentages). LASSO regression analysis was used for data dimensionality reduction and element selection. (Figure 1) Between-group heterogeneity was compared through the chi-square test. Using univariate and multivariate logistic regression analysis, odds ratios (ORs) and 95% confidence intervals (CIs) were generated, of which OR>1 results indicated that the variable was a risk factor. Differences achieved statistical significance if P was less than 0.05. In the final nomogram model, the indicators with P less than 0.05 were included into the multiple logistic regression, and the nomogram model was built. The ROC of the model was obtained

10.3389/fonc.2023.1053375

TABLE 1 Patients Characteristics.

Variables, n (%)	Level	Total (n=191)	ILM negative (n=62)	ILM positive (n=129)	p	
Gender	0	82 (42.932)	32 (51.613)	50 (38.760)	0.093	Chi-square test
	1	109 (57.068)	30 (48.387)	79 (61.240)		
Age	0	99 (51.832)	25 (40.323)	74 (57.364)	0.027	Chi-square test
	1	92 (48.168)	37 (59.677)	55 (42.636)		
Tumorsize	0	73 (38.220)	31 (50.000)	42 (32.558)	0.02	Chi-square test
	1	118 (61.780)	31 (50.000)	87 (67.442)		
CA125	0	80 (41.885)	31 (50.000)	49 (37.984)	0.115	Chi-square test
	1	111 (58.115)	31 (50.000)	80 (62.016)		
CA199	0	104 (54.450)	40 (64.516)	64 (49.612)	0.053	Chi-square test
	1	87 (45.550)	22 (35.484)	65 (50.388)		
ALP	0	56 (29.319)	28 (45.161)	28 (21.705)	<0.001	Chi-square test
	1	135 (70.681)	34 (54.839)	101 (78.295)		
ALB	0	118 (61.780)	30 (48.387)	88 (68.217)	0.008	Chi-square test
	1	73 (38.220)	32 (51.613)	41 (31.783)		
GLOB	0	150 (78.534)	42 (67.742)	108 (83.721)	0.012	Chi-square test
	1	41 (21.466)	20 (32.258)	21 (16.279)		
DBIL	0	68 (35.602)	30 (48.387)	38 (29.457)	0.011	Chi-square test
	1	123 (64.398)	32 (51.613)	91 (70.543)		
IBIL	0	36 (18.848)	19 (30.645)	17 (13.178)	0.004	Chi-square test
	1	155 (81.152)	43 (69.355)	112 (86.822)		
WBC	0	133 (69.634)	51 (82.258)	82 (63.566)	0.009	Chi-square test
	1	58 (30.366)	11 (17.742)	47 (36.434)		
NEU	0	128 (67.016)	48 (77.419)	80 (62.016)	0.034	Chi-square test
	1	63 (32.984)	14 (22.581)	49 (37.984)		
PCT	0	171 (89.529)	59 (95.161)	112 (86.822)	0.078	Chi-square test
	1	20 (10.471)	3 (4.839)	17 (13.178)		
MPV	0	64 (33.508)	25 (40.323)	39 (30.233)	0.167	Chi-square test
	1	127 (66.492)	37 (59.677)	90 (69.767)		
PDW	0	79 (41.361)	31 (50.000)	48 (37.209)	0.093	Chi-square test
	1	112 (58.639)	31 (50.000)	81 (62.791)		
TT	0	34 (17.801)	8 (12.903)	26 (20.155)	0.22	Chi-square test
	1	157 (82.199)	54 (87.097)	103 (79.845)		
APTT	0	59 (30.890)	24 (38.710)	35 (27.132)	0.105	Chi-square test
	1	132 (69.110)	38 (61.290)	94 (72.868)		
PT	0	92 (48.168)	26 (41.935)	66 (51.163)	0.232	Chi-square test
	1	99 (51.832)	36 (58.065)	63 (48.837)		
INR	0	129 (67.539)	38 (61.290)	91 (70.543)	0.201	Chi-square test
	1	62 (32.461)	24 (38.710)	38 (29.457)		
FBG	0	61 (31.937)	16 (25.806)	45 (34.884)	0.208	Chi-square test
	1	130 (68.063)	46 (74.194)	84 (65.116)		

(Continued)

TABLE 1 Continued

Variables, n (%)	Level	Total (n=191)	ILM negative (n=62)	ILM positive (n=129)	p	
LMR	0	181 (94.764)	54 (87.097)	127 (98.450)	<0.001	Chi-square test
	1	10 (5.236)	8 (12.903)	2 (1.550)		
LSR	0	48 (25.131)	24 (38.710)	24 (18.605)	0.003	Chi-square test
	1	143 (74.869)	38 (61.290)	105 (81.395)		

to evaluate its performance, a thousand bootstrapping was performed, a calibration curve was generated, and then a DCA curve was generated to evaluate the net benefit of the model. (R4.1.2 and SPSS26.0 were used for data processing and statistical analysis) Finally, risk stratification was performed by combining preoperative CT and existing models.

Results

Fundamental attributes and limit values of the variables

191 patients who had LPD between May 2016 to December 2021 at the First Affiliated Hospital of Jilin University were included, with 109 males and 82 females. Table 1 lists the clinical features of the patients. The size of the tumor was extracted from preoperative CT-enhanced scan reports (e.g., imaging data). 129 out of 191 patients had positive lymph node, whereas 62 did not.

Independent preoperative risk factors for LNM

In the univariate logistic regression evaluation, age less than 61 years old, tumor size was equals to or over 2.6cm, Alkaline phosphatase(ALP) was equals to or more than 186U/L, ALB was less than 40.5g/L, globulin was less than 30.1g/L, Direct Bilirubin (DBIL) was equals to or more than52.8μmol/L,Indirect bilirubin (IBIL) was equals to or more than12.5μmol/L, WBC was equals to or more than 6.71 10^9/L, neutrophil (NEU) was equals to or more than4.19 10^9/L, LMR was less than 8.125, and LSR was equals to or more than 1.1. After multivariate logistic regression analysis, only tumor size was equals to or more than 2.6cm (odds ratio [OR] was equals to 2.259, 95% CI: 1.126-4.598, P was equals to 0.023), ALB was less than 40.5g/L(odds ratio [OR] equals to 0.429, 95% CI: 0.203-0.893, P was equals to 0.024), LMR was less than 8.125(odds ratio [OR] was equals to 0.169, 95% CI: 0.022-0.831, P was equals to 0.044), which were the preoperative independent risk factors for LNM in patients with pancreatic head cancer. Tables 2 and 3 list the results of univariate and multivariate regression analysis.

Development and validation of the novel preoperative LNM prediction nomogram

Age, tumor size, WBC, LMR, and ALB were taken based on the multiple logistic regression analysis of the training group to generate a nomogram and Forest plot to predict LNM in patients with pancreatic head cancer before surgery (Figures 2, 3). The total score of the integral nomogram formula may be obtained by adding the scores for the respective element, and the probability of MVI can be predicted based on the sum of the integrals. Under the total score was higher than 188 points, it is considered a high risk of lymph node

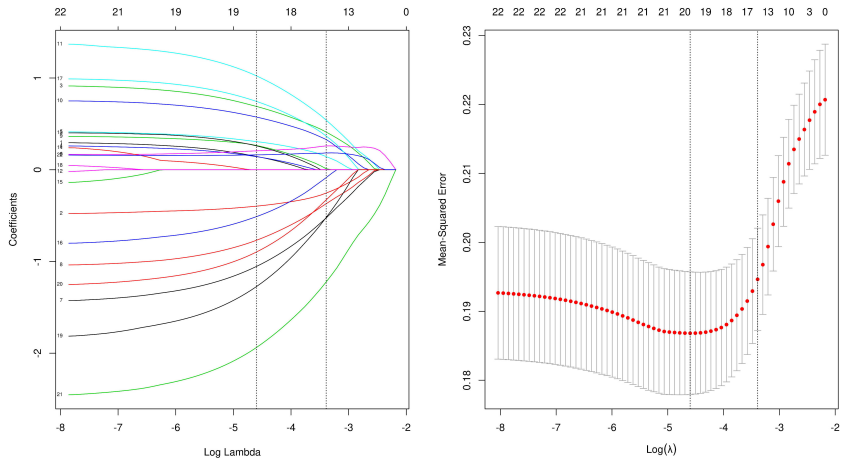


FIGURE 1
lasso analysis.

10.3389/fonc.2023.1053375

TABLE 2 Univariate regression analysis.

Variables	N	OR	95%CI	p	auc	cutoff
Gender						
0	82				0.564	1
1	109	1.685	[0.915,3.105]	0.094		
Age						
0	99				0.562	61
1	92	0.502	[0.271,0.930]	0.028		
Tumorsize						
0	73				0.565	2.6
1	118	2.071	[1.115,3.848]	0.021		
CA125						
0	80				0.538	11.57
1	111	1.633	[0.886,3.010]	0.116		
CA199						
0	104				0.548	219.33
1	87	1.847	[0.989,3.448]	0.054		
ALP						
0	56				0.594	186
1	135	2.971	[1.547,5.703]	0.001		
ALB						
0	118				0.601	40.5
1	73	0.437	[0.235,0.813]	0.009		
GLOB						
0	150				0.569	30.1
1	41	0.408	[0.201,0.829]	0.013		
DBIL						
0	68				0.6	52.8
1	123	2.245	[1.201,4.197]	0.011		
IBIL						
0	36				0.58	12.5
1	155	2.911	[1.385,6.119]	0.005		
WBC						
0	133				0.547	6.71
1	58	2.657	[1.263,5.591]	0.01		
NEU						
0	128				0.532	4.19
1	63	2.1	[1.050,4.201]	0.036		
PCT						
0	171				0.506	0.37
1	20	2.985	[0.841,10.600]	0.091		

(Continued)

10.3389/fonc.2023.1053375

TABLE 2 Continued

Variables	N	OR	95%CI	p	auc	cutoff
MPV						
0	64				0.514	10.9
1	127	1.559	[0.829,2.932]	0.168		
PDW						
0	79				0.522	13.3
1	112	1.687	[0.915,3.114]	0.094		
TT						
0	34				0.515	13.4
1	157	0.587	[0.249,1.384]	0.224		
APTT						
0	59				0.512	27.5
1	132	1.696	[0.893,3.222]	0.107		
PT						
0	92				0.53	11.3
1	99	0.689	[0.374,1.270]	0.233		
INR						
0	129				0.521	1.02
1	62	0.661	[0.350,1.249]	0.202		
FBG						
0	61				0.513	3.36
1	130	0.649	[0.331,1.274]	0.209		
LMR						
0	181				0.523	8.125
1	10	0.106	[0.022,0.517]	0.005		
LSR						
0	48				0.597	1.118787879
1	143	2.763	[1.405,5.436]	0.003		

TABLE 3 Multivariate regression analysis.

Variables	OR	Lower	Upper	p
Age	0.516	0.247	1.055	0.072
Tumorsize	2.259	1.126	4.598	0.023
ALP	1.224	0.411	3.485	0.709
ALB	0.429	0.203	0.893	0.024
GLOB	0.532	0.239	1.196	0.123
DBIL	0.974	0.336	2.632	0.959
IBIL	1.868	0.535	6.686	0.328
WBC	2.22	1.009	5.201	0.055
LMR	0.169	0.022	0.831	0.044
LSR	1.368	0.539	3.4	0.502

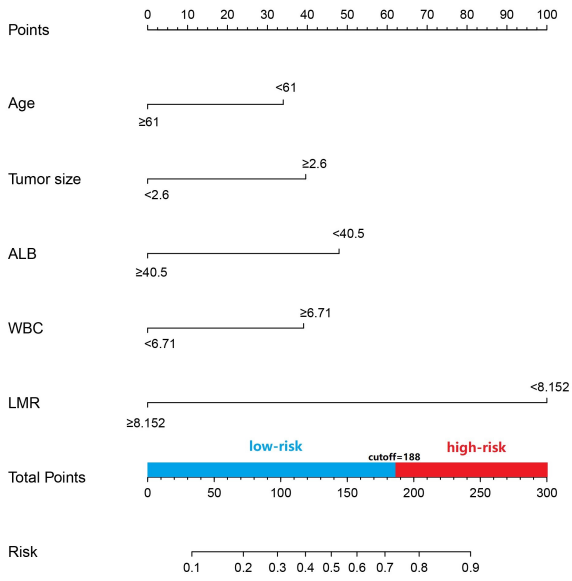


FIGURE 2
Prediction of LNM in patients with pancreatic head cncer using a nomogram. To get the position of each factor on the corresponding axis, draw lines on the point axis to represent the number of points. Add all the scores and find the place of the total score to determine the probability of LNM for that line in the nomogram.

metastasis. Under the total score of less than 188 points, it was considered a low risk of lymph node metastasis. The nomogram prediction model achieved a high degree of predictive capacity, as indicated by the result. The AUC area for this model was 0.745 (Figure 4). (95% CI 0.673-0.816). The result of the model indicated that the standard curve was well consistent with the predicted curve, thus suggesting agreement between the observed frequencies and projected probability of MVI (Figure 5). The result of DCA indicated that the predictive model had a high net benefit throughout almost the entire threshold probability range, thus suggesting that the new nomogram had considerable clinical use (Figure 6).

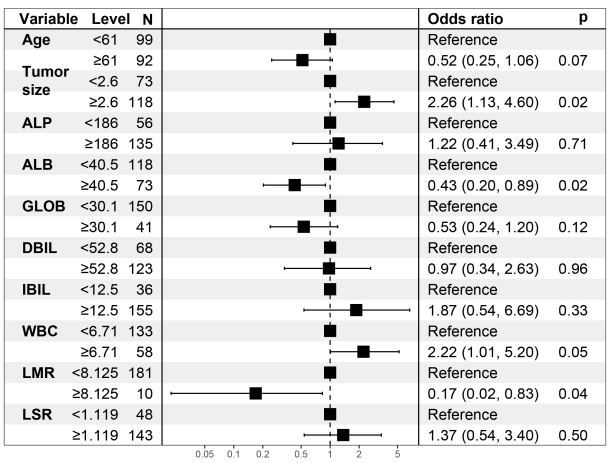


FIGURE 3
Model's forest plot.

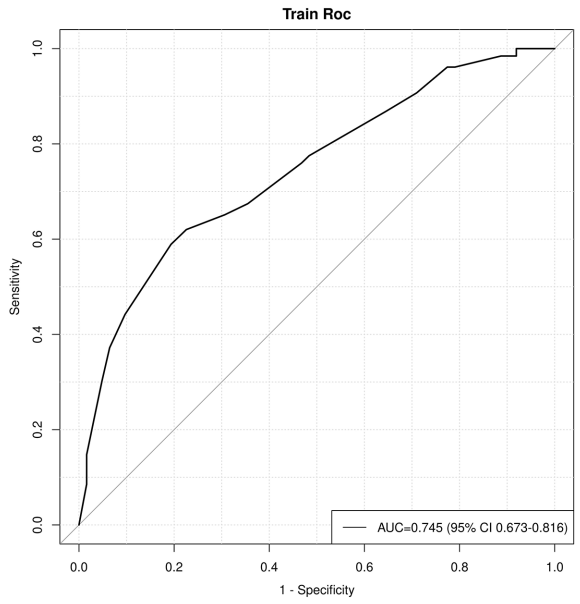


FIGURE 4
The ROC curve of the model.

The establishment of risk stratification model

Risk stratification was performed on the existing model with a low risk score of 0, a high risk score of 1, and a preoperative CT lymph node positive score of 1 and a negative score of 0, using a combination of preoperative CT examination and the model, if the cumulative

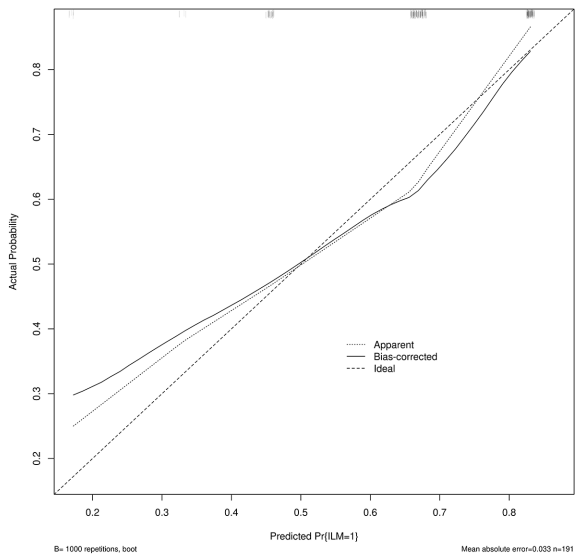


FIGURE 5
Calibration curve for predicting LNM. The nomogram predicted LNM is plotted on the x-axis, and the actual incidence of LNM is plotted on the y-axis. A plot along the 45° line will indicate a perfectly calibrated model where the predicted LNM is the same as the actual LNM. The expected probability distribution for the occurrence of LNM is shown at the top of the figure.

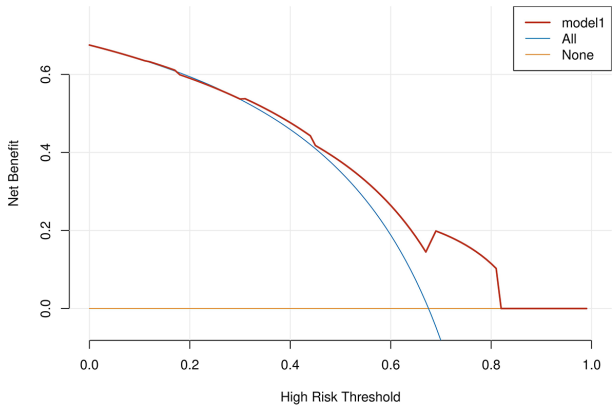


FIGURE 6
A decision curve analysis (DCA) was performed on the nomogram of the model. The solid black line assumes that all patients are LNM positive or negative, respectively. The dashed lines represent the net payoff of the nomogram at different threshold probabilities.

score≥1, the high-risk group, the cumulative score 0 for low-risk group, the final model has statistical significance. ($p < 0.05$)

Discussion

Pancreatic cancer is the "king of cancers" and overall survival rates for patients with ductal adenocarcinoma of the pancreas have barely improved over the last few decades (13–16). Statistics published in the United States (17) over the past few years have suggested that it is the fourth leading cause of death from cancer, one of the main reasons being its susceptibility to early metastasis through lymphatic drainage, and many studies have confirmed the adverse effects of lymphatic metastasis (18, 19). Thus, the stage of pancreatic cancer should be correctly evaluated, and lymph node status should be accurately reported, which is also conducive to evaluating the prognostic status of the patient and determining the best treatment plan. Through the analysis of the potential factors promoting lymph node metastasis (LNM), several positive indicators were identified, which can be evaluated preoperatively in conjunction with the American Joint Committee on Cancer (AJCC) 8th edition staging system for ductal adenocarcinoma of the pancreas (17) to determine whether a patient can undergo surgery or radiotherapy to achieve the optimal prognosis.

Studies have shown that neoadjuvant therapy enables a better survival rate than surgery alone (20), that lymph node positivity is a risk factor for poor outcomes in postoperative patients (20, 21), and that postoperative chemotherapy for patients with lymph node positivity has been shown to improve median survival and survival after surgery (22). In recent years, with the maturity of surgical techniques, postoperative chemotherapy has gradually achieved some results. Preoperative neoadjuvant chemotherapy has become a research hotspot for pancreatic cancer. This study is the first convincing demonstration of clear benefits of preoperative neoadjuvant therapy in node-positive patients (23), on the one hand, achieving reduced nodal staging and thus improved patient survival, and on the other hand, during this period of neoadjuvant therapy, patients with high-risk biological behavioral violations may develop distant metastases, avoiding unnecessary surgical treatment and waste of resources. One limitation of our study was the absence of patient survival data, which is currently being collected and will be analyzed in future studies, which currently require a large number of prospective studies to validate.

The best visualization is presented through simple statistical analysis by building a nomogram model. This model calculates a total score based on the values of individual predictor variables and uses the total score to infer the probability of a positive clinical event. It has been widely used in clinical practice in recent years (24), and it is proven to be effective.

In this study, tumor size (P=0.023), low levels of albumin (P=0.024), lymphocyte to monocyte ratio (P=0.044) were independent risk factors for LNM, with age (P=0.072) and white blood cells (P=0.055) (WBCs) slightly greater than 0.05, probably due to the small sample size in this experiment. It has also been previously demonstrated that younger age and higher WBC values are strongly correlated with the spread of tumor cells (25–30)

In our study, LNM was found to be significantly correlated with tumor size (p=0.023), which is consistent with the findings of most scholars (31–33). Previous findings have also focused on the correlation between LMN and tumor size, and although lymph node metastasis was also present in tumors smaller than 1 cm.In general,it appears that larger tumor volumes are more prone to LNM. Larger tumors are capable of directly invading the surrounding lymph nodes by invading the surrounding tissues besides metastasis through the lymphatic vessels since the pancreas lacks a complete envelope. We consider tumor volume to predict the probability of positive lymph nodes, which will help us to adopt an appropriate treatment plan. For smaller tumors, limited resection can be performed laparoscopically to avoid excessive lymph node dissection and damage to surrounding tissues and to improve the prognosis of the patient.

We also analyzed the correlation between the patient's serum in terms of total protein, albumin and other laboratory indicators and tumor development, and finally found that low protein levels may facilitate the growth and metastasis of tumor cells, which may ne beneficial to predict the probability of LNM (34). In a retrospective study of 207 cancer patients, Adam et al. (24) found that positive lymph nodes are significantly correlated with low albumin levels, and it was concluded that cancer patients are usually accompanied by hypoproteinemia and the subsequent production of ascites and tissue oedema may cause migration of tumor cells, leading to the development of LNM. A related discussion has been found in other

studies (35, 36). Alici et al. (37) have suggested that low preoperative serum albumin levels can indicate tumor malignant potential.

A higher probability of LNM occurrence was found in this study with a low lymphocyte to monocyte ratio (<8.152). The possible reason for this result is that monocytes secrete various pro-inflammatory cytokines that promote tumorigenesis, angiogenesis, and distant metastasis, whereas low lymphocyte levels are correlated with poorer tumor control (38). Macrophages are derived from monocytes, and considerable research (39, 40) has suggested that the presence of macrophages may facilitate the growth and migration of tumor cells, which may contribute to the promotion of LNM when the ratio of monocytes is high. Jeffrey W (40) has confirmed through clinical and experimental research that macrophages facilitate the progression of tumor cells. It is influenced by the tumor microenvironment and has a role in promoting angiogenesis, stromal breakdown, and cell motility, as well as producing various mutagenic oxygen and nitrogen radicals and angiogenic factors.

Several previous lineage table studies on the prediction of LNM in malignancy have shown that low age and high WBC counts are also potential independent risk factors. A considerable amount of research (41, 42) has suggested that inflammation is involved in tumor metastasis by altering the immune system status and local microenvironment, and that more WBCs are correlated with carcinogenesis, tumor progression and mortality. In this study, age and WBCs were found to be correlated with LNM with p-values of 0.072 and 0.055, respectively, slightly greater than 0.05.The possible reason for this result is that this study is a systematic review with a small single-center sample size, or possible bias in data collection and processing. However, it seems to be consistent with most scholars' views.

A review of the literature showed that CA199 is relevant for the diagnosis of early pancreatic cancer, and this has been verified in most studies (43) We suggest that CA199 may also be correlated with the development of LNM, In this study, however, no positive results were obtained. It has also been verified that higher CA199 is a risk factor for lymph node metastasis in early gastric cancer (35). Hopefully, larger medical centers will be able to conduct large sample, multicenter prospective studies to further validate the correlation between CA199 and LNM in pancreatic cancer.

This study also has the above drawbacks (e.g., the small sample size). Because all the information was collected retrospectively, there may have been errors and biases throughout the process. Second, there is sometimes randomness in the removal of peripancreatic lymph nodes when taking pathological tissue, which may result in a lower number of positive lymph nodes in the end than in reality. The 8th edition of the AJCC manual and the College of American Pathologists (CAP) protocol have recommended a minimum number of LNs (eln) of 12 examinations (44). The International Study Group on Pancreatic Surgery (ISGPS) recommends a minimum number of eln of 15 (45). The number of tissue lymph nodes obtained does not meet the above targets. Whereas this last study is a single-center retrospective analysis from northeastern China, further large-sample, multicenter studies and external validation are required to confirm the views of this study.

Conclusion

A line graph model was established based on the above indicators to predict the probability of LNM in pancreatic cancer. The model has some potential value and takes on a clinical significance in individualized clinical treatment. For patients at high risk of LNM, whether surgical resection and lymph node dissection are appropriate should be considered, and there is some guidance for the choice of radiotherapy.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding author.

Ethics statement

The study followed the Declaration of Helsinki. Because of the retrospective nature of the study, patient consent for inclusion was waived.

Author contributions

XG and BJ designed the research. XL, YL, YX, CX collected, analyzed, and interpreted the clinical data. XG and XS wrote and revised the manuscript. BJ revised the manuscript. All authors contributed to the article and approved the submitted version.

Funding

This work was supported by the Provincial Health Specific Project of Jilin Province (Grant No.2018SCZWSZX-019). The Science and Technology Development Program of Jilin Province (Grant No.20200201417JC).

Acknowledgments

We would like to thank the researchers and study participants for their contributions.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2023.1053375/full#supplementary-material

References

1. Siegel RL, Miller KD, Fuchs HE, Jemal A. Cancer statistics, 2021. *CA Cancer J Clin* (2021) 71(1):7–33. doi: 10.3322/caac.21654

2. Fogel EL, Shahda S, Sandrasegaran K, DeWitt J, Easler JJ, Agarwal DM, et al. A multidisciplinary approach to pancreas cancer in 2016: A review. *Am J Gastroenterol* (2017) 112(4):537–54. doi: 10.1038/ajg.2016.610

3. Katz MH, Wang H, Fleming JB, Sun CC, Hwang RF, Wolff RA, et al. Long-term survival after multidisciplinary management of resected pancreatic adenocarcinoma. *Ann Surg Oncol* (2009) 16(4):836–47. doi: 10.1245/s10434-008-0295-2

4. Luberice K, Downs D, Sadowitz B, Ross S, Rosemurgy A. Has survival improved following resection for pancreatic adenocarcinoma? *Am J Surg* (2017) 214(2):341–6. doi: 10.1016/j.amjsurg.2017.05.007

5. Tempero MA, Malafa MP, Al-Hawary M, Behrman SW, Benson AB, Cardin DB, et al. Pancreatic adenocarcinoma, version 2.2021, nccn clinical practice guidelines in oncology. *J Natl Compr Cancer Network* (2021) 19(4):439–57. doi: 10.6004/jnccn.2021.0017

6. Swords DS, Firpo MA, Johnson KM, Boucher KM, Scaife CL, Mulvihill SJ. Implications of inaccurate clinical nodal staging in pancreatic adenocarcinoma. *Surgery* (2017) 162(1):104–11. doi: 10.1016/j.surg.2016.12.029

7. Kumon RE, Repaka A, Atkinson M, Faulx AL, Wong RC, Isenberg GA, et al. Lymph node characterization in vivo using endoscopic ultrasound spectrum analysis with electronic array echo endoscopes. *Endoscopy* (2012) 44(6):618–21. doi: 10.1055/s-0032-1306774

8. Nanashima A, Sakamoto I, Hayashi T, Tobinaga S, Araki M, Kunizaki M, et al. Preoperative diagnosis of lymph node metastasis in biliary and pancreatic carcinomas: Evaluation of the combination of multi-detector ct and serum Ca19-9 level. *Digestive Dis Sci* (2010) 55(12):3617–26. doi: 10.1007/s10620-010-1180-y

9. Zhang Z, Procissi D, Li W, Kim DH, Li K, Han G, et al. High resolution mri for non-invasive mouse lymph node mapping. *J Immunol Methods* (2013) 400-401:23–9. doi: 10.1016/j.jim.2013.06.013

10. Wang S, Shi H, Yang F, Teng X, Jiang B. The value of (18)F-fdg Pet/Ct and carbohydrate antigen 19-9 in predicting lymph node micrometastases of pancreatic cancer. *Abdominal Radiol (New York)* (2019) 44(12):4057–62. doi: 10.1007/s00261-019-02248-0

11. Wang SC, Parekh JR, Porembka MR, Nathan H, D'Angelica MI, DeMatteo RP, et al. A pilot study evaluating serum Mmp7 as a preoperative prognostic marker for pancreatic ductal adenocarcinoma patients. *J Gastrointest Surg* (2016) 20(5):899–904. doi: 10.1007/s11605-015-3057-z

12. Lambin P, Leijenaar RTH, Deist TM, Peerlings J, de Jong EEC, van Timmeren J, et al. Radiomics: The bridge between medical imaging and personalized medicine. *Nat Rev Clin Oncol* (2017) 14(12):749–62. doi: 10.1038/nrclinonc.2017.141

13. Tonini V, Zanni M. Pancreatic cancer in 2021: What you need to know to win. *World J Gastroenterol* (2021) 27(35):5851–89. doi: 10.3748/wjg.v27.i35.5851

14. Beger HG, Rau B, Gansauge F, Poch B, Link KH. Treatment of pancreatic cancer: Challenge of the facts. *World J Surg* (2003) 27(10):1075–84. doi: 10.1007/s00268-003-7165-7

15. Yoshitomi H, Takano S, Furukawa K, Takayashiki T, Kuboki S, Ohtsuka M. Conversion surgery for initially unresectable pancreatic cancer: Current status and unresolved issues. *Surg Today* (2019) 49(11):894–906. doi: 10.1007/s00595-019-01804-x

16. Tsuchiya N, Matsuyama R, Murakami T, Yabushita Y, Sawada Y, Kumamoto T, et al. Role of conversion surgery for unresectable pancreatic cancer after long-term chemotherapy. *World J Surg* (2020) 44(8):2752–60. doi: 10.1007/s00268-020-05503-4

17. Shi S, Hua J, Liang C, Meng Q, Liang D, Xu J, et al. Proposed modification of the 8th edition of the ajcc staging system for pancreatic ductal adenocarcinoma. *Ann Surg* (2019) 269(5):944–50. doi: 10.1097/SLA.0000000000002668

18. Byun Y, Lee KB, Jang JY, Han Y, Choi YJ, Kang JS, et al. Peritumoral lymph nodes in pancreatic cancer revisited; is it truly equivalent to lymph node metastasis? *J Hepatobiliary Pancreat Sci* (2021) 28(10):893–901. doi: 10.1002/jhbp.940

19. Riediger H, Keck T, Wellner U, zur Hausen A, Adam U, Hopt UT, et al. The lymph node ratio is the strongest prognostic factor after resection of pancreatic cancer. *J Gastrointest Surg* (2009) 13(7):1337–44. doi: 10.1007/s11605-009-0919-2

20. Mokdad AA, Minter RM, Zhu H, Augustine MM, Porembka MR, Wang SC, et al. Neoadjuvant therapy followed by resection versus upfront resection for resectable pancreatic cancer: A propensity score matched analysis. *Off J Am Soc Clin Oncol* (2017) 35(5):515–22. doi: 10.1200/jco.2016.68.5081

21. Artinyan A, Anaya DA, McKenzie S, Ellenhorn JD, Kim J. Neoadjuvant therapy is associated with improved survival in resectable pancreatic adenocarcinoma. *Cancer* (2011) 117(10):2044–9. doi: 10.1002/cncr.25763

22. Merchant NB, Rymer J, Koehler EA, Ayers GD, Castellanos J, Kooby DA, et al. Adjuvant chemoradiation therapy for pancreatic adenocarcinoma: Who really benefits? *J Am Coll Surgeons* (2009) 208(5):829–38. doi: 10.1016/j.jamcollsurg.2008.12.020

23. Tran Cao HS, Zhang Q, Sada YH, Silberfein EJ, Hsu C, Van Buren G2nd, et al. Value of lymph node positivity in treatment planning for early stage pancreatic cancer. *Surgery* (2017) 162(3):557–67. doi: 10.1016/j.surg.2017.05.003

24. Lv J, Liu YY, Jia YT, He JL, Dai GY, Guo P, et al. A nomogram model for predicting prognosis of obstructive colorectal cancer. *World J Surg Oncol* (2021) 19(1):337. doi: 10.1186/s12957-021-02445-6

25. Shao Y, Tu X, Liu Y, Bao Y, Ren S, Yang Z, et al. Predict lymph node metastasis in penile cancer using clinicopathological factors and nomograms. *Cancer Manag Res* (2021) 13:7429–37. doi: 10.2147/CMAR.S329925

26. Yj L, Hr L, Cm N, Uk H, Sh J. White blood cell count and the risk of colon cancer. *Yonsei Med J* (2006) 47(5):646-56. doi: 10.1007/s00268-020-05770-1

27. Tian Z, Meng L, Wang X, Diao T, Hu M, Wang M, et al. Predictive nomogram and risk factors for lymph node metastasis in bladder cancer. *Front Oncol* (2021) 11:690324. doi: 10.3389/fonc.2021.690324

28. Min Y, Liu X, Hu D, Chen H, Chen J, Xiang K, et al. Risk factors, prognostic factors, and nomogram for distant metastasis in breast cancer patients without lymph node metastasis. *Front Endocrinol (Lausanne)* (2021) 12:771226. doi: 10.3389/fendo.2021.771226

29. Paiva GR, de Oliveira Araujo IB, Athanazio DA, de Freitas LA. Penile cancer: Impact of age at diagnosis on morphology and prognosis. *Int Urol Nephrol* (2015) 47 (2):295–9. doi: 10.1007/s11255-014-0875-y

30. Song J, Yin H, Zhu Y, Fei S. Identification of predictive factors for lymph node metastasis in Pt1 stage colorectal cancer patients: A retrospective analysis based on the population database. *Pathol Oncol Res* (2022) 28:1610191. doi: 10.3389/pore.2022.1610191

31. Nakao Y, Hayashi H, Yamashita YI, Takashi O, Matsumura K, Uemura N, et al. Risk factors for lymph node metastasis in patients with pancreatic neuroendocrine neoplasms. *World J Clin Oncol* (2022) 13(6):520–8. doi: 10.5306/wjco.v13.i6.520

32. Zhang M, Ding C, Xu L, Feng S, Ling Y, Guo J, et al. A nomogram to predict risk of lymph node metastasis in early gastric cancer. *Sci Rep* (2021) 11(1):22873. doi: 10.1038/s41598-021-02305-z

33. Pu N, Chen Q, Gan W, Shen Y, Gao S, Habib JR, et al. Lymph node metastatic patterns and survival predictors based on tumor size in pancreatic ductal adenocarcinoma. *Adv Ther* (2021) 38(8):4258–70. doi: 10.1007/s12325-021-01819-2

34. Brewczynski A, Jablonska B, Pawlicki K. Associations between nutritional parameters and clinicopathologic factors in patients with gastric cancer: A comprehensive study. *Nutr Cancer* (2017) 69(5):752–61. doi: 10.1080/01635581.2017.1324993

35. Li X, Shao L, Lu X, Yang Z, Ai S, Sun F, et al. Risk factors for lymph node metastasis in gastric neuroendocrine tumor: A retrospective study. *BMC Surg* (2021) 21(1):174. doi: 10.1186/s12893-021-01174-7

36. Gupta D, Lis CG. Pretreatment serum albumin as a predictor of cancer survival: A systematic review of the epidemiological literature. *Nutr J* (2010) 9:69. doi: 10.1186/1475-2891-9-69

37. Alici S, Kaya S, Izmirli M, Tuncer I, Doğan E, Ozbek H, et al. Analysis of survival factors in patients with advanced-stage gastric adenocarcinoma. *Med Sci Monit* (2006) 12 (5):CR221–229.

38. Song L, Heng Y, Hsueh CY, Huang H, Tao L, Zhou L, et al. A predictive nomogram for lymph node metastasis in supraglottic laryngeal squamous cell carcinoma. *Front Oncol* (2022) 12:786207. doi: 10.3389/fonc.2022.786207

39. Condeelis J, Pollard JW. Macrophages: Obligate partners for tumor cell migration, invasion, and metastasis. *Cell* (2006) 124(2):263–6. doi: 10.1016/j.cell.2006.01.007

40. Pollard JW. Tumour-educated macrophages promote tumour progression and metastasis. *Nat Rev Canc* (2004) 4(1):71–8. doi: 10.1038/nrc1256

41. Kaltenmeier C, Simmons RL, Tohme S, Yazdani HO. Neutrophil extracellular traps (Nets) in cancer metastasis. *Cancers (Basel)* (2021) 13(23):6131. doi: 10.3390/cancers13236131

42. Zhou C, Liu HS, Liu XH, Zheng XB, Hu T, Liang ZX, et al. Preoperative assessment of lymph node metastasis in clinically node-negative rectal cancer patients based on a nomogram consisting of five clinical factors. *Ann Transl Med* (2019) 7(20):543. doi: 10.21037/atm.2019.09.127

43. Chen J, Wang H, Zhou L, Liu Z, Tan X. A combination of circulating tumor cells and Ca199 improves the diagnosis of pancreatic cancer. *J Clin Lab Anal* (2022) 36(5): e24341. doi: 10.1002/jcla.24341

44. Nagaria TS, Wang H. Modification of the 8(Th) ajcc staging system of pancreatic ductal adenocarcinoma. *Hepatobiliary Surg Nutr* (2020) 9(1):95–7. doi: 10.21037/hbsn.2019.08.01

45. de Virgilio C. Question sets and answers. *Surgery* (2015), 156(3):591–699. doi: 10.1016/j.surg.2014.06.016

Frontiers in Oncology

Advances knowledge of carcinogenesis and tumor progression for better treatment and management

The third most-cited oncology journal, which highlights research in carcinogenesis and tumor progression, bridging the gap between basic research and applications to imrpove diagnosis, therapeutics and management strategies.

Discover the latest Research Topics

See more →

Frontiers

Avenue du Tribunal-Fédéral 34
1005 Lausanne, Switzerland
frontiersin.org

Contact us

+41 (0)21 510 17 00
frontiersin.org/about/contact