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Diagnosis and management of acute, chronic, and autoimmune pancreatitis

Edited by
Ravi Kumar Sharma, Francesco Vitali and Puneet Chhabra

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Diagnosis and management of acute, chronic, and autoimmune pancreatitis

Topic editors

Ravi Kumar Sharma — Chandigarh University, India

Francesco Vitali — University Medical Center, University of Greifswald, Germany

Puneet Chhabra — Calderdale Royal Hospital, United Kingdom

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EDITED AND REVIEWED BY
Angel Lanas,
University of Zaragoza, Spain

*CORRESPONDENCE
Ravi Kumar Sharma
ravisharma1j@gmail.com

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Editorial: Diagnosis and management of acute, chronic, and autoimmune pancreatitis

Ravi Kumar Sharma[1]*, Francesco Vitali[2] and Puneet Chhabra[3]

[1]Department of University Institute of Biotechnology, Chandigarh University, Mohali, Punjab, India, [2]Department of Internal Medicine, A University Hospital of Greifswald, Greifswald, Germany, [3]Calderdale Royal Hospital, Halifax, United Kingdom

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Editorial on the Research Topic
Diagnosis and management of acute, chronic, and autoimmune pancreatitis

Pancreatitis represents an inflammatory disease of the pancreatic gland with multiple genetic and environmental etiological factors that cause considerable clinical concern commonly associated with hospital admissions, increased morbidity, and mortality worldwide (1). According to the clinical course, pancreatitis is classified as acute and chronic, although sometimes an overlap between the two entities, like in recurrent pancreatitis, can be found (2). A progression from acute to chronic pancreatitis has also been described (3). Autoimmune pancreatitis (AIP) is a rare disease with a low annual incidence, which varies substantially between geographical regions (4, 5).

AP is defined by sudden pancreatic inflammation, involving the acini and the pancreatic ducts. It's an unpredictable and potentially fatal illness. The prognosis is primarily determined by the development of organ failure, pancreatic or peripancreatic necrosis and subsequent infection. Gallstones, alcohol usage, hyperlipidaemia, or certain drugs are the most common causes. Severe abdominal pain (often radiating to the back), high serum amylase and lipase levels, and signs of pancreatic edema or inflammation on imaging techniques such as abdominal ultrasonography or CT scans are common features (6).

AP is managed primarily by supportive treatment, which includes fluid resuscitation, pain management, and nutritional assistance. Severe cases may require radiological and endoscopic intervention in a step-up approach manner, especially if patients develop complications such as abscess or suprainfection of necrosis (7, 8).

CP results from fibro-inflammatory changes of the pancreatic gland, leading to irreversible damage and functional impairment. Etiologies often include long-term alcohol abuse, tobacco smoking, genetic factors, or metabolic disorders (9). Clinically, patients may present with abdominal pain, malabsorption, and diabetes. The diagnosis is established through a combination of history, imaging, and functional tests assessing pancreatic enzyme secretion. Management emphasizes pain relief, lifestyle modification and enzyme replacement therapy. In some cases, surgical options, such as ductal decompression or resection, may be indicated. CP patients have a lifetime risk of developing pancreatic ductal adenocarcinoma (10).

AIP, an entity often classified as a chronic pancreatitis, is characterized clinically by obstructive jaundice with or without a pancreatic mass. It is often associated with other autoimmune conditions. It is categorized into two types: Type 1, which is associated with IgG4-related disease, and Type 2, which is associated with inflammatory bowel disease and histologically characterized by granulocytic ductal infiltrations (GEL: granulocytic epithelial lesions)1 (11). Diagnosis relies on serological markers, imaging findings, and histological confirmation through biopsy. Management typically involves corticosteroids, which can lead to significant improvement. Long-term follow-up is critical, as relapse can occur (4, 5).

The current Research Topic on pancreatitis encompasses a wide range of significant contributions in the form of original articles. Yang et al. demonstrated that modulation of the PI3K/AKT signaling pathway can enhance apoptosis in pancreatic acinar cells while simultaneously reducing the inflammatory response, highlighting a potential therapeutic approach. Similarly, Li et al. identified smoking as an independent risk factor, showing that it can significantly increase the severity of pancreatitis. Xing et al. developed a nomogram that performed well in predicting persistent organ failure (POF) in patients with acute pancreatitis (AP). This tool can support clinical decision-making and improve personalized treatment strategies. Ma et al. explored the causal associations between specific lipidome types and pancreatitis, contributing to a deeper understanding of lipid metabolism in the disease, which may pave the way for more targeted interventions. Lin et al. used Mendelian randomization analysis to suggest that there is no causal association between glucocorticoid use and the risk of pancreatitis, which helps clarify conflicting data on this therapeutic approach.

Wiese et al. highlighted that malnourished patients with chronic pancreatitis can significantly benefit from intensified nutritional therapy. In addition to improving nutritional status, a multimodal intervention can enhance muscle function and improve overall disease prognosis. Frost et al. showed that the composition of the stent microbiome is associated with prolonged hospital stays and adverse events during endoscopic drainage therapy. This finding underscores the importance of infection control to optimize patient outcomes during such procedures. Jia et al. demonstrated that the combination of traditional Chinese medicine and modern medicine in treating patients with mild to moderate acute pancreatitis effectively reduces inflammatory indicators and shortens both symptom duration and hospitalization periods, ultimately promoting faster disease recovery. Jia et al. demonstrated that integrating traditional Chinese medicine with modern medical treatments significantly reduces inflammatory markers in patients with mild to moderate AP. This approach helps shorten the duration of symptoms, reduces hospital stay, and accelerates recovery. Zahariev et al. demonstrated that factors such as the severity and recurrence of AP, along with etiologies like alcohol consumption and hypertriglyceridemia, as well as conditions like organ failure, pancreatic necrosis, obesity, chronic kidney and liver diseases, and dyslipidemia, are linked to an increased risk of developing prediabetes or diabetes.

Research continues to evolve, focusing on understanding the underlying mechanisms of pancreatitis, identifying biomarkers for early diagnosis, and developing targeted therapies. Enhanced collaboration between gastroenterologists, radiologists, and pathologists is essential to improve patient outcomes.

In conclusion, a comprehensive understanding of the diverse forms of pancreatitis is crucial for clinicians. By advancing diagnostic methods and tailoring management strategies, we can significantly impact patient care, mitigate complications, and improve overall quality of life for those affected by this complex condition.

Author contributions

RS: Writing – original draft. FV: Writing – review & editing. PC: Writing – review & editing.

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.

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EDITED BY
Michael Chvanov,
University of Liverpool, United Kingdom

REVIEWED BY
Lu Ke,
Nanjing University, China
Hanna Sternby,
Lund University, Sweden

*CORRESPONDENCE
Péter Hegyi
✉ hegyi2009@gmail.com

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

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Risk factors for diabetes mellitus after acute pancreatitis: a systematic review and meta-analysis

Olga Julia Zahariev[1,2,3], Stefania Bunduc[1,4], Adrienn Kovács[1,5], Dóra Demeter[1,6], Luca Havelda[1,2], Bettina Csilla Budai[1,2], Dániel Sándor Veres[1,7], Nóra Hosszúfalusi[1,5], Bálint Mihály Erőss[1,2,3], Brigitta Teutsch[1,3], Márk Félix Juhász[3,8†] and Péter Hegyi[1,2,3,9*†]

[1]Centre for Translational Medicine, Semmelweis University, Budapest, Hungary, [2]Institute of Pancreatic Diseases, Semmelweis University, Budapest, Hungary, [3]Institute for Translational Medicine, Medical School, University of Pécs, Pécs, Hungary, [4]Carol Davila University of Medicine and Pharmacy, Bucharest, Romania, [5]Department of Internal Medicine and Hematology, Semmelweis University, Budapest, Hungary, [6]Dietetic Services, Central Hospital of Northern Pest - Military Hospital, Budapest, Hungary, [7]Department of Biophysics and Radiation Biology, Semmelweis University, Budapest, Hungary, [8]Heim Pál National Pediatric Institute, Budapest, Hungary, [9]Translational Pancreatology Research Group, Interdisciplinary Center of Excellence for Research Development and Innovation University of Szeged, Szeged, Hungary

Introduction: Within 5years of having acute pancreatitis (AP), approximately 20% of patients develop diabetes mellitus (DM), which later increases to approximately 40%. Some studies suggest that the prevalence of prediabetes (PD) and/or DM can grow as high as 59% over time. However, information on risk factors is limited. We aimed to identify risk factors for developing PD or DM following AP.

Methods: We systematically searched three databases up to 4 September 2023 extracting direct, within-study comparisons of risk factors on the rate of new-onset PD and DM in AP patients. When PD and DM event rates could not be separated, we reported results for this composite outcome as PD/DM. Meta-analysis was performed using the random-effects model to calculate pooled odds ratios (OR) with 95% confidence intervals (CI).

Results: Of the 61 studies identified, 50 were included in the meta-analysis, covering 76,797 participants. The studies reported on 79 risk factors, and meta-analysis was feasible for 34 risk factor and outcome pairs. The odds of developing PD/DM was significantly higher after severe and moderately severe AP (OR: 4.32; CI: 1.76–10.60) than mild AP. Hypertriglyceridemic AP etiology (OR: 3.27; CI: 0.17–63.91) and pancreatic necrosis (OR: 5.53; CI: 1.59–19.21) were associated with a higher risk of developing PD/DM. Alcoholic AP etiology (OR: 1.82; CI: 1.09–3.04), organ failure (OR: 3.19; CI: 0.55–18.64), recurrent AP (OR: 1.89; CI: 0.95–3.77), obesity (OR: 1.85; CI: 1.43–2.38), chronic kidney disease (OR: 2.10; CI: 1.85–2.38), liver cirrhosis (OR: 2.48; CI: 0.18–34.25), and dyslipidemia (OR: 1.82; CI: 0.68–4.84) were associated with a higher risk of developing DM.

Discussion: Severe and moderately severe AP, alcoholic and hypertriglyceridemic etiologies, pancreatic necrosis, organ failure, recurrent acute pancreatitis and comorbidities of obesity, chronic kidney disease liver disease, and dyslipidemia are associated with a higher risk of developing PD or DM.

Systematic review registration: https://www.crd.york.ac.uk/prospero/, identifier CRD42021281983.

KEYWORDS

diabetes mellitus, prediabetes, acute pancreatitis (AP), pancreatitis—complications, gastrointestinal disorders, risk factor (RF)

1 Introduction

Acute pancreatitis (AP) is characterized by premature activation of pancreatic enzymes leading to autodigestion and inflammation of the pancreatic tissue. Potential short-term complications include acute pancreatic fluid collection, pancreatic necrosis, and organ failure (1). Patients with preexisting diabetes mellitus (DM) have an increased risk of developing complications during an AP episode (2). Additionally, elevated glucose levels during hospitalization are associated with more severe AP episodes and increased mortality rates (3). Moreover, it is gaining recognition that DM might also develop after AP as a potential long-term complication (4, 5).

A large population-based study of 14,830 people found that compared to the general population the risk of DM is 2-fold having had a single episode of mild AP (6). Multiple meta-analyses found that within 5 years of an AP episode 18–20% of the patients develop DM, which later increases to approximately 37–40% (7, 8). New-onset prediabetes (PD) is also frequent. Das et al. found the combined incidence of PD and DM to be 35% in the first year following the first AP episode, increasing to 59% after 5 years (7). Not only is the risk of these conditions substantially increased in the context of AP, but their therapy is also challenging. Post-AP DM is recognized as a distinct subtype of DM (9) with more frequent hypoglycemic events (10, 11) and simultaneously greater insulin needs (5, 12, 13) than type 2 DM.

Studies focusing on acute pancreatitis patients with extended follow-up periods are limited (14) and investigations into the implications of developing post-AP DM are even more scarce. Compared to type 2 DM, post-AP DM carries a higher risk of cardiovascular and cerebrovascular disease based on cohort studies exceeding 150,000 patients (5, 11). A population-based matched cohort study of 10,549 individuals in New Zealand reported higher cancer-related deaths (not including pancreatic cancer) and increased mortality from gastrointestinal and infectious diseases in patients with post-AP DM compared to type 2 DM (15). Patients with post-AP DM also have an increased risk of all-cause mortality compared to patients with type 2 DM (5, 10, 11).

Therefore, it is essential to understand the risk factors of developing PD and DM after AP, to facilitate prompt diagnosis and treatment. Two previous meta-analyses provided data on possible risk increasing features, but with conflicting results (7, 8). One possible reason is that instead of pooling direct within-study comparisons these studies used analytical methods conferring a significantly higher risk of bias and less accurate estimations, i.e., meta-regression of PD and DM based on the proportion of a proposed risk factor, indirect comparison of PD and DM prevalence in individuals with different proposed risk factors. The number of analyzed variables was also very limited (to severity, alcoholic and biliary etiology, necrosis, age, sex, follow-up length, and publication year).

We aimed to conduct a comprehensive systematic review and meta-analysis of all available risk factors for PD and DM development after AP, including only studies where prognostic factors are directly compared, allowing for more reliable conclusions.

2 Methods

2.1 Protocol and reporting

Our review followed the Cochrane Handbook for Systematic Reviews (16) and adhered to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) 2020 guideline (Supplementary Table S1) (17). The study protocol was registered with the International Prospective Register of Systematic Reviews (PROSPERO, CRD42021281983).

2.2 Eligibility criteria

Our study aimed to investigate risk factors for developing PD and DM following AP, via analyzing all factors assessed during the hospitalization with AP, that were compared between new-onset PD or DM and normal glucose regulation groups. To establish the eligibility criteria, we used the PECOTS framework.

Population (P): adult AP patients without confirmed DM at discharge. Exposure and comparator (E): any factor assessed at the time of hospitalization with AP and (C) its control group, such as severe vs. non-severe AP, necrosis vs. absence of necrosis, smoking vs. not smoking, male vs. female. Classification of AP severity has changed over the years. Our study group's data in two ways firstly comparing severe AP (SAP) vs. moderately severe and mild AP as one group and alternatively comparing SAP and moderately severe AP as one group vs. mild AP. Some studies applied classification criteria with only two categories: severe and non-severe AP. These studies defined SAP based on 1992 Atlanta criteria (18–21), Scoring $\geq$8 on APACHE II (22), $\geq$3 Ranson score (23), and $\geq$2 Japanese severity score (24). We analyzed the findings of these studies using the categories of SAP vs. moderate and mild AP as one group.

Outcome (O): Number of AP patients who developed, after hospital discharge: DM or PD (impaired fasting glucose, impaired glucose tolerance, and HbA1c $\geq$5.7 and $<$6.5%) as reported by the study authors. Multiple studies provided the number of patients who developed PD or DM combined; we included this composite outcome in our analyses as PD/DM. In case of studies providing incomplete or no definition for glycemic outcomes or not stating explicitly that preexisting DM was excluded from the cohort, this uncertainty was taken into account during the risk of bias assessment.

Timing (T): Initially, we planned to include studies assessing the outcome at least 3 months after hospital discharge. However, we decided to deviate and include all studies that reported on the relevant outcomes after hospital discharge because of the limited and heterogeneous data on follow-up and diagnosis time intervals.

Study design (S): The analysis included interventional and observational studies that met the criteria of our review's PECO framework. Case reports, case series, and studies with less than 10 participants per outcome group or less than 10 participants in the exposed or comparator group were excluded. Conference abstracts were retained.

2.3 Search strategy and selection process

The systematic search was carried out in three databases: MEDLINE (via PubMed), Embase, and Cochrane Central Register of Controlled Trials (CENTRAL) from database inception to September 04, 2023 without any filters or restrictions. The main concepts in the search strategy were prediabetes, diabetes, acute, and pancreatitis. See Supplementary Table S2 for the detailed search key and selection process.

2.4 Data collection process and data items

Data collection process is detailed in Supplementary Table S2. Data on the following variables were collected when available: country, year of publication, study period, follow-up time, name and the number of centers, study design, sample size, age, sex and weight of participants, inclusion and exclusion criteria of participants, classification of AP severity, outcome domains reported and their assessment method, and risk factors during the initial AP episode and their definitions. For a complete list of the risk factors investigated in relation to new-onset PD, DM, or PD/DM by the included studies, see Supplementary Table S3.

2.5 Data synthesis

We calculated odds ratios (ORs) with 95% confidence intervals (CI). Refer to Supplementary Table S4 for detailed description.

2.6 Risk of bias

Two independent reviewers (OZ and AK) assessed each study for risk of bias using the Quality In Prognosis Studies (QUIPS) tool (25). Disagreements were resolved by discussion until reaching a consensus. Risk of bias analyses were conducted for each outcome and prognostic factor separately. To simplify and ease the interpretation of these results, three summary Risk of bias assessments were created for the three main outcomes (DM, PD, and PD/DM), taking into account the worst possible scenario for each study and each domain.

2.7 Publication bias

To assess the possibility of publication bias (small study effect), we created and visually assessed funnel plots for every analysis where at least six studies were included. Harbord modified Egger's test was performed in the case of 10 or more included studies (26), with a $p < 0.1$ indicating statistical significance for funnel plot asymmetry.

3 Results

3.1 Study selection

The systematic search yielded 14,977 results (Figure 1). Overall, 61 studies with 85 reporting articles were eligible for inclusion. The meta-analysis encompassed 50 studies and 76,797 patients.

3.2 Characteristics of included studies

Key study characteristics are summarized in Table 1. Approximately 68% of the studies were based on the general AP population, four included only SAP patients, six focused on necrotizing AP patients and in 10 studies participants were superselected for other criteria. The outcome was reported as PD, DM, and PD/DM in 6, 43, and 22 studies, respectively. A total of 79 prognostic factors were reported on by at least one study and the unique combinations of prognostic factors and outcomes numbered 137 different comparisons. Meta-analysis was possible in the case of 34 risk factor and outcome pairs.

3.3 Synthesis of results

Our findings of the 34 meta-analyses are summarized in an aggregated forest plot, which shows the pooled OR for each risk factor and outcome pair (Figure 2). In addition, per risk factor groups we present the original forest plots or more detailed aggregated forest plots. All other individual plots can be found in the Supplementary material.

3.3.1 AP severity and complications

Having SAP or moderately severe AP was associated with a significantly greater odds of developing PD/DM [OR: 4.32; CI: 1.76–10.60; Figure 3A; (27–34)] and DM [OR: 2.11; CI: 1.30–3.41; Figure 3C; (28, 29, 35–41)] compared to mild disease. SAP was associated with significantly increased odds of developing PD/DM [OR: 3.13; CI: 1.60–6.11; Figure 3B; (18, 22, 23, 27, 28, 30, 31, 33)] and DM [OR: 1.86; CI: 1.27–2.73; Figure 3D; (19–21, 24, 28, 35–37, 41–45)] compared to mild-or-moderate disease.

We found a significantly greater odds of developing PD/DM with necrotizing AP [OR: 5.53; CI: 1.59–19.21; (22, 31, 46–48)] and a statistically non-significant tendency with DM [OR: 3.09; CI: 0.98–9.72; (24, 30, 36, 40, 41, 49, 50)] compared to non-necrotizing AP (Figure 4). Sensitivity analysis revealed that leaving out Takeyama (24) from the analysis would lead to a statistically significant OR (4.17; CI: 2.08–8.37) of developing DM in AP patients who had necrosis compared to its absence (Supplementary Figure S28). In this study, the data collection of index AP episode—and thus the evaluation of necrosis—occurred in 1987, which was 24 years earlier than any other study included in the analysis. Notably, computer tomography imaging has improved significantly in that time (51).

A limited number of studies allowed for the analysis of the extent of pancreatic necrosis (Figure 4). Necrosis affecting over 50% of the pancreatic tissue was associated with a significantly higher odds of developing DM [OR: 4.12; CI: 1.83–9.30; (30, 36, 41)] compared to smaller proportions affected. We also observed a statistically

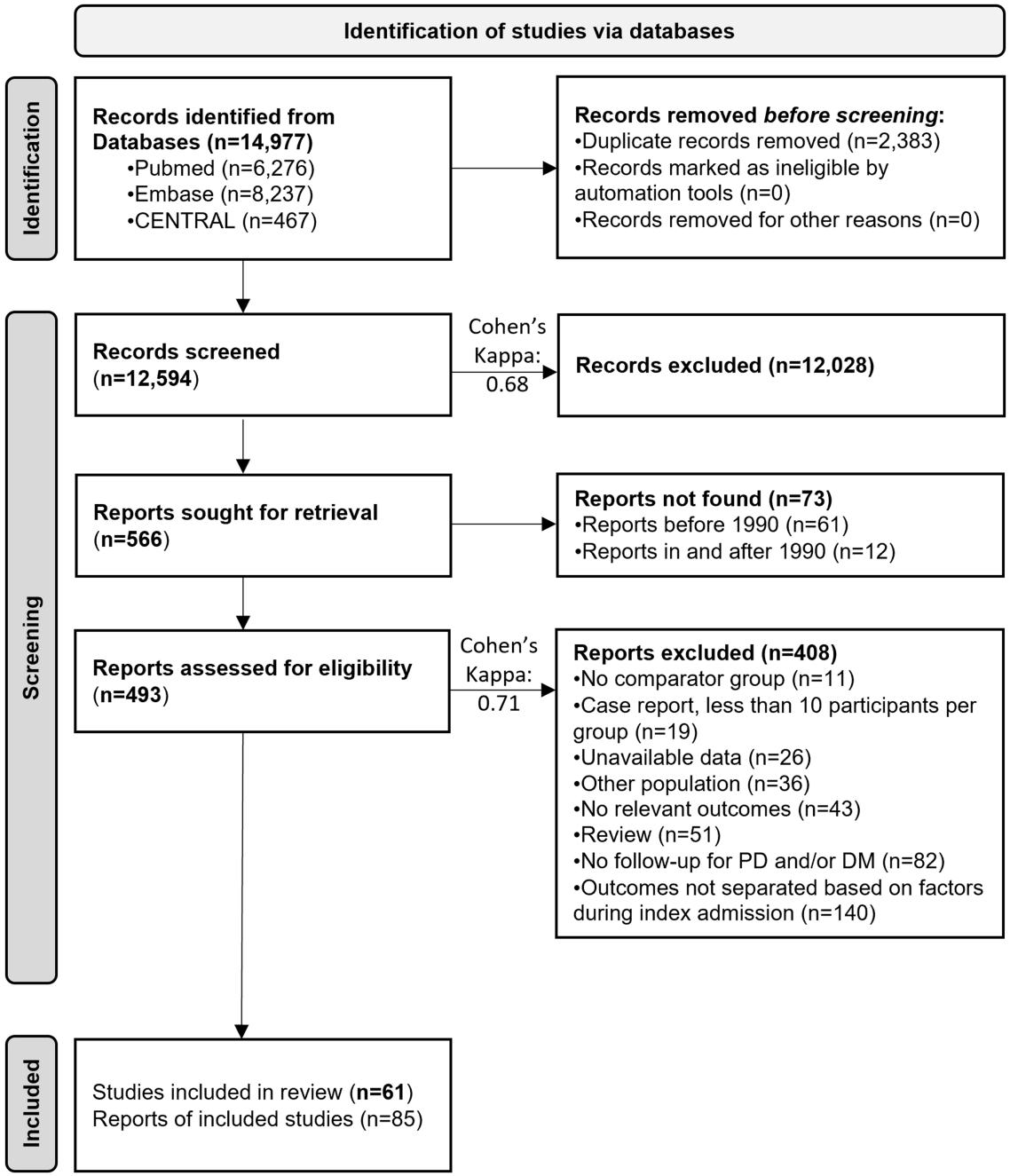


FIGURE 1
PRISMA flow diagram of screened and included studies. PD, Prediabetes; DM, Diabetes mellitus.

non-significant tendency for developing PD/DM in patients whose pancreas was at least 30% necrotic [OR: 5.44; CI: 0.19–157.71; (30–32)].

Similarly, only a statistically non-significant tendency could be observed in case of any organ failure (regardless of organ and duration of impairment) and DM [OR: 3.19; CI: 0.55–18.64; (36, 40, 45, 52)] or PD/DM [OR: 2.14; CI: 0.51–9.06; (31, 32, 46); Figure 4].

3.3.2 AP etiology and recurrent AP

We conducted quantitative syntheses assessing the risk of PD/DM after alcoholic, biliary, and hypertriglyceridemia-induced AP, and the risk of DM after alcoholic, biliary, and idiopathic AP (Figure 5). We found that alcoholic AP patients had a higher odds of developing DM [OR: 1.82; CI: 1.09–3.04; I^2 = 88%; (18, 20, 24, 35–40, 43, 45, 50, 53–57)] compared to patients with non-alcoholic AP. Moreover, after conducting a subgroup analysis based on follow-up time, we found reduced statistical heterogeneity (I^2 = 57%) as well as a possible increasing effect over time (Supplementary Figure S6). While not reaching statistical significance, we observed a tendency of increased risk of new-onset PD/DM following alcoholic [OR: 1.33; CI: 0.77–2.31; (23, 27, 31, 33, 53, 57–59)] and hypertriglyceridemic AP [OR: 3.27; CI: 0.17–63.91; (27, 31, 58)] as well. Biliary etiology was

TABLE 1 Basic characteristics of the included studies.

Study identifier	Country	Study design	Population	Total No. of participants (male %)	Age * (year)	Outcome type			Outcome assessment method	Mean time to follow-up (months)*
						PD	DM	PD/DM		
Akbar et al. (48)†	India	Prospective cohort	AP	86 (77%)	36§ ±12¶	23.3%	10.5%	33.7%	FPG, OGTT, HbA1c	12§§
Akbar et al. (32)	India	Prospective cohort	AP	86 (77%)	33§ (26–44.2)‖	23.3%	10.5%	33.7%	FPG, OGTT, HbA1c	12§§
Andersson et al. (18)	Sweden	Prospective cohort	AP	40 (40%)	61§ (48-68)‖	33.3%	23.1%	56.4%	FPG, OGTT	42 (36–53)
Angelini et al. (53)	Italy	Prospective cohort	ANP	27 (89%)	NA	44.4%	14.8%	59.3%	OGTT	12–36‖
Bharmal et al. (71)‡	New Zealand	Cross-sectional	AP	79 (62%)	50 (41–63)	34.2%	NA	NA	FPG, HbA1c	26 (6–47)
Bharmal et al. (50)	New Zealand	Prospective cohort	AP	120 (58%)	G1: 48 ± 16 ; G2: 54 ± 16 ; G3: 53 ± 20	NA	6.6%	NA	HbA1c	24§§
Bharmal et al. (72)‡	New Zealand	Prospective cohort	AP	68 (47%)	G1: 60 ± 20 ; G2: 55 ± 18 ; G3: 48 ± 15	20.5%	NA	NA	FPG, HbA1c	24 §§
Bojková et al. (55)	Czech Republic	Retrospective cohort	AP progressing to CP in 1–2 years	56 (52%)	52**	NA	21.4%	NA	NA	12–24‖
Boreham and Ammori (61)	United Kingdom	Prospective cohort	AP	23 (57%)	55 (21–77)	NA	17.4%	NA	FPG	3§§
Burge and Gabaldon-Bates (69)	New Mexico	Retrospective cohort	AP	887 (56%)	NA	NA	11.0%	NA	Diagnostic codes	NA
Buscher et al. (57)	Netherlands	Prospective case–control	ANP	20 (75%)	52** ±3††	30.0%	25.0%	55.0%	OGTT	63** (8–136)‖
Castoldi et al. (19)	Italy	Cross-sectional	AP	631 (50%)	61 ± 19	NA	3.5%	NA	Questionnaire	52 ± 8
Chandrasekaran et al. (73)‡	India	Prospective cohort	SAP	35 (83%)	37§ ±10¶	NA	48.6%	NA	OGTT	26 ± 18
Cho et al. (42)	New Zealand	Retrospective cohort	AP with gout	9,471 (48%)	56 ± 19	NA	5.9%	NA	Diagnostic codes Medication prescription	46 ± 34
Cho et al. (64)	New Zealand	Retrospective cohort	MAP, MSAP	10,870 (49%)	56 ± 19	NA	6.5%	NA	Diagnostic codes, medication prescription	G1: 107 ± 0.4 ; G2: 95 ± 0.6
Chowdhury et al. (38)†	USA	Prospective cohort	AP	723 (50.2%)	43 ± 14	NA	4.6%	NA	HbA1c	9–63‖
Doepel et al. (56)	Finland	Prospective cohort	SAP	37 (68%)	49** (26-90)‖	10.8%	54.1%	64.9%	FPG, OGTT, and HbA1c	74** (12–168)‖
Ermolov et al. (74)‡	Russia	Prospective cohort	ANP	210 (69%)	55 ± 13	NA	29.5%	NA	FPG	102 ± 36

(Continued)

TABLE 1 (Continued)

Study identifier	Country	Study design	Population	Total No. of participants (male %)	Age * (year)	Outcome type			Outcome assessment method	Mean time to follow-up (months)*
						PD	DM	PD/DM		
Firkins et al. (43)	United States	Retrospective case–control	AP	42,818 (47%)	53** ±0.2‡‡	NA	5.9%	NA	Diagnostic code	12§§
Frey et al. (54)	United States	Retrospective cohort	AP	306 (69%)	NA	NA	24.8%	NA	Medication prescription	NA
Garip et al. (22)	Turkey	Prospective cohort	AP	109 (53%)	57 ± 16	NA	NA	34.4%	OGTT	32** (6-48)‖
Gold-Smith et al. (39)	New Zealand	Cross-sectional	AP non-iatrogenic	93 (61%)	53 (42–65)	NA	12.9%	NA	FPG, HbA1c	22 (7–46)
Guo et al. (70)	China	Retrospective cohort	AP	492 (64%)	G1: 44 (35–54) ; G2: 52 (39–63)	NA	NA	31.0%	FPG, OGTT, HbA1c, random blood glucose	3–60‖
Halonen et al. (52)	Finland	Prospective cohort	SAP	145 (83%)	44** (20-78)‖	NA	41.4%	NA	Medical records and questionnaire	66 ± 32
Hietanen et al. (63)	Finland	Prospective cohort	AP	62 (84%)	G1: 49§ (21-73)‖ ; G2: 55§ (27-80) ‖	NA	8.1%	NA	NA	31§ (17-53)‖
Ho et al. (20)	Taiwan	Retrospective cohort	AP	12,284 (71%)	NA	NA	5.0%	NA	Diagnostic codes	12–120‖
Hochman et al. (60)	Canada	Prospective cohort	SAP	25 (64%)	59** (37-86)‖	NA	32.0%	NA	Questionnaire	24–36‖
Huang et al. (75)‡	China	Prospective cohort	ANP	50 (52%)	G1: 53 ± 16 ; G2: 51 ± 15	NA	Not stated	NA	FPG, random blood glucose	3–69‖
Koziel et al. (44)	Poland	Prospective cohort	MAP, SAP	150 (63%)	G1: 52 ± 17 ; G2: 57 ± 16	NA	13.5%	NA	HbA1c	G1: 14 ± 4 ; G2: 15 ± 4
Li et al. (47)	New Zealand	Cross-sectional	AP non-iatrogenic	72 (67%)	G1: 60 (47–67) ; G2: 51 (43–59)	NA	NA	50.0%	FPG, HbA1c	27** ±2‡‡
Lv et al. (37)	China	Retrospective cohort	AP	1,804 (63%)	48 (36–62)	NA	6.1%	NA	Questionnaire	37 (21–54)
Ma et al. (45)	China	Cross-sectional	AP non-iatrogenic	616 (63%)	47 (37–63)	NA	20.0%	NA	OGTT, HbA1c	3§§
Malecka-Panas et al. (67)	Poland	Prospective cohort	Alcoholic AP with pseudocyst	50 (68%)	46 ± 14	NA	NA	26.0%	OGTT	46 ± 20
Malecka-Panas et al. (23)	Poland	Prospective cohort	AP BMI ≤25 kg/m^2	82 (67%)	47 ± 8	4.9%	15.9%	20.3%	OGTT	56 ± 43
Man et al. (41)	Romania	Prospective cohort	AP	308 (54%)	G1: 60 § (18-90)‖ G2: 45.5 § (40-65)‖	NA	2.5%	NA	FPG, OGTT	12§§

(Continued)

TABLE 1 (Continued)

Study identifier	Country	Study design	Population	Total No. of participants (male %)	Age * (year)	Outcome type			Outcome assessment method	Mean time to follow-up (months)*
						PD	DM	PD/DM		
Miko et al. (28)†	Hungary	Prospective cohort	AP	178 (NA)	NA	34.3%	15.7%	50.0%	OGTT	12§§
Nikkola et al. (29)	Finland	Prospective cohort	Alcoholic AP	77 (90%)	48§ (25-71)‖	19.1%	19.1%	38.2%	FPG, OGTT, HbA1c	126§ (37–155)‖
Nikolic et al. (76)‡	Sweden, Italy	Retrospective cohort	AP	35 (48.6%)	41 § (26-NA)‖	NA	8.6%	NA	Diagnostic codes, medical records	54§
Norbitt et al. (65)	New Zealand	Cross-sectional	AP	69 (59.4%)	NA	NA	NA	53.6%	FPG, HbA1c	60§§
Norbitt et al. (77)‡	New Zealand	Cross-sectional	AP	69 (59.4%)	NA	NA	NA	53.6%	FPG, HbA1c	NA
Patra and Das (40)	India	Retrospective cohort	AP	100 (64%)	42** (14-88)‖	NA	17.0%	NA	FPG, OGTT	60§§
Pendharkar et al. (66)	New Zealand	Cross-sectional	AP non-iatrogenic	83 (60%)	G1: 47 ± 15 ; G2: 57 ± 13	NA	NA	36.1%	FPG, HbA1c	G1: 33 ± 30 ; G2: 23 ± 19
Pendharkar et al. (33)	New Zealand	Cross-sectional	AP non-iatrogenic	83 (60%)	NA	NA	NA	36.1%	FPG, HbA1c	30**
Robertson et al. (36)	UK	Prospective cohort	AP	337 (60%)	G1: 57 (17–90) G2: 58.5 (21–84)	NA	11.2%	NA	Insulin prescription	22 § (11-33)‖
Symersky et al. (21)	Netherlands	Prospective cohort	biliary and iatrogenic AP	34 (47%)	53** ±3‡‡	NA	35.3%	NA	OGTT	55** (12-90)‖
Takeyama (24)	Japan	Retrospective cohort	MSAP, SAP	714 (NA)	NA	NA	13.0%	NA	FPG	≥ 156
Thiruvengadam et al. (78)‡	USA	Retrospective cohort	AP	118,479 (NA)	NA	NA	10.6%	NA	Diagnostic codes, medication prescription	42§
Trgo et al. (34)	Croatia	Prospective cohort	MAP, MSAP	33 (100%)	NA	NA	NA	42.4%	OGTT	1§§
Trikudanathan et al. (79)†‡	USA	Prospective cohort	ANP	390 (66%)	51 (36–64)	NA	25.8%	NA	NA	13 (3–35)
Tu et al. (30)	China	Prospective cohort	AP	113 (66%)	47** ±1‡‡	29.2%	30.1%	59.3%	OGTT, HbA1c	43 ± 4
Tu et al. (46)	China	Prospective cohort	AP	256 (66%)	44** ±1‡‡	NA	NA	60.2%	FPG, random blood glucose, OGTT	43 ± 4
Tu et al. (4)	China	Cross-sectional	AP	88 (NA)	NA	NA	25.0%	NA	FPG, OGTT, HbA1c	6–90‖
Uomo et al. (68)	Italy	Prospective cohort	ANP	40 (43%)	48 ± 18	NA	15.8%	NA	FPG, OGTT	180 ± 13
Vujasinovic et al. (35)	Slovenia	Prospective cohort	AP developing PEI	21 (81%)	57 ± 12	NA	28.6%	NA	OGTT, HbA1c	32 ± 52
Walker et al. (62)	Scotland	Prospective cohort	AP	1,748 (49%)	NA	NA	13.3%	NA	Diagnostic codes, Medication prescriptions	73 (62–84)

(Continued)

TABLE 1 (Continued)

Study identifier	Country	Study design	Population	Total No. of participants (male %)	Age * (year)	Outcome type			Outcome assessment method	Mean time to follow-up (months)*
						PD	DM	PD/DM		
Wu et al. (58)	China	Prospective cohort	AP	59 (56%)	59 ± 14	NA	NA	30.5%	FPG, HbA1c	42** (12-72)‖
Wundsam et al. (80)‡	Austria	Retrospective cohort	AP	302 (59%)	60 ± 18	NA	3.3%	NA	NA	NA
Yu et al. (31)	China	Retrospective cohort	AP	361 (56%)	49 ± 13	NA	NA	41.6%	FPG, OGTT	24 ± 24
Yuan et al. (27)	China	Retrospective cohort	AP	310 (60%)	52 (41–63)	11.0%	11.3%	22.3%	FPG	36 (22–53)
Zhang et al. (81)†‡	China	Retrospective cohort	AP	946 (NA)	NA	NA	7.0%	NA	NA	0–48‖
Zhang et al. (49)	China	Retrospective cohort	AP	820 (61.3%)	50 (38–63)	NA	8.3%	NA	Diagnostic codes	3–57‖

NA, Not available; AP, Acute pancreatitis; ANP, Acute necrotizing pancreatitis; CP, Chronic pancreatitis; SAP, Severe acute pancreatitis; MSAP, Moderately severe acute pancreatitis; MAP, Mild acute pancreatitis; DM, Diabetes mellitus; PD, Prediabetes; FPG, Fasting plasma glucose; OGTT, Oral glucose tolerance test; HbA1c, Hemoglobin A1c; G, Group; PEI, Pancreatic exocrine insufficiency; BMI, Body mass index; *Data reported as mean with standard deviation or median with interquartile range, unless otherwise specified; †Conference abstract; ‡Study not included in the meta-analyses; §Median; ¶SD; ‖Range; **Mean; ††Standard error of the mean; ‡‡Standard error; and §§Predetermined follow-up time.

associated with a significantly lower odds of developing DM [OR: 0.70; CI: 0.52–0.95; (18, 20, 24, 35–38, 41–43, 45, 50, 54, 55, 57, 60–62)] and PD/DM [OR: 0.72; CI: 0.55–0.95; (23, 27, 31, 33, 47, 57, 58)] compared to other etiologies. A statistically non-significant reducing trend could be observed for idiopathic AP and DM development [OR: 0.79; CI: 0.46–1.37; (24, 37, 45, 54, 55)].

We observed a near statistically significant increased odds of DM [OR: 1.89; CI: 0.95–3.77; Figure 6A; (4, 20, 29, 35–37, 41–43, 50)] and PD/DM [OR: 1.72; CI: 0.92–3.20; Figure 6B; (23, 27, 29, 33, 47)] recurrent acute pancreatitis (RAP) compared to a single AP episode. Subgroup analysis for follow-up length found no effect of time; however, few studies made up each subgroup. Some studies explored the effect of different numbers of AP episodes. Three or more episodes of AP were associated with a near statistically significant increased odds of DM [OR: 2.53; CI: 0.95–6.74; (4, 20, 41)] compared to having one or two AP episodes (Supplementary Figure S10).

3.3.3 Demographic factors and comorbidities

Figure 7 displays the pooled OR for the remainder of the prognostic factors that were reported on by a sufficient number of included studies in a comparable manner for quantitative synthesis (see Supplementary Figures S11–S22 for individual forest plots). We found that obesity (29, 39, 41, 43, 49, 50, 62) and chronic kidney disease (36, 38, 43) were associated with a significantly higher odds of developing DM (OR: 1.85; CI: 1.43–2.38 and OR: 2.10; CI: 1.85–2.38, respectively). We observed a statistically non-significant tendency of increased odds of developing DM with liver cirrhosis (20, 38, 63), other liver disease (37, 43, 64), dyslipidemia (20, 37, 42, 43), and being overweight or obese (37, 41, 50). We found no association between new-onset DM and hypertension (20, 36, 37, 43), cardiovascular disease (20, 36–38, 43), or age (20, 38, 43). Smoking (29, 31, 36–38, 43, 64–66), alcohol consumption (29, 31, 36, 37, 64, 67), and male sex (20, 27, 31–33, 35–38, 41, 42, 47, 50, 61, 62, 68–70) were not associated with either new-onset DM or PD/DM.

3.3.4 Additional risk factors and outcomes

There were 55 additional prognostic factors investigated by the included studies that could not be meta-analyzed due to an insufficient number of reports or heterogeneity. See Supplementary Table S5 for the qualitative analysis, which includes the 11 eligible studies that could not be meta-analyzed (71–81).

3.4 Evaluation of bias and heterogeneity

Overall, the proportion of the high risk of bias studies was notable (32–44%) for all three outcome factors (Supplementary Figures S23–S25). This was primarily due to a lack of reporting on study attrition and suboptimal definitions of outcome measurements.

High heterogeneity was noted in several of our analyses. Subgroup analysis for follow-up length significantly reduced heterogeneity only for new-onset DM in relation to alcoholic etiology. For the other prognostic factors, heterogeneity remained high even after accounting for follow-up time.

Of the 34 risk factor and outcome pairs that could be meta-analyzed, sensitivity analysis was feasible in the case of 14 analyses (Supplementary Figures S26–S34). Leave-one-out analysis identified one study (24), whose omission would make a significant difference, which we reported in paragraph 3.3.1.

Publication bias assessment was limited to six meta-analyses on new-onset DM: severe AP, moderately severe and severe AP, alcoholic and biliary etiology, recurrent AP, and male sex (Supplementary Figures S35–S40). Possible small study publication bias was detected in the case of alcoholic etiology in relation to DM development based on Egger's test and visual inspection of the funnel plot.

4 Discussion

This is the first systematic review and meta-analysis of risk factors for developing new-onset PD and DM after AP that pooled direct,

Exposure	Control	Outcome	Studies	Total (N)	OR	95% CI
SAP+MSAP	MAP	PD/DM	8	1,211	4.32	1.76-10.60
SAP	MAP+MSAP	PD/DM	8	1,261	3.13	1.60-6.11
SAP+MSAP	MAP	DM	9	3,631	2.11	1.30-3.41
SAP	MAP+MSAP	DM	13	69,349	1.86	1.27-2.73
HTG etiology	non-HTG	PD/DM	3	730	3.27	0.17-63.91
alcoholic etiology	non-alcoholic	DM	17	60,083	1.82	1.09-3.04
alcoholic etiology	non-alcoholic	PD/DM	8	1,014	1.33	0.77-2.31
idiopathic etiology	known etiology	DM	5	3,469	0.79	0.46-1.37
biliary etiology	non-biliary	PD/DM	7	987	0.72	0.55-0.95
biliary etiology	non-biliary	DM	18	71,406	0.70	0.52-0.95
necrosis present	absent	PD/DM	5	871	5.53	1.59-19.21
necrosis present	absent	DM	7	2,299	3.09	0.98-9.72
necrosis >30%	<30% necrosis	PD/DM	3	384	5.44	0.19-157.71
necrosis >50%	<50% necrosis	DM	3	827	4.12	1.83-9.30
organ failure	no organ failure	DM	4	1,188	3.19	0.55-18.64
organ failure	no organ failure	PD/DM	3	703	2.14	0.51-9.06
RAP	single AP	DM	10	67,319	1.89	0.95-3.77
RAP	single AP	PD/DM	5	594	1.72	0.92-3.20
≥ 3 episodes of AP	1 or 2 episodes of AP	DM	3	12,680	2.53	0.95-6.74
liver cirrhosis	no liver cirrhosis	DM	3	13,069	2.48	0.18-34.25
CKD	no CKD	DM	3	43,878	2.10	1.85-2.38
obese	non-obese	DM	7	46,780	1.85	1.43-2.38
dyslipidemia	no dyslipidemia	DM	4	66,355	1.82	0.68-4.84
liver disease*	no liver disease	DM	3	55,492	1.73	0.32-9.46
overweight & obese	normal & underweight	DM	3	1,914	1.69	0.91-3.16
male	female	DM	12	27,766	1.41	0.92-2.14
hypertension	no hypertension	DM	4	60,295	1.38	0.91-2.08
male	female	PD/DM	6	1,404	1.29	0.91-1.83
smoking	non-smoking	PD/DM	4	560	1.27	0.64-2.51
smoking	non-smoking	DM	6	56,620	1.26	0.74-2.13
alcohol consumption	not drinking	DM	4	13,079	1.16	0.63-2.12
alcohol consumption	not drinking	PD/DM	3	456	1.13	0.02-65.68
age >65 years	age <65 years	DM	3	55,825	0.90	0.51-1.60
CVD	no CVD	DM	5	57,966	0.90	0.50-1.61

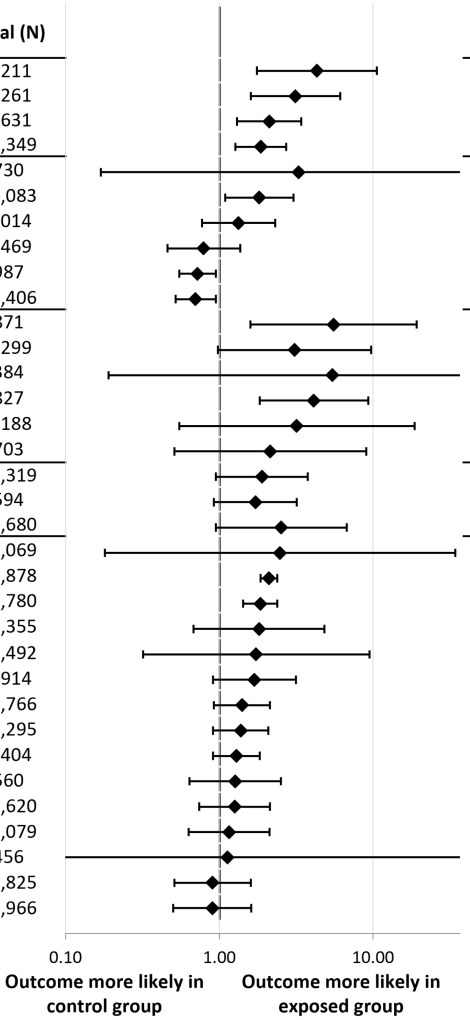


FIGURE 2
Aggregated forest plot summarizing our results for the 34 meta-analyses. Each row shows the pooled odds ratio for a risk factor and outcome pair. An odds ratio over 1.0 indicates that the given outcome (diabetes or PD/DM) is more likely to occur in the exposed group compared to the control group. Statistical significance is achieved if the line of null effect does not fall into the confidence interval. Black squares represent the pooled odds ratios and the lines represent the confidence intervals. PD, Prediabetes; DM, Diabetes mellitus; OR, Odds ratio; CI, Confidence interval; AP, Acute pancreatitis; SAP, Severe acute pancreatitis; MSAP, Moderately severe acute pancreatitis; MAP, Mild acute pancreatitis; HTG, Hypertriglyceridemic; RAP, Recurrent acute pancreatitis; CKD, Chronic kidney disease; and CVD, Cardiovascular disease. *Liver disease other than liver cirrhosis.

within-study comparisons. We found that severe AP, moderately severe AP, and necrosis are associated with a greater risk of developing DM and PD/DM. We also observed a significant association with alcoholic etiology, obesity, chronic kidney disease, and new-onset DM, whereas biliary etiology was associated with a lower risk of developing DM and PD/DM compared to other etiologies. Additionally, we observed a tendency for increased risk of developing DM or PD/DM with hypertriglyceridemic AP, organ failure, RAP, and comorbidities of liver disease or dyslipidemia.

4.1 Severity and local complications

Past meta-analyses applying indirect comparisons found conflicting results regarding the association of AP severity and new-onset PD or DM (7, 8). Our analysis of direct, within-study comparisons confirms a positive relationship between SAP, moderately severe AP, and new-onset DM. Classification of AP severity is based on the development of local complications (such as necrosis) and organ failure (1). Beta cell death secondary to local complications of AP is believed to be one of the possible mechanisms behind the ensuing DM (82). Our meta-analysis supports this hypothesis as necrosis was associated with significantly greater risk of developing DM and PD/DM. Moreover, patients with local complications might require interventions such as pancreatic debridement, lavage, drainage, necrosectomy, and partial pancreatectomy, during which further pancreatic tissue is lost (83).

Nevertheless, cell death is only one aspect of the complex pathomechanism of post-AP DM. It was proposed that the inflammation accompanying AP stimulates endogenous beta-cell proteins to undergo post-translational modifications (84). Such modified proteins could trigger autoimmune processes as seen in type 1 diabetes (85), which could explain the earlier and greater need for insulin therapy seen with post-pancreatitis DM compared to type 2 DM (12). The level of inflammatory

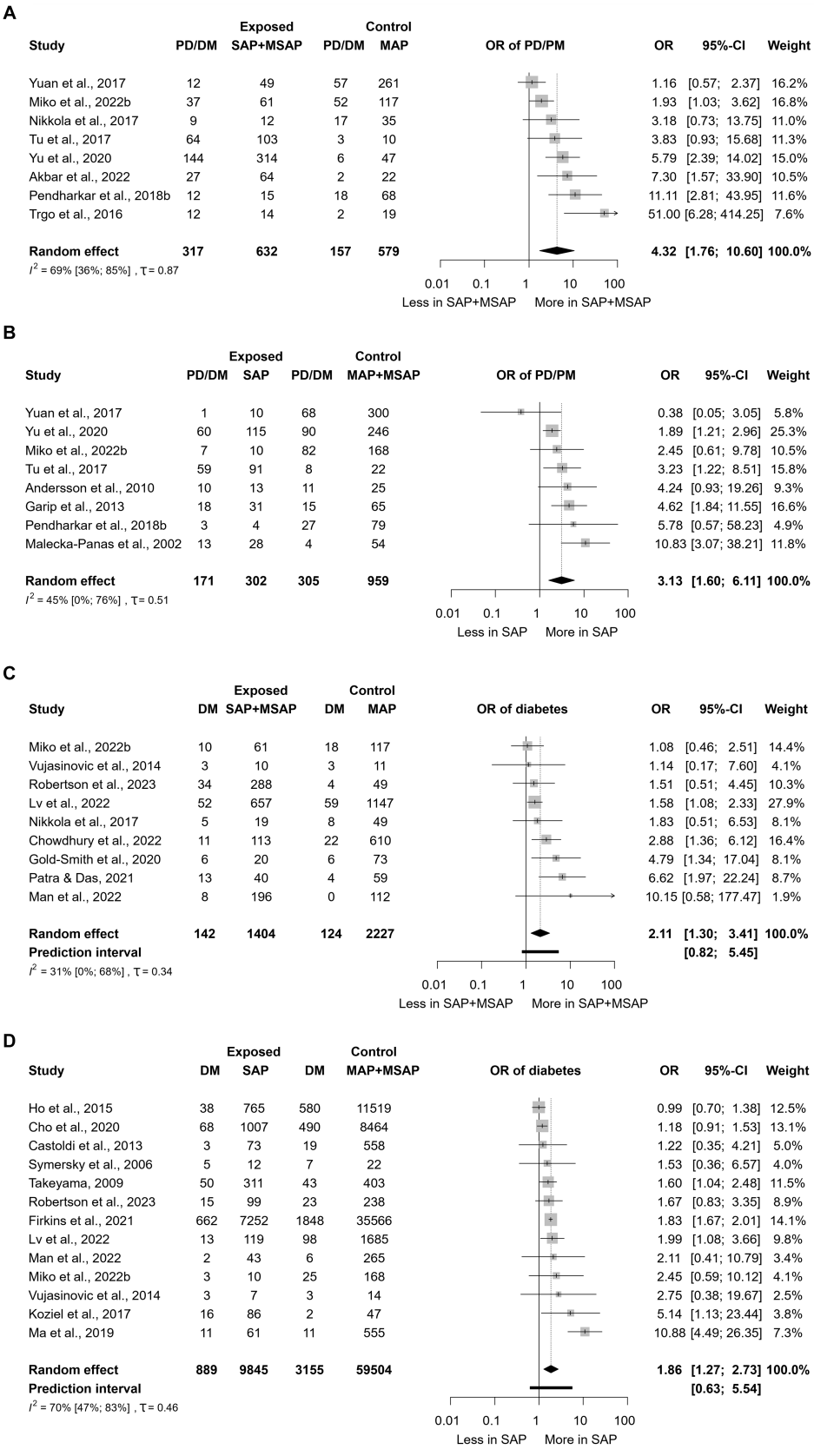


FIGURE 3
The association between severity grades of acute pancreatitis (AP) and subsequent development of prediabetes and diabetes. **(A)** Severe or moderately severe AP vs. mild AP in relation to new-onset prediabetes and diabetes. **(B)** Severe AP vs. mild or moderately severe AP in relation to new-onset prediabetes and diabetes. **(C)** Severe or moderately severe AP vs. mild AP and new-onset diabetes. **(D)** Severe AP vs. mild or moderately severe AP and new-onset diabetes. AP, Acute pancreatitis; OR, Odds ratio; CI, Confidence interval; PD, Prediabetes; DM, Diabetes mellitus; SAP, Severe acute pancreatitis; MSAP, Moderately severe acute pancreatitis; MAP, Mild acute pancreatitis; and vs., versus.

Exposure	Control	Outcome	Studies	Exposed (n/N)	Control (n/N)	Total (N)	OR	95% CI	I2
necrosis	no necrosis	PD/DM	5	265/396	137/475	871	5.53	(1.59-19.21)	91% (81-95%)
necrosis	no necrosis	DM	7	103/597	135/1,702	2,299	3.09	(0.98-9.72)	74% (45-88%)
necrosis >30%	<30% necrosis	PD/DM	3	209/274	40/110	384	5.44	(0.19-157.71)	87% (64-96%)
necrosis >50%	<50% necrosis	DM	3	77/220	37/607	827	4.12	(1.83-9.30)	0% (0-90%)
organ failure	No organ failure	DM	4	53/202	83/986	1,188	3.19	(0.55-18.64)	85% (62-94%)
organ failure	No organ failure	PD/DM	3	155/253	178/450	703	2.14	(0.51-9.06)	74% (14-92%)

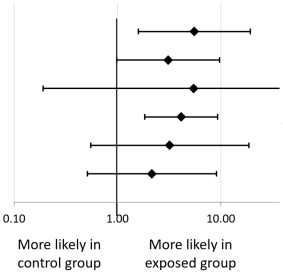


FIGURE 4
Aggregated forest plot showing the pooled odds ratios for various complications of acute pancreatitis and subsequent diabetes and prediabetes development. PD, Prediabetes; DM, Diabetes mellitus; OR, Odds ratio; and CI, Confidence interval.

cytokines correlate well with persistent organ failure, which is the hallmark of SAP (86). Our study found an increase in the odds of developing DM and PD/DM after AP with organ failure, albeit not-statistically significant. It is noteworthy that none of the included studies specified the duration of the organ failure, and few mentioned the affected organs or the number of organs affected.

We found a more pronounced association with severity in the case of PD/DM than with DM suggesting an even more substantial influence of AP severity on the development of PD. Moreover, comparing severe and moderate AP as one group vs. mild yielded a higher odds ratio than the comparison of SAP to moderate and mild AP as one group. This could imply that progresses from mild to moderate AP severity has a greater impact on PD and DM development compared to the step from moderate to severe disease progression.

4.2 Etiology

We found that alcoholic AP was associated with an increased risk of developing DM. Alcohol has a toxic effect on the pancreas. Its metabolites elicit sustained intracellular calcium overload, which disrupts beta-cell functioning and insulin secretion while also leading to oxidative stress (87), to which beta-cells are especially vulnerable due to their low antioxidation capacity (88).

The most likely explanation for the tendency seen with hypertriglyceridemic etiology is that hypertriglyceridemia itself is associated with DM (89). The two conditions often coexist in metabolic syndrome. Analysis of the Hungarian Study Group's registry data shows that 69% of the non-diabetic hypertriglyceridemic AP patients present with at least two factors of the metabolic syndrome on admission and they are at an increased risk of developing post-AP DM (90). Therefore, the development of DM might be a natural progression of the disease, possibly quickened by the AP episode.

Acute pancreatitis tends to be more severe if caused by excessive alcohol consumption or hypertriglyceridemia (91) and if metabolic syndrome is present (90). Toxic factors (e.g., alcohol and fatty acids) play a role in the development and severity of pancreatitis when they accumulate (92). This aligns with the multiple hits theory of AP severity documented for smoking, drinking (93), obesity, hypertension, and hyperlipidemia (90). The risk factors we identified—local complications, severity, alcoholic, and hypertriglyceridemic AP—often coexist (91, 94, 95). Suggesting that the development of post-AP DM might work on a similar multiple hits theory basis.

Both alcoholic and hypertriglyceridemic etiologies are linked to poor dietary habits that are difficult to change and the ongoing exposure conveys a high risk for RAP, progression of the disease, and development of complications (96). On the contrary, the recurrence of biliary AP is often prevented by cholecystectomy after the index episode (64). Without identifying a treatable or preventable etiology, there is a risk for RAP. However, we found no association between idiopathic AP and DM development, possibly due to the control group—containing alcoholic and hypertriglyceridemic etiology—demonstrating a positive association with DM.

4.3 Recurrence

Recurrent acute pancreatitis conveys repeated pancreatic inflammation and cellular insult or loss, leading to an assumed association with developing pancreatic endocrine dysfunction (96). Our analysis found a tendency of increased odds for new-onset DM and PD/DM with RAP, which neared statistical significance. It should be pointed out that there was considerable heterogeneity in the study designs. Some studies excluded patients presenting with RAP at the index AP episode while others included them. Importantly, those who had RAP and developed DM by the index AP episode were excluded from the analysis based on the premise of pre-existing DM. Moreover, 60% of the analyzed studies had a relatively short follow-up of less than 3 years. Finally, different distributions of the etiological factors among the included studies might influence the observed association between RAP and DM or PD/DM, as alcoholic and hypertriglyceridemic APs are associated with a greater risk of RAP (91). All four factors could influence the true relationship between disease recurrence and PD/DM development.

4.4 Other factors

Our study found that obesity was associated with a significantly greater risk of new-onset DM. Some of the other risk factors we identified for new-onset DM after AP (hypertriglyceridemic AP, AP-related complications, and SAP) tend to occur more frequently in obese individuals (90, 97). Moreover, excess weight is a known independent risk factor for type 2 DM. Therefore, it could be a natural progression of the disease or AP might even trigger DM in genetically or metabolically predisposed patients (7). At present, there is still a

Exposure	Outcome	Studies	Exposed (n/N)	Control (n/N)	Total (N)	OR	95% CI	I2
HTG	PD/DM	3	81/169	156/561	730	3.27	(0.17-63.91)	74% (15-92%)
Alcoholic	DM	17	1,267/19,755	2,424/40,328	60,083	1.82	(1.09-3.04)	88% (83-92%)
Alcoholic	PD/DM	8	69/166	278/848	1,014	1.33	(0.77-2.31)	20% (0-62%)
Idiopathic	DM	5	86/942	311/2,527	3,469	0.79	(0.46-1.37)	58% (0-84%)
Biliary	PD/DM	7	137/463	194/524	987	0.72	(0.55-0.95)	0% (0-71%)
Biliary	DM	18	1,034/19,613	3,418/51,793	71,406	0.70	(0.52-0.95)	75% (61-84%)

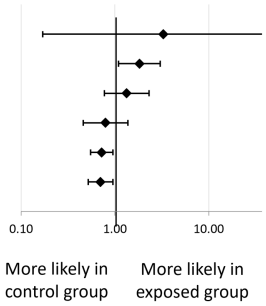
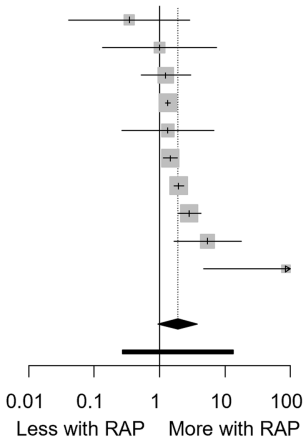


FIGURE 5
Aggregated forest plot showing the pooled odds ratios for different etiologies of acute pancreatitis and new-onset diabetes alone or in combination with prediabetes. Etiologies listed in the exposure column are compared to all other etiologies to provide an odds ratio for the outcome of interest. PD, Prediabetes; DM, Diabetes mellitus; HTG, Hypertriglyceridemic; OR, Odds ratio; and CI, Confidence interval.

A

Study	Exposed DM	Exposed RAP	Control DM	Control single AP	OR	95%-CI	Weight
Bharmal et al., 2022a	1	34	7	86	0.34	[0.04; 2.89]	5.2%
Vujasinovic et al., 2014	2	7	4	14	1.00	[0.13; 7.45]	5.6%
Robertson et al., 2023	7	53	31	284	1.24	[0.52; 2.99]	11.3%
Firkins et al., 2021	863	12257	1647	30561	1.33	[1.22; 1.45]	14.9%
Man et al., 2022	2	62	6	246	1.33	[0.26; 6.77]	7.1%
Cho et al., 2020	82	1027	476	8444	1.45	[1.14; 1.85]	14.6%
Ho et al., 2015	262	3463	356	8821	1.95	[1.65; 2.29]	14.8%
Lv et al., 2022	40	321	71	1483	2.83	[1.88; 4.26]	14.0%
Tu et al., 2023	18	48	4	40	5.40	[1.65; 17.69]	9.4%
Nikkola et al., 2017	13	26	0	42	85.00	[4.73; 1526.87]	3.3%
Random effect	**1290**	**17298**	**2602**	**50021**	**1.89**	**[0.95; 3.77]**	**100.0%**
Prediction interval						[0.27; 13.36]	

I^2 = 78% [60%; 88%] τ = 0.79



B

Study	Exposed PD/DM	Exposed RAP	Control PD/DM	Control single AP	OR	95%-CI	Weight
Yuan et al., 2017	12	44	57	266	1.38	[0.67; 2.84]	37.0%
Malecka-Panas et al., 2002	10	42	7	40	1.47	[0.50; 4.34]	16.6%
Pendharkar et al., 2018b	10	23	20	60	1.54	[0.58; 4.11]	20.1%
Li et al. 2021	14	23	22	49	1.91	[0.70; 5.24]	19.1%
Nikkola et al., 2017	12	14	14	33	8.14	[1.57; 42.33]	7.2%
Random effect	**58**	**146**	**120**	**448**	**1.72**	**[0.92; 3.20]**	**100.0%**

I^2 = 0% [0%; 79%] τ = 0

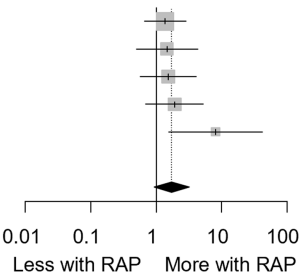
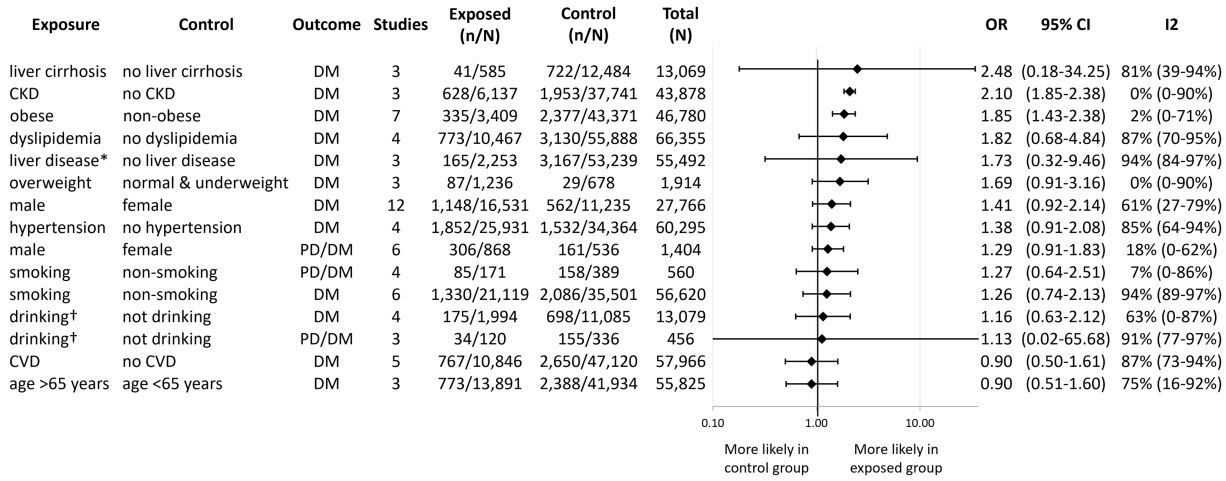


FIGURE 6
The association between recurrent acute pancreatitis and subsequent development of diabetes **(A)** and prediabetes or diabetes **(B)**. PD, Prediabetes; DM, Diabetes mellitus; OR, Odds ratio; CI, Confidence interval; AP, Acute pancreatitis; and RAP, Recurrent acute pancreatitis.

lack of consensus on differentiating type 2 DM from post-AP DM in patients who had an AP episode (7, 98). Most studies define post-AP DM as new-onset of hyperglycemia (using the standard cutoff values for DM as per the World Health Organization or American Diabetes Association recommendations) following an AP episode (12, 13, 99). The prospective, multi-center DREAM study (Diabetes RElated to



Exposure	Control	Outcome	Studies	Exposed (n/N)	Control (n/N)	Total (N)	OR	95% CI	I2
liver cirrhosis	no liver cirrhosis	DM	3	41/585	722/12,484	13,069	2.48	(0.18-34.25)	81% (39-94%)
CKD	no CKD	DM	3	628/6,137	1,953/37,741	43,878	2.10	(1.85-2.38)	0% (0-90%)
obese	non-obese	DM	7	335/3,409	2,377/43,371	46,780	1.85	(1.43-2.38)	2% (0-71%)
dyslipidemia	no dyslipidemia	DM	4	773/10,467	3,130/55,888	66,355	1.82	(0.68-4.84)	87% (70-95%)
liver disease*	no liver disease	DM	3	165/2,253	3,167/53,239	55,492	1.73	(0.32-9.46)	94% (84-97%)
overweight	normal & underweight	DM	3	87/1,236	29/678	1,914	1.69	(0.91-3.16)	0% (0-90%)
male	female	DM	12	1,148/16,531	562/11,235	27,766	1.41	(0.92-2.14)	61% (27-79%)
hypertension	no hypertension	DM	4	1,852/25,931	1,532/34,364	60,295	1.38	(0.91-2.08)	85% (64-94%)
male	female	PD/DM	6	306/868	161/536	1,404	1.29	(0.91-1.83)	18% (0-62%)
smoking	non-smoking	PD/DM	4	85/171	158/389	560	1.27	(0.64-2.51)	7% (0-86%)
smoking	non-smoking	DM	6	1,330/21,119	2,086/35,501	56,620	1.26	(0.74-2.13)	94% (89-97%)
drinking†	not drinking	DM	4	175/1,994	698/11,085	13,079	1.16	(0.63-2.12)	63% (0-87%)
drinking†	not drinking	PD/DM	3	34/120	155/336	456	1.13	(0.02-65.68)	91% (77-97%)
CVD	no CVD	DM	5	767/10,846	2,650/47,120	57,966	0.90	(0.50-1.61)	87% (73-94%)
age >65 years	age <65 years	DM	3	773/13,891	2,388/41,934	55,825	0.90	(0.51-1.60)	75% (16-92%)

FIGURE 7
Aggregated forest plot showing the pooled odds ratios for various comorbidities, demographic factors, and new-onset diabetes alone or in combination with prediabetes. PD, Prediabetes; DM, Diabetes mellitus; OR, Odds ratio; CI, Confidence interval; CKD, Chronic kidney disease; and CVD, Cardiovascular disease. *Liver diseases other than liver cirrhosis. †Drinking refers to alcohol consumption.

Acute Pancreatitis and Its Mechanisms) was recently designed to characterize the DM phenotypes after AP and their pathomechanism (100, 101).

We found no association between sex, smoking, alcohol consumption, and DM or PD/DM. The lack of association with alcohol consumption is paradoxical in light of the increased risk of DM with AP of alcoholic etiology. However, the included studies mostly compared alcohol consumption to not-drinking, not taking into account the amount and duration of alcohol consumption. Studies with follow-up length over 4 years were more likely to favor an association between alcohol consumption and new-onset DM or PD/DM (29, 64, 67) compared to shorter studies (31, 36, 37). Also, the analysis included only four and three studies for DM and PD/DM, respectively, with two of the studies containing unusually low proportions of alcoholic etiology (4 and 14%) and three studies including only patients with AP of alcoholic etiology.

Additionally, we observed a clinically relevant odds ratio for post-AP DM with liver disease and dyslipidemia. We believe that statistical significance was not achieved due to the low number of studies investigating these risk factors and their heterogeneous nature. In our analysis, chronic kidney disease was associated with a significantly higher risk of post-AP DM. It is notable that the analysis was based on three studies, of which Firkins et al. (43) accounted for 99.4% of the pooled results due to the large sample size. This is a retrospective nationwide database analysis, where only patients with a second hospital admission within one calendar year were included. Patients with chronic kidney disease are admitted more frequently to hospitals (102); thus, they were likely over-represented in the study by Firkins et al. (43).

4.5 Follow-up after AP

Timely translation of scientific data to clinical practice has crucial importance in healthcare (103, 104). Long-term complications of AP (exocrine and endocrine insufficiency) were documented as early as 1941 (105); nonetheless, the Chinese guideline in 2021 was the first to recommend follow-up visits after AP (59). They recommend that all AP patients should be monitored after rehabilitation, however, for different lengths of time depending on severity. They rated the strength of recommendation and supporting evidence weak.

While all AP patients should be followed up for the development of long-term complications after AP, financial and human resources are often limited in healthcare. Our study highlights the sub-populations of AP patients who are at a higher risk for developing PD or DM. Therefore, more frequent follow-ups of these patients increase the likelihood of preventing and reducing post-AP diabetes-related morbidity, mortality, and healthcare costs.

4.6 Strengths and limitations

Due to the broad search strategy and lack of constraints on the results, this is the first comprehensive systematic analysis of potential risk factors for new-onset PD/DM following AP, with the largest number of included studies (50 in total) covering 76,797 participants in the meta-analysis. Our study was based on direct, within-study comparisons; therefore, it is more representative of the true effect of risk factors compared to previous meta-analyses (7, 8). Due to the inclusive nature of our research, there was substantial heterogeneity between the studies, which we attempted to reduce by performing separate analyses for PD/DM and DM and conducting subgroup analysis for follow-up length. Almost a third of the meta-analyses were based on three studies. For these risk factor and outcome pairs, conclusions should be cautiously handled.

4.7 Implication for practice

All patients require medical follow-up for endocrine and exocrine insufficiency after AP. Our results show that patients who have suffered severe or moderately severe AP, alcoholic or hypertriglyceridemic AP, develop pancreatic necrosis or organ failure,

had multiple AP episodes, are obese or have pre-existing chronic kidney disease, liver disease or dyslipidemia are at a greater risk for developing PD or DM. Therefore, closer monitoring is warranted in these high-risk groups.

4.8 Implication for research

Further long-term follow-up studies of AP patients are needed to observe morbidity and mortality following single and multiple AP episodes as well. High-quality well-controlled observational studies with long follow-up duration are needed to establish an evidence-based follow-up schedule after AP to help identify patients early in a prediabetic state, where interventions could still prevent DM. Future studies should also explore interventions for preventing post-pancreatitis DM. In 2022 the Hungarian Pancreatic Study Group launched two longitudinal randomized controlled trials on dietary intervention (106) and smoking and alcohol cessation following hospitalization for AP (107).

4.9 Conclusion

We found that AP severity, alcoholic and hypertriglyceridemic etiologies, pancreatic necrosis, organ failure, RAP and comorbidities of obesity, chronic kidney disease, liver disease, and dyslipidemia are associated with a higher risk of developing PD or DM following AP. Glucose homeostasis should be regularly monitored in high-risk populations after hospital discharge. Further research is needed to establish an appropriate follow-up schedule and interventions for preventing DM after AP.

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

OZ: Conceptualization, Writing – review & editing, Data curation, Investigation, Methodology, Project administration, Visualization, Writing – original draft. SB: Conceptualization, Methodology, Project administration, Visualization, Writing – review & editing. AK: Investigation, Writing – review & editing. DD: Investigation, Writing – review & editing, Visualization. LH: Visualization, Investigation, Writing – review & editing. BB: Investigation, Writing - review & editing. DV: Data curation, Visualization, Conceptualization, Writing – review & editing, Formal analysis, Methodology. NH: Conceptualization, Writing – review & editing, Methodology. BE: Writing – review & editing, Conceptualization, Methodology. BT: Writing – review & editing, Methodology, Project administration, Visualization. MJ: Writing – review & editing, Conceptualization, Methodology, Supervision. PH: Conceptualization, Funding acquisition, Supervision, Writing – review & editing, Methodology.

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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 author(s) declared that they were an editorial board member of Frontiers, at the time of submission. This had no impact on the peer review process and the final decision.

Publisher's note

Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2023.1257222/full#supplementary-material

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Glossary

ANP	Acute necrotizing pancreatitis
AP	acute pancreatitis
BMI	Body mass index
CENTRAL	Cochrane central register of controlled trials
CI	95% Confidence interval
CKD	Chronic kidney disease
CP	Chronic pancreatitis
CVD	Cardiovascular disease
DM	Diabetes mellitus
DREAM	Diabetes RElated to Acute Pancreatitis and Its Mechanisms
FPG	Fasting plasma glucose
G	Group
HbA1c	Hemoglobin A1c
HTG	Hypertriglyceridemic
MAP	Mild acute pancreatitis
MSAP	Moderately severe acute pancreatitis
NA	Not available
OGTT	Oral glucose tolerance test
OR	Odds ratio
PD	Prediabetes
PECOTS	Population, exposure, comparator, outcome, timing, study design
PEI	Pancreatic exocrine insufficiency
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-Analyses
PROSPERO	International Prospective Register of Systematic Reviews
QUIPS	Quality In Prognosis Studies
RAP	Recurrent acute pancreatitis
SAP	Severe acute pancreatitis

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EDITED BY
Ravi Kumar Sharma,
Chandigarh University, India

REVIEWED BY
Vikas Kumar,
Chandigarh University, India
Gaurav Sharma,
Central Drug Research Institute (CSIR), India

*CORRESPONDENCE
Zhi Li
lizhi-swmu@126.com
Wen-Fu Tang
tangwf@scu.edu.cn

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

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Material basis and molecular mechanisms of Chaihuang Qingyi Huoxue Granule in the treatment of acute pancreatitis based on network pharmacology and molecular docking-based strategy

Jia Yang[1†], Yu-Hong Jiang[2†], Xin Zhou[3,4], Jia-Qi Yao[2], Yang-Yang Wang[1], Jian-Qin Liu[3,4], Peng-Cheng Zhang[2], Wen-Fu Tang[2*] and Zhi Li[3,4*]

[1]School of Integrated Traditional Chinese and Western Medicine, Southwest Medical University, Luzhou, Sichuan, China, [2]Department of Integrated Traditional Chinese and Western Medicine, National Clinical Research Center for Geriatrics, West China Hospital, Sichuan University, Chengdu, China, [3]Department of Spleen and Stomach Diseases, Chinese Medicine Hospital Affiliated to Southwest Medical University, Luzhou, Sichuan, China, [4]The Key Laboratory of Integrated Traditional Chinese and Western Medicine for Prevention and Treatment of Digestive System Diseases of Luzhou city, Affiliated Traditional Medicine Hospital of Southwest Medical University, Luzhou, China

Objectives: This study aimed to analyze active compounds and signaling pathways of CH applying network pharmacology methods, and to additionally verify the molecular mechanism of CH in treating AP.

Materials and methods: Network pharmacology and molecular docking were firstly used to identify the active components of CH and its potential targets in the treatment of AP. The pancreaticobiliary duct was retrogradely injected with sodium taurocholate (3.5%) to create an acute pancreatitis (AP) model in rats. Histological examination, enzyme-linked immunosorbent assay, Western blot and TUNEL staining were used to determine the pathway and mechanism of action of CH in AP.

Results: Network pharmacological analysis identified 168 active compounds and 276 target proteins. In addition, there were 2060 targets associated with AP, and CH had 177 targets in common with AP. These shared targets, including STAT3, IL6, MYC, CDKN1A, AKT1, MAPK1, MAPK3, MAPK14, HSP90AA1, HIF1A, ESR1, TP53, FOS, and RELA, were recognized as core targets. Furthermore, we filtered out 5252 entries from the Gene Ontology(GO) and 186 signaling pathways from the Kyoto Encyclopedia of Genes and Genomes(KEGG). Enrichment and network analyses of protein-protein interactions predicted that CH significantly affected the PI3K/AKT signaling pathway, which played a critical role in programmed cell death. The core components and key targets showed strong binding activity based on molecular docking results. Subsequently, experimental validation demonstrated that CH inhibited the phosphorylation of PI3K and AKT in

pancreatic tissues, promoted the apoptosis of pancreatic acinar cells, and further alleviated inflammation and histopathological damage to the pancreas in AP rats.

Conclusion: Apoptosis of pancreatic acinar cells can be enhanced and the inflammatory response can be reduced through the modulation of the PI3K/AKT signaling pathway, resulting in the amelioration of pancreatic disease.

KEYWORDS

acute pancreatitis, Chaihuang Qingyi Huoxue Granule, network pharmacology, molecular docking, pancreatic acinar cells, Traditional Chinese

1 Introduction

Acute pancreatitis (AP) is a digestive disorder that has a widespread occurrence and can be extremely serious, even leading to death. The condition is distinguished by abrupt abdominal ache and increased levels of pancreatic enzymes in the blood (1). Despite the progress made in comprehending the development of AP and enhancing its medical treatment, the occurrence and fatality rates of AP persistently rise (2, 3). Currently, Western medical treatments for AP mainly focus on acid suppression, inhibition of pancreatic enzyme secretion, antispasmodics, and pain relief. Further studies are required to explore multiple methods of treating AP. Several research studies have shown the effectiveness of traditional Chinese medicine (TCM) for treating AP. TCM is a cost-effective option with minimal side effects (4, 5), deserving further study.

The Department of Gastroenterology at the Affiliated Traditional Chinese Medicine Hospital of Southwest Medical University utilizes an in-house remedy called Chaihuang Qingyi Huoxue Granule (CH) to effectively treat cases of severe acute pancreatitis (6). It contains 14 Chinese herbs, Chaihu (Bupleurum), Houpu (Magnolia Bark), Chishao (Red Peony Root), Dahuang (Rhubarb), Taoren (Peach Kernel), Danshen (Salvia miltiorrhiza), Gancao (Licorice Root), Yanhusuo (Corydalis Yanhusuo), Huangqi (Astragalus Root), Huangqin (Scutellaria Baicalensis Root), Zhishi (Immature Bitter Orange), Zhizi (Gardenia Fruit), Baishao (White Peony Root), and Pugongying (Dandelion). For numerous years, CH has been utilized in clinical settings to treat AP. Evidence (7, 8) has shown that CH can regulate gastrointestinal motility and inhibit the production and release of pro-inflammatory factors, thereby alleviating AP. However, the precise chemical composition and exact mechanisms of action have yet to be fully elucidated.

Network pharmacology and molecular docking were effective methods that utilize advanced computer simulations to identify components and predict drug targets. In this study, the active ingredients of CH were identified through data mining, targeting identification, and the establishment of a comprehensive network. Additionally, enrichment analysis using GO and KEGG revealed the involvement of the PI3K/Akt signaling pathway, thereby predicting the potential mechanism of CH in treating AP. Subsequently, a series of animal experiments, including HE staining, WB, ELISA, TUNEL, etc., were conducted to validate that CH could inhibit the activation of the PI3K/Akt signaling pathway, induce apoptosis of pancreatic acinar cells, reduce inflammatory reactions, and mitigate damage in AP model rats. This suggests that network pharmacology can be an effective approach to elucidate the mechanism of action of CH in treating AP. The research route is illustrated in Figure 1.

2 Materials and methods

2.1 Network pharmacology predictions

2.1.1 Data mining for chemical ingredients

Information regarding the specific plants in CH was extracted from the Traditional Chinese Medicine System Pharmacology Database (TCMSP) (9). The molecular structures of every compound were obtained from the NCBI PubChem database (10) and confirmed through thorough research in literature. It is crucial to evaluate the absorption, distribution, metabolism, and excretion characteristics of every compound in order to identify the active components of CH and examine their functional foundation. For this investigation, we opted for oral absorption (OA) and drug similarity (DS). For the purpose of further analysis, molecules with OB $\geq$ 30% were chosen in this particular section (11). In TCM research, the DL threshold of 0.18, which is based on the average value of all compounds in the DrugBank database (12), is widely used as a screening standard for identifying 'drug-like' compounds.

2.1.2 Compounds target fishing

In this process, the active ingredients filtered out early were used as bait to identify the corresponding targets in the TCMSP and DrugBank databases. Afterwards, the UniProt KB (13) was used to map all stationary objectives and acquire their official gene symbols.

2.1.3 Disease targets database building

The Gene Cards database (14), OMIM database (15), PharmGkb database (16), TTD (17, 18), and DrugBank database were used to screen AP-related targets. We combined the targets

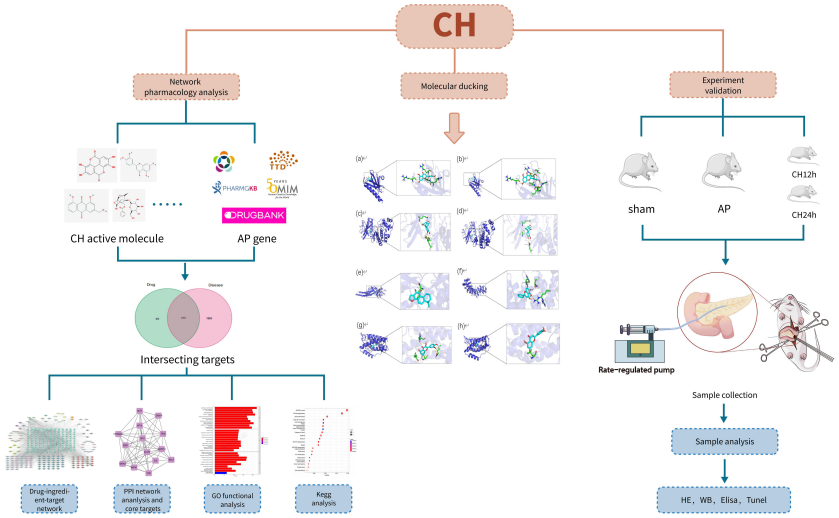

FIGURE 1
All technical approaches rely on network pharmacology and experimental verification.

from these five databases, removed duplicate targets, and identified the disease targets related to AP. In this section, the search was conducted using the keywords "acute pancreatitis" and selection of species condition for "homo sapiens" as a supplement. Subsequently, the retrieved targets were mapped to UniProt KB to obtain the corresponding official gene symbols.

In the end, we compared the projected goals of CH active components with the targets related to AP and chose the common targets as potential therapeutic targets for CH. A Venn diagram of the targets was plotted and visualized using FunRich software in the CH-AP network. Subsequently, the common targets were processed for additional analysis.

2.1.4 Enrichment analysis of gene ontology and KEGG pathway

In order to delve deeper into the possible molecular mechanism of CH, we conducted GO enrichment analysis and KEGG signaling pathway analysis. This involved connecting the chosen possible targets to the Database Visualization and Integrated Discovery system (DAVID) Bioinformatics Resources 6.8 (19). Only the terms with $P < 0.05$ significance level were taken into account in this particular section. The overlapping targets were plotted using R language, and the GO results were represented using a bar chart, while the KEGG analysis results were represented using a bubble chart.

2.1.5 Network construction and analysis

Drug-compound-target (D-C-T) network of CH was created by establishing connections between active compounds and their potential targets using Cytoscape v3.9.1 software (20), a robust tool frequently employed in bioinformatics research for data integration and visualization. Nodes in graphical networks depict compounds and targets, while edges represent depict interactions between the compound and target. Moreover, 'degree', a crucial topological parameter in network pharmacology, was examined using the CytoNCA plugin of Cytoscape. The degree of a node indicates the total number of other nodes that interact with it.

Construction of a network for the interaction between proteins (PPI) was carried out to identify potential therapeutic targets of the active components in CH. The STRING database (21) was utilized for this purpose. The settings for network analysis consisted of choosing the mode of analysis as 'Multiple proteins', selecting 'Homo sapiens' as the species, and setting a confidence score of at least 0.95. Isolated proteins were excluded, and the results were saved as a TSV file. Afterwards, we employed the Cytoscape 3.9.1 application to exhibit the protein-protein interaction (PPI) network. To pinpoint the main targets, we carried out network topology analysis through the "Network Analyzer" module in Cytoscape. Different parameters, including Betweenness (BC), Closeness (CC), Degree (DC), Eigenvector (EC), local average connectivity-based methods (LAC), Network (NC), and Information (IC), were used to establish the main objectives. During this process, the core targets were identified by assessing the degree of each node in the network.

After conducting the KEGG pathway analysis on the potential targets identified in the DAVID database, the Compound-Target-Pathway (C-T-P) network was built in Cytoscape 3.9.1. This network connected the top 30 KEGG signaling pathways with their respective targets. Nodes in graphical networks represent compounds, targets, and signaling pathways, while edges represent C-T-P interactions. A node's significance in the network is indicated by a higher degree value.

2.1.6 Molecular docking verification of core components and targets

The network known as the 'Compound-Target-Pathway (C-T-P)' was employed to discover compounds and their possible targets. These targets were subsequently analyzed through molecular docking investigations with AutoDock Vina (22). Molecular

docking involves several steps. Initially, the PDB database (23) was utilized to acquire the three-dimensional arrangement of the main protein of interest. Second, this structure was imported into PyMOL software to eliminate water molecules and inactive ligands. Following this, the structure was loaded into AutoDockTools software for hydrogenation and charging, and finally saved in PDB format. Third, the target proteins and the components of CH were transformed into the pdbqt file format, which is in line with AutoDock Vina's compatibility. To facilitate the docking of the target protein's active site, the size and coordinates of the Grid Box were adjusted. Subsequently, the active components of CH were docked onto the active site of the target protein. Finally, the components that had the best binding energy and strongest affinity towards the target proteins were selected. Using the PyMOL software, we visualized the docked complexes of the selected elements.

2.2 Experiment validation

2.2.1 CH preparation

Each bag of CH contains 7 g. The recommended dosage for adults with AP is 42 g, three times per day. In the case of rats, the equivalent dosage was 6.3 times that in adults. Therefore, the dosage of CH in the rats was approximately 0.7 g/kg.BW × 6.3 ≈ 4.4 g/kg.BW = 0.44 g/100g.BW. CH was dissolved in saline at a 0.44 g/mL concentration. The rats were gavaged with 1 mL of the solution per 100 g of body weight.

2.2.2 Reagents

Sodium taurocholate (Item No. The purity of 97-98% was acquired from Beijing Bioway Technology Co., Ltd. The CH ready-to-use granules were obtained from the Affiliated Traditional Chinese Medicine Hospital of Southwest Medical University. Ruixin Biotech was the source of the purchased ELISA kits (RX302856R, RX302869R, and RX302058R). An apoptosis detection kit (T-6013) was purchased from UElandy (China). Antibodies against p-PI3K (ab182651) were purchased from Abcam (Cambridge, USA). The β-actin antibody (AT0040) was purchased from Engibody (USA). Proteintech (USA) provided the antibodies for phosphoinositide 3-kinase (PI3K) (60225-1-Ig), Bcl-2 (68103-1-Ig), P-P65 (82335-1-RR), and P65 (10745-1-AP). Cell Signaling Technology (Danvers, MA, USA) was the source of the purchased antibodies targeting Bax (14796S), AKT (8200S), and p-Akt (8200S). All additional substances utilized in this research were of analytical quality and obtained from nearby vendors.

2.2.3 Animal experiment

2.2.3.1 Animals

A total of sixty male Sprague-Dawley rats, averaging 200 ± 20 g in weight, were acquired from the Animal Ethics Committee at Southwest Medical University. The animals were housed in a pathogen-free facility, where the humidity levels were kept between 40% and 70%, the temperature was sustained at 22 ± 2°C, and a 12-hour light-dark cycle was adhered to. The rats were given standard rodent feed and had unrestricted access to water during the experiment. The Animal Ethics Committee of the Southwest Medical University (NO.20221222-002) granted approval for all animal experiments. All rats were subjected to a 12h fasting period in both the pre-modeling and post-modeling phases.

2.2.3.2 Experimental design and induction of AP

The animals were allocated at random to two primary groups: a 12h group (n=30) and a 24h group (n=30). Each primary group was subsequently divided into three subgroups: sham (n=10), AP (n=10), and CH (n=10). AP was induced in the AP model group through the injection of 3.5% sodium taurocholate into the pancreaticobiliary duct in a retrograde manner (24). The control group received an identical surgical intervention, with the exception that they were given 0.9% saline instead. In the CH group, CH (1 mL/100 g.BW) was administered by gavage 6h after modeling. Samples of blood and pancreatic tissues were obtained at 12 and 24 hours following administration via gavage.

2.3 Experiment assay

2.3.1 Pancreatic histopathology and scoring

The pancreas tissue was preserved in a 4% solution of paraformaldehyde, underwent dehydration using a gradient method, was embedded in paraffin wax. Hematoxylin and eosin (HE) staining was performed on thick sections sliced to a 5 μm thickness. Pancreatic abnormalities following HE staining were evaluated by two pathologists using the Schmidt pathology scoring criteria (25) under a light microscope, employing a double-blind approach. Five randomly selected fields were assessed to determine the scores for edema, inflammation, and necrosis. Ultimately, the mean score was used to quantify the extent of damage.

2.3.2 TdT-mediated dUTP Nick-end labeling assay

The paraffin sections were deparaffinized in water. Proteinase K was used to permeabilize the tissues. The TUNEL reaction solution was applied to tissues and incubated in the dark. Finally, the sections were sealed after DAPI counterstaining. Fluorescence microscope was utilized to capture images and ImageJ software was employed for quantification.

2.3.3 Western blot

Protein specimens were obtained by utilizing RIPA lysis solution comprising of protease and phosphatase inhibitors, and protein concentration was ascertained utilizing a BCA assay kit. Following the electrophoretic separation of proteins, PVDF membranes were utilized to facilitate their transfer. Next, the antibodies were encapsulated with 5% BSA for a duration of 30 minutes. Subsequently, the membrane was incubated with the specified antibodies at 4°C overnight. After undergoing three 10-minute washes with TBST, the membrane underwent incubation with a secondary antibody conjugated with horseradish peroxidase for a duration of 2 hours. Subsequently, the membrane was washed

again and subjected to incubation with an ECL chemiluminescent substrate to facilitate imaging. The obtained images were analyzed using the ImageJ software.

2.3.4 The enzyme-linked immunosorbent assay and blood biochemistry

ELISA was used to detect the serum levels of interleukin (IL)-6, IL-1β, and tumor necrosis factor-alpha (TNF-α) in accordance with the manufacturer's guidelines. Additionally, an automated biochemical analyzer was utilized to measure the levels of serum amylase and lipase.

2.4 Statistics

We calculated the mean ± standard deviation using the statistical tool GraphPad Prism 9.5. The variance was calculated to satisfy the normal distribution of the data, and one-way ANOVA (One-Way ANOVA) was chosen when the variance was uniform; the Kruskal-Wallis H test was used to do a nonparametric test for multiple independent samples when none of the conditions were met. The statistical significance level was established to be below 0.05 and identified as $P < 0.05$.

3 Results

3.1 Network pharmacology prediction analysis

3.1.1 Active compounds and targets in CH

By screening the TCMSP database, a total of 168 active components and 276 targets were identified. The results indicate that most of the compounds interact with multiple targets (Supplementary Table S1). The active components of Baishao, Chaihu, Chishao, Dahuang, Danshen, Gancao, Houpu, Huangqin, Huangqi, Taoren, Yanhusuo, Zhishi, and Zhizi were 3, 7, 8, 5, 28, 40, 2, 13, 7, 16, 42, 4, and 7, respectively. The 10 molecules with the highest OB scores are listed in Table 1.

3.1.2 Disease target selection and Venn diagram

By eliminating duplicates, we acquired a grand total of 1986 disease targets from the GeneCards database using a relevance score of ≥10. Out of these, 2060 targets were obtained, including 143 targets from the OMIM database, 112 targets from the PharmGkb database, 9 targets from the TTD database, and 6 targets from the DrugBank database. We identified overlapping targets to select 177 potential therapeutic targets for treating AP between the compound and AP-related targets, as shown in Supplementary Table S2. The corresponding Venn diagram is shown in Figure 2A.

3.1.3 GO and KEGG pathway enrichment analysis

DAVID's GO analysis yielded three distinct categories: biological processes (BP), cellular constituents (CC), and molecular functions (MF), revealing a total of 4544, 269, and 439 identified terms in each category, respectively. The top 10 GO terms significantly enriched in each stratum can be found in Figure 2B. The results showed that BP was most related to epithelial cellular proliferation, CC was most related to the vesicle lumen, and MF was most related to mRNA blinding involved in post-transcriptional gene splicing.

In order to enhance our comprehension of the pharmacological mechanism of CH for AP treatment at the signaling pathway level, we conducted KEGG analysis. This analysis unveiled that the chosen 177 potential targets were significantly present in 186 pathways, encompassing the PI3K/AKT signaling pathway (hsa04151), AGE/AGE signaling pathway (hsa04933), JAK/TAT signaling pathway (hsa04630), and TNF signaling pathway (hsa04668). The top 30 most significantly enriched pathways are shown ($P<0.05$, ordered by gene count) in Figure 2C. A comprehensive overview of the PPI data is available in Supplementary Table S3.

3.1.4 Construction and analysis of the D-C-T network

Cytoscape 3.9.1 was used to construct and visualize the D-C-T network of the active CH compounds and potential targets, which includes common targets between compound targets and AP-related targets. In this section, the 181 compounds linked to potential targets are presented in the network diagram. Figure 2D illustrates a network consisting of 358 nodes, including 181 compounds and 177 potential targets, connected by 1832 edges. The key compounds of CH can be considered as the ones that showed strong interactions in the C-T network. These compounds are from Baishao, Chaihu, Chishao, Dahuang, Danshen, Gancao, Houpu, Huangqin, Huangqi, Taoren, Yanhusuo, Zhishi, and Zhizi, with the numbers 3, 7, 8, 5, 28, 40, 2, 13, 7, 16, 42, 4, and 7, respectively. 148 different herb ingredients targeted prostaglandin G/H synthase 2 (PTGS2), while heat shock protein (HSP) 90-alpha (HSP90AA1), sodium channel protein type 5 subunit alpha (SCN5A), prostaglandin G/H synthase 1 (PTGS1), and nuclear receptor coactivator 2 (NCOA2) were targeted by 89, 83, 82, and 74 herb ingredients, respectively. This implies that the synergistic therapeutic effect on AP is achieved by multiple CH compounds.

The ingredients of CH are represented by the outer nodes in Figure 3, while the common targets between the drug and the disease are represented by the middle nodes. The CH formula uses various colors to represent different herbal medicines: navy blue for Chaihu (Bupleuri Radix), crimson for Houpo (Magnoliae Officinalis Cortex), pale green for Chishao (Paeoniae Radix Rubra), vibrant green for Dahuang (Rhei Radix et Rhizoma), ochre yellow on the right for Taoren (Persicae Semen), dark green for Danshen (Salviae Miltiorrhizae Radix et Rhizoma), rose for Gancao (Glycyrrhizae Radix et Rhizoma), lavender for Yanhusuo (Corydalis Rhizoma), yellow on the left for Huangqi (Astragali Radix), golden yellow for Huangqin (Scutellariae Radix), deep violet for Zhishi (Aurantii Fructus Immaturus), pale yellow for Zhizi (Gardeniae Fructus), and sky blue for Baishao (Paeoniae Radix Alba).

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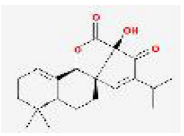
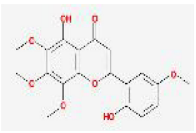
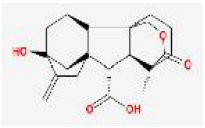
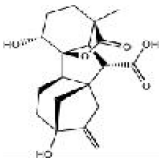
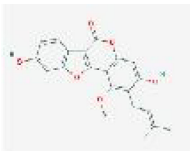
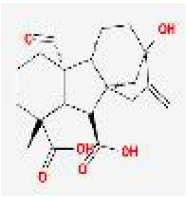
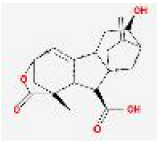
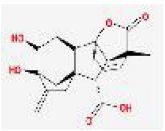
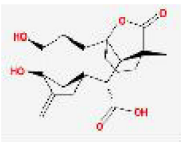
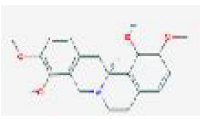
TABLE 1 Representative molecules from CH and their corresponding OB, DL, and structures.

Drug	MolId	MolName	MW	OB (%)	DL	structure
Danshen	MOL007064	przewalskin b	330.46	110.32	0.44	
Huangqin	MOL002934	NEOBAICALEIN	374.37	104.34	0.44	
Taoren	MOL001351	Gibberellin A44	346.46	101.61	0.54	
Taoren	MOL001353	GA60	348.43	93.17	0.53	
Gancao	MOL002311	Glycyrol	366.39	90.78	0.67	
Taoren	MOL001349	4a–formyl–7alpha–hydroxy–1–methyl–8–methylidene–4aalpha,4bbeta–gibbane–1alpha,10beta–dicarboxylic acid	362.46	88.6	0.46	
Taoren	MOL001344	GA122–isolactone	330.41	88.11	0.54	
Taoren	MOL001329	2,3–didehydro GA77	346.41	88.08	0.53	
Taoren	MOL001360	GA77	348.43	87.89	0.53	
Yanhusuo	MOL004193	Clarkeanidine	327.41	86.65	0.54	

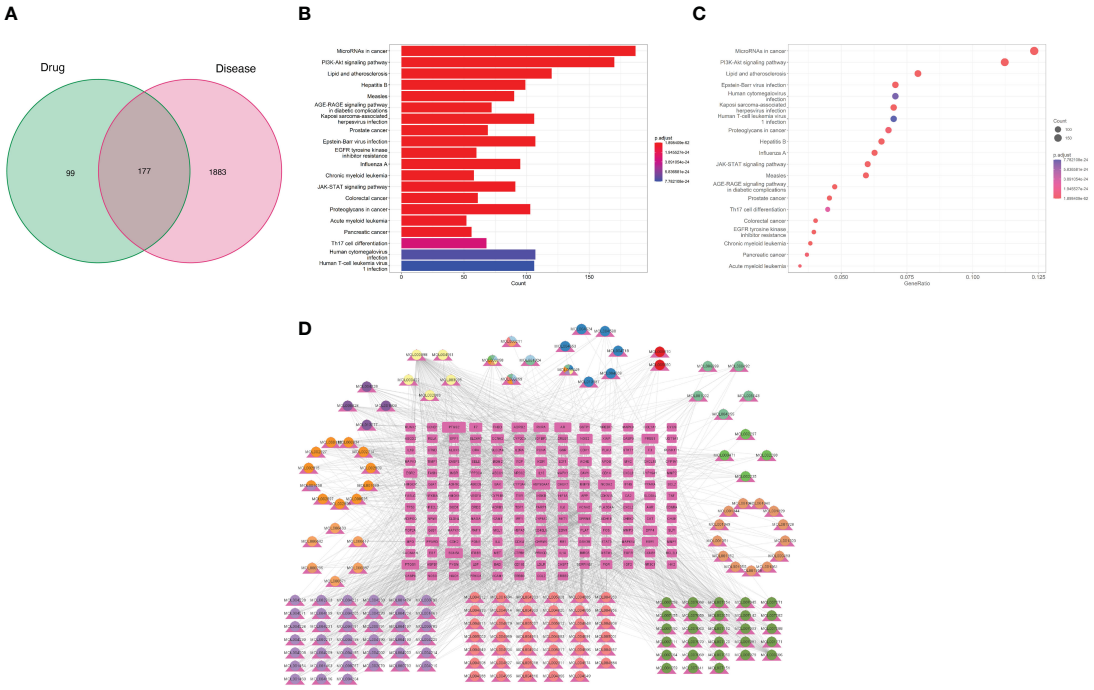


FIGURE 2
(A) Venn diagram of active compounds and AP–related targets. The green areas represent the number of active compound targets, the pink areas represent the number of AP–related targets, and the overlapping part is the number of their common target genes. **(B)** GO functional enrichment analysis to show the top 10 enriched BP, CC, and MF GO terms. **(C)** Generate a bubble chart illustrating the 20 most enriched KEGG pathways. **(D)** The "Drug–Ingredient–Target" network diagram predicts the active compounds of drugs and potential targets of CH effective in treating AP.

3.1.5 Construction and analysis of the PPI network

Utilization of the STRING database, a PPI network of the chosen 177 potential targets was established based on the aforementioned methods according to the minimum required interaction score ≥ 0.95 with isolated nodes hidden in the network in Figure 4B. In CytoNCA, different measures such as betweenness (BC), closeness (CC), degree (DC), eigenvector (EC), local average-connectivity-based methods (LAC), networks (NC), and information (IC) were utilized for the scoring and filtering procedures. Each gene was required to surpass its median value for retention. This scoring and filtering process was performed thrice in Figure 4A. Ultimately, 14 core target genes were identified (STAT3, IL6, MYC, CDKN1A, AKT1, MAPK1, MAPK3, MAPK14, HSP90AA1, HIF1A, ESR1, TP53, FOS, and RELA) comprising 14 nodes and 60 edges. Detailed information on PPI data is presented in Supplementary Table S4.

3.1.6 Construction and analysis of the C-T-P network

The 14 core targets identified in the PPI network were linked to their corresponding compounds in the DCT network through the top 30 KEGG pathways, as depicted in Figure 4C. The C-T-P network that was obtained consisted of 165 nodes, comprising 121 compounds, 14 targets with potential, and 30 pathways for signaling. Additionally, there were 485 edges in the network. Additional examination uncovered that the chosen possible objectives had a strong connection with the PI3K/AKT (hsa04151, degree = 15), TNF (hsa04668, degree = 12), IL-17 (hsa04657, degree = 9), and HIF-1 (hsa04066, degree = 9) signaling pathways. The involvement of these pathways is crucial in the advancement of AP and various other inflammatory conditions.

3.1.7 Molecular docking validation

From the C-T-P network, we identified the top three selected compounds and their corresponding target proteins, and conducted a molecular docking validation. Specifically, the target proteins chosen for docking overlapped with key target proteins in the PPI network. Table 2 displays the outcomes of molecular docking. A lower docking score indicates a stronger ligand-receptor binding affinity, signifying a higher likelihood of interaction. All the binding energy scores between the active compounds and the key target proteins were less than -5.0 kJ/mol. The 3D binding models of each target protein and compound are shown in Figure 3. Additionally, the docking diagrams of the compound-target proteins suggested possible intermolecular interactions between the core compounds and protein targets.

3.2 The experimental verification of the network pharmacology results

3.2.1 Effects of CH on pancreatic pathological changes

According to the findings shown in Figure 5A, the group with AP demonstrated significantly elevated serum amylase and lipase levels compared to the sham group. However, the administration of

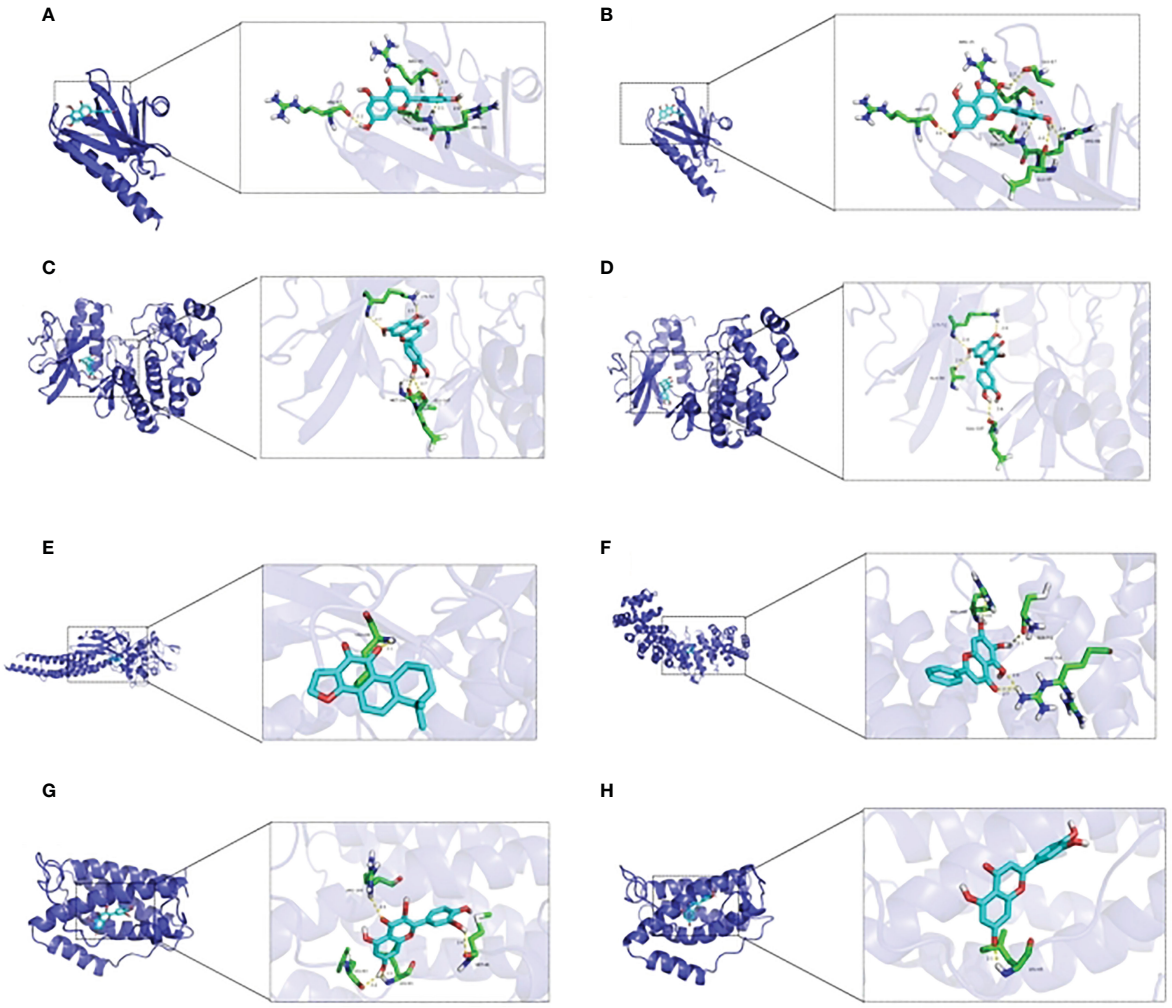


FIGURE 3
Molecular docking between essential elements of CH and crucial targets illustrated through schematic diagrams. The molecular docking diagrams of luteolin and AKT1, quercetin and AKT1, luteolin and MAPK1, quercetin and MAPK1, cryptotanshinone and STAT3, baicalein and HIF−1, quercetin and IL6, and luteolin and IL6 are represented by Note **(A–H)** respectively.

CH resulted in decreased levels. Moreover, the AP model group exhibited notably elevated levels of edema and inflammation compared to the group that underwent a sham operation. However, the administration of CH resulted in a decreased level of pancreatic pathological harm, which involved decreased swelling, inflammation, and tissue death, along with a decrease in pathological scores ($P < 0.05$) (Figures 5B, C). Collectively, these results demonstrate that CH alleviates pancreatic tissue inflammation and mitigates pathological damage in AP rats.

3.2.2 Effect of CH on the expression of TNF-α, IL-6, and IL-1β

According to Figure 6, the AP group showed significantly raised levels of serum TNF-α, IL-6, and IL-1β in comparison with the sham group. However, the administration of CH resulted in a decrease of these levels. Over time, the levels of TNF-α, IL-6, and IL-1β in the AP group exhibited a substantial growth ($P < 0.05$).

3.2.3 Effect of CH on the PI3K/AKT signaling pathway

The levels of PI3K, AKT, and NF-κB proteins did not show any notable differences among the sham, AP, and CH groups (Figure 7). However, the AP model group exhibited considerably elevated levels of protein phosphorylation compared to the sham group. Conversely, the intervention group CH demonstrated notably reduced levels of phosphorylation among the proteins PI3K, AKT, and NF-κB.

3.2.4 Effect of CH on cells apoptosis

TUNEL staining was used to analyze apoptosis of acinar cells. Pancreatic acinar cells underwent apoptosis upon the induction of sodium taurocholate, as depicted in Figure 8A. After CH treatment, the incidence of TUNEL-positive cells has significantly increased, unlike the AP group (Figure 8B). Furthermore, the western blot analysis demonstrated that the CH group exhibited markedly

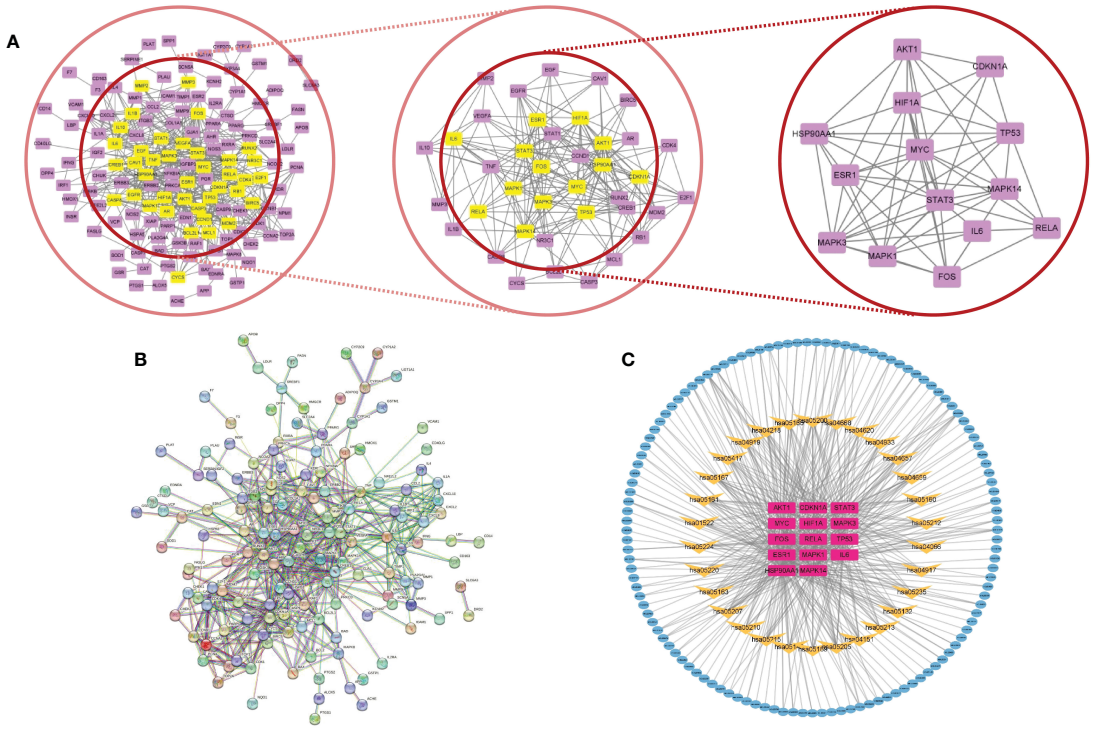


FIGURE 4
(A) Core subnetwork diagram of the target of CH for the treatment of AP. **(B)** PPI network of the target of CH for the treatment of AP. **(C)** The network called C–T–P is made up of 165 nodes and 485 edges. Blue nodes denote the active compounds. Yellow nodes denote the pathways, while purple nodes indicate core targets.

elevated levels of the pro-apoptotic protein Bax, while displaying notably reduced levels of the anti-apoptotic protein BCL-2 in comparison to both the sham and AP groups. This indicates that CH significantly enhanced apoptosis in acute pancreatitis. (Figures 8C–E)

4 Discussion

AP is a common disorder marked by the activation of trypsinogen and abrupt inflammation of the pancreas (26). In clinical practice, treating complex diseases using a single targeted drug is challenging (27). Currently, drugs specific to AP are lacking, and most available treatments focus on symptom relief and supportive care (26). TCM is a highly effective method for treating AP due to its various benefits, including its ability to target multiple areas and its minimal adverse reactions (28, 29). Previous animal and clinical experiments have confirmed the therapeutic impact of CH on AP, yet the precise molecular mechanisms behind it remain unclear. To investigate the therapeutic effects of CH on AP, we employed a blend of network pharmacology and experimental validation techniques to examine the active constituents, targets, networks, and pathways involved. This approach aided our understanding of the material basis and molecular mechanisms underlying its benefits. We then conducted a series of experiments to confirm our findings using network pharmacology analysis. Lately, there has been a global focus on utilizing network pharmacology for the treatment of illnesses. Network pharmacology is an efficient method for identifying ingredients and forecasting drug targets using advanced computer simulations. This study explored the potential use of network pharmacology to understand how CH can effectively and affordably treat AP. Using network pharmacology, we investigated the basic elements and mechanisms underlying the therapeutic effects of CH on AP.

TCMSP retrieval yielded 181 active compounds. The analysis of the D–C–T network demonstrated that there were 177 interconnected potential targets, indicating that CH might affect AP through mechanisms involving multiple compounds and targets. Additionally, PPI network analysis identified 14 core

TABLE 2 The affinity of compounds and targets.

Ligand	luteolin	quercetin	luteolin	quercetin	cryptotanshinone	baicalein	quercetin	luteolin
Protein	AKT1	AKT1	MAPK1	MAPK1	STAT3	HIF-1α	IL6	IL6
Affinity (kcal/mol)	−6.7	−7	−8	−7.9	−8.1	−7.2	−7.4	−7.4

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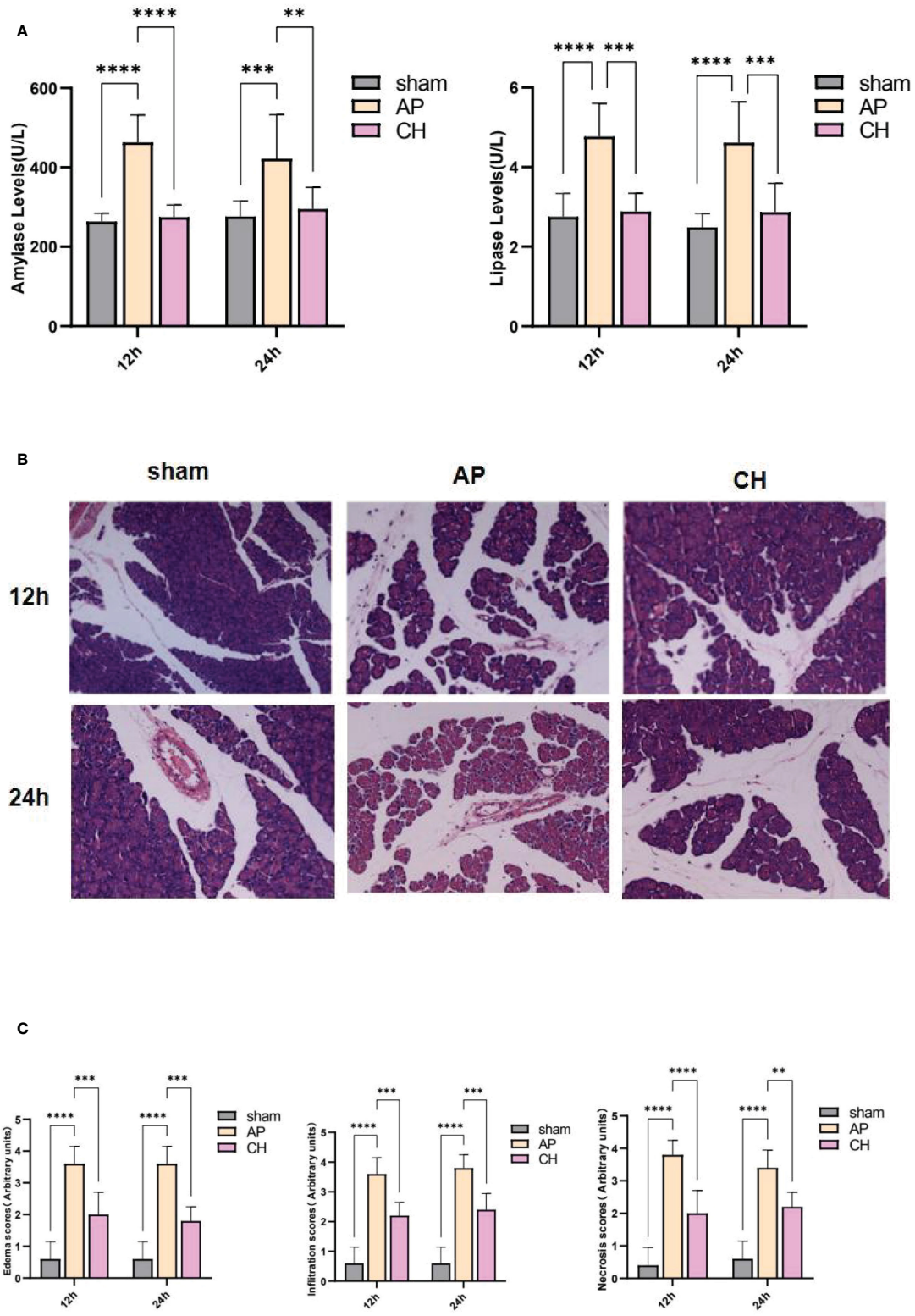

FIGURE 5
CH treatment attenuated AP. **(A)** Serum levels of amylase and lipase in different groups. The data presented above represent the average ± SD (n = 6) for each group, with **P < 0.01, ***P < 0.001, and ****P < 0.0001 compared to the AP group. **(B)** HE staining (200×) was used to examine the pancreatic pathology in rats from various groups. (n = 5). **(C)** Corresponding pathological score. The data presented above represent the average ± SD (n = 5) for each group, with **P < 0.01, ***P < 0.001, and ****P < 0.0001 compared to the AP group.

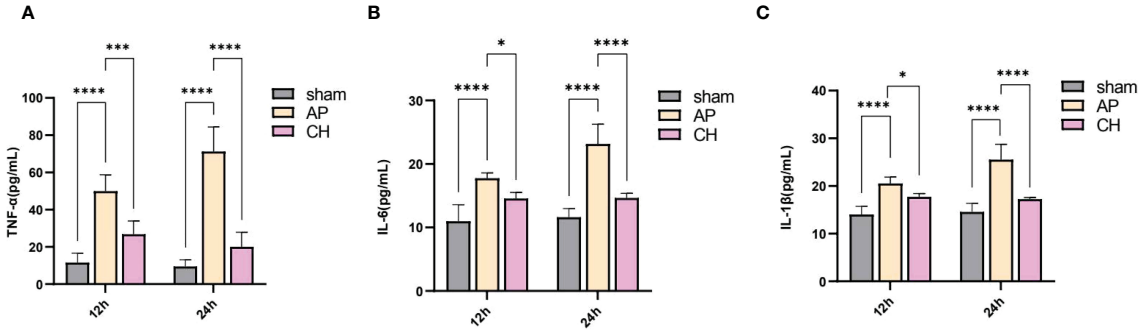

FIGURE 6
Effect of CH treatment on inflammation in AP rat model. **(A)** Serum levels of TNF−α among various groups. The data presented above are represented as the average ± SD (n = 5). ***P < 0.001 and ****P < 0.0001 compared to the AP group. **(B)** Serum levels of IL−6 among various groups. The data presented above are represented as the average ± SD (n = 5). *P < 0.05 and ****P < 0.0001 compared to the AP group. **(C)** Serum concentrations of IL−1β among various groups. The data presented above are represented as the average ± SD (n = 5). *P < 0.05 and ****P < 0.0001 compared to the AP group.

genes among the potential targets, including STAT3, IL6, MYC, CDKN1A, AKT1, MAPK1, MAPK14, HSP90AA1, HIF1, ESR1, TP53, FOS, and RELA. AKT1, MAPK1, MAPK3, MAPK14, IL6, STAT1, and HIF1 are closely related to AP based on wide−scale literature mining.

The precise pathophysiological mechanisms responsible for AP are still not fully understood. For this study, we conducted GO and KEGG investigations to uncover the potential molecular pathways involved in CH. Our findings validate the involvement of diverse biological processes and signaling pathways in the commencement and advancement of AP. The PI3K/AKT signaling pathway, which is one of the pathways enriched and connected to the network, is recognized as a significant contributor to inflammation associated with AP (27, 30, 31). Researchers (32–34) have also considered several other pathways as possible targets for AP treatment. These pathways include the AGE/RAGE signaling pathway (hsa04933), JAK/STAT signaling pathway (hsa04630), and TNF signaling pathway (hsa04668). The active compounds show favorable binding activity with the essential proteins of CH, as indicated by the molecular docking results.

Based on the above analysis, we performed further experimental verification and confirmed the protective effect of CH against AP. Elevated amylase and lipase are the hallmarks of AP. Combined with the pathological staining results, we also confirmed that CH reduced pancreatic injury in AP. Additionally, using western blot and Tunel staining, we further verified CH's ability to induce apoptosis in acinar cells through the PI3K/AKT pathway, thereby alleviating severe pancreatitis.

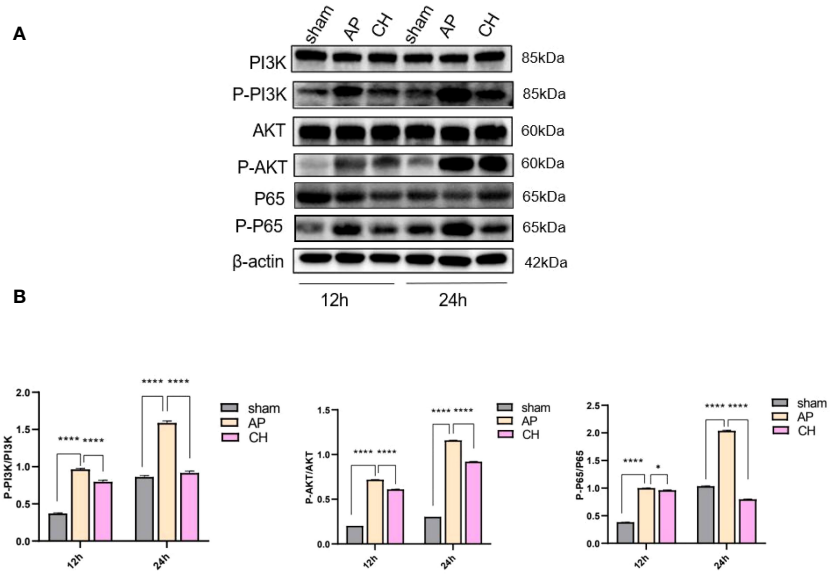

FIGURE 7
The impact of CH on the PI3K/AKT signaling pathway in the AP model. **(A)** Expression of PI3K, p−PI3K, AKT, p−AKT, P65, p−P65, and β−actin in different groups. (n = 3). **(B)** Ratios of p−PI3K/PI3K, p−AKT/AKT, and p−P65/P65 that correspond. The data presented above are represented as the average ± SD (n = 3). *P < 0.05 and ****P < 0.0001 compared to the AP group.

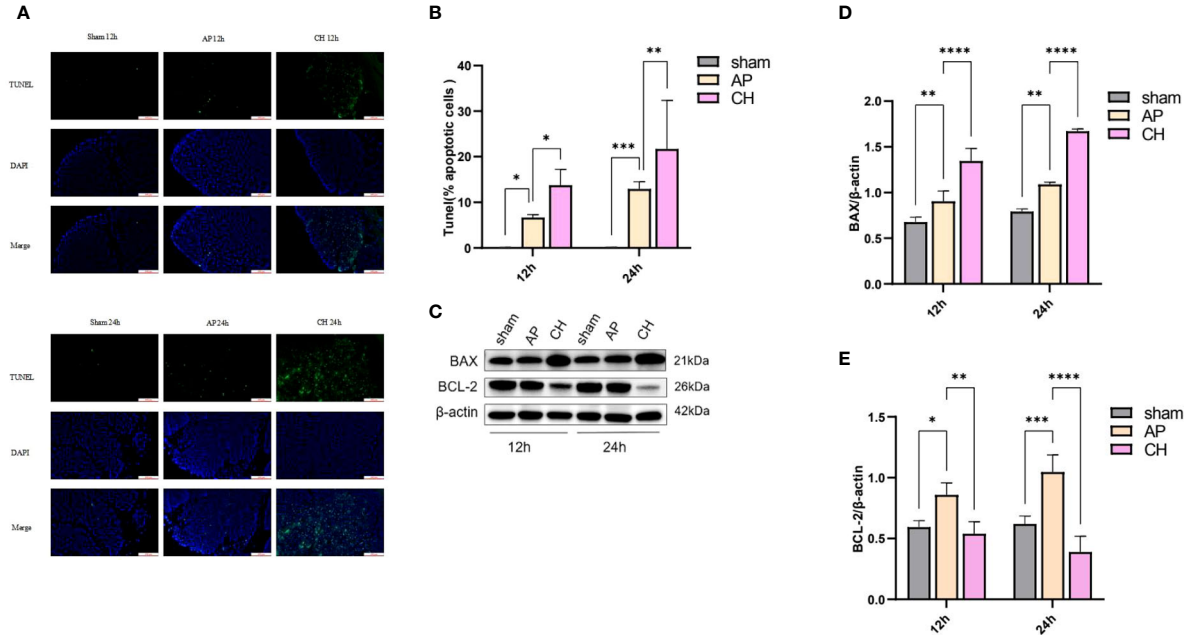

FIGURE 8
Administration of CH increases the apoptosis of pancreatic acinar cell in rats with AP. **(A)** Images from the TUNEL assay of pancreatic tissue, 100 μm scale bar. (n = 6). **(B)** Statistical results on the proportion of pancreatic acinar cells undergoing apoptosis in each group. Mean ± SD (n = 6) data were reported for each group, and statistical significance was observed. *P < 0.05, **P < 0.01, and ***P < 0.001 in comparison to the AP group. **(C)** Expression levels of BAX, BCL−2, and β−actin in various animal model groups.(n = 3). **(D)** Corresponding ratios of BAX/β−actin. Mean ± SD data were reported for each group, and statistical significance was observed (n = 3). **P < 0.01 and ****P < 0.0001 in comparison to the AP group. **(E)** Corresponding ratios of BCL−2/β−actin. Mean ± SD data were reported for each group, and statistical significance was observed (n = 3). *P < 0.05, **P < 0.01, ***P < 0.001, and ****P < 0.0001 in comparison to the AP group.

During the initial stages of AP, acinar cell death via necrosis and apoptosis significantly affects the progression of the condition. Recent research (35) has shown that apoptosis may serve as a protective response in AP. Furthermore, studies (26, 36) have shown that triggering apoptosis in acinar cells can decrease the activation and secretion of trypsin, consequently lowering the incidence of systemic inflammatory response syndrome (SIRS). Several signaling pathways are involved in apoptosis. The PI3K/AKT pathway is essential in averting cell death, and blocking it results in higher levels of programmed cell death in pancreatic cells. This, in turn, reduces pancreatic harm and enhances the chances of survival in AP rats (37). Previous studies (38) have extensively confirmed the role of the PI3K/AKT signaling pathway in the process of inflammation, which is strongly linked to NF−κB and IL−6. PI3Ks play vital roles in mediating inflammatory responses. Lupia et al. (39) revealed that by deleting the PI3K gene in rats, there was a significant reduction in the damage and mortality of pancreatic acinar cells compared to rats with the wild−type gene. Earlier research (40–43) has suggested that PI3K, by activating AKT, has the ability to boost the movement of NF−κB into the nucleus and its activity in transcription, which is crucial in the progression of Severe Acute Pancreatitis. Furthermore, activation of the PI3K/AKT pathway was observed in both *in vivo* experiments with AP−induced inflammation and *in vitro* studies with cytokine administration. Further investigations (44) revealed that inhibiting PI3K/AKT or NF−κB could enhance the survival rates of rats with AP. These results indicate that blocking PI3K may offer the potential for preventing and treating AP. Consistent with these research, our study has shown that CH intervention effectively suppressed the activation of the PI3K/AKT pathway, resulting in the promotion of apoptosis in pancreatic acinar cells.

This investigation has certain limitations that merit further investigation. The sample size of AP−related targets in the GEO database (45) should be increased. Additionally, the potentially active compounds identified in this study require independent validation of their therapeutic efficacy against AP.

5 Conclusions

Despite the unclear comprehension of the complex pharmacological mechanisms implicated in the effectiveness of CH for treating AP, this study sought to forecast the responsible mechanisms for the defensive benefits of CH against AP through a network pharmacology−driven approach. The findings from our study suggest that CH may achieve its healing benefits by utilizing a variety of elements, objectives, and routes. The network pharmacology method holds the potential to uncover the molecular mechanisms of Traditional Chinese Medicine (TCM), including CH, by presenting valuable insights for clinical application of this formulation.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Materials, further inquiries can be directed to the corresponding author/s.

Ethics statement

The animal study was approved by The Animal Ethics Committee of the Southwest Medical University. Affiliations: Southwest Medical University. The study was conducted in accordance with the local legislation and institutional requirements. No potentially identifiable images or data are presented in this study.

Author contributions

JY: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Writing – original draft, Writing – review & editing. Y–HJ: Conceptualization, Data curation, Visualization, Writing – original draft. XZ: Funding acquisition, Validation, Writing – review & editing. J–QY: Validation, Writing – review & editing. Y–YW: Investigation, Writing – original draft. J–QL: Methodology, Writing – review & editing. P–CZ: Writing – review & editing. W–FT: Writing – review & editing. ZL: Writing – review & editing.

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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.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fimmu.2024.1353695/full#supplementary-material

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EDITED BY
Ravi Kumar Sharma,
Chandigarh University, India

REVIEWED BY
Wenjian Mao,
Nanjing University, China
Neha Jakhete,
University of Maryland, United States

*CORRESPONDENCE
Lian Hu
✉ 2530605685@qq.com

†These authors have contributed equally to this work

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A dose–response correlation between smoking and severity of acute pancreatitis: a propensity score-matched study

Runzhuo Li[1,2†], Wanyun Tang[3†], Sun Yan[4], Xiaohan Yu[5] and Lian Hu[1*]

[1]Department of Digestion, First People's Hospital of Yibin, Yibin, China, [2]Department of Digestion, Dandong Central Hospital, China Medical University, Dandong, China, [3]Department of Orthopedics, Dandong Central Hospital, China Medical University, Dandong, China, [4]Department of Cardiology, The First Affiliated Hospital of Chongqing Medical University, Chongqing Medical University, Chongqing, China, [5]General Surgery Department, Dandong Central Hospital, China Medical University, Dandong, China

Background: Acute pancreatitis, among the most prevalent gastrointestinal disorders, exhibits a continual rise in its incidence recent years. This study endeavor to explore the correlation between smoking exposure and the severity of acute pancreatitis (AP).

Methods: Five hundred and eight patients diagnosed as acute pancreatitis (AP) were included in our data analysis. Patients were categorized based on their smoking pack-years into four groups: light, moderate, heavy, and non-smokers. Outcomes were classified as two: "mild acute pancreatitis (MAP)" and "moderately severe acute pancreatitis (MSAP) or severe acute pancreatitis (SAP)". We conducted propensity score matching (PSM) to adjust confounding factors and multivariable logistic regression analysis to determine adjusted odds ratios and 95% confidence intervals. Additionally, a dose-dependent association analysis between smoking exposure and the incidence rate of "MSAP or SAP" was performed.

Results: Smokers exhibited a higher risk of "MSAP or SAP" compared to non-smokers, both before (17.1 vs. 54.9%, $p < 0.001$) and after (9.4 vs. 24.7%, $p < 0.001$) PSM. With an area under the ROC curve of 0.708, smoking showed a moderate level of predictive ability. Furthermore, propensity score matching analysis showed that patients who smoked compared to non-smokers had significantly higher risks of "MSAP or SAP" for light smoking (OR 3.76, 95% CI 1.40–10.07, $p = 0.008$), moderate smoking (OR 4.94, 95% CI 2.23–10.92, $p < 0.001$), and heavy smoking (OR 8.08, 95% CI 3.39–19.25, $p < 0.001$).

Conclusion: Smoking is an independent risk factor that can raise the severity of pancreatitis. Moreover, the severity of acute pancreatitis escalates in tandem with the accumulation of pack-years of smoking.

KEYWORDS

smoking, acute pancreatitis, propensity score-matching, severity grading, dose–response relationship

Introduction

Acute pancreatitis (AP) is an intense inflammatory leading to edema, hemorrhage, and potentially necrosis (1, 2). With an incidence rate estimated at 110–140 cases per 100,000 population, AP has become one of the most prevalent gastrointestinal illnesses requiring hospitalization in the United States (3). Between 2002 and 2013, the number of hospitalizations attributed to AP rose from 9.48 per 1000 cases to 12.19 per 1000 cases (4). According to the 2012 Atlanta guidelines, AP fall under the category as mild (MAP), moderately severe (MSAP), or severe (SAP) in accordance with its severity of onset. The latter two categories, MSAP and SAP, are more severe and often lead to local or systemic complications including pancreatic leakage, pancreatic necrosis, pancreatic abscess, systemic inflammatory response syndrome (SIRS), even multi-organ dysfunction syndrome (MODS) (5). These complications not only prolong hospitalization and increase medical costs but may also result in long-term disabilities and mortality. Approximately 80% of patients present with MAP to MSAP, with one-fifth progressing to severe disease, and a mortality rate of about 20% (6–8). Traditional causes of AP include gallstones, alcohol abuse, and hypertriglyceridemia. Many studies (9–12) has reported smoking also accounting for the incidence of AP. However, within Asian populations, there is limited research on the relationship between smoking and AP, particularly regarding its impact on the severity of the disease.

Currently, smoking is a well-known risk factor for various digestive diseases (13). Nicotine and other harmful substances in tobacco smoke can cause pancreatic damage, inflammation, and impaired pancreatic function. Smoking has been shown to inhibit pancreatic secretion and increase the risk of leakage of pancreatic enzymes into the bloodstream. Prolonged exposure to nicotine increases the content of pancreatic zymogen within cells and induces vacuolization of acinar cells in rats (14). These factors may exacerbate the development of AP and its complications. Therefore, elucidating the link between smoking exposure and the severity of AP is crucial for preventing AP from progressing into an irreversible and lethal disease. The goal in our study is to figure out the link between smoking exposure measured in pack-years and the severity of AP through propensity score matching analysis.

Methods

Data sources

This retrospective investigation utilized anonymized clinical data from electronic medical records of patients admitted for AP at Dandong Central Hospital, China Medical University from October 2017 to October 2023. The Dandong Hospital Ethics Committee approved the study and granted a consent waiver for this retrospective cohort study. In order to protect personal health information, online medical records were limited to the range of anonymized data analysis, which included demographics, message of laboratory findings, imaging analysis results, and various complications.

Data collection

In this study, we collected variables including demographic characteristics: age, gender, smoking history, drinking history, hypertension, diabetes, hyperlipidemia, cardiovascular disease; and serological indicators: white blood cell count (WBC), red blood cell count (RBC), hemoglobin concentration (HGB), platelet count (PLT), potassium ion (K+), sodium ion (Na+), calcium ion (Ca+), creatinine (Cr), blood glucose (BG), alanine aminotransferase (ALT), lactate dehydrogenase (LDH), total bilirubin (TBIL), albumin (ALB), amylase (AMY), lipopolysaccharide (LPS), D-dimer, C-reactive protein (CRP), prothrombin time (PT), and activated partial thromboplastin time (APTT).

All serological indicators for the patients were obtained from blood samples collected within 24 h of admission.

Patient selection

Both the diagnosis and severity grading of AP referred to the 2012 Atlanta guidelines (5). We included patients in our study who satisfied at least two of the following criteria. (1) Acute, persistent, intense pain in the upper abdomen, with or without radiation to the back; (2) The activity of serum lipase (or amylase) beyond three times the upper limit of normal; (3) Imaging findings match characteristics of AP.

Patients were excluded from the study if meeting any of following criteria. (1) Presence of other pancreatic diseases, such as chronic pancreatitis; (2) Presence of other serious diseases, such as tumors, autoimmune diseases, etc.; (3) Incomplete or unavailable data; (4) Taking medications that may affect the severity of pancreatitis; (5) History of pancreatic surgery; (6) Previous hospitalization for AP.

Before the commencement of the study, four researchers received professional training in data collection, including the diagnosis and severity grading of AP. Three researchers independently collected the variables. If necessary, any differences in variable identification were discussed with or determined by the senior researcher.

Exposure and outcome

Our study's main exposure factor was smoking pack-years, calculated by multiplying the daily pack count by the total number of smoking years. The patients were categorized into smoking and non-smoking groups according to their smoking history. Smokers included in the study were those who had been continuously smoking and were still smoking within 1 month of disease onset. To assess the link between dosage and response of smoking pack-years and AP severity, study population was further split into four groups on the basis of pack-years: nonsmokers (Never smoking), smokers with pack-years $\leq$ 10 (Light smoking), smokers with pack-years $>$10 but $\leq$20 (Moderate smoking), and smokers with pack-years $>$20 (Heavy smoking).

The primary outcome assessed was the severity of AP, graded referring to the 2012 Atlanta guidelines. AP severity categorization was determined on patients' clinical complaints and symptoms,

serological indicators, imaging findings, presence of organ failure, and complications, dividing AP into MAP, MSAP, and SAP. Based on previous research (15), we defined the outcome variable as "MAP" and "MSAP or SAP", categorized as binary variables "yes" or "no", for our statistical analysis.

Analytical statistics

When analyzing patients' baseline characteristics, various types of data necessitate specific methods. Percentages (%) are applicable to categorical variables, with the chi-square test used for group comparisons. Mean ± standard deviation (SD) is suitable for normally distributed continuous measurements, with independent sample *t*-tests employed for group comparisons. Median and interquartile range are utilized for non-normally distributed data, with the Mann-Whitney *U*-test used for group comparisons.

We investigate the linkage between smoking and the severity of AP using logistic regression modeling. After possible confounding factors with p-value ≥ 0.05 are excluded using univariable logistic regression, factors with p-value < 0.05 are then included in the multivariable regression analysis. Additionally, we computed the area under the ROC curve (AUC) to ascertain discriminative capacity of smoking pack-years for distinguishing between "MAP" and "MSA or SAP". Then we assessed the relationship between smoking pack-years and AP severity through graphical representations illustrating the association between observed rates and predicted probabilities across various levels of smoking pack-years.

To further mitigate the influence of confounding variables, a propensity score matching (PSM), of which matching ratio was 1:1, was performed by the nearest neighbor algorithm, to make sure the most appropriate balanced distribution of covariates between groups. With a caliper width of 0.25 standard deviations, the matching effect was assessed by standardized mean differences (SMDs). Following PSM, we stratified all covariates and then conducted univariable logistic regression analysis to explore the linkage between smoking pack-years and the severity of AP. Finally, for assessing the strength of the link. we calculated odds ratios (ORs) and 95% confidence intervals (CIs).

SPSS version 27 (IBM Corp., Armonk, NY, USA) and R software version 4.0.3 (R Foundation for Statistical Computing, Vienna, Austria) were our main statistical tools that we used.

Results

Baseline characteristics

Four hundred and twenty-one patients were disqualified in total based on the exclusion criteria. In the end, 508 patients involved into the eventual data analyzing (eFigure). The smoking group had more male patients and a higher proportion of patients with alcohol consumption. Laboratory testing revealed the smoking group had higher levels of LDH, CRP, and WBC count, as well as their median and interquartile range. The two groups had no valid statistical discrepancy in terms of age, hypertension, hyperlipidemia, or cardiovascular disease history. The baseline characteristics of the smoking and non-smoking groups at admission are displayed in Table 1.

Multivariable analysis and propensity score matching

To explore the relationship between the 27 factors and "MSAP or SAP", we conducted single-factor and multi-factor analyses (Table 2). After adjusting for any confounding variables, we found that seven factors emerged as independent predictors of "MSAP or SAP": smoking, WBC, HGB level, BG, Ca^{+} AMY, and PT. Even after adjusting for other variables, these factors remained statistically significant ($P < 0.05$).

Table 3 displays the baseline characteristics of the smoking and non-smoking groups of patients both before and after 1:1 PSM. A satisfactory balance between the smoking and non-smoking groups was attained by PSM, as most variables had standardized mean differences of <0.1.

The correlation between smoking pack-years and "MSAP or SAP"

The average smoking pack-years in the "MSAP or SAP" group were higher than those in the MAP group, both before PSM (3.28 vs. 12.71, $p < 0.001$) and after PSM (6.64 vs. 14.62, $p < 0.001$) (Figure 1). The proportion of patients with "MSAP or SAP" in the smoking group was larger than that in the non-smoking group, both before PSM (17.1 vs. 54.9%, $p < 0.001$) and after PSM (9.4 vs. 24.7%, $p < 0.001$) (Table 4). The risk of "MSAP or SAP" was higher in the smoking group than in the non-smoking group (unadjusted OR 5.86, 95% CI 3.85–8.94, $p < 0.001$). After adjusting for confounding factors, this result remained significant (adjusted OR 5.01, 95% CI 2.76–9.09, $p < 0.001$). With minimized selection bias by PSM, consistent finding was obtained (adjusted OR after PSM: 4.18, 95% CI 2.18–8.03, $p < 0.001$) (Table 5). Additionally, results from examining smoking pack-years as an ongoing factor were in coincidence: the unadjusted odds increased by 8% and the adjusted odds increased by 7% for every unit increase in smoking pack-years.

Compared to non-smoking patients, after adjusting for confounding factors, patients with varying degrees of smoking (light to heavy) exhibited an increased risk of developing "MSAP or SAP" (light: adjusted OR 3.76, 95% CI 1.40–10.07, $p = 0.008$; moderate: adjusted OR 4.94, 95% CI 2.23–10.92, $p < 0.001$; heavy: adjusted OR 8.08, 95% CI 3.39–19.25, $p < 0.001$). This linkage persisted even after PSM (light: adjusted OR after PSM 1.72, 95% CI 0.52–5.69, $p = 0.374$; moderate: adjusted OR after PSM 2.63, 95% CI 1.18–5.88, $p = 0.018$; heavy: adjusted OR after PSM 3.25, 95% CI 1.21–8.67, $p = 0.019$) (Table 5).

Additionally, the AUC of the ROC curve for smoking predicting the severity of AP was 0.708 (Figure 2), showing a risk of "MSAP or SAP" with a moderate predictive value.

TABLE 1 Baseline characteristics of the study population by smoking history.

Characteristics	Total patients ($n = 508$)	Smoking status		*P*-value
		Never ($n = 355$)	Current ($n = 153$)	
Demographic				
Age, years [median (Q1, Q3)]	52 (38, 6)	55 (38, 6)	48 (37, 6)	0.057
Male gender (*n*, %)	279 (55)	139 (39)	140 (92)	<0.001
Drinking history, (*n*, %)	90 (18)	25 (7)	65 (42)	<0.001
Hypertension, (*n*, %)	148 (29)	106 (30)	42 (27)	0.659
Diabetes, (*n*, %)	101 (20)	68 (19)	33 (22)	0.614
Hyperlipemia, (*n*, %)	31 (6)	20 (6)	11 (7)	0.638
Cardiovascular disease, (*n*, %)	63 (12)	44 (12)	19 (12)	0.994
Laboratory findings				
WBC count, $\times 10^9$/L, [median (Q1, Q3)]	12.57 (9.5, 15.9)	11.2 (9.0, 14.0)	14.85 (12.4, 18.0)	<0.001
RBC count, $\times 10^{12}$/L, (mean ± SD)	4.59 ± 0.7	4.48 ± 0.68	4.84 ± 0.67	<0.001
HGB level, ×g/L, (mean ± SD)	143.12 ± 24.05	138.57 ± 23.14	153.67 ± 22.87	<0.001
PLT count, $\times 10^9$/L [median (Q1, Q3)]	226.5 (180.0, 277.5)	227 (182.0, 272.5)	224 (176.0, 280.0)	0.779
K+, ×mmol/L [median (Q1, Q3)]	3.9 (3.6, 4.2)	3.9 (3.6, 4.2)	4 (3.7, 4.2)	0.081
Na+, ×mmol/L, [median (Q1, Q3)]	138 (135.0, 141.0)	138 (136.0, 141.0)	138 (135.0, 140.0)	0.023
Ca+, ×mmol/L, [median (Q1, Q3)]	2.22 (2.1, 2.3)	2.23 (2.1, 2.3)	2.19 (2.0, 2.3)	0.015
Cr, ×μmol/L, [median (Q1, Q3)]	59 (47.7, 75.0)	56 (45.0, 72.0)	70 (56.0, 83.0)	<0.001
BG, ×mmol/L, [median (Q1, Q3)]	8 (6.4, 11.6)	7.8 (6.3, 10.7)	8.6 (6.5, 13.6)	0.009
ALT, ×U/L, [median (Q1, Q3)]	41 (23.0, 136.7)	43 (23.5, 139.0)	38 (23.0, 112.0)	0.343
LDH, ×U/L, [median (Q1, Q3)]	283 (208.7,463.0)	269 (203.0, 443.5)	305 (218.0, 529.0)	0.032
TBIL, ×μmol/L, [median (Q1, Q3)]	19 (13.0, 35.0)	19 (12.0, 33.0)	20 (15.0, 35.0)	0.047
ALB, ×g/L, [median (Q1, Q3)]	41 (37.0, 44.0)	41 (37.3, 44.0)	41.1 (36.0, 44.0)	0.539
AMY, ×U/L, [median (Q1, Q3)]	609 (200.7, 1,359.5)	611 (197.5, 1,344.0)	604 (248.0, 1,491.0)	0.928
LPS, ×U/L, [median (Q1, Q3)]	2,148 (1,005.7, 4,336.2)	2,081 (901.5, 4,262.0)	2,318 (1,282.0, 4,535.0)	0.239
D-Dimer, ×μg/ml, [median (Q1, Q3)]	7.5 (2.8, 16.2)	7.4 (2.9, 16.2)	7.6 (2.5, 16.5)	0.522
CRP, ×mg/ml, [median (Q1, Q3)]	52.7 (9.4, 127.5)	38.9 (8.2, 122.0)	66.7 (15.6, 142.0)	0.003
PT, ×second, [median (Q1, Q3)]	13.2 (12.5, 14.4)	13.1 (12.4, 14.2)	13.5 (12.8, 15.1)	0.019
APTT, ×second, [median (Q1, Q3)]	30.3 (26.9,34.1)	30 (26.7, 33.5)	31.5 (27.4, 35.1)	0.012

SD, standard deviation; WBC, white blood cell count; RBC, red blood cell count; HGB, hemoglobin level; PLT, platelet; K+, potassium; Na+, sodium; Ca+, calcium; Cr, creatinine; BG, blood glucose; ALT, glutamic-pyruvic transaminase; LDH, lactate dehydrogenase; TBIL, total bilirubin; ALB, albumin; AMY, amylase; LPS, lipase; CRP, C-reactive protein; PT, prothrombin Time; APTT, activated partial thromboplastin time.

Dose-response linkage and interaction analysis

In the AP patients studied, both before PSM (17.1% in non-smokers, 37.5% in light smokers, 56.6% in moderate smokers, and 66% in heavy smokers) and after PSM (18.9% in non-smokers, 31% in light smokers, 56.8% in moderate smokers, and 55.5% in heavy smokers), the incidence of "MSAP or SAP" increased with increasing smoking pack-years (Figure 3). Before and after PSM, there was a dose-response linkage between the risk of "MSAP or SAP" and smoking pack-years (before PSM: Figure 4; after PSM: Figure 5). Non-smokers had a lower risk of "MSAP or SAP" compared to smokers (Figures 4A, 5A). According to the level of smoking pack-years, the predicted and observed rates of MSAP or SAP increased with higher smoking pack-years, and the predicted and observed rates of MSAP or SAP were consistent (Figures 4B, 5B), indicating a dose-response relationship. Additionally, possible interactions between smoking and other factors were assessed in this study (Figure 6). There were no discernible interactions between smoking and the other factors ($p > 0.05$). Colinearity analysis shows that no collinearity exists between variables (eTable). All in all, these findings suggest that smoking is an independent risk factor for exacerbating AP.

TABLE 2 Univariable and multivariable analysis for "MSAP or SAP" in AP patients.

Variables	Univariable			Multivariable		
	OR	95%CI	*P*-value	OR	95%CI	*P*-value
Demographic						
Age	1.00	0.99–1.01	0.998	<NA>	<NA>	<NA>
Male gender	1.44	0.97–2.14	0.065	<NA>	<NA>	<NA>
Smoking	5.86	3.85–8.94	<0.001	5.01	2.76–9.09	<0.001
Drinking history	1.58	0.97–2.55	0.061	<NA>	<NA>	<NA>
Hypertension,	1.13	0.74–1.72	0.551	<NA>	<NA>	<NA>
Diabetes	1.88	1.19–2.97	0.006	1.37	0.65–2.88	0.395
Hyperlipemia	1.13	0.74–1.72	0.551	<NA>	<NA>	<NA>
Cardiovascular disease	1.29	0.73–2.27	0.369	<NA>	<NA>	<NA>
Laboratory findings						
WBC count	1.25	1.19–1.32	<0.001	1.21	1.12–1.29	<0.001
RBC count	1.58	1.19–2.11	0.002	1.26	0.65–2.43	0.485
HGB level	1.02	1.00–1.02	0.005	0.97	0.96–0.99	0.016
PLT count	1.00	1.00–1.01	0.014	1.00	0.99–1.01	0.375
K+	1.64	1.16–2.32	0.005	1.24	0.68–2.29	0.474
Na+	0.99	0.97–1.01	0.811	<NA>	<NA>	<NA>
Ca+	0.01	0.01–0.38	<0.001	0.01	0.00–0.04	<0.001
Cr	1.00	1.00–1.01	0.001	1.00	0.99–1.01	0.380
BG	1.17	1.11–1.23	<0.001	1.12	1.04–1.21	0.001
ALT	1.00	0.99–1.00	0.660	<NA>	<NA>	<NA>
LDH	1.00	1.00–1.01	0.001	1.00	1.00–1.00	0.414
TBIL	1.01	1.00–1.01	0.030	1.00	0.99–1.01	0.415
ALB	0.91	0.88–0.95	<0.001	1.02	0.96–1.08	0.486
AMY	1.00	1.00–1.00	0.043	1.00	1.00–1.00	0.039
LPS	1.00	1.00–1.00	0.149	<NA>	<NA>	<NA>
D-Dimer	1.00	0.99–1.00	0.943	<NA>	<NA>	<NA>
CRP	1.01	1.00–1.00	<0.001	1.00	0.99–1.00	0.295
PT	1.21	1.10–1.34	<0.001	1.14	1.02–1.29	0.021
APTT	1.04	1.01–1.07	0.010	1.00	0.95–1.04	0.985

P-value is from univariable and multivariable ($P < 0.05$ indicates statistical significance).

Discussion

In our study, we found that smoking is independently associated with the severity of AP. Compared to AP patients who have never smoked, smokers with AP have a more severe condition and a higher incidence of "MSAP or SAP". Moreover, there is a dose-response linkage between smoking pack-years and the risk of "MSAP or SAP", with the risk increasing with higher smoking pack-years. Even accounting for possible demographic and clinical variables, the association between smoking and the occurrence of "MSAP or SAP" remained significant. This suggests that smoking itself exacerbates AP. PSM also yielded consistent results, confirming that the linkage between smoking and the severity of pancreatitis cannot be attributable solely to baseline variation within the two groups.

Previous studies have indicated that smoking increases the risk of AP. A cohort study by Tolstrup et al. (10), involving 17,905 patients, observed that 46% of AP cases during the follow-up period could be attributed to smoking. They noted a dose-response relationship between smoking pack-years (15–29 pack-years) and the hazard ratio (HR) for AP (HR 2.2; 95% CI 1.2–3.8). Similarly, a large-scale study by Lee et al. (16) ($N = 4{,}238{,}822$) found that compared to non-smokers, current smokers had an adjusted HR of 1.66 (CI, 1.53–1.8) for acute pancreatitis, higher than that of former smokers (HR 1.34; CI, 1.17–1.54). China's largest prospective cohort study (17) ($n = 512{,}891$) also demonstrated an increased

TABLE 3 Patient baseline characteristics before and after PSM by smoking history.

Variables	Before PSM			After PSM		
	Never smoking ($n = 355$)	Current smoking ($n = 153$)	SMD	Never smoking ($n = 95$)	Current smoking ($n = 95$)	SMD
Demographic						
Age, ×years [mean (SD)]	53.73 (18.03)	50.79 (17.93)	0.163	50.21 (18.35)	50.98 (17.90)	0.042
Male gender (*n*, %)	139 (39.2)	140 (91.5)	1.317	85 (89.5)	83 (87.4)	0.066
Drinking history (*n*, %)	25 (7.0)	65 (42.5)	0.900	24 (25.3)	27 (28.4)	0.071
Hypertension (*n*, %)	106 (29.9)	42 (27.5	0.053	24 (25.3)	23 (24.2	0.024
Diabetes (*n*, %)	68 (19.2)	33 (21.6)	0.060	16 (16.8)	15 (15.8)	0.028
Hyperlipemia (*n*, %)	20 (5.6)	11 (7.2)	0.06	3 (3.2)	2 (2.1	0.066
Cardiovascular disease (*n*, %)	44 (12.4)	19 (12.4)	0.001	9 (9.5)	11 (11.6)	0.069
Laboratory findings						
WBC count, $\times 10^9$/L [mean (SD)]	11.89 (4.40)	15.52 (5.29)	0.748	14.09 (4.69)	14.23 (4.22)	0.033
RBC count, $\times 10^{12}$/L [mean (SD)]	4.48 (0.68)	4.84 (0.67)	0.531	4.83 (0.67)	4.80 (0.70)	0.045
HGB level, ×g/L [mean (SD)]	138.57 (23.14)	153.67 (22.87	0.657	152.12 (20.83)	151.26 (23.13)	0.039
PLT count, $\times 10^9$/L [mean (SD)]	229.36 (73.11)	228.45 (76.22)	0.012	228.16 (63.73)	224.53 (71.37)	0.054
K^+, ×mmol/L [mean (SD)]	3.92 (0.48)	4.04 (0.69)	0.208	4.00 (0.53)	3.91 (0.53)	0.170
Na^+, ×mmol/L [mean (SD)]	137.97 (8.20)	136.52 (11.58)	0.145	136.29 (14.27)	136.31 (14.24)	0.001
Ca^+, ×mmol/L [mean (SD)]	2.21 (0.24)	2.15 (0.24)	0.267	2.19 (0.23)	2.17 (0.21)	0.083
Cr, ×μmol/L [mean (SD)]	64.68 (49.15)	91.04 (117.40)	0.293	71.72 (32.56)	70.51 (24.92)	0.042
BG, ×mmol/L [mean (SD)]	8.97 (4.01)	10.37 (5.06)	0.307	9.33 (3.92)	9.53 (4.81)	0.046
ALT, ×U/L [mean (SD)]	127.09 (188.77)	129.16 (229.55)	0.010	98.81 (134.79)	119.64 (187.10)	0.128
LDH, ×U/L [mean (SD)]	404.68 (359.59)	488.22 (546.71)	0.181	401.82 (330.68)	395.21 (319.08)	0.020
TBIL, ×μmol/L [mean (SD)]	29.13 (31.80)	34.64 (38.44)	0.156	30.04 (26.74)	32.43 (37.95)	0.073
ALB, ×g/L [mean (SD)]	40.56 (5.53)	39.85 (6.18)	0.122	40.23 (6.52)	40.27 (6.20)	0.006
AMY, ×U/L [mean (SD)]	996.43 (1,096.61)	1,093.03 (1,300.16)	0.080	838.94 (996.26)	912.34 (1,022.56)	0.073
LPS, ×U/L [mean (SD)]	3,063.51 (2,960.17)	3,132.59 (2,561.67)	0.025	2,707.83 (2,575.56)	2,805.33 (2,353.06)	0.040
D-Dimer, ×μg/ml [mean (SD)]	15.05 (20.76)	16.93 (22.15)	0.088	15.45 (20.12)	17.77 (23.01)	0.107
CRP, ×mg/ml [mean (SD)]	68.74 (75.35)	100.93 (107.91)	0.346	75.22 (84.66)	82.35 (97.02)	0.078
PT, ×second [mean (SD)]	13.67 (2.77)	14.03 (2.45)	0.135	14.04 (3.70)	13.73 (1.82)	0.10
APTT, ×second [mean (SD)]	30.76 (7.37	32.02 (6.89)	0.176	32.52 (11.61)	32.10 (6.06)	0.045

SMD, standardized mean difference, used to evaluate the balance before and after PSM ≥ 0.5 indicates imbalance; PSM, propensity score matching; SD, standard deviation; WBC, white blood cell count; RBC, red blood cell count; HGB, hemoglobin level; PLT, platelet; K+, potassium; Na+, sodium; Ca+, calcium; Cr, creatinine; BG, blood glucose; ALT, glutamic-pyruvic transaminase; LDH, lactate dehydrogenase; TBIL, total bilirubin; ALB, albumin; AMY, amylase; LPS, Lipase; CRP, C-reactive protein; PT, prothrombin time; APTT, activated partial thromboplastin time.

risk of AP among current male smokers (HR 1.45; 95% CI 1.28–1.64; $P = 0.02$). While aforesaid studies revealed that smoking increased the prevalence of AP, few have focused on the linkage between smoking and the severity of AP. Kim et al. (18) conducted a retrospective analysis of 905 AP patients and found that smoking was an independent risk factor for the development of SAP (OR: 7.22; 95% CI: 1.05–49.69; $P = 0.04$).

Several mechanisms may underlie the observed exacerbation of AP severity by smoking. Firstly, smoking directly damages pancreatic tissue through intricate mechanisms. Overall, chronic smoking exposure promotes pancreatic fibrosis, calcification, and chronic inflammation. This leads to premature activation of pancreatic enzymes and reduced secretion, causing retention of active digestive enzymes within acinar cells, exacerbating pancreatic autodigestion (14, 19, 20). This may increase the risk of complications in AP patients, such as pancreatic leakage, accumulation of necrotic pancreatic material, pseudocysts, and pancreatic abscesses. Additionally, pancreatic necrosis exacerbates systemic inflammation by releasing various inflammatory mediators into the bloodstream, potentially leading to multi-organ

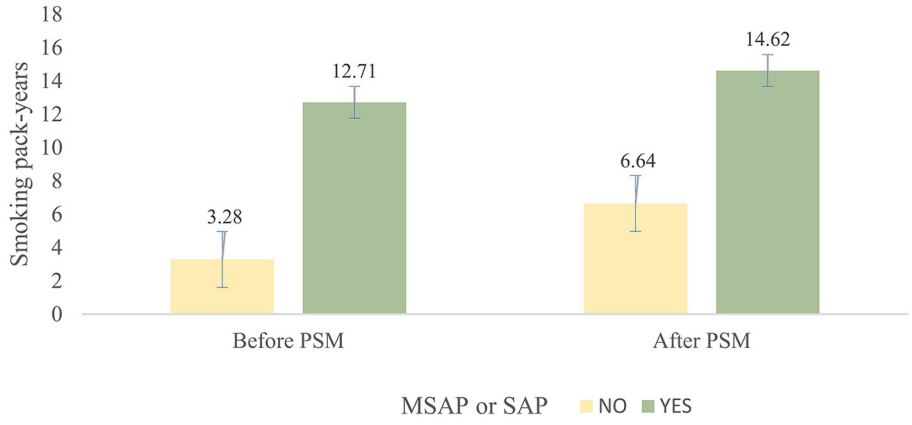


FIGURE 1
Mean and standard deviation of pack-years between the smoking group and non-smoking group before and after PSM.

TABLE 4 Comparison of the incidence of "MSAP or SAP" after PSM based on smoking status.

MSAP or SAP	Before PSM		*P**-value	After PSM		*P*-value
	Never smoking ($n = 355$)	Current smoking ($n = 153$)		Never smoking ($n = 95$)	Current smoking ($n = 95$)	
Yes	61 (17.1%)	84 (54.9%)	<0.001	18 (9.4%)	47 (24.7%)	<0.001
No	294 (82.8%)	69 (45.0%)		77 (40.5%)	48 (25.2%)	

*P-value is from chi-squared test to indicate significant differentiation ($P < 0.05$ means significant differentiation).

failure (21–24). For instance, Garg et al. (25) demonstrated a bidirectional relationship between the severity of pancreatic necrosis and organ failure. The extent of pancreatic necrosis influences the severity of organ failure, while organ failure exacerbates the progression of pancreatic necrosis. In a meta-analysis by Petrov et al. (26) based on 1,478 AP patients, it was found that the impact of organ failure and pancreatic necrosis on mortality was comparable; the presence of either indicates severe disease. Multi-organ dysfunction syndrome, extent of pancreatic necrosis, infection, and sepsis are major determinants of mortality in AP.

Secondly, ingredients of tobacco that can lead to inflammation in a number of different disorders. When acute pancreatitis (AP) occurs, this effect may exacerbate the severity of AP. For instance, a prospective study by Colak et al. (27) involving 98,085 participants found that plasma C-reactive protein levels increased by 4.8% (95% CI 4.4–5.2%) for every 10 pack-years and by 1.6% (95% CI 0.4–2.8%) for each T allele, indicating that both genetically and observably, greater tobacco use is linked to increased systemic inflammation. According to Liu et al. (28), nicotine can cause inflammatory reactions by triggering signaling pathways including STAT3 and NF-κB that are linked to inflammation. This causes increased intracellular inflammation, inflammatory cells to be recruited, inflammatory mediators to be induced, and tissue damage and inflammation to worsen. Nicotine also can activate AChR expressed on immune cell surfaces, thereby reducing the immunological response and blocking macrophages and lymphocytes from functioning (29). This may make inflammation and infection more likely.

Lastly, Smoking itself can act as a risk factor for various organ diseases (30), such as chronic obstructive pulmonary disease (COPD), atherosclerosis, coronary artery disease, myocardial infarction, heart failure, and stroke. Moreover, both moderately MSAP and SAP can result in varying degrees of organ failure. Prolonged exposure to tobacco may exacerbate this process, leading to an increased risk of susceptible organ infections and failure. For instance, NNK in tobacco suppresses the production of IL-8, which plays a crucial role in acute inflammation by recruiting and activating neutrophils. A reduction in IL-8 may lead to an increased incidence of pulmonary infections (31, 32). Munzel et al. (33) reported that smoking can lead to endothelial dysfunction, increased oxidative stress, and cardiovascular events.

It's worth noting that in our study, the increase in risk and incidence rate of "MSAP or SAP" after PSM exhibits a lower inflection point compared to pre-PSM (1 pack-year before PSM, 2 pack-years after PSM). However, irrespective of pre or post PSM, when smoking pack-years are ≤2, the corresponding OR values remain close to 1, consistent with our post-PSM findings regarding the risk of "MSAP or SAP" (smoking pack-years ≤10, OR 1.72, 95% CI 0.52–5.69, $P = 0.374$). This suggests that at lower levels of smoking pack-years, the risk of "MSAP or SAP" occurrence isn't significantly different from that of non-smokers. Smoking needs a certain accumulation time to noticeably exacerbate the severity of AP, with the specific threshold currently unknown. Similar cumulative effects of smoking have been documented in other studies. For instance, a comprehensive study by Tolstrup et al. (10) revealed no significant difference in pancreatitis incidence between smokers consuming 1–14 g/day and never-smokers, yielding a HR

TABLE 5 Comparison of the unadjusted and risk-adjusted OR by different smoking status.

Type	Smoking status	Events *n* (%)	Unadjusted OR	*P*-value	Multivariable regression adjusted OR	*P*-value	PSM adjusted OR	*P*-value
Continuous	Per 1	NA	1.08 (1.05–1.10)	<0.001	1.07 (1.04–1.10)	<0.001	NA	NA
Cut off	Never	61 (3.3)	1 (Reference)	NA	1 (Reference)	NA	1 (Reference)	NA
	Current	84 (16.5)	5.86 (3.85–8.94)	<0.001	5.01 (2.76–9.09)	<0.001	4.18 (2.18–8.03)	<0.001
Smoking degree	Never	61 (3.3)	1 (Reference)	NA	1 (Reference)	NA	1 (Reference)	NA
	Light	15 (2.9)	2.89 (1.44–5.08)	0.003	3.76 (1.40–10.07)	0.008	1.72 (0.52–5.69)	0.374
	Moderate	34 (6.6)	6.30 (3.52–11.26)	<0.001	4.94 (2.23–10.92)	<0.001	2.63 (1.18–5.88)	0.018
	Heavy	35 (6.8)	9.37 (4.98–17.62)	<0.001	8.08 (3.39–19.25)	<0.001	3.25 (1.21–8.67)	0.019

The factors of the multivariable regression: age, gender, drinking history, hypertension, diabetes, hyperlipemia, cardiovascular disease, WBC count, RBC count, HGB level, PLT count, K+, Na+, Ca+, Cr, BG, ALT, LDH, TBIL, ALB, AMY, LPS, D-Dimer, CRP, PT, APTT.
CI, confidence interval; OR, odds ratio; PSM, propensity scores matching.

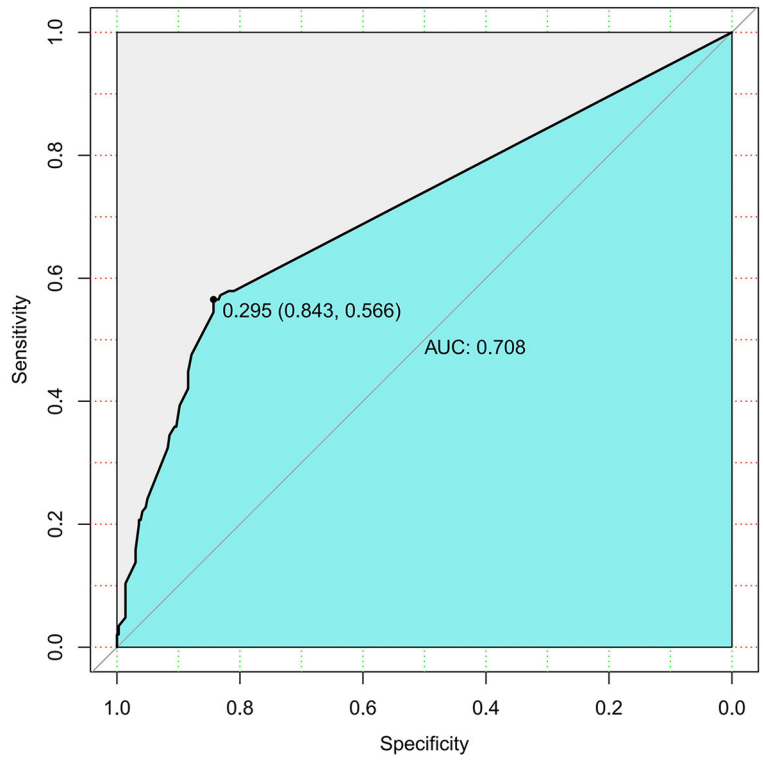


FIGURE 2
Smoking history prediction model for "MASP or SAP" rate in AP (AUC).

of 1.5 (95% CI 0.9–2.5). Pancreatitis incidence attained statistical significance only with increased smoking to 15–24 g/day, showing an HR of 2.5 (95% CI 1.5–3.9). Similarly, Hansen et al. (34), based on a prospective study of 108,438 individuals, reported HRs of 1.1 (95% CI 0.8–1.7, with no statistical significance) and 3.6 (95% CI 1.8–2.5) for smoking pack-years of 0.1–9 and 9.1–24, respectively, for chronic pancreatitis risk. Large-scale prospective studies are imperative to elucidate the underlying mechanisms and determine the threshold for cumulative effects.

Alcohol is considered an independent risk factor for the occurrence of AP (35). According to calculations based on weekly alcohol consumption, drinking ≥5 drinks per day significantly increases the risk of developing AP (36, 37). However, in our study, we did not find a significant correlation between a history of alcohol

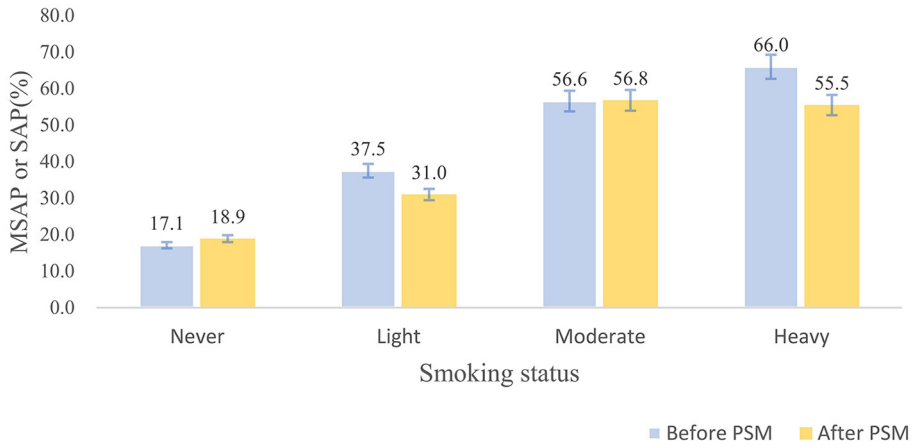


FIGURE 3
Rate of "MSAP or SAP" in each group by smoking status (never, light, moderate, and heavy smoking group).

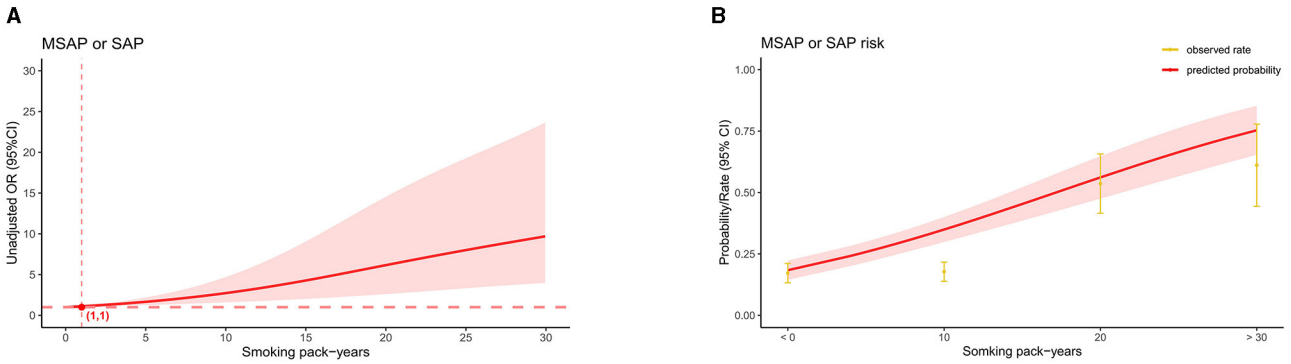


FIGURE 4
Linkage between smoking pack-years and "MASP or SAP" in patients with AP before PSM. **(A)** Adjusted odds ratios (ORs) and 95% confidence intervals (CIs) are shown for every five smoking pack-years interval. **(B)** Predicted probabilities and the observed rate of "MASP or SAP".

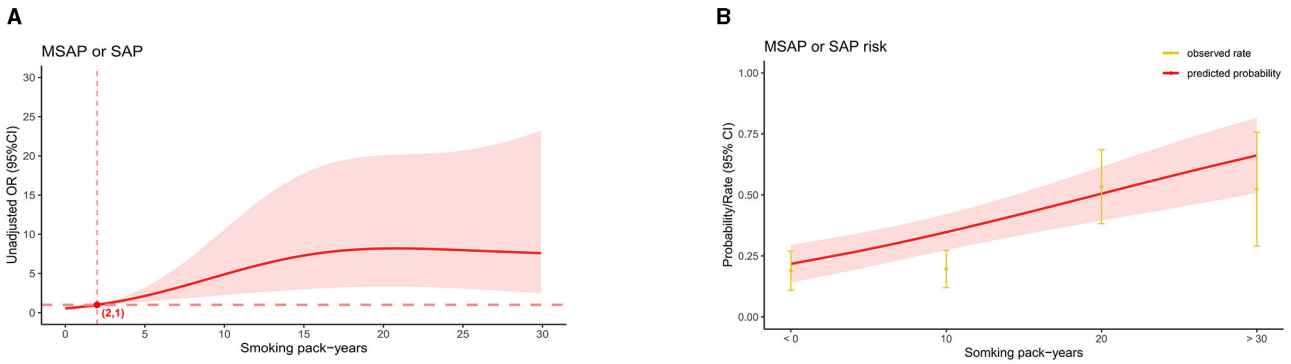
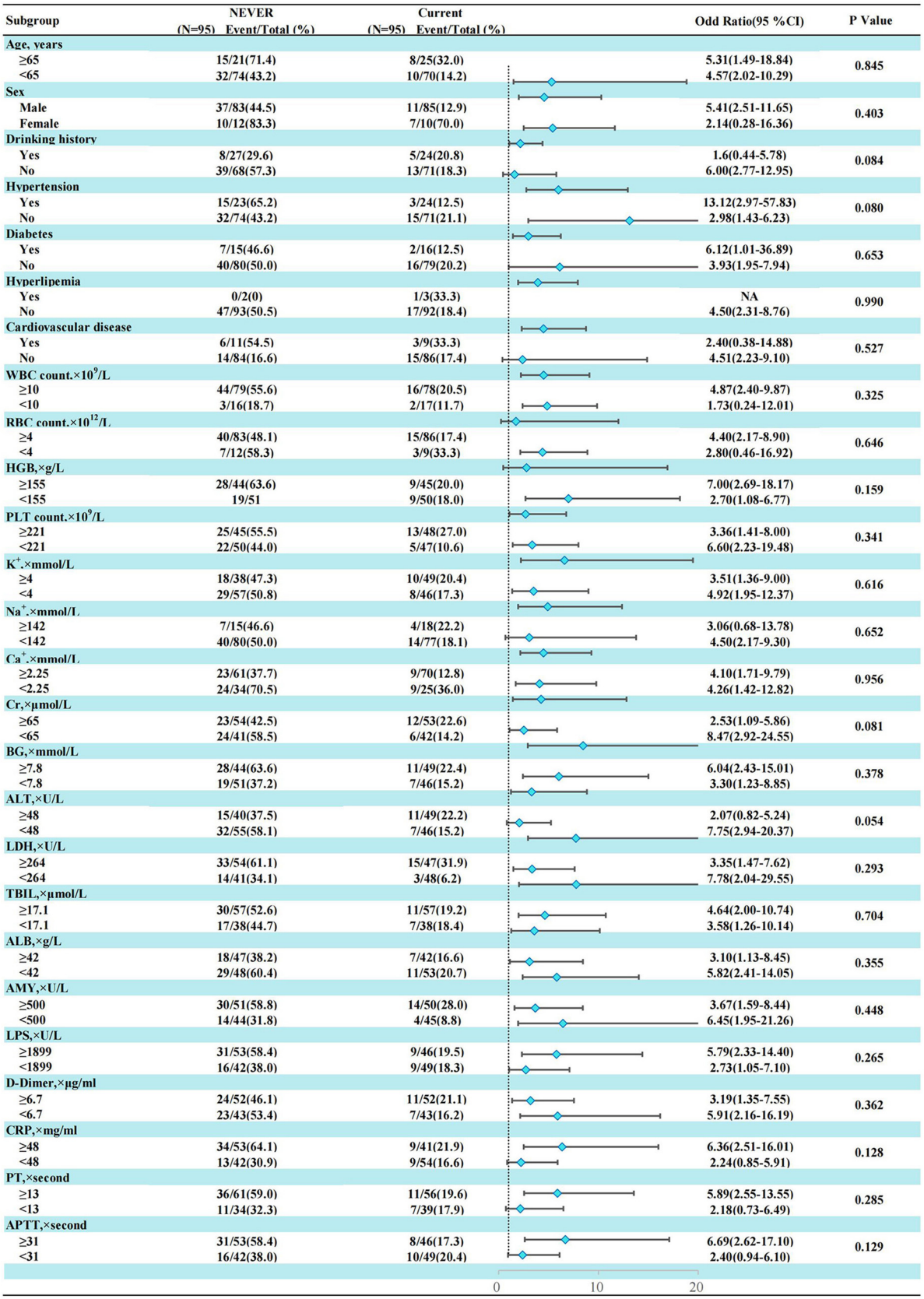


FIGURE 5
Linkage between smoking pack-years and "MASP or SAP" in patients with AP after PSM. **(A)** Adjusted odds ratios (ORs) and 95% confidence intervals (CIs) are shown for every five smoking pack-years interval. **(B)** Predicted probabilities and the observed rate of "MASP or SAP".

consumption and the severity of AP ($P = 0.061$). On one hand, alcohol consumption may be a factor in the onset of pancreatitis rather than exacerbating its severity. On the other hand, this lack of correlation may be related to the drinking patterns of the patients included in our study. We observed a higher proportion of occasional drinkers rather than daily alcohol abusers among our



Subgroup	NEVER (N=95) Event/Total (%)	Current (N=95) Event/Total (%)	Odd Ratio(95 %CI)	P Value
Age, years				
≥65	15/21(71.4)	8/25(32.0)	5.31(1.49-18.84)	0.845
<65	32/74(43.2)	10/70(14.2)	4.57(2.02-10.29)	
Sex				
Male	37/83(44.5)	11/85(12.9)	5.41(2.51-11.65)	0.403
Female	10/12(83.3)	7/10(70.0)	2.14(0.28-16.36)	
Drinking history				
Yes	8/27(29.6)	5/24(20.8)	1.6(0.44-5.78)	0.084
No	39/68(57.3)	13/71(18.3)	6.00(2.77-12.95)	
Hypertension				
Yes	15/23(65.2)	3/24(12.5)	13.12(2.97-57.83)	0.080
No	32/74(43.2)	15/71(21.1)	2.98(1.43-6.23)	
Diabetes				
Yes	7/15(46.6)	2/16(12.5)	6.12(1.01-36.89)	0.653
No	40/80(50.0)	16/79(20.2)	3.93(1.95-7.94)	
Hyperlipemia				
Yes	0/2(0)	1/3(33.3)	NA	0.990
No	47/93(50.5)	17/92(18.4)	4.50(2.31-8.76)	
Cardiovascular disease				
Yes	6/11(54.5)	3/9(33.3)	2.40(0.38-14.88)	0.527
No	14/84(16.6)	15/86(17.4)	4.51(2.23-9.10)	
WBC count.×10^9/L				
≥10	44/79(55.6)	16/78(20.5)	4.87(2.40-9.87)	0.325
<10	3/16(18.7)	2/17(11.7)	1.73(0.24-12.01)	
RBC count.×10^{12}/L				
≥4	40/83(48.1)	15/86(17.4)	4.40(2.17-8.90)	0.646
<4	7/12(58.3)	3/9(33.3)	2.80(0.46-16.92)	
HGB,×g/L				
≥155	28/44(63.6)	9/45(20.0)	7.00(2.69-18.17)	0.159
<155	19/51	9/50(18.0)	2.70(1.08-6.77)	
PLT count.×10^9/L				
≥221	25/45(55.5)	13/48(27.0)	3.36(1.41-8.00)	0.341
<221	22/50(44.0)	5/47(10.6)	6.60(2.23-19.48)	
K^+.×mmol/L				
≥4	18/38(47.3)	10/49(20.4)	3.51(1.36-9.00)	0.616
<4	29/57(50.8)	8/46(17.3)	4.92(1.95-12.37)	
Na^+.×mmol/L				
≥142	7/15(46.6)	4/18(22.2)	3.06(0.68-13.78)	0.652
<142	40/80(50.0)	14/77(18.1)	4.50(2.17-9.30)	
Ca^+.×mmol/L				
≥2.25	23/61(37.7)	9/70(12.8)	4.10(1.71-9.79)	0.956
<2.25	24/34(70.5)	9/25(36.0)	4.26(1.42-12.82)	
Cr,×μmol/L				
≥65	23/54(42.5)	12/53(22.6)	2.53(1.09-5.86)	0.081
<65	24/41(58.5)	6/42(14.2)	8.47(2.92-24.55)	
BG,×mmol/L				
≥7.8	28/44(63.6)	11/49(22.4)	6.04(2.43-15.01)	0.378
<7.8	19/51(37.2)	7/46(15.2)	3.30(1.23-8.85)	
ALT,×U/L				
≥48	15/40(37.5)	11/49(22.2)	2.07(0.82-5.24)	0.054
<48	32/55(58.1)	7/46(15.2)	7.75(2.94-20.37)	
LDH,×U/L				
≥264	33/54(61.1)	15/47(31.9)	3.35(1.47-7.62)	0.293
<264	14/41(34.1)	3/48(6.2)	7.78(2.04-29.55)	
TBIL,×μmol/L				
≥17.1	30/57(52.6)	11/57(19.2)	4.64(2.00-10.74)	0.704
<17.1	17/38(44.7)	7/38(18.4)	3.58(1.26-10.14)	
ALB,×g/L				
≥42	18/47(38.2)	7/42(16.6)	3.10(1.13-8.45)	0.355
<42	29/48(60.4)	11/53(20.7)	5.82(2.41-14.05)	
AMY,×U/L				
≥500	30/51(58.8)	14/50(28.0)	3.67(1.59-8.44)	0.448
<500	14/44(31.8)	4/45(8.8)	6.45(1.95-21.26)	
LPS,×U/L				
≥1899	31/53(58.4)	9/46(19.5)	5.79(2.33-14.40)	0.265
<1899	16/42(38.0)	9/49(18.3)	2.73(1.05-7.10)	
D-Dimer,×μg/ml				
≥6.7	24/52(46.1)	11/52(21.1)	3.19(1.35-7.55)	0.362
<6.7	23/43(53.4)	7/43(16.2)	5.91(2.16-16.19)	
CRP,×mg/ml				
≥48	34/53(64.1)	9/41(21.9)	6.36(2.51-16.01)	0.128
<48	13/42(30.9)	9/54(16.6)	2.24(0.85-5.91)	
PT,×second				
≥13	36/61(59.0)	11/56(19.6)	5.89(2.55-13.55)	0.285
<13	11/34(32.3)	7/39(17.9)	2.18(0.73-6.49)	
APTT,×second				
≥31	31/53(58.4)	8/46(17.3)	6.69(2.62-17.10)	0.129
<31	16/42(38.0)	10/49(20.4)	2.40(0.94-6.10)	

FIGURE 6
Subgroup analysis of smoking history and "MASP or SAP" in AP patients after propensity score matching.

patients. Additionally, in our study, we did not find a significant interaction between a history of alcohol consumption and smoking ($P = 0.084$).

This study boasts several strengths: we employed propensity score matching and multivariable logistic regression to rigorously control for confounding factors. The dose-response linkage analysis visually depicted the correlation between smoking pack-years and the severity of AP. Nonetheless, certain limitations should be acknowledged: the sample size was relatively small; the single-center retrospective design precludes establishing causality, necessitating further prospective and multicenter studies to validate these findings; our study only included non-smokers and current smokers, excluding former smokers, which limits our ability to determine whether smoking cessation can mitigate the exacerbating effect of smoking on the severity of AP. Additionally, Relative confounding from unmeasured factors may continue even after corrections.

Conclusion

In conclusion, our study provides evidence suggesting that smoking is associated with an increased risk of developing "MSAP or SAP" in patients with AP. We also observed a dose-response relationship between smoking pack-years and the severity of pancreatic involvement, indicating that the impact of smoking on the severity of AP may require a certain amount of time to accumulate before becoming evident. Our findings lay the groundwork for future longitudinal studies. However, whether smoking cessation can mitigate this effect requires further confirmation through new multicenter prospective randomized studies.

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 humans were approved by the Ethics Committee of Dandong Central Hospital. The studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent for participation was not required from the participants or the participants' legal guardians/next of kin in accordance with the national legislation and institutional requirements.

Author contributions

RL: Supervision, Writing – original draft, Writing – review & editing. WT: Data curation, Writing – original draft, Writing – review & editing. SY: Conceptualization, Supervision, Writing – original draft. XY: Methodology, Writing – original draft. LH: Project administration, Writing – original draft.

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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/fmed.2024.1397111/full#supplementary-material

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EDITED BY
Ravi Kumar Sharma,
Chandigarh University, India

REVIEWED BY
Vishal G. Shelat,
Tan Tock Seng Hospital, Singapore
Shubhransu Patro,
KIIT University, India
Alexandra Mikó,
University of Pécs, Hungary

*CORRESPONDENCE
Fang He
✉ zxhxb2013@126.com

†These authors have contributed equally to this work

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Development and validation of a nomogram combining pain score with laboratory indicators for predicting persistent organ failure in acute pancreatitis: a retrospective cohort study

Jiayu Xing[1,2†], Musen Xu[1†], Jiale Xu[1], Jiao Liu[1,2] and Fang He[1,2*]

[1]Shanxi Bethune Hospital, Shanxi Academy of Medical Sciences, Tongji Shanxi Hospital, Third Hospital of Shanxi Medical University, Taiyuan, China, [2]Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China

Background: Acute pancreatitis is an inflammatory disease that can lead to persistent organ failure (POF), which is associated with increased morbidity and mortality. Early prediction of POF in AP can significantly improve patient outcomes.

Objective: To develop and validate a nomogram that combines pain score with laboratory indicators for predicting POF in patients with AP.

Methods: A retrospective cohort study was conducted, including patients diagnosed with AP. Pain score and laboratory indicators were collected within the first 24 h of admission. A nomogram was developed using logistic regression models and validated in a separate cohort.

Results: There were 807 patients in the training cohort and 375 patients in the internal validation cohort.Multivariate logistic regression demonstrated that pain score, serum creatinine, hematocrit, serum calcium, and serum albumin were independent risk factors for the incidence of POF in patients with AP. The area under the curve of the nomogram constructed from the above factors were 0.924, respectively. The model demonstrated good calibration and discrimination in both the development and validation cohorts.

Conclusion: The nomogram had a good performance in predicting POF in patients with AP and can be used to guide clinical decision-making.

KEYWORDS

acute pancreatitis, persistent organ failure, nomogram, pain score, prediction model

Introduction

Acute pancreatitis (AP) is a common clinical emergency of the digestive system (1). It refers to an acute abdomen characterized by a local inflammatory reaction in the pancreas caused by abnormal activation of pancreatic enzymes that have a digestive effect on the pancreas itself and surrounding organs (2). In severe cases, it can lead to other organ dysfunction. Its typical clinical symptoms are acute onset of persistent upper abdominal pain

that can radiate to the back. In AP, persistent organ failure (POF) refers to the failure of at least one major organ system for more than 48 h. In the latest Atlanta classification criteria, severe acute pancreatitis (SAP) is defined as persistent organ failure (defined as >48 h).Therefore, POF is an important determinant of the severity of AP and is also closely related to the high mortality rate of severe acute pancreatitis (3). Studies have pointed out that the mortality rate in patients with AP combined with POF is as high as 20–30% (1). The clinical manifestations of AP are unreliable and nonspecific, and its sensitivity for predicting adverse outcomes is less than 40% (4). Therefore, early assessment of whether AP patients will develop POF is crucial to improve the prognosis of AP patients and reduce mortality.

Clinically, common scoring systems used to assess the severity of AP include: Ranson's criteria, BISAP score, modified Marshall scoring system, and SOFA score. Ranson's criteria is one of the earliest AP scoring systems, developed by Dr. John Ranson in the early 1970s. This scoring includes laboratory parameters at admission and after 48 h. In the study by Mikó A et al., the AUC of Ranson's criteria for predicting SAP was 0.81 (5). Gao et al. also clearly pointed out that the Ranson score has a quite high AUC (0.83) in identifying SAP (6). Venkatesh reported that the prognostic accuracy of Ranson's criteria for predicting SAP increased from 57.3% at admission to 73.8% after 48 h (7). However, this scoring system has its limitations; it only incorporates serological indicators during data collection, with no radiological parameters included. Furthermore, calculating the score requires at least 48 h, with an accuracy rate of approximately 75% (8). However, Ong Y pointed out that the 48-h waiting time for the Ranson score is not a disadvantage, but rather an inherent advantage, as the progression of the inflammatory response can be better assessed within 48 h (9).The BISAP score is a simple and practical scoring tool used to assess the severity of illness in patients with acute pancreatitis. It aims to provide a straightforward and rapid method to predict the mortality and complication risks in patients with acute pancreatitis. The BISAP scoring system is based on the following five clinical parameters: (1) Blood Urea Nitrogen; (2) Impaired consciousness: Glasgow Coma Scale; (3) Systemic Inflammatory Response Syndrome; (4) Age; (5) Pleural effusion. The BISAP score ranges from 0 to 5, with higher scores indicating more severe illness and increased risks of mortality and complications. A BISAP score of $\geq$3 is a statistically significant threshold value, with the AUC for predicting SAP and mortality being 0.875 and 0.740, respectively (10). Kapadia NN et al. pointed out that the specificity of BISAP in predicting SAP is 94.62% (11). However, the assessment of the patient's mental state in the BISAP score is often subjective. If rigorousness is pursued, it needs to be compared with the patient's baseline mental state, but these are unknown. Additionally, pleural effusion is a complication that may develop in SAP over time and may not be present at the time of admission. The modified Marshall scoring system was originally designed to assess organ dysfunction and predict outcomes in critically ill patients in intensive care and has also been utilized for predicting SAP. The design of the modified Marshall scoring system includes the following three organ systems: (1) respiratory system; (2) circulatory system; (3) renal function. C et al. found that the modified Marshall score's sensitivity, specificity, and AUC for predicting SAP were 83.33, 87.5%, and 0.938, respectively (12). SOFA score is a scoring tool used to evaluate the extent of organ function failure in patients, widely utilized in intensive care units. Initially proposed by Vincent and colleagues in 1996, its purpose is to describe and quantify the severity of organ failure and predict the prognosis of critically ill patients. The SOFA scoring system includes functional assessments of six organ systems: (1) respiratory; (2) coagulation; (3) liver; (4) cardiovascular; (5) central nervous; (6) renal functions. Studies indicate that the SOFA score has an AUC of 0.966 for predicting SAP (13). While the diversity of variables increases the sensitivity and specificity of the SOFA score, it complicates its use in clinical practice. These scoring systems lack sufficient research evidence for patients with POF. A recent review highlights that current early predictive markers for POF are not sufficiently accurate for individualized patient predictions. The ideal predictive marker should be applicable at the time of admission or within 24 h of onset, with an accuracy rate exceeding 90% (14).

The genesis of the pain scoring system can be traced back to the International Association for the Study of Pain (IASP) in 1979, defining pain as 'an unpleasant sensory and emotional experience associated with actual or potential tissue damage.' Over time, this definition has evolved to emphasize the influence of cognitive and social factors on the perception of pain. In clinical practice, pain scoring assists physicians and nurses in accurately assessing the severity of a patient's pain, devising appropriate treatment plans, and monitoring therapeutic outcomes (15). Professional societies recommend that pain should be used as a biological indicator similar to other vital signs to jointly assess a patient's vital status (16).

A nomogram is a statistical graphic constructed using clinical data, commonly employed to evaluate the prognosis of diseases. It generates the probability of an event's occurrence by integrating clinical variables. Compared to traditional scoring charts, nomograms provide a more intuitive representation of the likelihood of outcomes based on various clinical indicators, facilitating numerical calculations (17).

This study aims to develop a predictive model that combines pain scoring with laboratory indicators to forecast the occurrence of POF in patients with AP. Utilizing diagnostic markers within the first 24 h of admission, the model seeks to promptly identify individuals at high risk for POF, thereby guiding clinicians to implement personalized diagnostic and therapeutic measures to improve prognosis.

Materials and methods

Study design and population

This study is a retrospective cohort study. It encompasses patients hospitalized and initially diagnosed with AP at Shanxi Bethune Hospital from January 1, 2012, to January 1, 2022. As a retrospective study, all data were derived from our hospital's Hospital Information System, negating the need for informed consent from each patient (in accordance with the ethical requirements of clinical retrospective research). Moreover, the study design and implementation strictly adhered to the principles of the 1975 Declaration of Helsinki and received approval from the Ethics Committee of Shanxi Bethune Hospital (Ethical Approval Number: YXLL-2023-237). All AP patients in the study cohort were randomly divided into a training cohort and an internal validation cohort at a ratio of 7:3. Within the training set, patients were grouped based on the presence or absence of POF, and through univariate and multivariate analyses, independent risk factors for the occurrence of POF in AP patients were identified to establish a clinical prediction model, which was then validated in the validation group.

Inclusion and exclusion criteria

Inclusion Criteria: (1) Meets the diagnostic criteria for AP; (2) Age ≥ 18 years; (3) Admission within 48 h of onset; (4) Hospitalized at Shanxi Bethune Hospital and initially diagnosed with AP.

Exclusion Criteria: (1) Incomplete clinical data or missing medical records; (2) Recurrent AP; (3) Chronic pancreatitis, trauma, or pregnancy-related pancreatitis; (4) Cancer patients; (5) Pre-existing severe dysfunction of the heart, brain, lungs, kidneys, etc., diagnosed before the onset; (6) Age < 18 years.

Data collection and definitions

Data were retrospectively retrieved from electronic medical records, encompassing variables such as demographics: age, gender, Body Mass Index (BMI), smoking status, alcohol consumption, hypertension, length of hospital stay, and laboratory indicators within 24 h of admission: complete blood count, liver and kidney function, pancreatic function, coagulation profile, among others.

The diagnostic criteria for Acute Pancreatitis (AP) adhere to the 2012 revision of the Atlanta Classification: (1) Persistent upper abdominal pain; (2) Serum amylase and/or lipase levels more than three times the upper limit of normal; (3) Abdominal imaging findings consistent with changes of acute pancreatitis.A diagnosis of AP can be established if two of the above three criteria are met.

The diagnostic criteria for organ failure are based on the modified Marshall scoring system, where a score of ≥2 for any organ is defined as organ failure. If the condition persists for more than 48 h, it is classified as persistent organ failure.

Statistical analysis

Statistical analyses were conducted using SPSS 26.0 and R version 4.3.2. Quantitative data following a normal distribution are presented as mean ± standard deviation, and comparisons between groups were performed using independent samples t-tests. Non-normally distributed quantitative data are expressed as median [IQR, P25; P75], with group comparisons conducted using the Mann–Whitney U test. Counts are presented as the number of cases and percentages. Comparisons between groups were made using the Chi-square (X^2) test. A p-value <0.05 was considered statistically significant.

Variables deemed significant in the univariate analysis were incorporated into a binary logistic regression equation for multivariate analysis. The results will be used to construct a nomogram for predicting the occurrence of POF in patients with AP. The area under the curve (AUC) and 95% confidence intervals for the predictive models in the training and validation cohorts were determined using the receiver operating characteristic (ROC) curve. Additionally, calibration curves and clinical decision curves (DCA) were plotted.

Results

Between January 1, 2012, and January 1, 2022, a total of 1,489 patients were diagnosed with AP at Shanxi Bethune Hospital. Following the inclusion and exclusion criteria, 1,182 patients were ultimately eligible for the study (Figure 1).

Comparison of basic characteristics and clinical parameters between patients grouped by POF

In the training set of 807 patients, they were grouped according to the occurrence of POF, with 261 cases in the POF group and 546 cases in the non-POF group (Table 1). A comparison was made between the two groups regarding basic clinical characteristics upon admission, pain scoring, and laboratory test indicators.

Basic Clinical Characteristics: There were no significant differences between the two groups in terms of gender, BMI, smoking, alcohol consumption, hypertension, etc. ($p > 0.05$). However, there were statistical differences in age, hospital stay duration, and pain scores ($p < 0.05$).

Laboratory Tests: Statistically significant differences were observed between the two groups in AST, ALB, DB, Urea, SCr, AMY, LPS, Ca, PT, FIB, WBC, NEUT, LYMPH, HCT, RDW, PLT, MPV, PDW, PLR, and NLR ($p < 0.05$). No significant differences were noted in the remaining laboratory parameters.

Construction of predictive model

The indicators that differed between the two groups mentioned above were subjected to univariate and multivariate logistic regression analyses (Table 2). Pain scores, ALB, SCr, Ca, and HCT were identified as independent risk factors for predicting the occurrence of POF in AP.

Development of a predictive model nomogram

Based on these results, a predictive model nomogram was established (Figure 2). Each indicator in the figure corresponds to its test result, allowing for the determination of respective predictive scores. By aggregating the scores of each indicator, a total predictive score can be ascertained, which corresponds to the probability of POF.

Performance evaluation of the predictive model

The model was validated using a validation dataset. Its performance was assessed in terms of discrimination, calibration, and clinical utility. As illustrated in Figures 3A,B, the ROC curves for both the training and validation sets demonstrated the model's capability to differentiate outcome events: the AUC for the training set was 0.924, and for the validation set, it was 0.941. The calibration curves in Figures 4A,B further supported the model's validity, showing a close correlation between the predicted outcome events and actual occurrences. To further elucidate the clinical utility of the model, Decision Curve Analysis (DCA) was plotted in Figures 5A,B, indicating that our model provides a significant clinical net benefit. The results confirm that our model can accurately predict whether patients with AP will develop

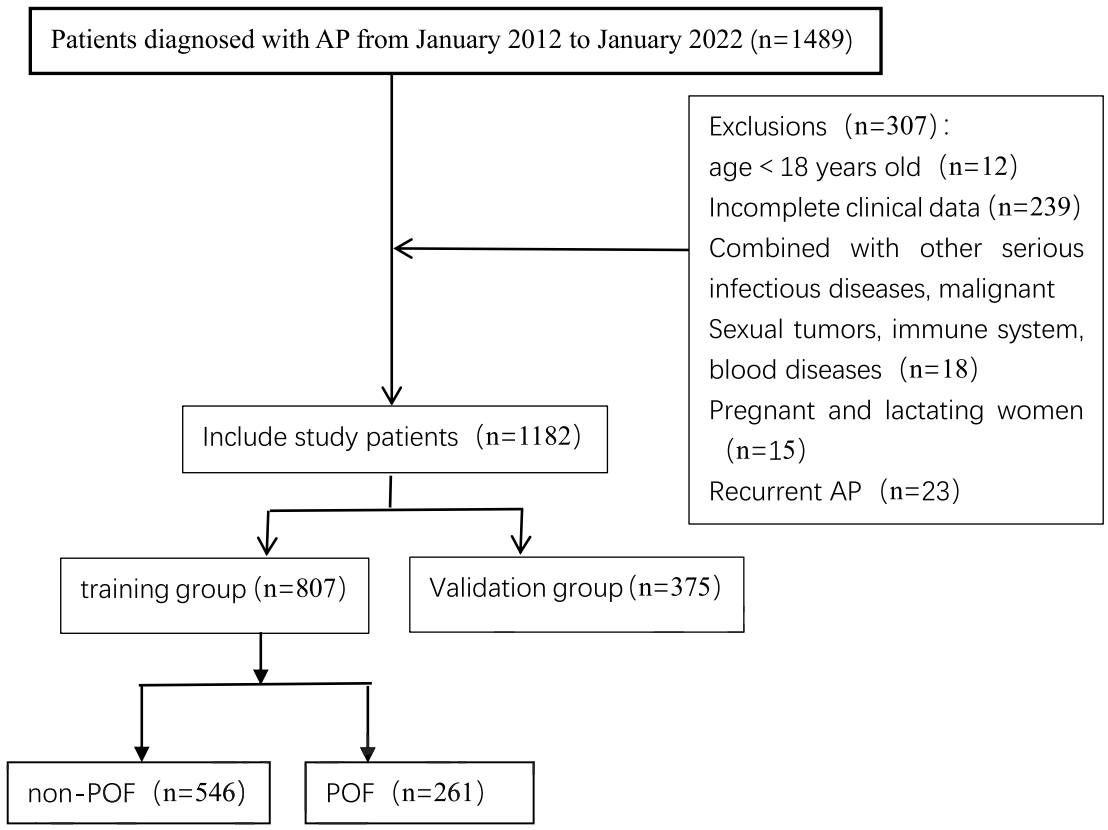

FIGURE 1
Flowchart for patient selection.

POF, underscoring its positive clinical implications. In addition, We validated our prediction model using the Hosmer-Lemeshow test and the coefficient of determination (R^2). Hosmer-Lemeshow test on the internal validation cohort: The p-value was 0.086 ($p > 0.05$), indicating that there was no significant difference between the observed and predicted values. Coefficient of determination (R^2) for the internal validation cohort: The R^2 value was 0.546. These results indicate that our model demonstrates a good fit.

Web-based calculator

Although nomograms are intuitive, convenient, and cost-effective, they cannot provide precise numerical values during the calculation process. Therefore, we have developed a web-based calculator founded on nomogram principles to streamline the calculation process and obtain more accurate predictive values (https://xu-123.shinyapps.io/DynNomapp/). Select the appropriate variable values on the left, then click 'Predict'. The probability of POF occurrence and the confidence interval will be displayed in the graph on the right. Please remember to click the 'quit' button after each use (Figure 6).

Discussion

The occurrence of POF in conjunction with AP often signifies a poor prognosis for the patient. Studies indicate that organ failure lasting ≤48 h is usually associated with a lower risk of complications and mortality. However, patients with AP who develop POF lasting >48 h have a mortality rate as high as 50% (1). Early identification of risk factors associated with POF is crucial for the prognostic management of AP. The indicators collected in this study were obtained from the initial examination within 24 h of patient admission, minimizing interference from subsequent clinical treatments. Furthermore, we offer two visualization models for clinicians to choose from: a nomogram and a web-based calculator. The nomogram is straightforward, while the web-based calculator is convenient and precise. Our model has been validated and exhibits excellent predictive capabilities for AP with POF, facilitating early intervention by clinicians and reducing the mortality rate associated with POF.

Abdominal pain is an indispensable evaluation criterion in the diagnostic standards for acute pancreatitis. The latest prognostic scoring system for pancreatitis, the PASS system, also incorporates abdominal pain into its evaluation criteria (18). Pain is a subjective experience that is influenced by a variety of factors, both physiological and psychological. Additionally, there are individual differences in the understanding and perception of pain (19). Accurate and objective assessment of pain is crucial in clinical practice for the diagnosis and subsequent treatment of patients. At our institution, Assess using the Numeric Rating Scale (NRS) before implementing any pain management measures upon patient admission. Patients rate their pain intensity on a scale from 0 (no pain) to 10 (most severe pain). This scale can be rapidly implemented following training of medical staff and is considered the gold standard for pain assessment due to its

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TABLE 1 Comparison of general characteristics and indicators of patients.

Variables	Total (*n* = 807)	non-POF (*n* = 546)	POF (*n* = 261)	*p*
Sex, *n* (%)				0.229
Female	281 (35)	182 (33)	99 (38)	
Male	526 (65)	364 (67)	162 (62)	
Age, IQR	47 (35, 61)	44 (34, 57)	52 (38, 68)	< 0.001
Time, IQR	12.53 (8.92, 17.45)	11.51 (8.28, 15.52)	15.28 (11, 23.36)	< 0.001
BMI, IQR	25.82 (23.32, 29.3)	25.65 (23.15, 28.73)	26.35 (23.71, 29.41)	0.133
Smoking, *n* (%)				0.34
No	502 (62)	333 (61)	169 (65)	
Yes	305 (38)	213 (39)	92 (35)	
Drinking, *n* (%)				0.493
No	523 (65)	349 (64)	174 (67)	
Yes	284 (35)	197 (36)	87 (33)	
Diabetes, *n* (%)				0.218
No	699 (87)	479 (88)	220 (84)	
Yes	108 (13)	67 (12)	41 (16)	
Hypertension, *n* (%)				0.112
No	599 (74)	415 (76)	184 (70)	
Yes	208 (26)	131 (24)	77 (30)	
Pain, *n* (%)				< 0.001
2	85 (11)	85 (16)	0 (0)	
3	311 (39)	298 (55)	13 (5)	
4	146 (18)	96 (18)	50 (19)	
5	131 (16)	48 (9)	83 (32)	
6	89 (11)	19 (3)	70 (27)	
7	24 (3)	0 (0)	24 (9)	
8	21 (3)	0 (0)	21 (8)	
Liver function (IQR)				
ALT (U/L)	45.1 (22.5, 112.25)	45.55 (23, 113.6)	44 (21, 111.66)	0.536
AST (U/L)	36.6 (22.2, 114.1)	33.45 (21.6, 114.1)	46.4 (25.2, 114.1)	0.005
ALP (U/L)	108.9 (74.8, 108.9)	108.9 (78.25, 108.9)	108.9 (70.2, 108.9)	0.05
GGT (U/L)	219.5 (78.6, 219.5)	219.5 (80.68, 219.5)	219.5 (73.2, 219.5)	0.8
ALB (g/L)	36.3 (31.3, 40.8)	36.8 (33.5, 41.7)	34 (28.7, 37.9)	< 0.001
TB (μmol/L)	22.4 (13.3, 31.75)	21.8 (13.22, 30.25)	25.1 (13.7, 35.9)	0.065
DB (μmol/L)	8.3 (3.7, 13.4)	7.4 (3.3, 13.4)	10.5 (4.9, 13.4)	0.002
TC (mmol/L)	4.57 (3.21, 4.97)	4.63 (3.37, 4.97)	4.37 (2.85, 4.99)	0.197
TG (mmol/L)	1.62 (0.89, 3.9)	1.75 (0.92, 3.9)	1.43 (0.85, 3.9)	0.414
Kidney function (IQR)				
Urea (mmol/L)	5.4 (4.1, 6.4)	5.15 (4, 5.8)	5.8 (4.4, 7.6)	< 0.001
SCr (μmol/L)	75.7 (62.85, 86.5)	75 (62.5, 84.4)	77.4 (63.6, 95.2)	0.005
Pancreatic function (IQR)				
AMY (U/L)	207.5 (80.95, 599.65)	179.8 (70.2, 537.7)	298 (105.6, 756.1)	< 0.001
LPS (U/L)	275.6 (93.6, 756.35)	237.1 (88.32, 695.65)	388.7 (111, 897.2)	0.005
Ca (mmol/L)	2.16 (2.04, 2.28)	2.19 (2.09, 2.29)	2.08 (1.93, 2.2)	< 0.001
Coagulation function (IQR)				
PT (s)	12.9 (12, 13.4)	12.75 (11.9, 13.2)	12.9 (12.1, 13.6)	0.008
APTT (s)	30.3 (28.4, 31.65)	30.3 (28.4, 31.7)	30.3 (28.4, 31.3)	0.321
FIB (g/L)	4.52 (3.39, 5.2)	4.4 (3.33, 5.2)	4.83 (3.58, 5.2)	0.01

(Continued)

TABLE 1 (Continued)

Variables	Total (n = 807)	non-POF (n = 546)	POF (n = 261)	p
Blood routine (IQR)				
WBC (10^9/L)	11 (7.7, 14.3)	10.54 (7.33, 13.6)	11.9 (8.7, 15.6)	< 0.001
NEUT (10^9/L)	9.11 (5.81, 12.09)	8.29 (5.31, 11.53)	10.14 (7.24, 13.4)	< 0.001
LYMPH (10^9/L)	1.1 (0.78, 1.59)	1.23 (0.84, 1.71)	0.9 (0.63, 1.26)	< 0.001
RBC (10^{12}/L)	4.53 (4.06, 5)	4.54 (4.08, 4.98)	4.48 (4.01, 5.06)	0.86
HCT (L/L)	0.45 (0.4, 13.95)	0.44 (0.39, 0.52)	0.48 (0.4, 34)	0.005
MCV (fL)	91.9 (89.16, 95.82)	91.8 (89.08, 95.38)	92.3 (89.3, 97)	0.055
MCH (pg)	31.3 (30.2, 32.5)	31.3 (30.1, 32.4)	31.3 (30.3, 32.7)	0.284
MCHC (g/L)	338 (332.78, 343.4)	338 (333, 344)	338.58 (332, 343)	0.52
RDW (%)	13.3 (12.5, 14)	13.2 (12.43, 13.97)	13.6 (12.8, 14.2)	0.005
PLT (10^9/L)	202 (163, 253)	207 (170.25, 255.75)	188 (146, 242.5)	< 0.001
MPV (fL)	16.8 (16.2, 17.4)	16.7 (16.1, 17.3)	16.9 (16.5, 17.5)	< 0.001
PDW (%)	8.6 (7.95, 9.5)	8.6 (7.9, 9.5)	8.7 (8.1, 9.6)	0.038
PLR	178.26 (127.65, 258.36)	167.52 (121.09, 244.73)	204.11 (146.2, 300.79)	< 0.001
NLR	7.84 (4.36, 13.4)	6.68 (3.76, 11.35)	11.43 (6.57, 17.52)	< 0.001

IQR, median and interquartile range [P25; P75]; BMI, body mass index; WBC, white blood cell count; NEUT, neutrophil count; LYMPH, lymphocyte count; RBC, red blood cell count; MCV: mean corpuscular volume; MCH: mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; RDW, red cell distribution width; PLT, platelet count; PDW, platelet distribution width; MPV, mean platelet volume; AST, aspartate aminotransferase; ALT, alanine aminotransferase; ALP, alkaline phosphatase; GGT, gamma-glutamyltransferase; ALB, albumin; TB, total bilirubin; DB, direct bilirubin; TC, total cholesterol; TG, triglycerides; BUN, blood urea nitrogen; SCr, serum creatinine; AMY, amylase; LPS, lipase; Ca, calcium; APTT, activated partial thromboplastin time; PT, prothrombin time; FIB, fibrinogen; PLR, platelet to lymphocyte ratio; NLR, neutrophil to lymphocyte ratio.

TABLE 2 Univariable and multivariable logistic regression: risk factors for POF.

Variables	Univariable ($P < 0.05$) OR (95% CI)	P	Multivariable ($P < 0.05$) OR (95% CI)	P
Age	1.02(1.01–1.03)	<0.001		
Time	1.06(1.04–1.08)	<0.001		
Pain	4.65(3.82–5.75)	<0.001	4.58(3.67–5.84)	<0.001
AST	1(1–1)	0.03		
ALB	0.99(0.97–1.00)	0.03	0.99(0.97–1.00)	0.02
DB	1(0.99–1.01)	0.21		
Urea	1.21(1.14–1.29)	<0.001		
SCr	1.01(1.00–1.02)	<0.001	1.01(1.00–1.02)	0.02
AMY	1(1–1)	0.03		
LPS	1(1–1)	0.12		
Ca	0.03(0.01–0.08)	<0.001	0.09(0.03–0.34)	<0.001
PT	1.16(1.05–1.29)	0.004		
FIB	1.13(1.03–1.23)	0.01		
WBC	1.06(1.03–1.10)	<0.001		
NEUT	1.09(1.05–1.12)	<0.001		
LYMPH	0.48(0.37–0.63)	<0.001		
HCT	1.003(0.997–1.008)	0.16	1.007(1.00–1.01)	0.04
RDW	1.06(0.98–1.15)	0.13		
PLT	1.00(0.99–1.00)	0.002		
MPV	1.13(1.04–1.23)	0.006		
PDW	1.10(0.98–1.23)	0.11		
PLR	1.002(1.001–1.004)	<0.001		
NLR	1.06(1.04–1.08)	<0.001		

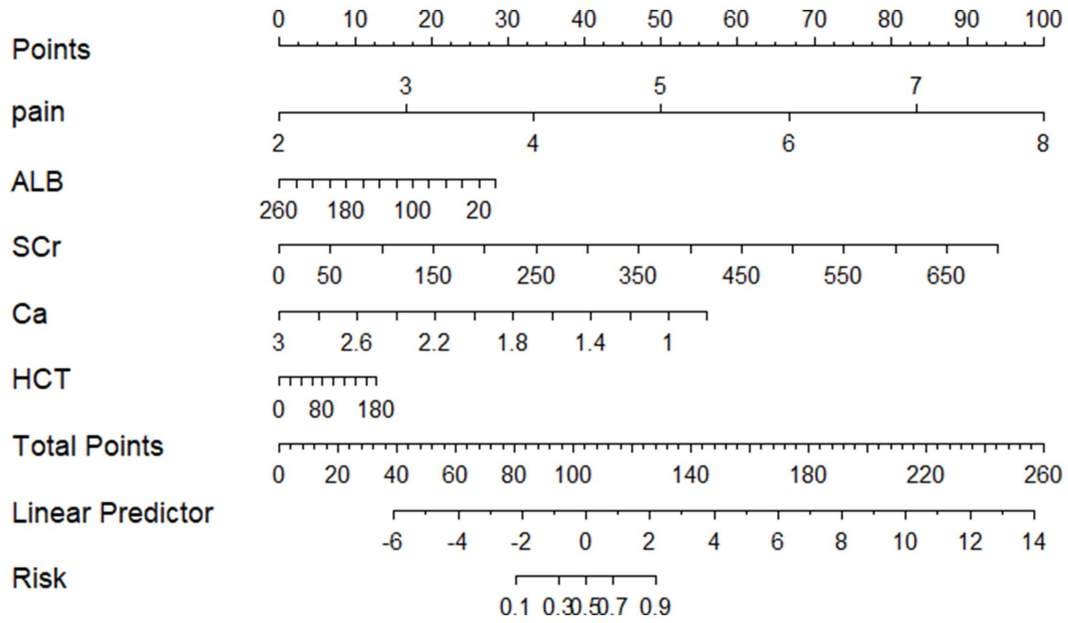

FIGURE 2
Nomogram of an early prediction model for POF in AP.

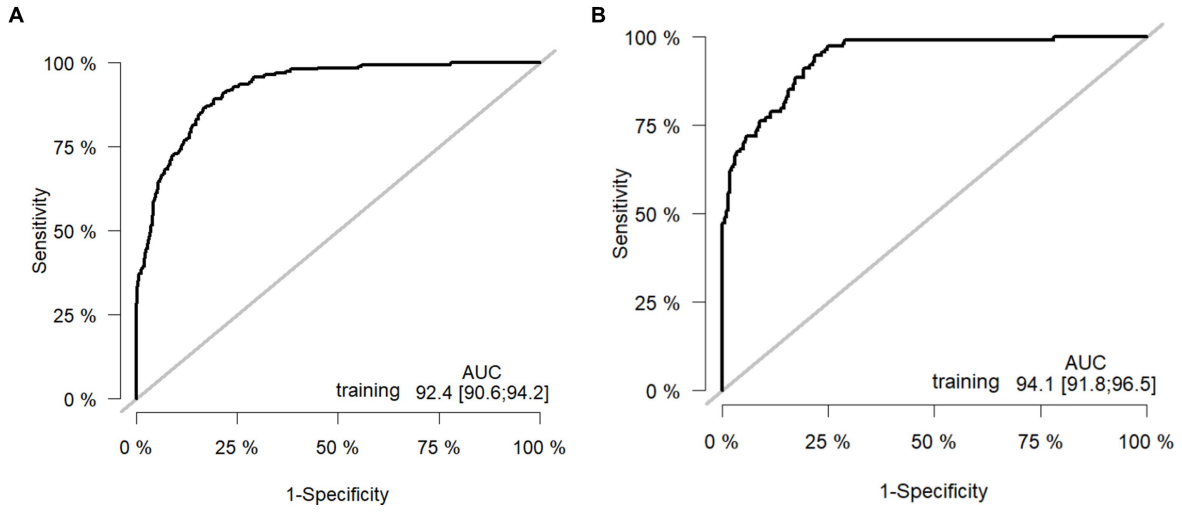

FIGURE 3
Receiver operating curves: **(A)** Training cohort. **(B)** Internal validation cohort. AUC: area under the ROC (receiver operating characteristic) curve.

speed, convenience, and low cost. The patient's pain response is one of the most direct indicators of disease progression. Clinicians can evaluate the progression of a patient's condition based on the clinical manifestations caused by the pain. Studies have shown that tolerable pain is associated with a favorable prognosis for the patient (20). Few studies have linked pain scores with adverse patient outcomes. This study concludes that pain scores at the time of admission are independent risk factors for the development of POF in patients with AP. This underscores the importance in clinical practice of not solely relying on laboratory test indicators but also incorporating the patient's subjective experiences into the assessment of their condition, which aids in better evaluating the patient's status and taking timely necessary actions.

ALB is an important biochemical marker for assessing a patient's nutritional status and the severity of their illness. Since albumin has a half-life of 21 days, it may not be the optimal acute phase reactant. However, hypoalbuminemia is still associated with poor prognosis in various diseases. The occurrence of POF is often accompanied by a severe inflammatory response, leading to increased capillary permeability and the leakage of albumin from the vasculature into the interstitial space, resulting in the consumption of serum albumin. Patients with AP complicated by

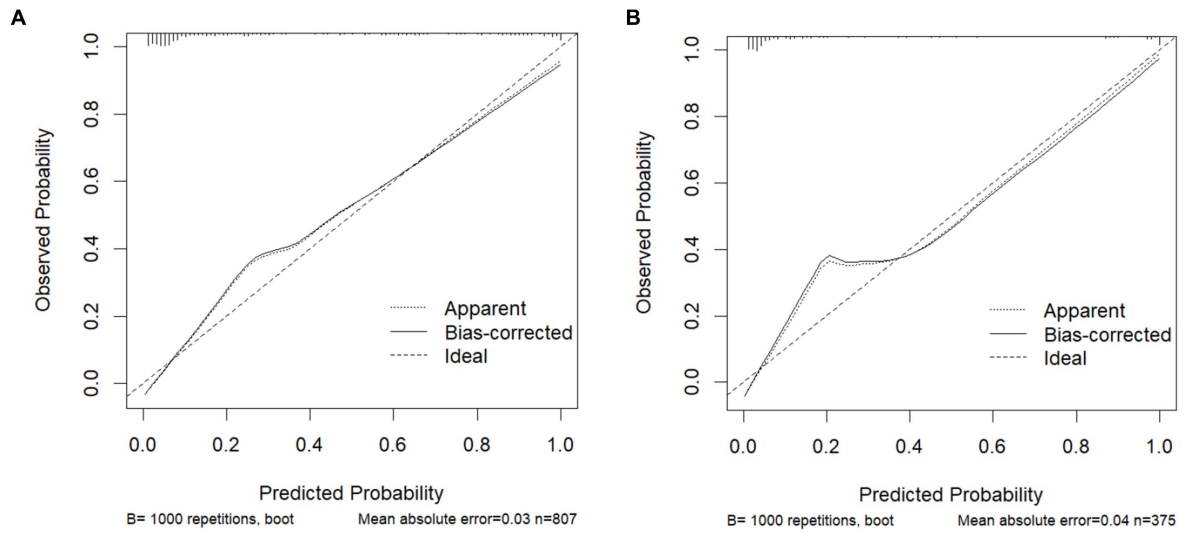

FIGURE 4
Calibration curves for predicting the POF: **(A)** Training cohort. **(B)** Internal validation cohort.

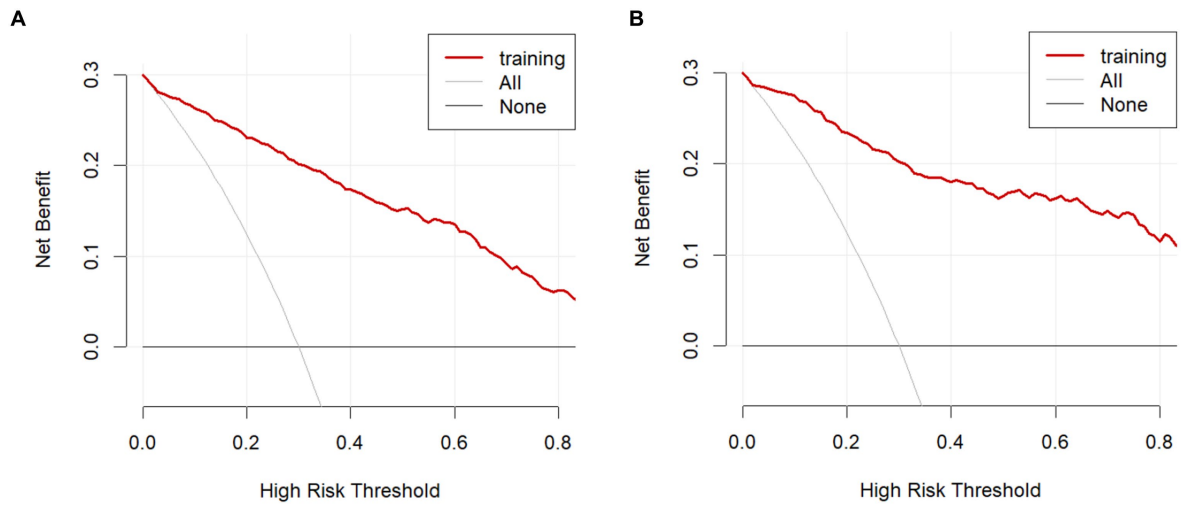

FIGURE 5
Decision curve analysis in the prediction of POF. **(A)** Training cohort. **(B)** Internal validation cohort.

POF often have limited intestinal absorption capabilities, leading to inadequate protein intake. Additionally, the high metabolic changes within the body increase the demand for energy and protein, further reducing ALB levels. Studies have indicated that lower plasma albumin levels are associated with an increased number of complications, higher rates of subsequent infections, and increased mortality (21). Early low ALB levels are associated with poor prognosis in patients with AP (22). Patients with SAP who have higher serum albumin levels often have a better prognosis and subsequent quality of life (23). Hypoalbuminemia reflects poor nutritional status, enhanced inflammatory response, or reduced hepatic synthesis function, all of which may lead to a decreased resistance to disease and an increased risk of POF.Therefore, ALB levels can serve as an important indicator for assessing the severity of a patient's condition and prognosis.

SCr is a widely recognized and commonly used renal function marker in the assessment of organ failure. This study indicates that serum creatinine can independently predict the onset of POF, consistent with the results of previous research (24). In patients with POF, the release of inflammatory signals from pancreatic acinar cells leads to the accumulation of fluid in the third space, resulting in renal ischemia. Concurrently, the influx of leukocyte interleukins, platelet-activating factors, and inflammatory mediators into the bloodstream exacerbates renal ischemia, causing sustained renal damage, which in turn leads to renal injury and associated increases in SCr (25). Studies have shown that acute kidney injury is often accompanied by organ failure in patients with AP, and the mortality rate in patients with acute kidney injury and AP exceeds 25% (26). Pete et al. pointed out that renal failure for more than 48 h in patients with SAP is a strong predictor of poor prognosis (27).

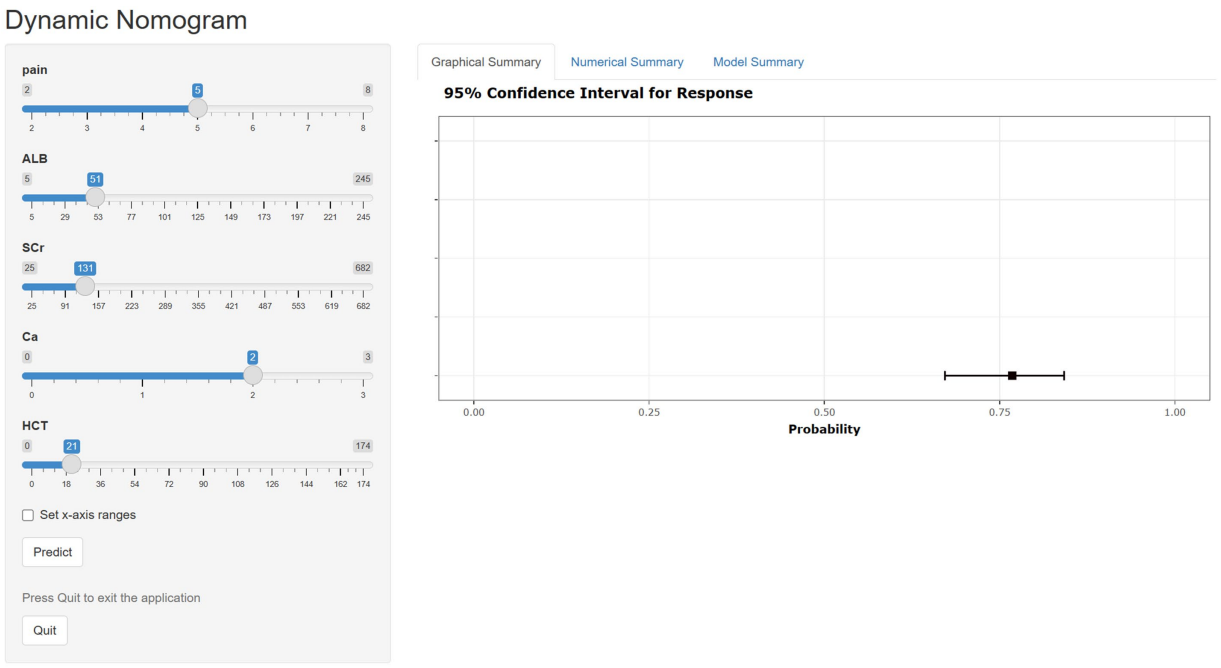

FIGURE 6
Dynamic web-based calculator for predicting POF in AP patients.

Serum Ca has been shown to be associated with clinical outcomes in pancreatitis (28). (1) Calcium ions can abnormally activate pancreatic enzymes, initiating autodigestion of the pancreas (29). (2) In the context of POF, specific calcium channels known as SOCE (store-operated calcium entry) are abnormally activated, facilitating the influx of calcium ions into cells and resulting in a decrease in serum calcium concentration. Concurrently, an excessive intracellular concentration of Ca^{2+} alters the permeability of mitochondria (30). The consumption of ATP is required to maintain toxic concentrations of Ca^{2+}. Excessive accumulation of calcium ions within cells can disrupt the normal function of mitochondria, which may be a cause of pancreatic acinar cell death. Furthermore, calcium overload itself can trigger autodigestion and necrosis of pancreatic acinar cells (31). At the same time, fat necrosis in AP patients with POF can bind free calcium and further lead to hypocalcemia.

Studies have demonstrated that the HCT levels measured upon admission have the same sensitivity as the Ranson scores obtained after 48 h (32). Meanwhile,HCT is an independent risk factor for predicting mortality in AP (33). When admission HCT ≥44%, the incidence of POF reached 53.6% (34). This is similar to the results obtained in this study. Higher HCT values may be associated with inflammation and increased capillary permeability due to bradykinin-like substances. This can lead to peripheral vasodilation and the shift of fluid from the vasculature to the interstitial space, reducing circulating blood volume and relatively increasing the proportion of red blood cells.

In this study, we combined five selected predictive factors, each belonging to different systems: mental status, liver function, renal function, routine blood indicators, and pancreatic function. Consequently, this predictive model can comprehensively and accurately forecast the incidence of POF in patients with AP. Additionally, we have created visual nomograms and web-based calculators. These tools can be conveniently applied in clinical settings and provide valuable guidance for clinicians. However, the data for this study comes from a single center and employs a retrospective method, which has some limitations. In the future, validation should be conducted in multicenter, large-sample prospective studies.

Conclusion

Pain score, serum creatinine, hematocrit, serum calcium, and serum albumin were independent predictors of acute pancreatitis complicated by persistent organ failure. A prediction model was developed based on these 5 clinical risk indicators and a nomogram and network calculator were constructed. The model had good prediction performance.

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 humans were approved by the Shanxi Bethune Hospital (Ethical Approval Number: YXLL-2023-237). The studies were conducted in accordance with the local legislation and institutional requirements. The human samples used in this study were acquired from primarily isolated as part of your previous study for which ethical approval was obtained. Written informed consent for participation was not required from the participants or the

participants' legal guardians/next of kin in accordance with the national legislation and institutional requirements.

Author contributions

JXi: Data curation, Formal analysis, Writing – original draft. MX: Data curation, Methodology, Writing – original draft, Writing – review & editing. JXu: Data curation, Formal analysis, Investigation, Writing – review & editing. JL: Investigation, Methodology, Writing – review & editing. FH: Data curation, Supervision, Writing – review & editing.

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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

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EDITED BY
Ravi Kumar Sharma,
Chandigarh University, India

REVIEWED BY
Ran Cheng,
Baylor College of Medicine, United States
Atul Kabra,
Chandigarh University, India

*CORRESPONDENCE
Wandong Hong
xhnk-hwd@163.com

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The causality between use of glucocorticoids and risk of pancreatitis: a Mendelian randomization study

Wenfeng Lin[1], Qiqi Zheng[2], Xiaorong Wang[3], Xiaolu Lin[4], Xixi Ni[1], Jingye Pan[3], Maddalena Zippi[5], Sirio Fiorino[6] and Wandong Hong[1*]

[1]Department of Gastroenterology and Hepatology, The First Affiliated Hospital of Wenzhou Medical University, Wenzhou, Zhejiang, China, [2]Department of Infection and Liver Diseases, The First Affiliated Hospital of Wenzhou Medical University, Wenzhou, Zhejiang, China, [3]Department of Intensive Care Unit, The First Affiliated Hospital of Wenzhou Medical University, Wenzhou, Zhejiang, China, [4]Department of Digestive Endoscopy Center, Fujian Provincial Hospital, Shengli Clinical Medical College of Fujian Medical University, Fuzhou, Fujian, China, [5]Unit of Gastroenterology and Digestive Endoscopy, Sandro Pertini Hospital, Rome, Italy, [6]Unit of Internal Medicine, Budrio Hospital, Local Health Unit of Bologna, Bologna, Italy

Background and aim: To date, the association between glucocorticoid use and the risk of pancreatitis remains controversial. The aim of this study was the investigation of this possible relationship.

Methods: We carried out a two-sample Mendelian randomization (MR) analysis using GWAS data from European ancestry, East Asian descendants and the FinnGen Biobank Consortium to evaluate this potential causal relationship. Genetic variants associated with glucocorticoid use were selected based on genome-wide significance ($p < 5\times10\text{-}8$).

Results: Our MR analysis of European ancestry data revealed no significant causal relationship between glucocorticoid use and AP (IVW: OR=1.084, 95% CI= 0.945-1.242, P=0.249; MR-Egger: OR=1.049, 95% CI= 0.686-1.603, P=0.828; weighted median: OR=1.026, 95% CI= 0.863-1.219, P=0.775) or CP (IVW: OR=1.027, 95% CI= 0.850-1.240, P=0.785; MR-Egger: OR= 1.625, 95% CI= 0.913-2.890, P= 0.111; weighted median: OR= 1.176, 95% CI= 0.909-1.523, P= 0.218). Sensitivity analyses, including MR-Egger and MR-PRESSO, indicated no evidence of pleiotropy or heterogeneity, confirming the robustness of our findings. Multivariable MR analysis adjusted for alcohol consumption, BMI, cholelithiasis and C-reactive protein levels supported these findings. Replicated analysis was performed on datasets from the FinnGen Biobank Consortium and East Asian descendants, and similar results were obtained.

Conclusions: This MR analysis suggests that there is no causal association between glucocorticoid use and the risk of pancreatitis.

KEYWORDS

acute pancreatitis, chronic pancreatitis, glucocorticoid, Mendelian randomization, risk factor

1 Introduction

Inflammation of the exocrine pancreas, often associated with acute abdominal pain, can lead to multiple organ failure (1, 2). About 80% of cases are classified as mild to moderate with no organ failure after 48 hours, while the remaining 20% progress to severe pancreatitis with a mortality rate of approximately 20% (1, 2). With an increasing global incidence (3), pancreatitis is now the leading cause of hospitalizations related to gastrointestinal disorders worldwide (1). In particular, acute pancreatitis can result from a number of recognized causes, with gallstones and alcohol consumption being the most common. However, the etiology of this condition remains elusive in some cases (2), with a definitive cause being lacking in around 20 per cent of cases (2, 4, 5).

Historically, drug-induced acute pancreatitis has been considered a rare etiology. Recent studies indicate that it may be the third most common cause of the disease, accounting for between 0.1 per cent and 2 per cent of all cases (6, 7). Glucocorticoids (GCs), a widely used group of medications, are prescribed to roughly 2-6% of the population (8). These drugs are known to have a number of negative effects, such as increased diabetes mellitus, osteoporosis and peptic ulcers diseases (9, 10). Furthermore, several case reports have highlighted the onset of pancreatitis in patients receiving glucocorticoid therapy (11–21). Despite this, the pathophysiology and occurrence of glucocorticoid-induced pancreatitis remains poorly investigated and rarely reported. It is difficult to establish a causal relationship between glucocorticoids and pancreatitis (11, 16). Glucocorticoids-induced pancreatitis is remarkably rare, accounting for only 3% of all reported cases of drug-induced pancreatitis according to a literature review (22). This condition is primarily identified by a process of exclusion, and is often considered when there's a history of glucocorticoid use and after other most common causes of pancreatitis have been ruled out (11, 23). In many of these few reports suggesting glucocorticoids as a potential cause of pancreatitis, the presence of other contributing factors cannot be definitively excluded, making it difficult to attribute the etiology solely to glucocorticoids (24). This difficulty is increased in patients with multiple comorbidities and underlying risk factors, where ruling out more common causes of drug-induced pancreatitis becomes increasingly complex (25). Crucially, some conditions treated with glucocorticoid therapy, such as inflammatory bowel disease (26), systemic lupus erythematosus (27) and Wegener's granulomatosis (28), may act as risk factors for pancreatitis, leading to confusion in the indication. In addition, the definitive association of pancreatitis with glucocorticoid use is often unconfirmed due to the lack of possibility to repeat tests, especially for ethical reasons (22). A retrospective analysis of patients with systemic lupus erythematosus suggested that glucocorticoids were not responsible for the development of pancreatitis in these cases (29). Conversely, a handful of studies have shown that glucocorticoids may be useful in the prevention and treatment of pancreatitis (24, 30). Nonetheless, the current understanding of glucocorticoid-induced pancreatitis is largely based on theories derived from limited case reports, animal studies and other experimental data (11, 16, 17, 31). The evidence linking glucocorticoids to pancreatitis remains weak, with a significant risk of false-positive results (11, 16, 17, 31). It is therefore essential that large studies are carried out to establish the cause and effect link between the use of glucocorticoids and the risk of pancreatitis.

Mendelian randomization (MR) is a method that uses genetic variation as instrumental variables (IVs) to determine whether an observed association between a risk factor and an outcome is consistent with a causal effect (32). A two-sample MR approach identifies causal effects when exposure and outcome data come from different sources (33). This approach significantly limits residual confounding and is less vulnerable to reverse causation, as genetic variants are inherited at conception. As a result, a trait will typically remain unaffected by other traits (potential confounders or environmental elements). No previous study has investigated the causal relationship between glucocorticoid use and the risk of pancreatitis using MR to our knowledge. Thus, this study attempts to investigate the causal relationship between the use of glucocorticoids and the occurrence of pancreatitis using two-sample MR analysis.

2 Methods

2.1 Study design and instrument variable selection

Using summary-level data, we conducted a two-sample Mendelian Randomization (MR) study to investigate the causal relationship between glucocorticoid use and pancreatitis employing specific glucocorticoid-related single-nucleotide polymorphisms (SNPs) as instrumental variables (IVs). The main results of the MR analysis in the current study were based on GWAS summary datasets of European ancestry obtained from the study by Sakaue S et al. (34). Replicated analysis was performed on datasets from the FinnGen Biobank Consortium and East Asian descendants.

A multivariable MR assessment, adjusting for potential confounders such as preexisting alcohol use, body mass index (BMI), cholelithiasis (gallstones), and C-reactive protein values, was performed to determine the direct causal effect of glucocorticoid use on pancreatitis. The first three factors were identified as etiological contributors to pancreatitis, while the last one assessed the severity of the inflammation. To accurately assess the effects of confounding within the MR framework, the selected IVs must meet three criteria: (I) they should show an association with the exposure variable (the 'relevance' assumption); (II) they should not be associated with confounding factors (the 'independence' assumption); (III) their influence on the outcome should be mediated solely by the exposure variable, with no additional pathways involved (the 'exclusion' restriction). The selection criteria for identifying instrumental variables from SNPs were defined as follows (1): genome-wide significance with P values less than 5×10^-8 was required to ensure the robustness and reliability of these genetic instruments. However, a higher threshold of 5×10^-5 was used for East Asian descendants due to limited qualified data; (2) absence of linkage disequilibrium in SNPs, specified by a default r^2 = 0. 001 within a radius of 10,000 kb, ensuring their independence; and (3) to address potential bias from weak instruments, we calculated the Cragg-Donald F-statistic for each SNP using the formula F-statistic = β^2/SE^2 and excluded SNPs with an F-

statistic below 10. In this context, β is the estimate of the exposure effect, while SE is its standard error. The conceptual and analytical flow of this study is illustrated in Figure 1.

2.2 Data source

Supplementary Table 1 (34) provides an overview of Genome-Wide Association Studies (GWAS) on various exposures and outcomes. The detailed summary data on glucocorticoid use, acute pancreatitis and chronic pancreatitis in European and East Asian ancestry were extracted from the GWAS conducted by Sakaue S et al. (34). In European ancestry, this study included 17,352 cases of individuals using glucocorticoid (GWAS ID: ebi-a-GCST90019000) with 188,348 controls and analyzed 14,256,400 SNPs. This study also included 3,798 cases of acute pancreatitis (GWAS ID: ebi-a-GCST90018789) and 476,104 controls, analyzing a total of 24,190,697 SNPs. Summary statistics for chronic pancreatitis were extracted from the same GWAS (GWAS ID: ebi-a-GCST90018821). It included 1,424 patients and 476,104 controls, with a total of 24,195,431 SNPs examined. In East Asian descendants, this study included 13,102 cases of glucocorticoid use (GWAS ID: ebi-a-GCST90018780) and 165,624 controls, analyzing 12,454,705 SNPs. This study also included 827 cases of acute pancreatitis (GWAS ID: ebi-a-GCST90018569) and 177,471 controls, evaluating a total of 12,454,648 SNPs. Summary statistics for chronic pancreatitis were extracted from the same GWAS (GWAS ID: ebi-a-GCST90018601), which included 457 patients and 177,471 controls, with a total of 12,454,540 SNPs examined.

The detailed summary level data for acute pancreatitis and chronic pancreatitis were also extracted from the FinnGen Consortium GWAS. For acute pancreatitis, this study included 3,022 patients and 195,144 controls, with a total of 16,380,428 SNPs being investigated (GWAS ID: finn-b-K11_ACUTPANC). Similarly, for chronic pancreatitis, the study included 1,737 patients and 195,144 controls, with 16,380,413 SNPs examined (GWAS ID: finn-b-K11_CHRONPANC).

To clarify direct causal relationships and to reduce potential confounding, genetic instruments for variables such as frequency of alcohol consumption (sample size: 462,346), body mass index (BMI, sample size: 532,396), cholelithiasis (gallstones, sample size: 404,405) and C-reactive protein levels (sample size: 353,466) were acquired from the most comprehensive and recent studies (34–39). The first three variables above serve as etiological contributors to pancreatitis, with the last variable indicating the severity of the inflammatory response.

Detailed data sources for glucocorticoid administration, acute and chronic pancreatitis, frequency of alcohol consumption, BMI,

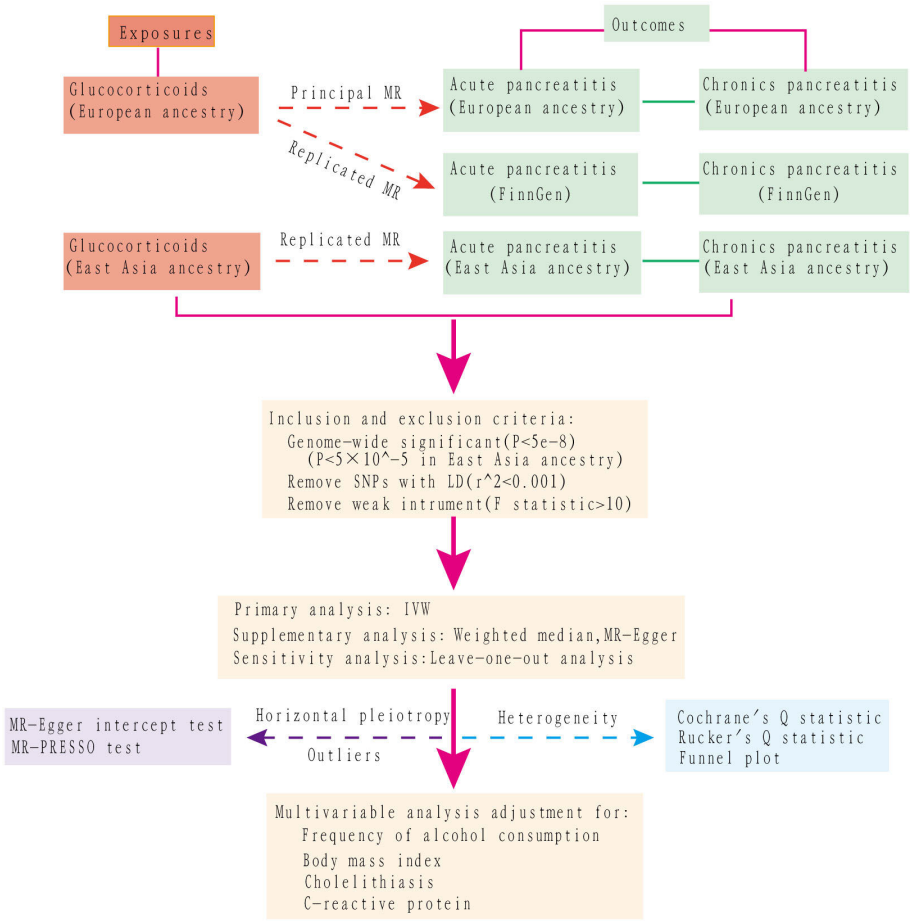

FIGURE 1
The work flow of this study.

cholelithiasis and C-reactive protein are meticulously documented in Supplementary Table 1.

2.3 Statistical analysis

The primary analytical approach applied in this study was the Inverse Variance Weighted (IVW) method, which assesses the effect of SNPs associated with glucocorticoid use on pancreatitis risk by aggregating individual Wald ratios to achieve unbiased causality in the absence of horizontal pleiotropy (40). Supplementary analyses using the weighted median and MR-Egger methods have also been performed to corroborate these findings (41, 42).

The influence of horizontal pleiotropy on risk estimation and the identification of potential confounders was evaluated by means of the MR-Egger intercept test (41). Heterogeneity of results was assessed using Cochrane's Q statistic for IVW analysis and Rucker's Q statistic for MR-Egger analysis (43). In addition, we performed a leave-one (SNP)-out analysis to identify and exclude outliers, potentially biasing a causal relationship and we systematically omitted each SNP and recalculated effect sizes, using the IVW method. Funnel plots were generated to visually assess the heterogeneity of the results, with a symmetric distribution around the vertical axis. This type of configuration indicated the absence of bias. The Mendelian randomization pleiotropy residual sum and outlier (MR-PRESSO) test was also used to identify horizontal pleiotropic outlier SNPs, providing identical results to IVW after outlier removal (44). To minimize the impact of horizontal pleiotropy on the results, each individual SNP was examined individually in the LDtrait human genotype-phenotype databases (45). This process allowed us to identify and exclude risk factors shared with glucocorticoid use, such as serum triglyceride levels (46), cholangitis (46) and alcohol consumption (46).

Multivariable MR analysis can be used to investigate the causality of multiple exposures imposed by a genetic tool on the same outcome variable. In clinical practice, alcohol and cholelithiasis are known to be common etiologies of pancreatitis, while BMI and CRP are risk and predictive factors of disease severity in patients with pancreatitis, respectively (46–49). These indexes may act as possible confounding factors that bias the results of the MR analysis. Therefore, we performed multivariable MR analysis to remove potential confounding bias. All Mendelian randomization analyses were performed using the TwoSampleMR package in R version 4.1.2, with P values less than 0.05 considered statistically significant.

3 Results

3.1 MR analysis of GWAS summary datasets of European ancestry

3.1.1 Causal association of glucocorticoid usage with AP

In this analysis, we employed 27 SNPs as instrumental variables to assess the impact of glucocorticoid use through MR analysis. Each SNP had an F-statistic greater than 10, exceeding the threshold for a 'weak instrumental variable' (F-statistic value less than 10), thereby mitigating concerns about weak instrument bias in our results.

There was no statistically significant causal relationship between glucocorticoid use and the development of acute pancreatitis (AP) using the inverse variance weighted (IVW) method (odds ratio (OR) = 1.084, 95% confidence interval (CI) = 0.945-1.242, P = 0.249), as depicted in Figure 2. Similarly, MR-Egger regression analysis (OR = 1.049, 95% CI = 0.686-1.603, P = 0.828) and the weighted median method (OR=1.026, 95% CI= 0.863-1.219, P=0.775) supported these findings, as presented in Figure 3. No horizontal pleiotropic outlier SNPs were identified by MR-PRESSO Global test ($P_{Global\ test}$ =0.123).

3.1.2 Causal association of glucocorticoid usage with CP

In this investigation, we included 26 SNPs as instrumental variables to assess the effect of glucocorticoid use in an MR analysis. All SNPs had F-statistics greater than 10, exceeding the threshold for weak instrumental variables. Therefore, concerns about weak instrumental bias in our results are considered negligible. The IVW method, as shown in Figure 4, did not reveal a substantial causal relationship between glucocorticoid use and the incidence of CP, with an OR of 1.027 and a 95% CI ranging from 0.850 to 1.240, resulting in a P value of 0.785. Similarly, both the MR-Egger regression yielded an OR of 1.625 (95% CI: 0.913-2.890; P=0.111) and the weighted median approach indicated an OR of 1.176 (95% CI: 0.909-1.523; P=0.218), supporting these findings (Figure 5). No horizontal pleiotropic outlier SNPs were identified by MR-PRESSO Global test ($P_{Global\ test}$ = 0.493).

3.1.3 Heterogeneity and sensitivity analysis

Cochran's Q statistics indicated the absence of significant heterogeneity in our results, as all P values exceeded 0.05 (Supplementary Table 2). In addition, the symmetric funnel plots generated for individuals with AP and CP further confirmed the lack of heterogeneity in our results (Supplementary Figures 1, 2). To assess potential pleiotropy, we used the MR Egger intercept test, which yielded intercepts that were not statistically different from zero (all p-values > 0.05; p=0.874 for AP and p=0.243 for CP), suggesting no evidence of horizontal pleiotropy in our investigation. Furthermore, the leave-one-out analysis showed that no single SNP significantly influenced the overall causal estimate (Supplementary Figures 3, 4). Detailed information on the MR analyses can be found in Supplementary Table 2.

3.1.4 Multivariable MR analysis

Furthermore, following adjustment for frequency of alcohol consumption, body mass index (BMI), cholelithiasis (gallstones) and C-reactive protein levels, multivariable MR analysis showed that there was no direct effects of glucocorticoid use either on the risk of AP (OR = 1.074, 95% CI = 0.948-1.216, P = 0.263, Figure 6; Supplementary Table 3) or risk of CP (OR =1.176, 95% CI =0.962-1.438, P =0.114; Figure 6, Supplementary Table 3).

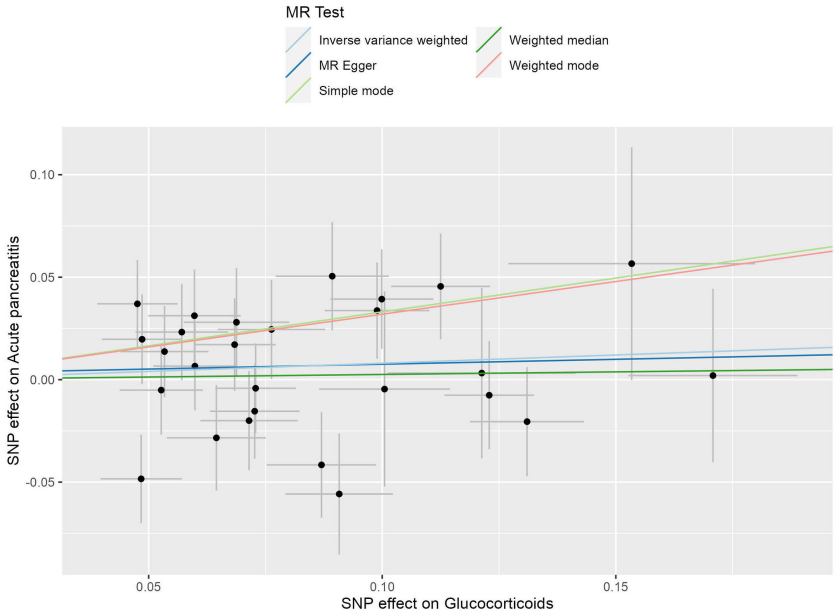

FIGURE 2
The scatter plot illustrates the causal effect of glucocorticoid usage on the risk of acute pancreatitis (AP) using GWAS summary data sets of European ancestry. The slope of the line indicates the strength of this causal relationship. MR denotes Mendelian randomization.

3.2 Replicated MR analysis of GWAS summary datasets from the FinnGen Biobank Consortium and East Asian descendants

3.2.1 Causal association of glucocorticoid use in relation to AP

Using the same instrumental variables of European ancestry, there was no statistically significant causal relationship between glucocorticoid administration and development of acute pancreatitis (AP) using the inverse variance weighted (IVW) method (OR = 1.130, 95% CI = 0.921-1.386, P = 0.243), as shown in Supplementary Figure 5 in the FinnGen Biobank Consortium. Similarly, the MR-Egger regression analysis (OR = 1.094, 95% CI = 0.567-2.110, P = 0.791) and the weighted median method (OR = 0.963, 95% CI = 0.771-1.202, P = 0.737) supported these findings, as shown in Supplementary Figure 6. The MR-PRESSO method identified one outlier SNP (rs10905284, $P_{Global\ test}$ <0.001). However, outlier correction shows similar OR estimates to the IVW method after removal of this outlying SNP (OR = 1.088, 95% CI = 0.917-1.2191, P = 0.343).

When employing the instrumental variables of the East Asian descendants, there was no statistically significant causal relationship between glucocorticoid administration and the development of acute pancreatitis (AP), by means of the inverse variance weighted (IVW) method (OR = 0.859, 95% CI = 0.682-1.083, P = 0.199), as shown in Supplementary Figure 7 in East Asian descendants. Similarly, MR-Egger regression analysis (OR = 0.796, 95% CI = 0.357-1.775, P = 0.580) and the weighted median method (OR = 0.962, 95% CI = 0.698-1.325, P = 0.812) supported these findings, as illustrated in Supplementary Figure 8. No horizontal pleiotropic outlier SNPs were identified by the MR-PRESSO Global test (($P_{Global\ test}$ = 0.602).

3.2.2 Causal association of glucocorticoid usage with CP

Using the same instrumental variables of European ancestry, the IVW method, as shown in Supplementary Figure 9, indicated no substantial causal relationship between glucocorticoid use and incidence of CP, with an OR of 0.982 and a 95% CI ranging from 0.798 to 1.209 and with a P value of 0.864 in the FinnGen Biobank Consortium. Similarly, both the MR-Egger regression showed an OR of 1.429 (95% CI: 0.748-2.730; P=0.291) and the weighted median approach showed an OR of 0.996 (95% CI: 0.750-1.324; P=0.979), supporting these findings (Supplementary Figure 10). No horizontal pleiotropic outlier SNPs were identified by the MR-PRESSO Global test ($P_{Global\ test}$ = 0.269).

The evaluation of the instrumental variables in East Asian descendants produced no statistically significant causal relationship between glucocorticoid administration and the development of CP, using the inverse variance weighted (IVW) method (OR = 1.038, 95% CI = 0.761-1.415, P = 0.816), as shown in Supplementary Figure 11 in East Asian descendants. Similarly, MR-Egger regression analysis (OR = 0.660, 95% CI = 0.223-1.930, P = 0.452) and the weighted median method (OR = 1.064, 95% CI = 0.674-1.679, P = 0.791) supported these findings, as illustrated in Supplementary Figure 12. No horizontal pleiotropic outlier SNPs were identified by the MR-PRESSO Global test ($P_{Global\ test}$ = 0.602).

3.2.3 Heterogeneity and sensitivity analysis

Significant heterogeneity was identified by Cochran's Q statistic for AP in the FinnGen Biobank Consortium ($P_{Q.Egger}$=0.0012; $P_{Q.IVW}$=0.0019). Cochran's Q statistic indicated the absence of significant heterogeneity in our other results, as all P values exceeded 0.05. In addition, the symmetric funnel plots generated for individuals with AP and CP further confirmed the lack of heterogeneity in our

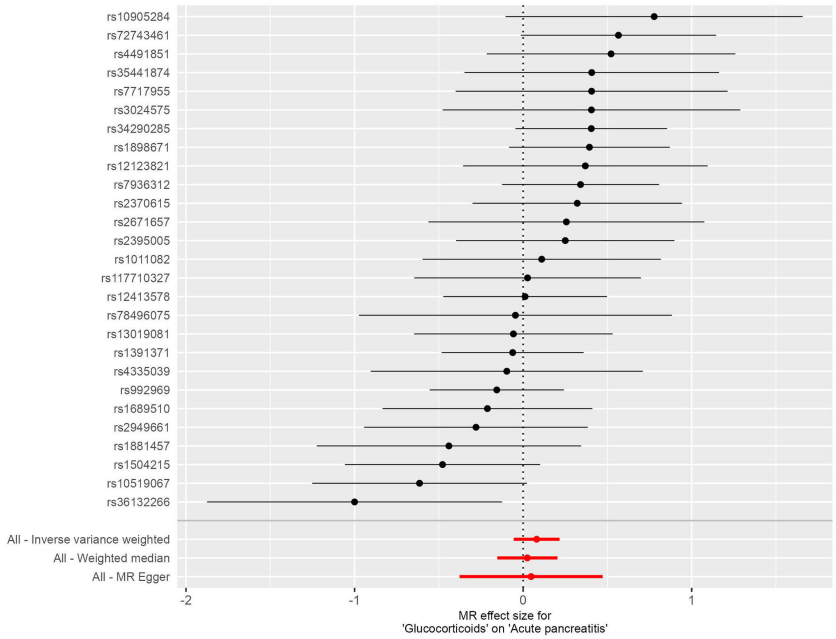

FIGURE 3
Forest plots illustrating the causal relationship between individual SNPs and the risk of acute pancreatitis (AP) using GWAS summary data sets of European ancestry.

results except for AP in the FinnGen Biobank Consortium (Supplementary Figures 13, 14). To assess potential pleiotropy, we used the MR Egger intercept test, which yielded intercepts that were not statistically different from zero (all p-values > 0.05), suggesting no evidence of horizontal pleiotropy in our investigation. Furthermore, the leave-one-out analysis showed that no single SNP significantly influenced the overall causal estimate (Supplementary Figures 15, 16). Detailed information on the MR analyses can be found in Supplementary Table 4.

In East Asian descendants, Cochran's Q statistic also indicated no significant heterogeneity, with all P values above 0.05 (Supplementary Table 5). Symmetric funnel plots for individuals with AP and CP further supported the absence of heterogeneity (Supplementary Figures 17, 18). The MR Egger intercept test

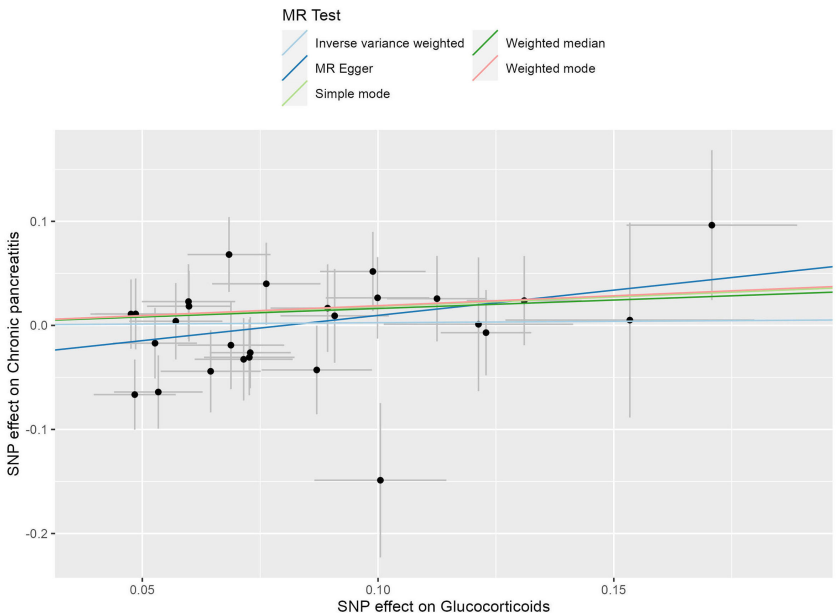

FIGURE 4
The scatter plot illustrates the causal effect of glucocorticoid usage on the risk of chronic pancreatitis (CP) using GWAS summary data sets of European ancestry. The slope of the line indicates the strength of this causal relationship. MR denotes Mendelian randomization.

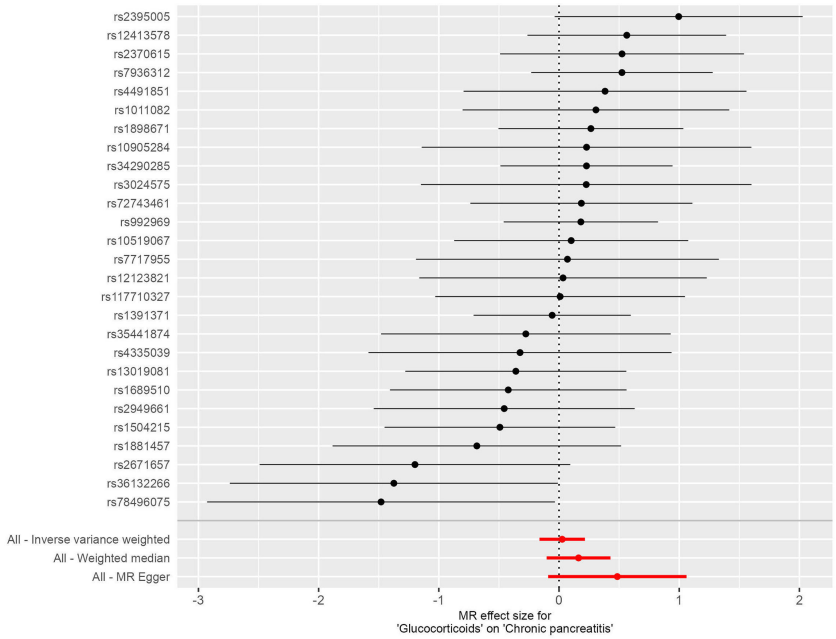

FIGURE 5
Forest plots illustrating the causal relationship between individual SNPs and the risk of chronic pancreatitis (CP) using GWAS summary data sets of European ancestry.

yielded intercepts that were not statistically different from zero (all P values > 0.05; P=0.845 for AP and P=0.392 for CP), indicating no horizontal pleiotropy. The leave-one-out analysis showed that no single SNP significantly influenced the overall causal estimate (Supplementary Figures 19, 20). Detailed information on the MR analyses can be found in Supplementary Table 5.

3.2.4 Multivariable MR analysis

Furthermore, after adjustment for frequency of alcohol consumption, body mass index (BMI), cholelithiasis (gallstones) and C-reactive protein levels, multivariable MR analysis showed no direct effect of glucocorticoid use on the risk of either AP (OR = 1.065, 95% CI = 0.911-1.244, P = 0.429, Supplementary Figures 21, Supplementary Table 6) or the risk of CP (OR =1.090, 95% CI =0.885-1.343, P =0.418; Figure 6; Supplementary Table 6) in the FinnGen Biobank Consortium. Multivariable MR analysis was not performed in East Asian offspring due to limited qualifying data.

4 Discussion

Establishing a diagnosis for drug-induced pancreatitis poses significant diagnostic challenges. This pathological condition is quite rare and it may present with different clinical course and severity.

Therefore, it is often difficult or ethically unjustifiable to use rechallenge to test for a causal relationship between a potentially dangerous drug and the development of pancreatitis, mainly in its acute form (1, 2, 50, 51). The hypothesis that glucocorticoids contribute to or correlate with pancreatitis in humans has been

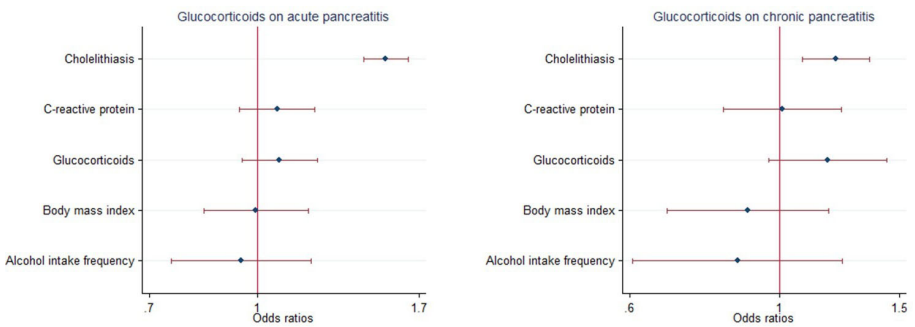

FIGURE 6
Multivariable Mendelian randomization of glucocorticoid usage on the risk of pancreatitis using GWAS summary data sets of European ancestry. Error bars represent 95% confidence intervals. AP, acute pancreatitis; CP, chronic pancreatitis.

emphasized by several Authors for many years (11–21). However, our current understanding of glucocorticoid-induced pancreatitis primarily relies on limited individual case series, animal research and other experimental findings (11, 16, 17, 24, 31). Consequently, studies assessing the possible association between glucocorticoids and pancreatitis provide no definitive conclusions, due to the risk of potential false positive results (11, 16, 17, 24, 31). Furthermore, few robust and large-scale studies investigating glucocorticoid-induced pancreatitis are available to date. Therefore, this circumstance makes the specific mechanisms associated with this condition largely unexplored and understood (13, 52). Among the few large-scale investigations to date, a population-based nested case-control study (13) examined 6,161 cases of acute pancreatitis along with 61,637 controls to explore the relationship between oral glucocorticoid use and incidence rates of acute pancreatitis. This study showed an increased probability of acute pancreatitis development in individuals currently using oral glucocorticoids compared to non-users (OR 1.53; 95% CI 1.27-1.84), suggesting that taking these drugs increases the risk of this disease. Nonetheless, the investigators also underlined that their study was subject to limitations, including the potential misestimation of prescribed medication use and the inability to adjust for confounding variables. The association with the use of glucocorticoids and the incidence of acute pancreatitis was investigated in another large study using the US Food and Drug Administration Adverse Event Reporting System (FAERS) (14). In this study, 8,437,343 cases were analyzed and 44,893 cases of acute pancreatitis were identified in patients who were taking various medications, including glucocorticoids. A pharmacological and epidemiological approach was used in this study. It concluded that glucocorticoid treatment was associated with an increased risk of having acute pancreatitis. This circumstance introduces some limitations to this study, such as susceptibility to underreporting, selective reporting bias and an inability to adjust for all confounding factors, thereby precluding definitive conclusions on causal relationship between glucocorticoids and acute pancreatitis. Furthermore, individuals suffering from this disease while on this medication frequently exhibit prominent predisposing factors for the development of this pathological condition, such as alcohol abuse, systemic vasculitis, due to immunological responses, and concurrent use of pharmacological substances recognized to induce pancreatitis, in addition to the drug under suspicion (22, 24, 31). It is also suggested that glucocorticoids may be involved in the onset of acute pancreatitis in people receiving this type of therapy to treat autoimmune diseases such as systemic lupus erythematosus (SLE) (53). However, it is worth noting that a significant proportion (approximately 8%) of SLE patients experience acute pancreatitis regardless of whether they have received glucocorticoids (53). Likewise, the development of acute pancreatitis in cancer patients has been linked to the use of glucocorticoids given as an anti-emetic during chemotherapy (54). However, it should be noted that these patients are often taking antineoplastic medications at the same time. These drugs are known to independently cause acute pancreatitis (54).

The pathophysiological mechanisms underlying glucocorticoid-induced pancreatitis remain poorly understood, although several theories have been proposed to elucidate its etiology (24). Some studies suggest that alterations in calcium metabolism within pancreatic cells may contribute to the development of this condition (19, 21), while others hypothesize that glucocorticoids promote the production of viscous protein-rich secretions, leading to blockage of pancreatic ductules and subsequent localized inflammation (55). Additional evidence indicates that intravenous administration of ACTH, hydrocortisone, or prednisolone can decrease pancreatic volume as well as bicarbonate and amylase secretion (56). It has also been postulated that glucocorticoids may increase total lipid levels, potentially triggering acute pancreatitis (57–60). However, these hypotheses are primarily based on individual animal experiments or clinical observations; several studies have produced conflicting or negative results (61–69). For example, high-dose methylprednisolone has been shown to reduce pancreatic inflammation and edema in animal models by inhibiting cytokine release and leukocyte activation (70). Dexamethasone has been shown to protect pancreatic tissue through its anti-inflammatory effects and inhibition of several inflammatory mediators (71). These inconsistencies cast doubt on the validity and strength of these clinical and laboratory deductions.

Furthermore, emerging research suggests that glucocorticoids exhibit therapeutic potential in the management of pancreatitis, particularly during its early phase. This step is characterized by the development of a significant phlogosis (72), a process which may trigger systemic inflammatory responses and impairs organ functionality (73). As potent anti-inflammatory agents, glucocorticoids have demonstrated efficacy across several inflammatory conditions (74–76). Notably, in animal models of AP, glucocorticoid treatment has shown promising therapeutic outcomes by improving survival rates (71, 77–81), although the underlying mechanisms remain unclear. The speculated pathophysiological pathways through which glucocorticoids may exert their effects in treating pancreatitis include suppression of inflammatory mediators (82), attenuation of endotoxin-induced damage (83), enhancement of microcirculation (84), scavenging oxygen free radicals (85), reduction of nitric oxide levels (86) and NF-kappa B activities (87, 88), as well as induction of acinar cell apoptosis (89–91). These insights underscore the potential role of glucocorticoids in improving outcomes associated with pancreatitis. For decades, there has been extensive research into the use of glucocorticoids in the treatment of AP (92), particularly this medication is considered a conventional treatment in autoimmune pancreatitis (93). Based on the best we know, Stephenson et al. were the first to report the therapeutic benefits of glucocorticoids in human hemorrhagic acute pancreatitis (AP) in 1952 (94). Subsequently, numerous corroborating clinical trials and case reports published in the literature. For example, one study demonstrated how combining dexamethasone with a traditional Chinese herbal concoction reduced the risk of acute respiratory distress syndrome (ARDS) in SAP (severe acute pancreatitis) patients (95), while a meta-analysis of six Chinese trials suggested that corticosteroids could improve patient outcomes in SAP cases (96). The aforementioned studies collectively suggest that glucocorticoids may confer therapeutic benefits in the management of pancreatitis, thereby raising questions about the causal relationship between glucocorticoid use and the potential initiation of pancreatitis.

It is crucial to acknowledge that the majority of existing research primarily includes observational studies. Besides the

aforementioned studies (13, 97), several smaller observational studies have discussed the effect of glucocorticoids on pancreatitis. For example, Iqbal et al. (98) reported a case of pancreatitis induced by high-dose glucocorticoids in a patient being treated for optic neuritis. This case highlighted the need for vigilance on the part of doctors, but its applicability was limited by the fact that it was a single case. Similarly, Ataallah et al. (17) documented a case of acute pancreatitis in a patient with idiopathic immune purpura who had recently been treated with steroids. This report highlights the diagnostic challenges in such patients, but being a single case study, its wider implications are limited. Observational studies are inherently susceptible to biases such as confounding, selection, recall, measurement and reporting bias, and temporal issues (99–101). Considering the inherent limitations of observational studies in establishing causation or fully accounting for confounding factors, caution must be taken into account when these findings are interpreted. To address this limitation and establish a causal link between glucocorticoid use and pancreatitis risk, we conducted a MR study. Unlike traditional observational studies, this method minimizes bias and reduces the risk of reverse causality by using genetic variants as instrumental variables (41, 102–104). This approach provides stronger evidence of causality and allows for a more robust assessment of the long-term effects of glucocorticoid use (41, 102–104). This methodological rigor increases the reliability of our findings and provides clearer insights into the true impact of glucocorticoid use on the risk of pancreatitis (41, 102–104). Our investigation found no significant evidence of a causal association between glucocorticoid use and the risk of acute pancreatitis (AP) and chronic pancreatitis (CP), as determined by rigorous statistical methods including inverse variance weighted (IVW), MR Egger regression, weighted median approach and MR-PRESSO. Across the GWAS pooled datasets from European ancestry, the FinnGen Biobank Consortium and East Asian descendants, Cochran's Q statistics indicated no significant heterogeneity in most outcomes (all P values > 0.05), except for AP in the FinnGen Biobank Consortium (PQ.Egger=0.0012; PQ.IVW=0.0019). Symmetric funnel plots for individuals with AP and CP further confirmed the lack of heterogeneity in these populations (Supplementary Figures 1, 2, 13, 14, 17, 18). To assess potential pleiotropy, we used the MR Egger intercept test, which yielded intercepts that were not statistically different from zero (all P values > 0.05), suggesting no evidence of horizontal pleiotropy. In addition, MR-PRESSO identified one outlier SNP (rs10905284, PGlobal test < 0.001), but showed similar OR estimates to the IVW method after removing this outlier (OR = 1.088, 95% CI = 0.917-1.2191, P = 0.343), reinforcing the robustness of our findings. The leave-one-out analysis further demonstrated that no single SNP significantly influenced the overall causal estimate across all datasets (Supplementary Figures 3, 4, 15, 16, 19, 20). Detailed information on the MR analyses can be found in Supplementary Tables 2, 4 and 5. Overall, these results demonstrate the robustness of our findings, which are consistent across different populations and methodologies. The inclusion of the MR-PRESSO results further validates our findings by addressing potential pleiotropy and confirming the stability of our estimates after outlier correction. Our findings differ from observational studies suggesting an increased risk of pancreatitis with glucocorticoid use. These discrepancies may be due to methodological differences, residual confounding, or limitations of observational data.

Our MR study used genetic instruments to investigate the causal relationship between glucocorticoid administration and the risk of pancreatitis. As far as we know, this is the first reported study to apply the MR method and visual representations in order to explore the causality effects of glucocorticoid usage on pancreatitis risk. The primary strength of our investigation lies in its employment of MR analytical approach, which effectively mitigates confounding biases inherent in retrospective studies and provides more compelling evidence. Unlike traditional observational studies, MR analysis significantly reduces the possibility of reverse causation (41, 102–104). However, it is important to recognize some limitations within our study. Our study does not take into account variations in glucocorticoid dosage, duration of use, or treatment regimens for different conditions. Future studies should take these factors into account to provide a more complete understanding of the relationship between glucocorticoid use and the risk of pancreatitis. Moreover, our findings are based on summary level data and should be interpreted with caution, given the assumptions about genetic tools and potential biases inherent in MR analyses. Although the MR-Egger intercept test showed no evidence of directional pleiotropy and the weighted median method provided consistent estimates, residual confounding cannot be completely excluded. Specifically, the absence of subgroup analysis was due to limited availability of comprehensive clinical data for participants. As a result, our study does not investigate possible sex-specific effects of glucocorticoid use on the risk of pancreatitis. Furthermore, the study population consisted predominantly of individuals with European descendants (34–39), potentially limiting generalizability across diverse ethnic backgrounds such as African populations. It is important to note that possible potential genetic heterogeneity within the European population may also affect the validity of our genetic instruments and MR findings. Therefore, further research with larger sample sizes, more genetically diverse populations or ethnic groups, more detailed sex-stratified analyses and longitudinal follow-up is imperative to conclusively validate the causal relationship between glucocorticoid use and pancreatitis risk. Although our selected genetic variants have been rigorously assessed for robustness and independence, it is acknowledged that they may not capture the entirety of glucocorticoid exposure. Future studies could consider expanding the range of genetic tools or incorporating alternative methodological approaches to comprehensively capture the complexity of glucocorticoid use. Despite the fact that our Mendelian randomization analysis effectively mitigates confounding by measured covariates and is sufficiently powered to detect moderate to large effects, it may not be sensitive enough to identify smaller effect sizes. In addition, unmeasured or residual confounders, such as a Western diet or diabetes, may have influenced our results. Another potential limitation of our study is the possibility of type II error. Type II error occurs when the study fails to detect a true effect due to insufficient statistical power. Given the complexity and multifactorial nature of the etiology of pancreatitis, it is possible that our non-significant results may have been influenced by type II error. Future

studies with larger sample sizes and more comprehensive data may help to mitigate this issue and provide a clearer understanding of the relationship between glucocorticoid use and pancreatitis risk. Moreover, our study does not consider possible interactions between glucocorticoid use and other medications or treatments that may affect the risk of developing pancreatitis. For instance, glucocorticoids and non-steroidal anti-inflammatory drugs (NSAIDs) are often used together, especially for conditions involving inflammation and pain (8, 105, 106). However, there are numerous case reports linking NSAIDs such as indomethacin, piroxicam, ketoprofen, naproxen, rofecoxib and celecoxib with acute pancreatitis (107). Interestingly, naproxen is often considered the preferred analgesic to limit the risk of developing acute pancreatitis (107). Studies have also suggested that widespread prophylactic use of NSAIDs may significantly reduce the risk of acute pancreatitis following therapeutic endoscopic retrograde cholangiopancreatography (ERCP) (107–109). Post-ERCP pancreatitis is a known complication, and glucocorticoids have been investigated for their potential role in preventing this condition (30). Some studies suggest that glucocorticoids may reduce inflammation and edema, potentially decreasing the incidence of post-ERCP pancreatitis (30). However, the evidence is mixed and sometimes contradictory, suggesting that more research is needed to establish their effectiveness in this setting (30, 108, 110–112). Future research should consider these interactions between glucocorticoid use and other medications or treatments to provide a more comprehensive understanding of pancreatitis risk. In addition, the ability of our study to detect small but clinically significant effects may be limited by several factors, most notably the limited number of SNPs used as instrumental variables (IVs). In Mendelian randomization (MR) studies, statistical power is highly dependent on both the strength and number of IVs (113–115). The limited number of SNPs in this study may reduce the ability to detect associations between the IVs and the exposure variable, which may explain the non-significant results. The minimum detectable effect size (MDES) is also crucial; a study with limited power may fail to detect small but meaningful effects (114, 116). To address this concern, we carried out additional replicated MR analyses using GWAS summary datasets from the FinnGen Biobank Consortium and East Asian descendants, in addition to the original European ancestry GWAS data. The consistent results across these different datasets suggest a degree of clinical significance and increase the credibility of our findings. Moreover, the MR-Egger method is designed to detect and correct for directional pleiotropy, which occurs when genetic variants influence outcome through pathways other than the exposure of interest. The key assumption of MR-Egger is the Instrument Strength Independent of Direct Effect (InSIDE) assumption, which states that the strength of the association of the genetic instrument with the exposure is independent of its direct effect on the outcome (115–117). However, this assumption may not always hold in practice, potentially leading to biased estimates. For instance, if the genetic variants have pleiotropic effects that are not independent of their associations with exposure, the MR-Egger intercept test may indicate the presence of pleiotropy even when it is absent, or fail to detect it when it is present (115–118). This may complicate the interpretation of causal estimates derived from MR-Egger analysis. Also, MR-Egger has less statistical power than other MR methods, such as inverse variance weighted (IVW) regression, especially when the number of genetic variants is small or the genetic instruments are weak (115–117, 119). This reduced power can lead to wider confidence intervals and less precise estimates of the causal effect, which should be taken into account when interpreting the results (114, 120). In our study, the MR-Egger intercept test showed no significant evidence of directional pleiotropy (all P values > 0.05), suggesting that pleiotropy is unlikely to significantly bias our causal estimates. Nevertheless, the limitations of MR-Egger, including its reduced precision, must be acknowledged. To address these limitations and validate the robustness of our findings, we conducted several sensitivity analyses, including the Cochran's Q test for heterogeneity and the MR-PRESSO method to detect and correct for pleiotropic outliers. These additional analyses help to provide a more comprehensive assessment of the potential bias due to pleiotropy and increase the transparency and reliability of our results (114, 117, 119, 120).

In conclusion, although our MR-Egger results suggest minimal pleiotropic bias, the inherent limitations of this method and the assumptions upon which it is based must be explicitly acknowledged. To improve the power of future studies and mitigate the inherent limitations of the MR-Egger method, it is essential to increase sample sizes and identify stronger genetic tools. Larger sample sizes can improve the ability to detect associations, thereby increasing the overall power of the study (114, 120). In addition, identifying and using multiple stronger genetic variants as IVs can strengthen the instruments and improve the precision of the estimates, thereby reducing bias and increasing power (115, 116). These strategies are essential to accurately assess the causal relationship between glucocorticoid use and the risk of pancreatitis. These limitations should be taken into account when interpreting the results.

Gene-environment interactions occur when environmental factors such as smoking, diet and concomitant medication use interact with genetic predispositions to influence disease risk (121). For example, oxidative stress from alcohol and smoking may exacerbate genetic mutations associated with pancreatitis, such as those in the SPINK1 and CFTR genes (122). Research suggests that genetic variants may influence how individuals respond to environmental factors (121). Thus, epigenetic modifications induced by environmental exposures may affect the expression of genes involved in glucocorticoid metabolism and stress responses, further complicating the relationship between glucocorticoid use and pancreatitis (123). Future research should focus on identifying specific gene-environment interactions that contribute to the risk of pancreatitis in glucocorticoid users. This can be achieved through genome-wide association studies (GWAS) and epigenome-wide association studies (EWAS), which examine the combined effects of genetic variants and environmental factors on disease risk. Such studies should involve large, diverse populations to capture a wide range of genetic and environmental exposures, thereby increasing the generalizability of the findings.

Additionally, our findings have significant implications for healthcare policy regarding glucocorticoid administration and pancreatitis management. Given the widespread prescription of glucocorticoids and the serious consequences associated with

pancreatitis development, establishing a definitive causal link is crucial for establishing public health strategies towards early prevention and intervention efforts. Despite the fact that our study found no evidence of an association between glucocorticoid use and an increased incidence of acute pancreatitis, clinicians must remain vigilant when prescribing these drugs because of their well-documented side effects. Healthcare providers should assess the risk-benefit profile of glucocorticoid therapy on a case-by-case basis, particularly in patients with additional risk factors for pancreatitis. Standard preventive measures for pancreatitis should continue to be used in clinical practice. Encouraging lifestyle changes, such as maintaining a healthy diet, regular exercise and avoiding excessive alcohol consumption, is crucial for overall health and may indirectly reduce the risk of pancreatitis in patients with complex medical histories (49, 124, 125). Our findings suggest that routine screening for pancreatitis in glucocorticoid users may not be warranted. However, clinicians should remain vigilant for pancreatitis symptoms in patients with multiple risk factors, particularly those with pre-existing conditions that predispose them to pancreatitis. Based on our findings, future guidelines for glucocorticoid therapy should emphasize targeted monitoring rather than broad screening. Although our study found no statistically significant association between glucocorticoid use and the risk of pancreatitis, even a small increase in risk could raise public health concerns due to the widespread use of these drugs and the potential severity of pancreatitis (1, 2, 8).

Given the high prevalence of glucocorticoid use (8), the absolute number of people affected could be substantial. Although our MR study does not support an association between glucocorticoid use and an increased incidence of acute pancreatitis, vigilant clinical practice and adherence to guidelines are essential to mitigate other potential risks. Glucocorticoid-induced pancreatitis can lead to serious complications, including systemic inflammatory response syndrome (SIRS), multiple organ failure and increased mortality (22, 46). For example, Iqbal et al. (98) reported a case of steroid-induced pancreatitis in a patient receiving high-dose steroids for optic neuritis, highlighting the importance of clinician vigilance. Similarly, Ataallah et al. (17) highlighted the diagnostic challenges of glucocorticoid-induced pancreatitis, particularly in patients with multiple risk factors. These cases suggest that although the incidence may be low, the clinical outcomes can be severe, highlighting the need for a public health strategy to mitigate the risks.

From a patient management perspective, it is important to identify high-risk individuals and monitor them closely during glucocorticoid therapy. Clinicians should exercise caution and carefully weigh the benefits of glucocorticoid therapy against the potential risk of pancreatitis, especially when prescribing glucocorticoids to patients with known risk factors such as a history of pancreatitis, alcohol use or metabolic disorders (17, 126). In these high-risk patients, regular monitoring of pancreatic function and prompt treatment of early symptoms may help prevent severe pancreatitis. Previous studies (21) have shown that glucocorticoid-induced pancreatitis can develop in a dose-dependent manner, suggesting that reducing the dose and duration of glucocorticoid therapy may reduce the risk. In addition, glucocorticoids are associated with a number of other adverse effects, including hyperglycemia, hypertension, osteoporosis, neuropsychiatric adverse effects and immunosuppression (127–130).

As an example, a systematic review and meta-analysis found an increased risk of cataract and glaucoma in patients using systemic glucocorticoids (127). Another study reported significant associations between short-term systemic glucocorticoid use and an increased risk of infection and hyperglycemia (128). Understanding the mechanisms underlying these glucocorticoid-induced adverse effects is essential for the development of safer medication strategies. The implementation of regular monitoring, dose reduction, shorter duration of therapy and, where appropriate, alternative treatments in high-risk patients may also help to reduce these risks (129, 131–133). In addition, our study uses Mendelian randomization (MR) to investigate the causal relationship between glucocorticoid use and the risk of pancreatitis. This approach helps to control for confounding while providing more robust evidence of causality (41, 102–104).

The application of MR to the understanding of glucocorticoid-related adverse effects may facilitate the development of targeted mitigation strategies to improve patient outcomes. Further research is needed to identify biomarkers that predict susceptibility to glucocorticoid-related adverse effects and to develop targeted interventions. Studies using pharmacogenomic approaches may provide insight into individual variability in response to glucocorticoid therapy. We recommend that future research should focus on the development and validation of risk assessment tools that integrate genetic, clinical and lifestyle factors to identify patients at high risk of glucocorticoid-related pancreatitis and thus develop safer drug use strategies.

5 Conclusion

This study represents the first MR investigating the causal relationship between glucocorticoid use and pancreatitis. However, our MR results do not provide evidence, supporting an association between glucocorticoid use and increased incidence of pancreatitis.

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 in the article/Supplementary Material.

Ethics statement

The studies involving humans were approved by the Ethics Committee of the First Affiliated Hospital of Wenzhou Medical University (KY2023-R270). The studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent for participation was not required from the participants or the participants' legal guardians/next of kin in accordance with the national legislation and institutional requirements.

Author contributions

WL: Data curation, Formal analysis, Writing – original draft, Writing – review & editing. QZ: Data curation, Writing – review & editing. XW: Conceptualization, Supervision, Writing – review & editing. XL: Data curation, Writing – review & editing. XN: Data curation, Writing – review & editing. JP: Conceptualization, Supervision, Writing – review & editing. MZ: Writing – review & editing. SF: Writing – review & editing. WH: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Supervision, Writing – original draft, Writing – review & editing.

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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.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fimmu.2024.1420840/full#supplementary-material

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EDITED BY
Ravi Kumar Sharma,
Chandigarh University, India

REVIEWED BY
Mateusz Jagielski,
Nicolaus Copernicus University in Toruń, Poland
Namrata Gupta,
University of Nevada, Reno, United States

*CORRESPONDENCE
Fabian Frost
✉ fabian.frost@med.uni-greifswald.de

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

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The composition of the stent microbiome is associated with morbidity and adverse events during endoscopic drainage therapy of pancreatic necroses and pseudocysts

Fabian Frost[1]*, Valeria Khaimov[2], Volkmar Senz[3], Stefan Weiss[1,4], Bastian Klußmann-Fricke[2], Malte Rühlemann[5], Corinna Bang[5], Andre Franke[5], Tilman Pickartz[6], Christoph Budde[1], Ali A. Aghdassi[1], Stefan Siewert[2], Frank U. Weiss[1], Niels Grabow[3], Markus M. Lerch[1,7†] and Matthias Sendler[1†]

[1]Department of Medicine A, University Medicine Greifswald, Greifswald, Germany, [2]Institute for Implant Technology and Biomaterials E. V., Rostock, Germany, [3]Institute for Biomedical Engineering, Rostock University Medical Center, Rostock, Germany, [4]Department of Functional Genomics, Interfaculty Institute for Genetics and Functional Genomics, University Medicine Greifswald, Greifswald, Germany, [5]Institute of Clinical Molecular Biology, Christian Albrechts University of Kiel, Kiel, Germany, [6]Department of Internal Medicine IV, Klinikum Südstadt Rostock, Rostock, Germany, [7]Ludwig Maximilian University Hospital, Ludwig Maximilian University of Munich, Munich, Germany

Background: Development of pancreatic necroses or pseudocysts are typical complications of pancreatitis and may require endoscopic drainage therapy using metal or plastic stents. Microbial infection of these lesions poses a major challenge. So far, the composition and significance of the microbial colonization on drainage stents are largely unknown although it may impact outcomes during endoscopic drainage therapy.

Methods: A total of 26 stents used for drainage of pancreatic lesions were retrieved and the stent microbiome was determined by 16S rRNA gene sequencing. Additional analysis included comparison of the stent microbiome to the intracavitary necrosis microbiome as well as scanning electron microscopy (SEM) and micro-computed tomography (μCT) imaging of selected metal or plastic stents.

Results: The stent microbiome comprises a large proportion of opportunistic enteric pathogens such as *Enterococcus* (14.4%) or *Escherichia* (6.1%) as well as oral bacteria like *Streptococcus* (13.1%). Increased levels of opportunistic enteric pathogens were associated with a prolonged hospital stay ($r = 0.77$, $p = 3e{-}06$) and the occurrence of adverse events during drainage therapy ($p = 0.011$). Higher levels of oral bacteria were associated ($r = -0.62$, $p = 8e{-}04$) with shorter durations of inpatient treatment. SEM and μCT investigations revealed complex biofilm networks on the stent surface.

Conclusion: The composition of the stent microbiome is associated with prolonged hospital stays and adverse events during endoscopic drainage therapy, highlighting the need for effective infection control to improve patient outcomes. In addition to systemic antibiotic therapy, antimicrobial stent coatings

could be a conceivable option to influence the stent microbiome and possibly enhance control of the necrotic microflora.

KEYWORDS

acute pancreatitis, bacteria, pancreatic necrosis, necrosis microbiome, microbiota, LAMS, WON, WOPN

1 Introduction

Acute and chronic pancreatitis are common reasons for hospital admissions to gastroenterological wards (1), with abdominal pain being the primary symptom. The most frequent causes are excessive alcohol consumption or biliary obstruction, the latter only for acute pancreatitis. The development of pancreatic necrosis or pseudocysts are a feared complication in patients with pancreatitis, which is associated with significant morbidity and mortality (2). The revised Atlanta classification (3) categorizes necrosis and fluid collections of the pancreas into four different categories. Areas of pancreatic necrosis are called acute necrotic collection for up to 4 weeks, until they develop a thickened wall and are then named walled-off necrosis (WON). Liquid lesions without solid contents are called peripancreatic fluid collections within the first 4 weeks after the initial pancreatitis episode. When these lesions mature, they develop a well-defined wall and are called pseudocysts. Pancreatic necrosis or pseudocysts may become superinfected or cause complications such as biliary or gastric outlet obstruction or analgesics-resistant pain. In such cases, drainage may be indicated which is mostly being performed endoscopically via transgastric or transduodenal drainage, as these methods are associated with lower morbidity or mortality compared to (open) surgical approaches (2, 4–6). These procedures involve creating an opening through the gastric or duodenal wall to connect the necrosis or fluid collection with the gastrointestinal tract, thus allowing its drainage. Lumen-apposing metal stents (LAMS) or multiple pigtail plastic stents are then placed into the opening to avoid its closure or blockage. In cases of necrosis, this opening can then be used to perform repeated endoscopic necrosectomies as needed. One of the most important factors influencing the success of endoscopic drainage therapy and its complication rate is the presence of bacteria within the pancreatic collections (7). When a superinfection is present, rates of stent dislocations or obstructions, as well as residual lesions requiring repeated interventions are more common. This highlights the need of adequately controlling superinfections during endoscopic drainage therapy. Presently, there is some knowledge about the composition of microbial communities within pancreatic necrosis or pseudocysts but very little about the microbiome colonizing the stents used to drain them. We have recently shown that the necrosis microbiome usually consists of a multitude of different bacteria with a strong presence of gram-negative opportunistic enteric pathogens like *Escherichia*, *Klebsiella*, or *Citrobacter* and gram-positive opportunistic pathogens like *Enterococcus* as well as anaerobic bacteria like *Bacteroides* (8). These opportunistic enteric pathogens are part of the natural gut microbiome and usually do not cause disease. However, they can become pathogenic when the gut barrier is impaired, especially when they translocate to areas with compromised immune control, such as encapsulated cystic or necrotic lesions. To date, the composition and significance of the microbial colonization on stents used for drainage of pancreatic necrosis or pseudocysts are largely unknown. Investigations of the biofilm on drainage stents in the hepatopancreaticobiliary tract have so far been limited to those used to treat biliary obstruction, where diverse microbial communities have been identified (9). In the present study, we characterized the microbiome detectable on the stents used for endoscopic drainage therapy of pancreatic necroses or pseudocysts. We investigated the clinical significance of the stent microbiome, and analyzed possible differences to the necrosis microbiome. Moreover, we performed scanning electron microscopy and micro-computed tomography (CT) analyses of explanted drainage stents to determine microbial growth and stent degradation patterns.

2 Methods

2.1 Study participants

Patients who underwent endoscopic drainage therapy of pancreatic necrosis or pseudocysts were prospectively recruited at the University Medicine Greifswald (Germany) in the period of March 2019 to June 2021. All participants provided written informed consent, and the study was approved by the ethics committee of the University Medicine Greifswald (III UV 91/03b). All methods were carried out in accordance with the relevant guidelines and regulations (Declaration of Helsinki).

2.2 Endoscopic drainage therapy and sample collection

Endoscopic ultrasound-guided transluminal drainage of pancreatic necroses or pseudocysts was performed in all 26 independent cases. The inclusion in this observational study had no impact on the modality of endoscopic drainage therapy. LAMS (HotAxios, Boston Scientific, Marlborough, MA, United States or SPAXUS, TaeWoong Medical, Ilsan, Korea) were used for the initial drainage in 23 cases and double-pigtail plastic stents in further three cases, according to the endoscopists choice. The stents were collected after a median time of 18.5 (10.8–45.5, 1st–3rd quartile) days. In case of WONs, additional tissue from the necrotic cavity was collected shortly before the removal of the stents. In four WON cases, however, no residual necrotic debris was left to collect before stent extraction.

2.3 16S rRNA gene sequencing and taxonomic annotation

Debris was collected from the inner stent surface and DNA was isolated using the PureLink Quick Plasmid Miniprep Kit (Invitrogen, Thermo Fisher Scientific, Waltham, MA, United States). 16S rRNA

gene sequencing was then performed as previously described (10). In brief, amplification of the V1 and V2 regions of bacterial 16S rRNA genes was performed using the primer pair 27F and 338R and samples subsequently sequenced on a MiSeq platform (Illumina, San Diego, United States) using a dual-indexing approach. The open-source software package DADA2 (v.1.10) (11) was used for amplicon-data processing following the authors' recommended procedure for large datasets[1] as described before (12). This approach allows for single-nucleotide resolution of amplicons (amplicon sequence variants, ASVs). Data processing was adapted to the V1-V2 amplicon. Five bases were truncated from the 5′ end of the sequence on both reads. Forward and reverse reads were truncated to a length of 200 and 150 bp, respectively. A shorter resulting read length after truncation was possible if the sequence quality dropped below five. Read-pairs were discarded if they contained ambiguous bases, expected errors higher than 2 and when originating from PhiX spike-in. Error profiles were inferred using 1 million reads of the respective sequencing run, followed by dereplication, error correction and merging of forward and reverse reads. After creation of ASV abundance tables of all samples, chimeric amplicon sequences were identified and removed using the removeBimeraDenovo() function in consensus mode. For taxonomic annotation, a Bayesian classifier and the Ribosomal Database Project (RDP) training set version 16 were used. The resulting median read count of the stent microbiome samples was 10,531 (6,004-17,942; first-third quartile).

2.4 Scanning electron microscopy

After extraction, stents were cut into two pieces along the longitudinal axis. One half of the stent was used for microbiological analysis as described above. The other half was prepared for scanning electron microscopy described before in detail (13). This included rinsing in 0.1 M sodium phosphate buffer (pH 7.4) and fixation with 2.5% glutaraldehyde/2% formaladehyde over night at 4°C. After fixation, the samples were rinsed in phosphate buffer before dehydration in ascending ethanol series (70–80% to 96–100%). Afterward, all samples were chemically dried using hexamethyldisiliazane. Scanning electron microscopy was performed on a Quanta FEG 250 (FEI Company, Germany). Prior to scanning, samples were sputter coated with gold.

2.5 Micro-computed tomography

The analysis of the distribution of necrotic debris on drainage stents was performed using a Skyscan 1273 (Bruker, United States) microCT system. To enhance contrast between the necrotic debris and the metal stent parts in the microCT, each stent was transferred to and kept overnight in Lugol's solution (Carl Roth, Germany) at room temperature. Afterwards, excess solution was removed by placing the stent on tissue paper followed by airdrying overnight under the fume hood. For scanning, stents were placed on a specimen holder using double sided sticky tape. Stents were scanned using voltages between 55 and 100 kV and currents between 40 and 272 μA with an exposure time of 200 ms. Achieved resolutions were between 13 and 17 μm voxel size. For visualization of the acquired datasets either ImageJ (NIH, United States) or CTVox (Bruker, United States) were used.

2.6 Phenotypic data

Body mass index (BMI) was calculated by dividing the body weight in kilogram by the square of the body height. Patients were considered as smokers if they consumed at least one cigarette daily. The diameter of the necrotic or fluid lesions before drainage was measured using available imaging data (computed tomography or magnetic resonance imaging). Antibiotic treatment states if any course of antibiotics was taken during initial drainage therapy, not including single shot antibiotic periinterventional prophylaxis. The initial duration of hospital stay indicates the days of the first hospital stay, whereas the total duration of hospital stay also includes follow-up inpatient treatments that were directly linked to the endoscopic drainage therapy. Laboratory values for white blood cells, hemoglobin, platelet count, estimated glomerular filtration rate (eGFR), urea, and bilirubin were obtained on admission. In one pseudocyst drainage case, the bilirubin level was not available. For C-reactive protein we documented the highest value within the first 48 h.

2.7 Data and statistical analysis

All statistical analyses were performed using the statistical language "R" (v.4.3.2, https://www.R-project.org/). For microbiome related analyses, the bacterial read counts resulting from 16S rRNA gene sequencing were transformed into relative abundance data. The index "Bray–Curtis dissimilarity" was computed prior to ordination using the R package "vegan" (function "vegdist") (14). Principal coordinate analysis (PCoA) was performed using the "vegan" function "cmdscale." The "vegan" function "adonis" was used to perform permutational analysis of variance (PERMANOVA, 1,000 permutations) based on a Bray–Curtis dissimilarity. The two-sided Mann–Whitney test (MW, "stats" function "wilcox.test") was applied for assessment of statistical significance in case of unpaired microbiome data, whereas the Wilcoxon signed-rank test was employed for paired data (WSR, "stats" function "wilcox.test," paired = true). Spearman correlations between continuous phenotypes and microbial taxa were calculated using the "cor.test" function ("stats" package). p values <0.05 were considered significant.

3 Results

The study cohort comprised 26 patients who underwent endoscopic drainage of pancreatic necroses or pseudocysts using LAMS or plastic stents. Table 1 shows the characteristics of these drainage cases of which 16 were performed to treat pancreatic necroses and 10 for pancreatic pseudocysts. Suspicion of infection was the most common indication (73.1%) for drainage. The median age was 58.0 years and 73.1% of patients were males. Alcohol abuse was the most common etiology for development of the underlying acute or chronic pancreatitis in 50.0% of cases. A total of 30.8% of patients were treated in an intensive care unit at some point during treatment. The mortality rate was 7.7%.

1 https://benjjneb.github.io/dada2/bigdata.html

TABLE 1 Case characteristics.

	All cases ($n = 26$)	WON ($n = 16$)	Pseudocysts ($n = 10$)
Age (years)	58.0 (50.0–66.2)	56.5 (45.0–67.8)	58.0 (56.0–62.8)
Female sex (%)	26.9	37.5	10.0
Body mass index (kg/m^2)	25.1 (23.1–29.9)	26.5 (23.9–31.8)	23.9 (23.1–25.6)
Active smoking (%)	73.1	68.8	80.0
Diabetes (%)	26.9	18.8	40.0
Exocrine pancreatic insufficiency (%)	11.5	6.2	20.0
History of cancer (%)	3.8	0	10.0
Proton-pump inhibitor usage (%)	76.9	68.8	90.0
Etiology of pancreatitis (%)			
Alcoholic	50.0	50.0	50.0
Biliary	15.4	25.0	0
Idiopathic	23.1	25.0	20.0
Post-ERC	7.7	0	20.0
Traumatic	3.8	0	10.0
Indication for drainage (%)			
Suspicion of infection	73.1	81.2	60.0
Gastric outlet obstruction	11.5	18.8	0
Continuous enlargement/pain	15.4	0	40.0
Diameter of lesion (cm)	7.9 (5.6–12.3)	7.7 (5.9–13.4)	8.4 (6.0–10.8)
Antibiotic treatment (%)	84.6	100.0	60.0
Duration of hospital stay, initial (days)	24.0 (12.0–40.0)	29.0 (17.2–68.2)	20.0 (7.5–26.2)
Duration of hospital stay, total (days)	31.5 (18.0–56.2)	32.5 (18.0–71.5)	25.5 (15.5–45.2)
Highest level of care (%)			
Regular ward	53.8	37.5	80.0
Intermediate care	15.4	12.5	20.0
Intensive care	30.8	50.0	0
Mortality (%)	7.7	12.5	0
White blood cells (cells/nL)	13.6 (10.6–18.2)	15.2 (11.0–23.2)	12.6 (10.3–15.0)
Hemoglobin (mmol/l)	7.6 (6.6–18.2)	7.8 (6.3–8.5)	7.4 (7.3–7.8)
Platelet count (cells/nL)	361.0 (242.0–500.0)	301.5 (212.0–494.8)	373.5 (302.8–543.2)
eGFR (ml/min)	68.0 (51.8–96.5)	77.5 (41.0–101.5)	68.0 (61.0–84.2)
Urea (mmol/l)	5.2 (3.9–10.8)	8.0 (4.3–15.5)	4.9 (3.9–5.2)
Bilirubin (µmol/l)	8.0 (5.3–11.4)	8.9 (6.3–19.5)	5.7 (5.2–8.0)
CRP (mg/l), highest within first 48 h	148.5 (70.4–251.0)	201.5 (101.8–272.2)	79.8 (47.8–216.5)

Continuous variables are given as median (1st–3rd quartile). Categorical variables are stated as percentages. All numbers were rounded to one decimal place. Laboratory values were obtained on admission if not indicated otherwise. CRP, C-reactive protein; eGFR, Estimated glomerular filtration rate; WON, Walled-off necrosis; n, Number of cases.

3.1 The stent microbiome largely consists of oral microbes and opportunistic enteric pathogens

A total of 23 LAMS and three plastic stents were collected after being removed during pancreatic necrosis or pseudocyst drainage therapy and the stent microbiome was determined using 16S rRNA gene sequencing. Figure 1 shows that the most frequently occurring bacteria were *Enterococcus*, *Streptococcus*, *Prevotella*, *Lactobacillus*, and *Escherichia* together accounting for 52.2% of the total abundance (Supplementary Table S1). Taken together, typical gut microbial opportunistic enteric pathogens such as *Enterococcus* as well as gram-negative *Escherichia*, *Citrobacter*, *Klebsiella*, and *Enterobacter* comprised 24.8% of the bacteria. Microbes that are found in high abundance in the oral cavity (15) such as *Streptococcus*, *Veillonella*, *Fusobacterium*, *Lactobacillus*, *Prevotella*, and *Haemophilus* made up 42.4% of the stent microbiome. Permutational ANOVA revealed some differences between the stent microbiome of necrosis and pseudocyst stents ($r^2 = 7.9\%$, $p = 0.024$). Specifically, stents used for necrosis drainage showed higher

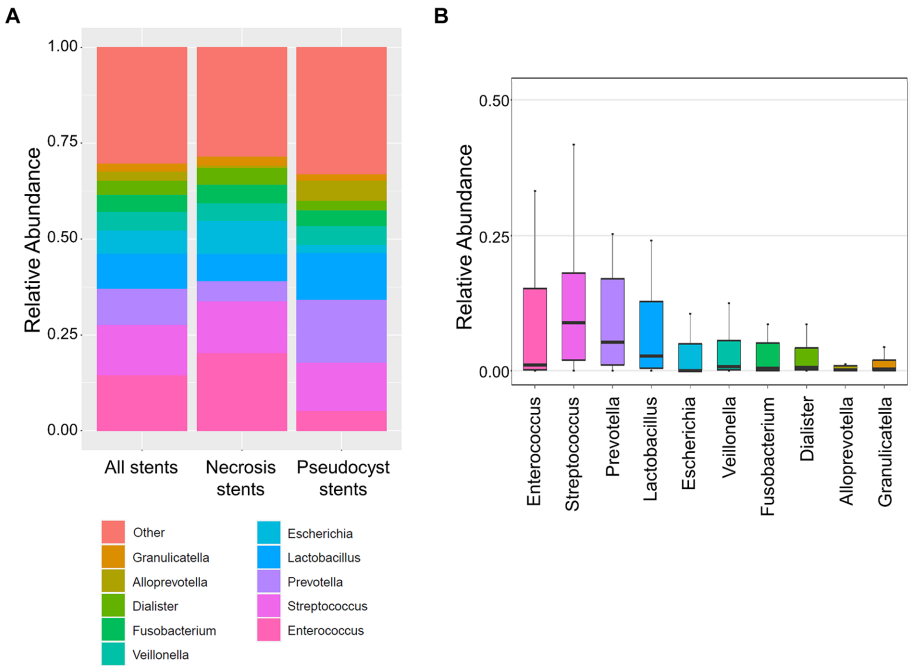

FIGURE 1
Stent microbiome composition. **(A)** Stacked bar plots show the average composition of the stent microbiome in all stents (left), necrosis stents (middle), and pseudocyst stents (right). **(B)** Boxplot shows the distribution of the 10 most abundant bacteria within the stent microbiome (all stents). The *y*-axis limits were set from 0 to 0.5 (50% abundance) for better display of the lesser abundant taxa.

abundance of *Enterococcus* (mean 20.2% vs. 5.1%, $p = 0.007$) and opportunistic enteric pathogens in general (mean 33.8% vs. 10.3%, $p = 0.016$) as compared to pseudocyst stents. Oral microbes were less abundant in necrosis stents with a mean abundance of 35.6% vs. 53.3% in pseudocyst stents, however, this was not significant ($p = 0.109$).

3.2 The composition of the stent microbiome correlates with length of hospital stay and the occurrence of adverse events during endoscopic drainage

The stent microbiome composition was associated with the initial ($r^2 = 14.2\%$, $p < 0.001$) and total length of hospital stay ($r^2 = 13.3\%$, $p < 0.001$). The associations with the initial and total length of hospital stay were also replicated in the subgroups of necrosis ($r^2 = 12.1\%$, $p = 0.042$ and $r^2 = 12.4\%$, $p = 0.035$, respectively) and pseudocyst drainage cases ($r^2 = 25.9\%$, $p < 0.001$ and $r^2 = 23.3$, $p = 0.008$, respectively). More specifically, the 10 most abundant stent microbiome bacteria, as well as the groups of opportunistic enteric pathogens and typical oral microbes, were correlated with the length of hospital stay (Figure 2A; Supplementary Table S2). The analysis revealed a strong positive correlation between the presence of *Enterococcus* (rho = 0.72, $p < 0.001$ and rho = 0.69, $p < 0.001$) or in general opportunistic enteric pathogens (rho = 0.80, $p < 0.001$ and rho = 0.78, $p < 0.001$) with the initial and total length of hospital stay. The presence of oral bacteria (rho = −0.62, $p < 0.001$ and rho = −0.62, $p < 0.001$) was associated with shorter hospital stay durations. Specific bacteria from the oral microbiome such as *Veillonella*, *Prevotella*, or *Streptococcus* exhibited the same inverse associations with the initial or total length of hospital stay in the overall group or the subgroups of necrosis or pseudocyst drainage stents (Figure 2A; Supplementary Table S2).

Adverse events such as instant or delayed bleeding, stent dislocation, buried LAMS, or residual lesions requiring a surgical intervention occurred in seven out of 26 cases undergoing endoscopic drainage therapy. These adverse events cases exhibited a different stent microbiome composition than the other uncomplicated cases ($r^2 = 8.0\%$, $p = 0.022$). Specifically, we found a higher abundance of opportunistic enteric pathogens (mean 53.3% vs. 14.3%, $p = 0.011$) and a lower abundance of oral bacteria (mean 25.0% vs. 48.7%, $p = 0.026$) in cases where adverse events occurred (Figure 2B).

3.3 The stent microbiome consists of more oral microbes as compared to the necrotic cavity microbiome

A direct comparison of the stent microbiome with the corresponding necrotic cavity microbiome was performed in 12 patients from whom paired stent and necrosis sample could be obtained. Figure 3 shows a high similarity between the stent and the necrosis microbiome of these patients. PCoA indicated that the microbiomes of paired necrosis stent and necrotic cavity samples were positioned in closer proximity to each other as compared to unrelated samples. According to PERMANOVA,

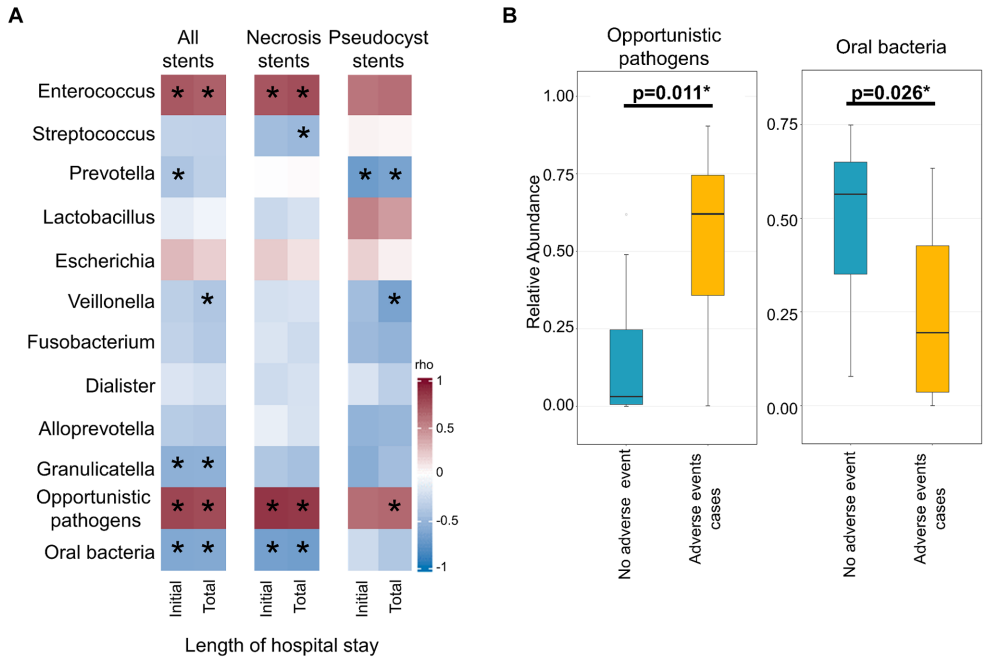

FIGURE 2
Association of the stent microbiome with length of hospital stay and occurrence of adverse events during endoscopic drainage therapy. **(A)** Heatmap depicts the strength of the Spearman correlation (rho) between bacteria from the stent microbiome in all stents (left), necrosis stents (middle), and pseudocyst stents (right) and the length of the initial and total hospital stay. **(B)** Boxplots show the distribution of opportunistic enteric pathogens or oral bacteria of the stent microbiomes of cases without adverse events as compared to cases where adverse events occurred during endoscopic drainage therapy. *Indicates significant result ($p < 0.05$).

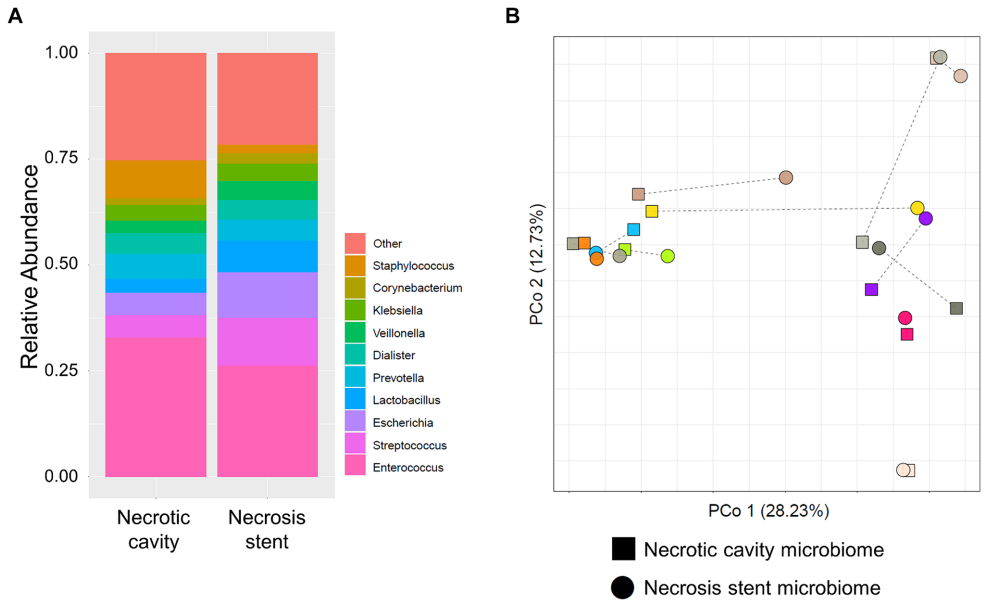

FIGURE 3
Microbiome comparison of necrosis drainage stents and paired necrotic cavity samples. **(A)** Stacked bar plots show the composition of the microbiome in the necrotic cavity (left) and necrosis stents (right). **(B)** Principal coordinate analysis (PCoA) depicts necrotic cavity and necrosis drainage stents microbiomes. Paired samples are connected via a thin line and share the same color. They are located more closely to each other than unrelated samples.

there was no major difference between the stent and the necrosis microbiome ($r^2 = 2.1\%$, $p = 0.119$). The stent microbiome merely yielded a higher abundance of *Lactobacillus* (mean 7.4% vs. 3.4%, $p = 0.029$) or typical oral microbes (mean 28.9% vs. 18.0%, $p = 0.029$) as compared to the necrosis microbiome (Supplementary Table S3).

3.4 Drainage stents have a homogenous microbiome composition on the in-and outside

To investigate whether the stent microbiome differs between the outer and inner surface, the microbiomes of both stent sides were determined in a subsample of six stents. There was no significant difference in the microbial community composition in terms of the most abundant microbes of the stent microbiome when comparing both sides as shown in Figure 4 and Supplementary Table S4.

3.5 Imaging of pancreatic necrosis or pseudocyst drainage stents

To investigate the distribution of necrotic material and the bacterial biofilm on the stent surface, micro-computed tomography (μCT) analyses were performed on six extracted LAMS used for pancreatic necrosis drainage. Figure 5 shows a scattered distribution of the necrotic debris along the inner surfaces of the stents with the largest amount of necrotic content at both flanges.

To further elucidate a possible link between the stent microbiome and the intensity of the degradation of the stent cover, scanning electron microscopy (SEM) of different LAMS ($n = 10$) and plastic stents ($n = 3$) was performed. As shown in Supplementary Figures S1, S2, degradation of the stent cover is present in all explanted LAMS as well as plastic stents. There was no apparent difference in the visual intensity of degradation in relation to the microbiome of the stent. Most stents showed dense bacterial populations. The dominating bacterial taxa, however, varied widely in the investigated stent explants, as demonstrated by the visually detectable different shapes and growth patterns of the stent microbial flora. Figure 6 shows exemplary SEM images of one double-pigtail plastic stent and three LAMS stents.

4 Discussion

We analyzed the bacterial composition on stents used for endoscopic drainage therapy of pancreatic necroses or pseudocysts to determine the stent-attached microbiome. The analyses showed that the stent microbiome mainly consists of two groups of bacteria: The first one consists of those that are highly abundant in the oral cavity (15) such as *Streptococcus*, *Veillonella*, *Prevotella*, *Lactobacillus*, or *Fusobacterium* whereas the second group comprises opportunistic enteric pathogens such as *Enterococcus* or *Escherichia*. We suggest that the group of opportunistic enteric pathogens colonized the stent surfaces secondarily from the necrotic cavity or fluid collection. Translocation of bacteria into areas of pancreatic necrosis or pseudocysts may occur via translocation of luminal intestinal bacteria, via bloodstream or ascending through the lymphatic system. Patients with acute or chronic pancreatitis are particularly vulnerable to translocation events because their dysbiotic gut microbiome is enriched with opportunistic enteric pathogens, such as *Enterococcus* and other gram-negative bacteria like *Escherichia* (16–20). This can partly be explained by the prominent role of the exocrine pancreas in regulating the gut microbiome composition (10, 12). Moreover, during the course of acute pancreatitis local und systemic immunosuppression and a disturbed gut barrier function promote translocation of gut bacteria into areas of necrosis (8).

We have recently shown that a higher proportion of *Enterococcus* in the pancreatic necrosis microbiome correlates with longer hospital stays of patients with necrotizing pancreatitis (8). Also, the rate of adverse events during endoscopic drainage therapy is associated with infected pancreatic necrosis (based on necrosis culture) (7). In the present analysis, patients harboring a stent microbiome with high abundance of opportunistic enteric pathogens had longer hospital stays and were more likely to experience adverse events such as instant or delayed bleeding, stent dislocation, buried LAMS, or residual lesions during endoscopic drainage therapy. The presence of opportunistic enteric pathogens in areas of pancreatic necrosis or pseudocyst can have multiple detrimental effects. First, the local infection drives systemic inflammation and may trigger recurring septicemia. This is complicated by the fact that the host's immune system is severely compromised in these encapsulated lesions, which is why endoscopic drainage may be required. Another challenge posed by these bacteria, even after drainage, is their ability for agglutination and biofilm formation. Lipopolysaccharides (LPS), which are an integral part of the outer membrane of gram-negative *Enterobacteria* such as *Escherichia*, play an important role in the initiation of biofilm formation (21, 22), which in turn may explain the increased rates of stent obstruction and residual lesions associated with infected pancreatic necrosis during endoscopic drainage therapy (7). Likewise the gram-positive bacterium *Enterococcus faecalis* induces local inflammation, protects itself from immune clearance via the multiple peptide resistance factor and delays wound healing (23). Taken together, these mechanisms may also delay contraction of the necrotic cavity or pseudocyst and possibly promote events of stent dislocation.

While a greater abundance of opportunistic enteric pathogens correlated with longer disease duration, we found that stent microbiomes enriched with bacteria that are frequently found in the oral microbiome, such as *Streptococcus*, *Veillonella*, *Fusobacterium*, *Lactobacillus*, *Prevotella*, and *Haemophilus* (15), were associated with shorter hospital stays and the absence of adverse events during endoscopic drainage therapy. These bacteria also account for the largest proportion of bacteria found in the gastric juice (24). Therefore, we hypothesize that oral bacteria secondarily colonize the stent surfaces, and to some extent the necrotic or pseudocyst cavity, after a connection to the gastrointestinal tract is being established by transgastric or transduodenal stent placement. Correspondingly, the stent microbiome contained a higher abundance of oral bacteria as compared to matched pancreatic necrotic cavity samples, the probable reason being the luminal proximity of the stent. Whether the presence of oral microbes among the stent microbiome is of pathophysiological relevance is currently unclear. On the one hand, it would be conceivable that these oral microbes suppress the growth of opportunistic enteric pathogens if present in abundance, promote successful endoscopic drainage therapy and lead to shorter hospital stays. On the other hand, a strong presence of oral bacteria in the stent microbiome could result from the absence of competing opportunistic enteric pathogens and have now pathophysiological relevance.

Tackling infected pancreatic necrosis or fluid collections has always been a therapeutic challenge and various approaches have tried to alleviate the disease burden. Attempts to avoid infection of pancreatic necrosis in the first place using antibiotic prophylaxis did not result in a significant reduction of infected pancreatic necrosis or mortality as

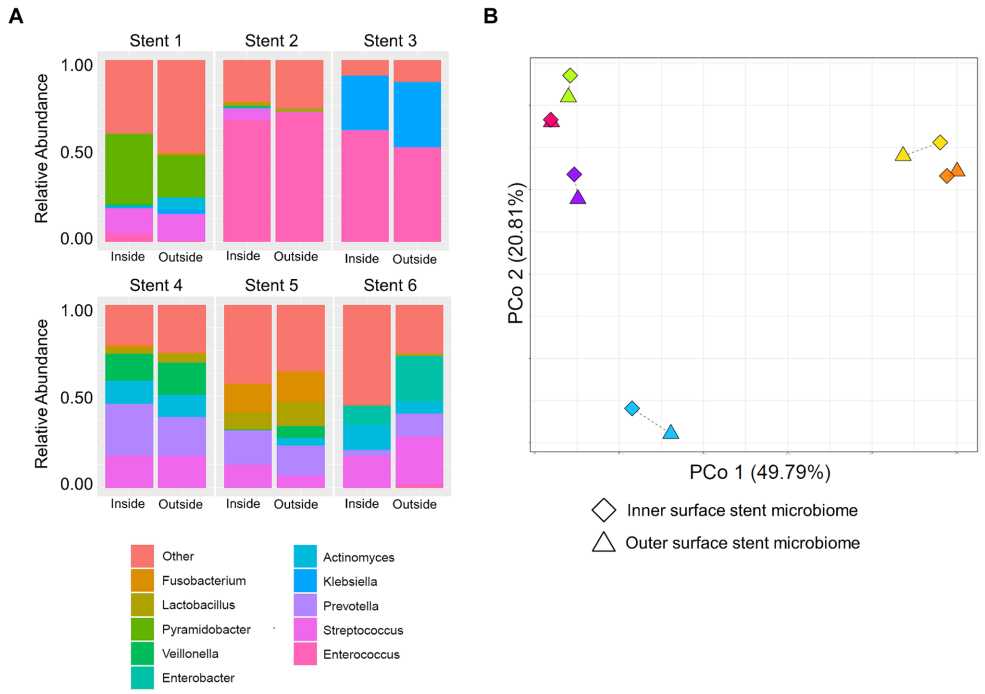

FIGURE 4
Comparison of the inner and outer surface stent microbiome. **(A)** Stacked bar plots show the composition of the microbiome of the inner (left bars) and outer (right bars) stent surface of six necrosis or pseudocyst drainage stents. **(B)** Principal coordinate analysis (PCoA) displays the microbiomes of the inner and outer surface of different drainage stents. Paired samples are connected via a thin line and share the same color. They are located very closely to each other, indicating high similarity, as compared to unrelated samples.

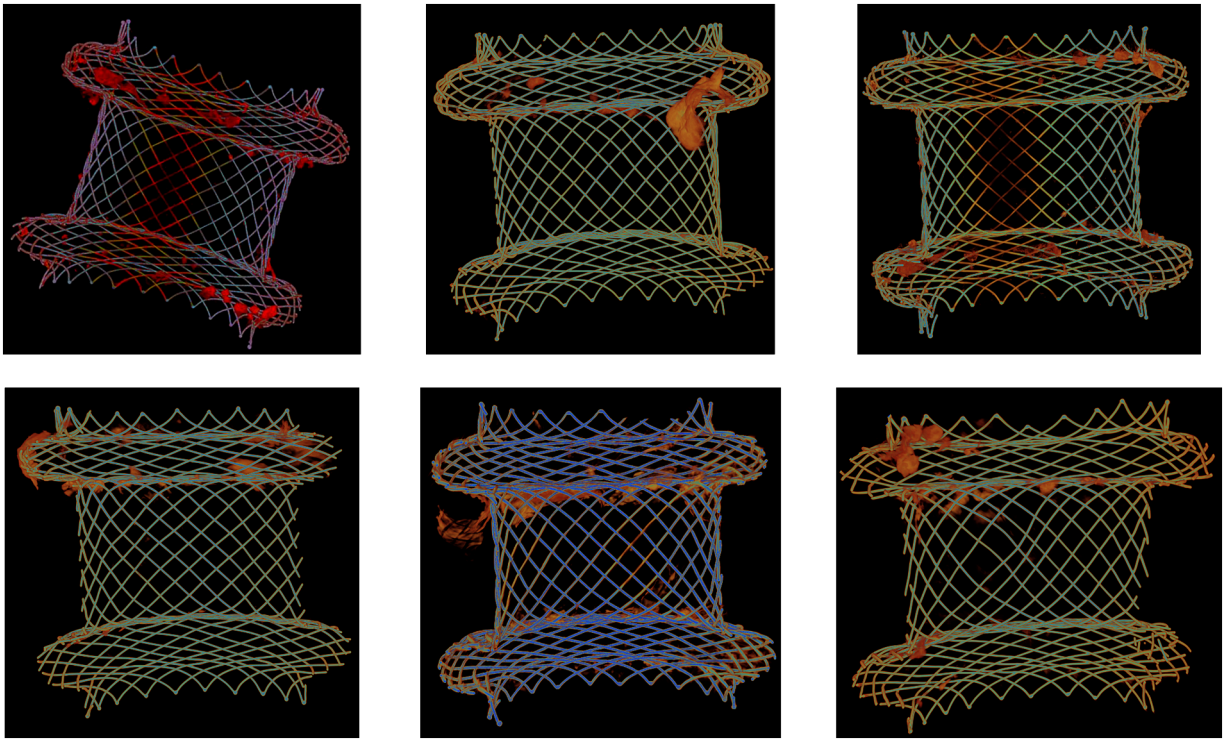

FIGURE 5
Micro-computed tomography (μCT) analysis of six extracted lumen-apposing metal stents (LAMS). The stents were used for drainage of pancreatic necrotic collections. Necrotic debris (orange-red color) is mostly located at both flanges.

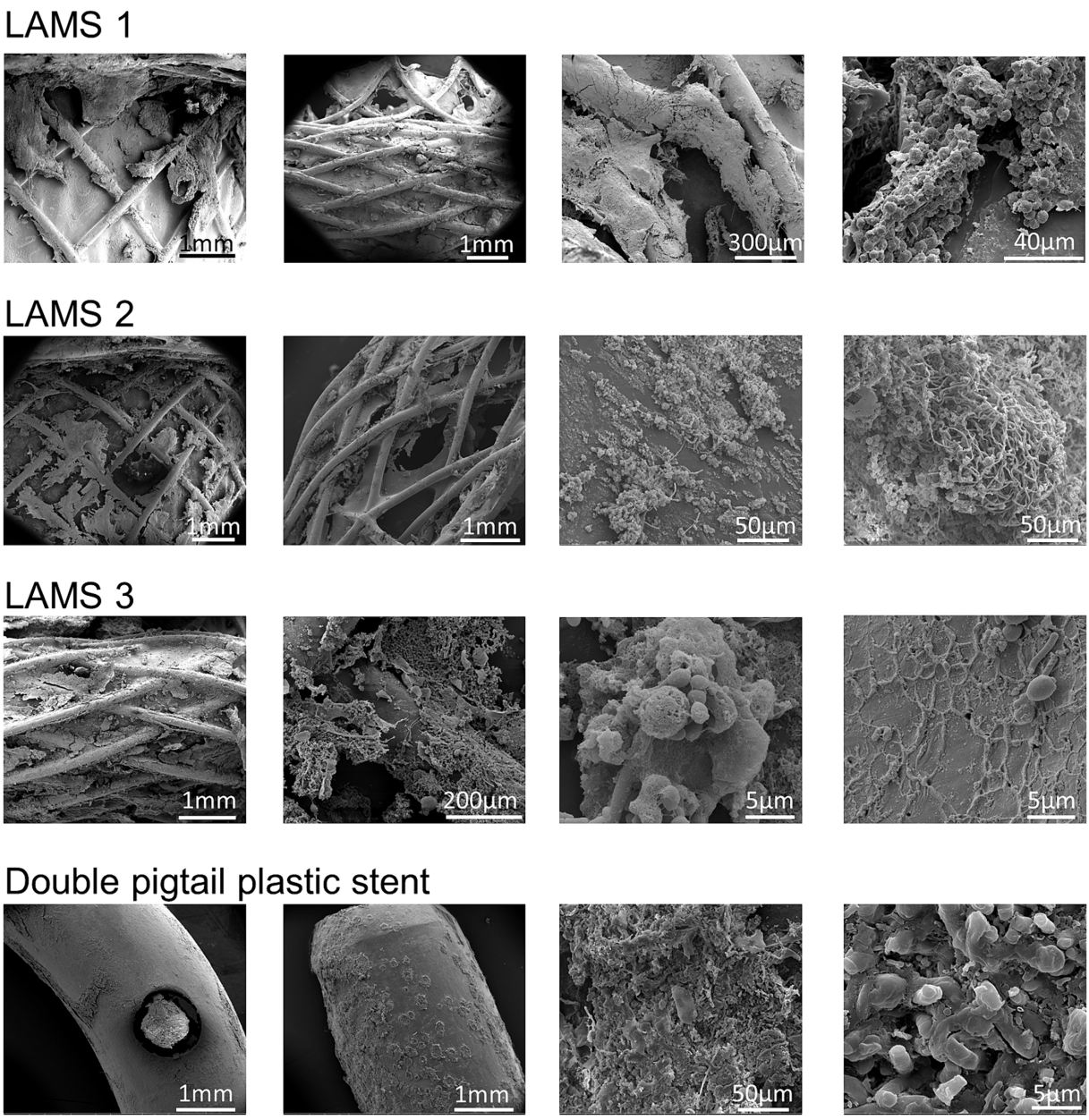

FIGURE 6
Examples of scanning electron microscopy investigations of stent surfaces. Shown are exemplary scanning electron microscopy images of three lumen-apposing metal stents (LAMS) and one double-pigtail plastic stent. All stents show distinct signs of surface degradation as well as bacterial overgrowth resulting in biofilms with complex meshworks.

shown in a recent meta-analysis (25). However, there is no debate that broad-spectrum antibiotics should be administered when there is suspicion of infected pancreatic necrosis or septicemia (26). Yet, in case of infected pancreatic collections, antibiotics alone are often insufficient and additional endoscopic drainage is required (26). LAMS have become the first choice in drainage of pancreatic necrosis containing large amounts of necrotic debris, whereas multiple plastic stents are still used for lesions with little solid content (27). The obvious advantage of LAMS is its larger diameter that may facilitate clearance of larger pieces of necrotic debris and also enables the endoscopist to perform repeated necrosectomies without having to exchange stents. Yet, even with an optimal antibiotic and endoscopic drainage therapy, morbidity and mortality in infected pancreatic necrosis remain significant (28). One possible concept to improve the clinical results of endoscopic drainage therapy is the utilization of antimicrobial stent coatings which have already been proposed for biliary stents e.g., to avoid cholangitis after endoscopic retrograde cholangiography (ERC) (29, 30). Similarly, stents for endoscopic drainage therapy of pancreatic necrosis or fluid collections could be coated with antimicrobial agents, potentially improving control of infection within the necrotic cavity, thus reducing the rate of drainage therapy associated complications. For such an approach, it is important to determine where on the stent the coating should be applied and to define the bacterial spectrum that needs to be effectively covered. Our SEM micrographs showed complex network-like biofilm formation on almost all stent explants. Further µCT analyses showed that adhesive necrotic debris can be found on

various spots along all of the stent surface on the inside as well as on the outside. Comparative analysis of the composition of the stent microbiome on the inner and outer surface showed no major differences. Therefore, a homogenous antimicrobial coating that covers all stent surfaces could be conceived. Its antimicrobial activity would primarily need to cover gram-negative *Enterobacteriaceae* like *Escherichia* or *Citrobacter* as well as *Enterococcus* as these were the most abundant opportunistic enteric pathogens present. To this end, an antimicrobial coating e.g., with gentamicin, which has already been preclinically tested for use with plastic biliary stents (29), could be an option. Gentamicin possesses antimicrobial bactericidal activity against the aforementioned bacteria (31) and it works synergistically with other antimicrobial compounds (e.g., ß-lactam antibiotics) that may be used for systemic therapy (32, 33). A localized gentamicin application would also allow to achieve higher local concentrations than would be tolerable if administered systemically. However, before this approach could be implemented in clinical practice, an antimicrobial coating system for drainage stents would have to be developed that ensures a uniform release of the antimicrobial agent into the stent environment. Further preclinical trials will need to investigate the duration of the antimicrobial effect to determine the appropriate replacement intervals for the stents. Last, it would have to be shown *in vivo* that stents with an antimicrobial coating can in fact influence the stent microbiome and possibly also the microbiome of the necrotic cavity before investigating its effect on clinical outcomes in a randomized-controlled trial.

In the present study, we investigated the composition of the stent microbiome, elucidated differences between the microbiome of the stent and that of the pancreatic necrotic cavity and performed SEM and µCT imaging of stent explants to examine the distribution patterns of pancreatic debris and biofilm formation. However, despite these thorough analyses, this study also has some limitations: First, this is a single-center study, which limits the sample size and reduces the statistical power to detect small differences or weaker clinical associations. Secondly, the composition of the stent microbiome prior to the intervention remains unknown, as the stents are usually retrieved several weeks after initial placement. However, since the microbiome of pancreatic necrosis is relatively stable over time, as we have shown before (8), the same could be assumed for the microbiome of the stent.

In summary, we have determined the microbiome composition on stents used for drainage therapy of pancreatic necrosis or pseudocysts. The resulting microbiome consisted predominantly of opportunistic enteric pathogens as well as oral bacteria. The presence of opportunistic enteric pathogens was associated with prolonged hospitalization whereas stent microbiomes dominated by oral bacteria indicated uncomplicated disease. Our data not only highlight the need for infection control in patients undergoing pancreatic endoscopic drainage therapy, but also suggests the possibility of applying antimicrobial coatings to drainage stents to improve local control of infection.

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 in the article/Supplementary material.

Ethics statement

The studies involving humans were approved by Ethics committee of the University Medicine Greifswald. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

Author contributions

FF: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. VK: Writing – review & editing, Writing – original draft, Methodology, Formal analysis, Data curation. VS: Writing – review & editing, Writing – original draft, Methodology, Formal analysis, Data curation. SW: Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Formal analysis. BK-F: Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis. MR: Writing – review & editing, Writing – original draft, Methodology, Investigation, Data curation. CBa: Writing – review & editing, Writing – original draft, Project administration, Methodology, Investigation. AF: Writing – review & editing, Writing – original draft, Supervision, Resources, Project administration, Funding acquisition. TP: Writing – review & editing, Writing – original draft, Supervision, Investigation. CBu: Writing – review & editing, Writing – original draft, Investigation. AA: Writing – review & editing, Writing – original draft, Investigation. SS: Writing – review & editing, Writing – original draft, Investigation, Formal analysis, Data curation. FW: Writing – review & editing, Writing – original draft, Supervision, Resources, Project administration, Methodology. NG: Writing – review & editing, Writing – original draft, Supervision, Resources, Project administration, Funding acquisition. ML: Writing – review & editing, Writing – original draft, Supervision, Resources, Project administration, Funding acquisition, Conceptualization. MS: Writing – review & editing, Writing – original draft, Supervision, Resources, Project administration, Methodology, Investigation, Formal analysis, Conceptualization.

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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.

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Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2024.1462122/full#supplementary-material

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EDITED BY
Ravi Kumar Sharma,
Chandigarh University, India

REVIEWED BY
Bo Cao,
People's Liberation Army General Hospital, China
Preeti Solanki,
Chandigarh University, India

*CORRESPONDENCE
Wanxing Zhang
zhangwx12@hotmail.com

†These authors have contributed equally to this work

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Causal relationship between plasma lipidome and four types of pancreatitis: a bidirectional Mendelian randomization study

Runzhou Ma[1†], Chengming Chen[2†], Ziyi Wang[1], Huaibin Guo[1] and Wanxing Zhang[1*]

[1]Department of Hepatobiliary Surgery, Hebei General Hospital, Shijiazhuang, China, [2]Department of Ophthalmology, Tangdu Hospital, The Air Force Military Medical University, Xi'an, China

Background: Pancreatitis is a serious and complex inflammatory disease that imposes a severe effect on quality of life. Links between plasma lipidome and pancreatitis have been reported, some of which have not yet been clearly elucidated.

Methods: Therefore, our study aimed to investigate the causal relationships between plasma lipidome and four types of pancreatitis by conducting a bidirectional, two-sample Mendelian randomization (MR) analysis. We obtained genetic variants associated with 179 lipid species from a Genome-wide association analysis of plasma lipidome. The aggregated statistical data of acute pancreatitis (AP), alcohol-induced acute pancreatitis (AAP), chronic pancreatitis (CP), and alcohol-induced chronic pancreatitis (ACP) from the FinnGen consortium were exploited as the outcome. The inverse variance weighted (IVW) technique as the main method was used for MR analysis and sensitivity analyses were used to evaluate heterogeneity and pleiotropy.

Results: After FDR correction, SE (27:1/20:4) (OR = 0.938, 95%CI = 0.906-0.972, P = 4.38×10^{-4}, PFDR = 0.039) was identified to be significantly associated with AP risk. Eight lipid species were identified to be significantly associated with CP risk: SE (27:1/20:4) (OR = 0.911, 95%CI = 0.869-0.954, P = 8.89×10^{-5}, PFDR = 0.016), LPC (20:4) (OR = 0.892, 95%CI = 0.843-0.945, P = 9.74×10^{-5}, PFDR = 0.009), PC (16:0_22:5) (OR = 0.880, 95%CI = 0.818-0.947, P = 6.29×10^{-4}, PFDR = 0.028), PC (17:0_20:4) (OR = 0.893, 95%CI = 0.842-0.948, P = 1.76×10^{-4}, PFDR = 0.010), PC (18:0_20:4) (OR = 0.920, 95%CI = 0.874-0.969, P = 1.70×10^{-3}, PFDR = 0.038), PC (O-16:0/20:4) (OR = 0.871, 95%CI = 0.804-0.943, P = 6.95×10^{-4}, PFDR = 0.025), PC (O-16:1/20:4) (OR = 0.890, 95%CI = 0.832-0.953, P = 7.85×10^{-4}, PFDR = 0.023), and PE (O-18:1/20:4) (OR = 0.866, 95%CI = 0.791-0.947, P = 1.61×10^{-3}, PFDR = 0.041). Furthermore, genetically predicted increased LPC (20:4) (OR = 0.862, 95%CI = 0.796-0.934, P = 3.00×10^{-4}, PFDR = 0.027) and SM (34:2;O2) (OR = 0.753, 95%CI = 0.659-0.860, P = 2.97×10^{-5}, PFDR = 0.005) levels were associated with decreased risk of ACP.

Conclusions: Our findings provide evidence of causal associations between the specific types of lipidome and pancreatitis, offering new insights into future clinical research.

KEYWORDS

lipidome, pancreatitis, causal inference, bidirectional Mendelian randomization, sensitivity

1 Introduction

Pancreatitis is an inflammatory disease characterized by severe abdominal pain and pancreatic exocrine and/or endocrine dysfunction, including acute pancreatitis (AP), acute recurrent pancreatitis and chronic pancreatitis (CP) (1). AP has become one of the most common gastrointestinal diseases requiring hospitalization, and its incidence continues to increase worldwide. Most AP is self-limiting, partly because repeated inflammation may progress to severe AP or even CP (2). Gallstone migration and alcohol abuse are the main causes of AP and CP (3). Recent study has shown that 43.5% of patients with alcoholic pancreatitis are readmitted due to recurrent pancreatitis, which is much greater than the 22.1% of patients with non-alcoholic pancreatitis. In addition, alcoholic acute pancreatitis (AAP) is more likely to progress to CP than non-alcoholic pancreatitis (4, 5). It is generally accepted that plasma lipid levels are also associated with the risk of pancreatitis. A systematic review has shown that hypertriglyceridemia has become a common cause of AP following alcohol as well as gallstone disease, accounting for 10% of all pancreatitis episodes (6). Plasma lipids are usually measured via high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), triglycerides (TG), and total cholesterol (TC) (7). In recent years, advances in modern efficient lipidomics techniques have expanded our understanding of the width of circulating lipids. The plasma lipidome encompasses a comprehensive profile of lipids present in the plasma, critically contributing to energy storage, cellular membrane formation, and intercellular communication. Its significance in diagnosing and managing a variety of health conditions, as well as its role in uncovering the underlying biochemical mechanisms, has been increasingly recognized (8). A more detailed classification of circulating lipids, including glycerolipids, glycerophospholipids, sphingolipids, and sterols, presumably ameliorates the risk and severity estimation of pancreatitis compared with the traditional four lipid panels.

The advent of Genome-wide association studies (GWAS) techniques and high-throughput technologies has advanced our understanding of the complex genetic factors behind diseases (9). Mendelian randomization (MR) analysis is an approach using genetic variants as instrumental variables (IVs) for exposure to investigate the causal relationship between the exposure and outcome. Compared with traditional observational studies, it has the advantage of overcoming the problems of confounding and reverse causality (10). Previous MR studies have reported that triglyceride levels and fatty acid unsaturated levels have a causal effect on pancreatitis risk (11). Yet, there is still a dearth of systematic evaluation of a broader range of plasma lipidome to pancreatitis predisposition.

In this study, we performed a large-scale bidirectional two-sample MR analysis to explore and establish protective causality for different types of plasma lipidome in the diversity of pancreatitis. Further understanding of the relationship between these plasma lipidome and the pathophysiology of pancreatitis will provide a new perspective for the diagnosis and treatment of pancreatitis.

2 Materials and methods

2.1 Study design

We assessed the bidirectional causal relationship between 179 lipid levels and four types of pancreatitis based on a two-sample MR approach. To perform MR analysis, three core assumptions must be followed by IVs: Firstly, there is a strong correlation between exposure and genetic variants; Secondly, there should be no association between genetic variants and potential confounding factors; Thirdly, genetic variants can only affect outcome through exposure. To ensure that MR analysis meets the three core assumptions. The overall research design is depicted in Figure 1.

2.2 Data sources

For the exposure instrument, we used the summary statistics from the recent large-scale GWAS on plasma lipidome, which are publicly available from the GWAS Catalog (https://www.ebi.ac.uk/gwas/, accession numbers from GCST90277238 to GCST90277416) for each lipid species. This GWAS analysis was performed based on 7174 Finnish individuals to test around 13 million variants. By using shotgun lipidomics, 179 lipid species were divided into four categories, including glycerolipids (GL) (n = 44), glycerophospholipids (GP) (n = 104), sphingolipids (SL) (n = 15), and sterols (ST) (n = 16). Specifically, GL included triacylglycerol (TAG) and diacylglycerol (DAG), GP included lysophosphatidylcholine (LPC), lysophosphatidylethanolamine

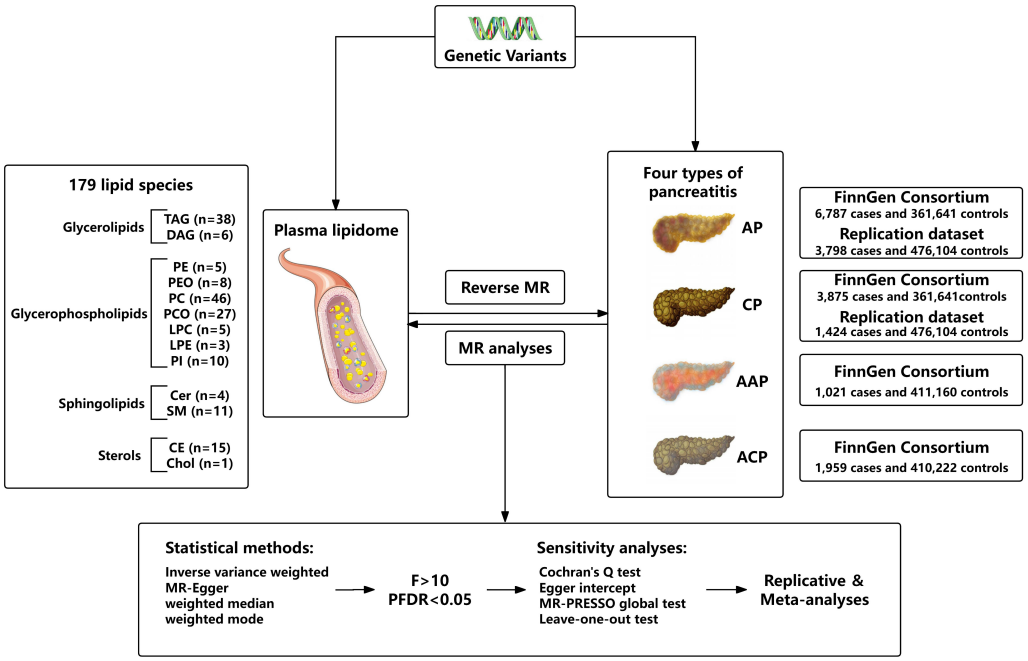

FIGURE 1
Flow chart of this study.

(LPE), phosphatidylcholine (PC), phosphatidylcholine-ether (PCO), phosphatidylethanolamine (PE), phosphatidylethanolamine-ether (PEO), phosphatidylinositol (PI), SL included ceramide (Cer) and sphingomyelin (SM), and ST included cholesterol ester (CE)/sterol ester (SE) and cholesterol (Chol) (Supplementary Table 1). The lipid species are named in the following notation: class name (sum of carbon atoms: sum of double bonds; sum of hydroxyl groups). The annotation of lipid subspecies includes information on their acyl moieties and, if available, on their sn-position. The acyl chains are separated either by "_" if the sn-position on the glycerol cannot be resolved or else by "/" (12).

GWAS summary data for pancreatitis were derived from the FinnGen consortium (https://r10.finngen.fi/). The latest R10 release of the FinnGen consortium data was used, which contains 6,787 cases and 361,641 controls for AP (K11_ACUTPANC), 3,875 cases and 361,641controls for CP (K11_CHRONPANC), 1,021 cases and 411,160 controls for AAP (ALCOPANCACU), 1,959 cases and 410,222 controls for ACP (ALCOPANCCHRON). The replication analyses for pancreatitis utilized GWAS data by Sakaue et al. from the IEU Open GWAS database (https://gwas.mrcieu.ac.uk/). For AP (ebi-a-GCST90018789), there were 3,798 cases and 476,104 controls of European individuals. For CP (ebi-a-GCST90018821), there were 1,424 cases and 476,104 controls of European individuals (13).

2.3 Selection of instrumental variables

Only a small amount of SNPs were chosen as IV at the strict threshold ($P < 5 \times 10^{-8}$). According to recent research, we use the more inclusive threshold ($P < 1 \times 10^{-5}$) to obtain more IVs. To ensure the independence of each IV, SNPs within the genomic window of 10,000 endonuclides were pruned, and a threshold of $r^2 = 0.01$ was applied to mitigate the effects of linkage disequilibrium (LD). Then, remove the palindromic SNPs and duplicate SNPs from IV. In addition, to avoid bias from weak instrumental variables, we used the F-statistic to assess the statistical strength of the association between each SNP and exposure. F > 10 was generally considered a threshold for powerful IVs. Typically, IVs with low F statistics (< 10) were removed from our analysis (14, 15). In reverse MR analysis, to ensure sufficient SNPs for alcohol-related pancreatitis, we selected a genome-wide significance level (5×10^{-6}) to identify potential causality.

2.4 Statistical analysis

The random-effect inverse-variance weighted (IVW) method was used as the main MR approach, which can obtain the most accurate and reliable causal effect between plasma lipidome and pancreatitis (16). For exposures containing one or two SNPs, IVW with fixed effects was used. Multiplicative random effects were used for MR analysis with more than three SNPs or heterogeneity in the data. Other methods including MR-Egger and weighted median were also used as additional analysis (17, 18). Through the integration of multiple MR methods to improve the accuracy and stability of estimating. For statistical analysis, Cochran's Q test ($P < 0.05$) was performed to test the heterogeneity and the MR-Egger regression intercept ($P < 0.05$) was used to identify horizontal pleiotropy (19). Meanwhile, MR-PRESSO was used to detect the presence of SNP outliers and corrected estimates by removing outliers if necessary. Leave-one-out analysis was performed to exclude the outlier variants one by one and evaluate the degree of dependence of the results on a specific variant, further

excluding possible horizontal pleiotropy (20). To avoid increased Type 1 errors from multiple hypothesis testing, we corrected the significance threshold using the false discovery rate (FDR) correction (21). Only results with FDR-corrected P-values less than 0.05 were included in the final analysis. Finally, we repeated the MR analyses with the other two pancreatitis GWAS summary datasets and conducted meta-analyses to verify the reliability of the results. All MR analyses were done via the TwoSampleMR package (version 0.5.8) in the R (4.3.2) software, and meta-analyses were performed in the R-based "meta" package.

3 Results

3.1 Exploring the impact of plasma lipidome on the causal relationship of AP

As the primary analysis methods, we employed the two-sample MR analysis and IVW approach to look into the causal association between plasma lipidome and AP. After FDR test adjustment (PFDR < 0.05), SE (27:1/20:4) was found to have a protective causal relationship to AP: (OR = 0.938, 95%CI = 0.906-0.972, P = 4.38×10^{-4}, PFDR = 0.039). Results from other MR methods were similar: MR-Egger (OR = 0.936, 95%CI = 0.884-0.991, P = 0.027), weighted median (OR = 0.944, 95%CI = 0.898-0.994, P = 0.027), and weighted mode (OR = 0.944, 95% CI = 0.900-0.990, P = 0.020) (Figure 2; Supplementary Table 2). The results of sensitivity analysis and the leave-one-out plot are detailed in the Supplementary Materials (Supplementary Table 2; Supplementary Figure 1). Moreover, replication analyses and meta-analyses have further bolstered the credibility of these results (Supplementary Table 2). In the reverse MR results of plasma lipidome and AP, all MR analysis p-values are greater than 0.05 after FDR test adjustment (PFDR > 0.05), indicating that AP does not affect the included lipidome (Supplementary Table 2).

3.2 Exploring the impact of plasma lipidome on the causal relationship of CP

After FDR adjustment (PFDR < 0.05), we detected protective effects of eight lipid levels on CP: one in the CE class, one in the LPC class, three in the PC class, two in the PCO class, and one in the PEO class. The IVW analysis results for all plasma lipidome were: SE (27:1/20:4) (OR = 0.911, 95%CI = 0.869-0.954, P = 8.89×10^{-5}, PFDR = 0.016), LPC (20:4) (OR = 0.892, 95%CI = 0.843-0.945, P = 9.74×10^{-5}, PFDR = 0.009), PC (16:0_22:5) (OR = 0.880, 95%CI = 0.818-0.947, P = 6.29×10^{-4}, PFDR = 0.028), PC (17:0_20:4) (OR = 0.893, 95%CI = 0.842-0.948, P = 1.76×10^{-4}, PFDR = 0.010), PC (18:0_20:4) (OR = 0.920, 95%CI = 0.874-0.969, P = 1.70×10^{-3}, PFDR = 0.038), PC (O-16:0/20:4) (OR = 0.871, 95%CI = 0.804-0.943, P = 6.95×10^{-4}, PFDR = 0.025), PC (O-16:1/20:4) (OR = 0.890, 95%CI = 0.832-0.953, P = 7.85×10^{-4}, PFDR = 0.023), PE (O-18:1/20:4) (OR = 0.866, 95%CI = 0.791-0.947, P = 1.61×10^{-3}, PFDR = 0.041). Furthermore, the MR-Egger methods of four of them were: SE (27:1/20:4) (OR = 0.943, 95%CI = 0.875-1.016, P = 0.129), PC (16:0_22:5) (OR = 0.887, 95%CI = 0.773-1.018, P = 0.096), PC (17:0_20:4) (OR = 0.915, 95%CI = 0.829-1.011, P = 0.088) and PC (O-16:0/20:4) (OR = 0.887, 95%CI = 0.764-1.029, P = 0.123). Although P > 0.05 of MR-Egger in these results, the beta values of the four MR analysis methods were all < 0, indicating that they were all in the same direction (Figure 3; Supplementary Table 3). The biggest difference between the MR-Egger method and IVW is that the MR-Egger method considers the existence of intercept term. In the absence of heterogeneity and horizontal pleiotropy, the IVW method provides a more accurate estimate than the MR-Egger method. We will give priority to the results of IVW. Therefore, above all, the results of MR analysis were still statistically significant. The results of the auxiliary analysis of the remaining four types were consistent with the observed results. All sensitivity analyses confirmed the robustness of the observed causal relationship, and further validation through subsequent replication studies and meta-analyses provided additional support for these results (Supplementary Table 2). In the reverse MR results of lipidome and CP, all MR analysis p-values are greater than 0.05 after FDR test adjustment (PFDR > 0.05), indicating that CP has no effect on the included lipidome. All results are detailed in the Supplementary Materials (Supplementary Table 3; Supplementary Figure 2).

3.3 Exploring the impact of plasma lipidome on the causal relationship of AAP

Based on the IVW test estimates, no lipid level was identified at a significance of 0.05. In the reverse MR results of lipidome and AAP, the p-value of seven plasma lipid levels was less than 0.05. However, after adjusting for FDR testing, all MR analysis p-values

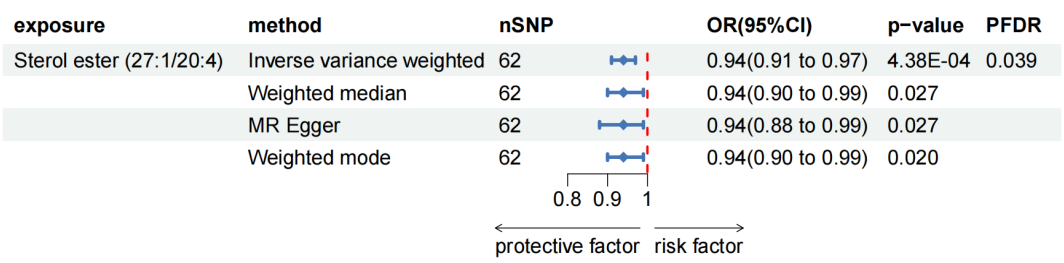

FIGURE 2
Forest plot for the causal effect of circulating immune cells on the risk of AP using different methods; nSNP, number of single nucleotide polymorphisms; OR, odds ratio; CI, confidence interval.

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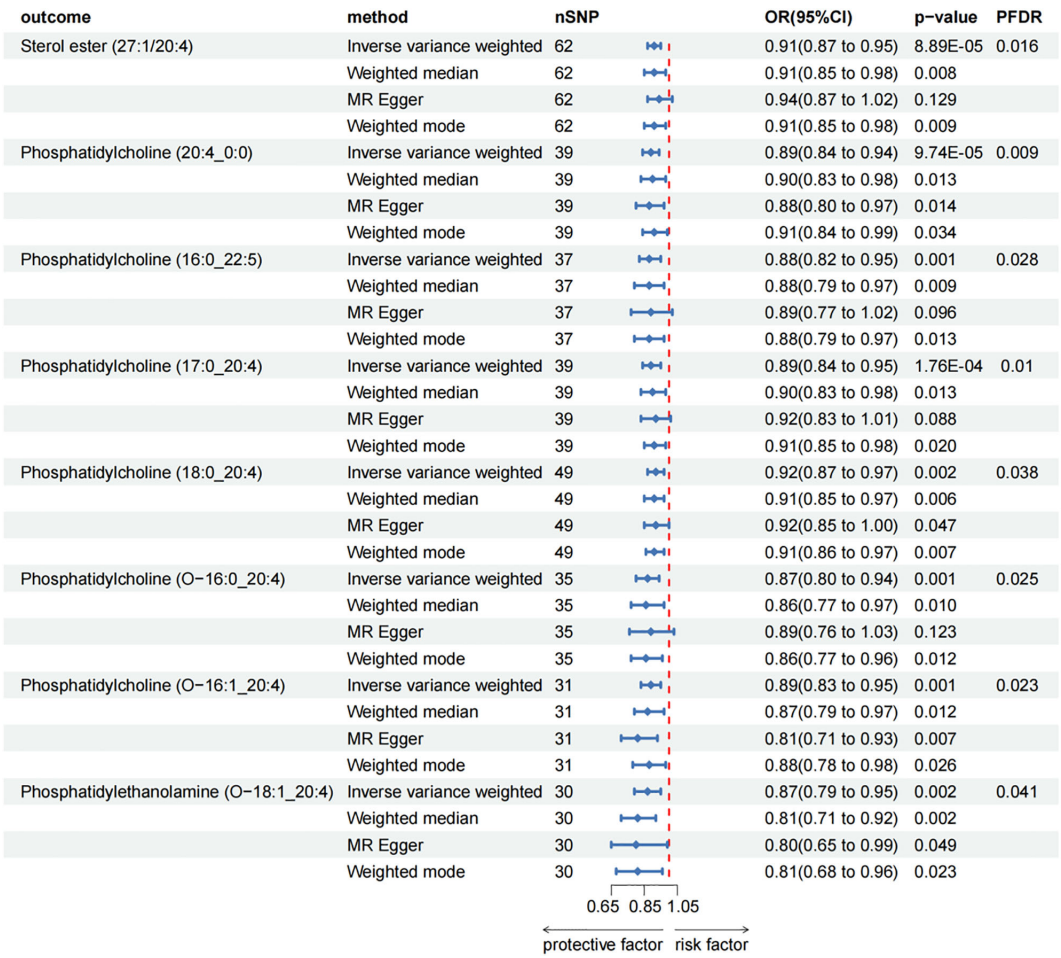

outcome	method	nSNP	OR(95%CI)	p−value	PFDR
Sterol ester (27:1/20:4)	Inverse variance weighted	62	0.91(0.87 to 0.95)	8.89E-05	0.016
	Weighted median	62	0.91(0.85 to 0.98)	0.008	
	MR Egger	62	0.94(0.87 to 1.02)	0.129	
	Weighted mode	62	0.91(0.85 to 0.98)	0.009	
Phosphatidylcholine (20:4_0:0)	Inverse variance weighted	39	0.89(0.84 to 0.94)	9.74E-05	0.009
	Weighted median	39	0.90(0.83 to 0.98)	0.013	
	MR Egger	39	0.88(0.80 to 0.97)	0.014	
	Weighted mode	39	0.91(0.84 to 0.99)	0.034	
Phosphatidylcholine (16:0_22:5)	Inverse variance weighted	37	0.88(0.82 to 0.95)	0.001	0.028
	Weighted median	37	0.88(0.79 to 0.97)	0.009	
	MR Egger	37	0.89(0.77 to 1.02)	0.096	
	Weighted mode	37	0.88(0.79 to 0.97)	0.013	
Phosphatidylcholine (17:0_20:4)	Inverse variance weighted	39	0.89(0.84 to 0.95)	1.76E-04	0.01
	Weighted median	39	0.90(0.83 to 0.98)	0.013	
	MR Egger	39	0.92(0.83 to 1.01)	0.088	
	Weighted mode	39	0.91(0.85 to 0.98)	0.020	
Phosphatidylcholine (18:0_20:4)	Inverse variance weighted	49	0.92(0.87 to 0.97)	0.002	0.038
	Weighted median	49	0.91(0.85 to 0.97)	0.006	
	MR Egger	49	0.92(0.85 to 1.00)	0.047	
	Weighted mode	49	0.91(0.86 to 0.97)	0.007	
Phosphatidylcholine (O−16:0_20:4)	Inverse variance weighted	35	0.87(0.80 to 0.94)	0.001	0.025
	Weighted median	35	0.86(0.77 to 0.97)	0.010	
	MR Egger	35	0.89(0.76 to 1.03)	0.123	
	Weighted mode	35	0.86(0.77 to 0.96)	0.012	
Phosphatidylcholine (O−16:1_20:4)	Inverse variance weighted	31	0.89(0.83 to 0.95)	0.001	0.023
	Weighted median	31	0.87(0.79 to 0.97)	0.012	
	MR Egger	31	0.81(0.71 to 0.93)	0.007	
	Weighted mode	31	0.88(0.78 to 0.98)	0.026	
Phosphatidylethanolamine (O−18:1_20:4)	Inverse variance weighted	30	0.87(0.79 to 0.95)	0.002	0.041
	Weighted median	30	0.81(0.71 to 0.92)	0.002	
	MR Egger	30	0.80(0.65 to 0.99)	0.049	
	Weighted mode	30	0.81(0.68 to 0.96)	0.023	

FIGURE 3
Forest plot for the causal effect of circulating immune cells on the risk of CP using different methods; nSNP, number of single nucleotide polymorphisms; OR, odds ratio; CI, confidence interval.

were greater than 0.05 after FDR test adjustment (PFDR > 0.05), indicating that AAP has no effect on the included lipidome. In summary, we did not find a causal relationship between plasma lipidome and AAP. The results are shown in the Supplementary Materials (Supplementary Table 4).

3.4 Exploring the impact of plasma lipidome on the causal relationship of ACP

After FDR adjustment (PFDR < 0.05), we detected protective effects of LPC (20:4) and SM (34:2;O2) levels on ACP. Among them, the odds ratio (OR) of LPC (20:4) on ACP risk estimated by IVW method was 0.862 (95%CI = 0.796-0.934, P = 3.00×10^{-4}, PFDR = 0.027). Results from other MR methods were similar: MR-Egger (OR = 0.857, 95%CI = 0.747-0.983, P = 0.034), weighted median (OR = 0.873, 95%CI = 0.780-0.977, P = 0.019), and weighted mode (OR = 0.884, 95%CI = 0.784-0.996, P = 0.051). The ratio of SM (34:2;O2) levels to ACP risk (OR) was assessed with the IVW method and was 0.753 (95% CI = 0.659-0.860, P = 2.97×10^{-5}, PFDR = 0.005). Results from other MR methods were similar: MR-Egger (OR = 0.888, 95%CI = 0.665-1.186, P = 0.428), weighted median (OR = 0.789, 95%CI = 0.650-0.957, P = 0.016), and weighted mode (OR = 0.811, 95%CI = 0.616-1.068, P = 0.148) (Figure 4; Supplementary Table 4). In the reverse MR results of lipidome and ACP, all MR analysis p-values were greater than 0.05 after FDR test adjustment (PFDR > 0.05), indicating that ACP had no effect on the included lipidome (Supplementary Table 4).

The results of the other three methods and sensitivity analyses confirmed the stability of the observed causal associations. Specifically, the Cochran's Q test, MR-Egger intercept and MR-PRESSO test were examined to rule out the heterogeneity and horizontal pleiotropy (Supplementary Table 4). In addition, leave-one-out plots showed the stability of the results (Supplementary Figure 3). All scatter plots regarding the causal relationship between plasma lipidome and the risk of pancreatitis can be found in Figure 5.

4 Discussion

Based on a large amount of publicly available genetic data, our study explored the causal relationships between 179 plasma lipidome and four types of pancreatitis. In this study, we

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exposure	method	nSNP	OR(95%CI)	p−value	PFDR
Phosphatidylcholine (20:4_0:0)	Inverse variance weighted	39	0.86(0.80 to 0.93)	3.00E-04	0.005
	Weighted median	39	0.87(0.78 to 0.98)	0.019	
	MR Egger	39	0.86(0.75 to 0.98)	0.034	
	Weighted mode	39	0.88(0.78 to 1.00)	0.051	
Sphingomyelin (d34:2)	Inverse variance weighted	29	0.75(0.66 to 0.86)	2.97E-05	0.027
	Weighted median	29	0.79(0.65 to 0.96)	0.016	
	MR Egger	29	0.89(0.66 to 1.19)	0.429	
	Weighted mode	29	0.81(0.62 to 1.07)	0.148	

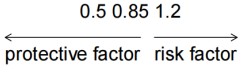

FIGURE 4
Forest plot for the causal effect of circulating immune cells on the risk of ACP using different methods; nSNP, number of single nucleotide polymorphisms; OR, odds ratio; CI, confidence interval.

discovered potential causal relationships between one type of plasma lipid level and AP, eight different types of plasma lipid levels and CP, and two types of plasma lipid levels and ACP.

Our study found that the risk of AP and CP decreased with an increase of SE (27:1/20:4), a cholesterol ester belonging to the CE (20:4) species. The synthesis and hydrolysis of CEs play important roles in the maintenance of cholesterol homeostasis. CEs are formed by the esterification of cholesterol and fatty acids, which is the main form of cholesterol storage and transport. The free cholesterol in plasma is catalyzed by lecithin cholesterol acyltransferase (LCAT) to accept the fatty acyl group on the lecithin molecule to form cholesterol ester and lysed lecithin (22). The newly formed CEs are

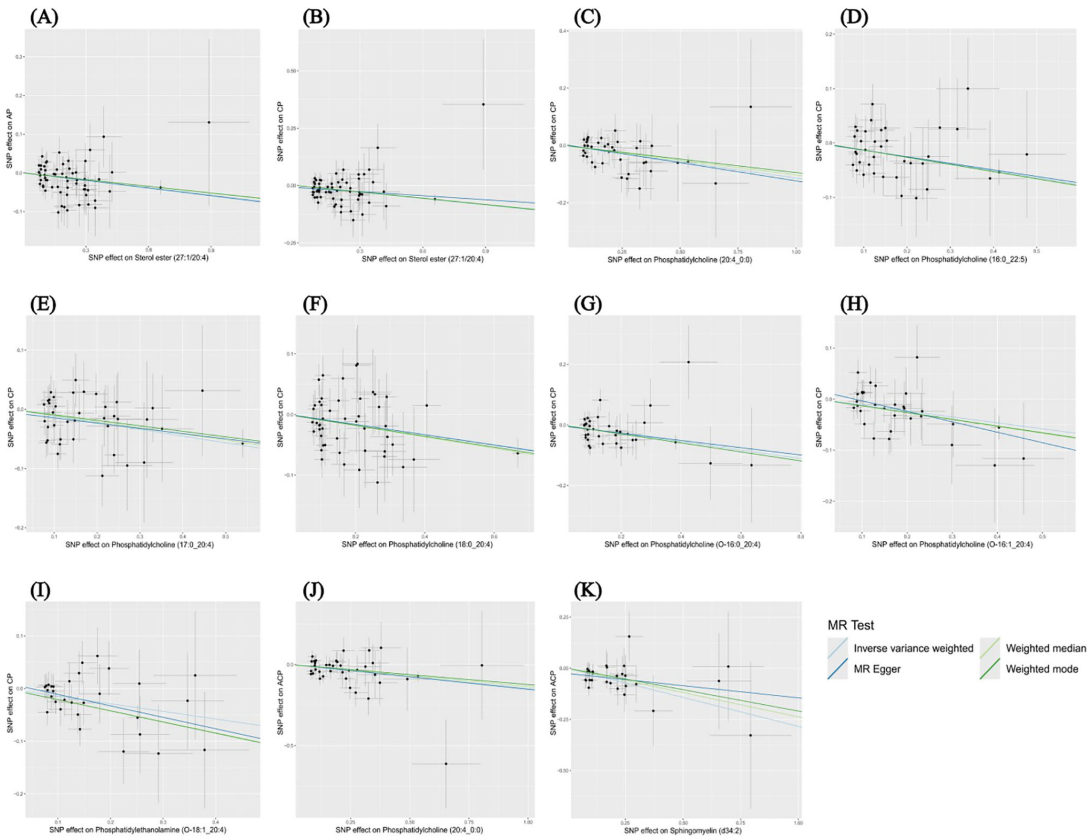

FIGURE 5
Scatter plots depicting the causal relationship between the effect sizes of SNPs on plasma lipidome components (x-axis) and the corresponding effect sizes for pancreatitis (y-axis). Each plot focuses on a specific lipid species: **(A)** Sterol ester (27:1/20:4) on AP. **(B)** Sterol ester (27:1/20:4) on CP. **(C)** Phosphatidylcholine (20:4_0:0) on CP. **(D)** Phosphatidylcholine (16:0_22:5) on CP. **(E)** Phosphatidylcholine (17:0_20:4) on CP. **(F)** Phosphatidylcholine (18:0_20:4) on CP. **(G)** Phosphatidylcholine (O-16:0_20:4) on CP. **(H)** Phosphatidylcholine (O-16:1_20:4) on CP. **(I)** Phosphatidylethanolamine (O-18:1_20:4) on CP. **(J)** Phosphatidylcholine (20:4_0:0) on ACP. **(K)** Sphingomyelin (d34:2) on ACP.

subsequently incorporated into the hydrophobic core of HDL, forming a concentration gradient on the free cholesterol content between its surface and plasma and surrounding cells, which is conducive to driving the net outflow or removal of cholesterol from the cells (23, 24). Limited research has been conducted on the role of plasma CEs in pancreatitis as of yet. The mechanism may be related to the maintenance of cholesterol metabolic homeostasis and participation in reverse cholesterol transport to reduce cholesterol accumulation, thus inhibiting inflammation and alleviating pancreatic toxicity (25).

Glycerophospholipids not only constitute biofilms but also regulate a variety of biological processes including cell proliferation, apoptosis, immunity, angiogenesis and inflammation through related pathways (26). According to research, glycerophospholipid metabolic pathway was identified as the key metabolic pathway of CP (27). In this study, phosphatidylcholine (20:4_0:0), which belongs to lysophosphatidylcholine (LPC), has been proven to be significantly associated with a reduced risk of CP and ACP. During CP development, apoptotic cells induce M2a macrophages to exert an anti-inflammatory response and relieve fibrosis by releasing LPC as a "find me" signal (28). Besides, the acyl chain length and degree of saturation of LPC lead to its different roles in the development of inflammatory diseases. Saturated LPC (16:0) is a potential inflammatory mediator that induces the release of pro-inflammatory cytokines. Polyunsaturated acyl LPC (20:4) acts as an anti-inflammatory lipid mediator, inhibiting inflammation caused by saturated LPC. Its anti-inflammatory effect is related to reducing plasma leakage and inflammatory cell activation, inhibiting the production of inflammatory mediators (IL-6, NO, PGE, etc.), and increasing anti-inflammatory factors (IL-10 and IL-4) (29). The result is consistent with our findings of this study.

According to this study, the risk of CP decreased as the three kinds of PC increased: (16:0_22:5), (17:0_20:4), and (18:0_20:4). Phosphatidylcholine (PC) is the predominant phospholipid found in cell membranes and has been linked to inflammatory processes by shaping membrane composition and fluidity (30). Previous studies have shown that PC is associated with improved insulin resistance and abnormal lipid accumulation and inhibits the synthesis and release of multiple inflammatory factors, such as IL-1β and TNF-α (31, 32). PC metabolites can also inhibit endoplasmic reticulum stress and subsequent oxidative stress response, maintain Th17/Treg cell immune balance, and improve tissue inflammation (33). Serum metabolomic studies have shown that PC metabolites can be used to diagnose CP and are associated with pancreatic exocrine insufficiency in CP (27, 34).

Ether lipids are a unique class of glycerophospholipids that have an alkyl chain connected to the sn-1 position by an ether bond. They are initially synthesized in peroxisomes and then processed into PCO and PEO in the endoplasmic reticulum (ER) (35). Ether lipids regulate cell signaling and act as antioxidants, mediating the relationship between oxidative stress and inflammation. The Daniel Hornburg team found that PEOs were significantly associated with health phenotypes, including low SSPG levels and high HDL levels (36). And we found that three plasma ether lipids (Phosphatidylcholine (O-16:0_20:4), Phosphatidylcholine (O-16:1_20:4) and Phosphatidylethanolamine (O-18:1_20:4))could reduce the risk of CP.

The synthesis of SM begins with ER, goes through a series of enzymatic reactions in the Golgi apparatus and plasma membrane, and is finally synthesized by the substrate ceramide and PC. Sphingomyelin has a similar structure to glycerol phospholipids, which gives it similar properties and functions (37). Like glycerophospholipid metabolism, sphingolipid metabolism is also thought to be related to CP. However, research on the role of sphingomyelin in ACP is limited. Recent studies have shown differential expression of sphingomyelin in the plasma of patients with CP and pancreatic cancer (38). Additionally, sphingosine 1-phosphate (S1P), a metabolite of sphingomyelin, is associated with the severity of AP (39). According to this study, we discovered a correlation between sphingomyelin (d34:2) levels and a decreased risk of ACP.

This study conducted a two-sample MR analysis based on large published GWAS datasets and performed causal inferences through a variety of powerful MR analysis techniques. The results were robust and not influenced by horizontal pleiotropy or other factors. Additionally, to control for false positive results in multiple hypothesis tests, we used FDR to control for statistical bias caused by multiple comparisons. However, there are some limitations to the study. First, the GWAS data used in our study was limited to European populations, limiting the generalizability of the findings to other ethnic groups. Second, due to the lack of individual information, we are unable to conduct further stratified analysis of the population (e.g., sex, age, etc.). Third, although multiple sensitivity analyses have been performed to evaluate the hypotheses of MR studies, it is not possible to completely rule out confounding bias or horizontal pleiotropy. Finally, we used a looser threshold to evaluate the results, which may increase some false positives despite FDR correction. However, the strong association between plasma lipidome and pancreatitis can be evaluated more comprehensively.

5 Conclusion

Overall, we have demonstrated the causal associations between plasma lipidome and four types of pancreatitis through comprehensive bidirectional MR analyses. Besides, we highlighted the different structures of lipids and their potential differential effects across various molecular subtypes. Our findings may provide new avenues for researchers to explore the biological mechanisms of pancreatitis and help explore early intervention and treatment. The pathogenesis of pancreatitis is very intricate, and the unique biological effects of lipid subclasses beyond the traditional lipid spectrum on pancreatitis need to be further studied.

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/s.

Ethics statement

Ethical review and approval was not required for the study on human participants in accordance with the local legislation and

institutional requirements. Written informed consent from the patients/participants or patients/participants' legal guardian/next of kin was not required to participate in this study in accordance with the national legislation and the institutional requirements.

Author contributions

RM: Data curation, Formal analysis, Supervision, Writing – original draft, Writing – review & editing. CC: Formal analysis, Supervision, Writing – review & editing. ZW: Data curation, Methodology, Writing – review & editing. HG: Data curation, Supervision, Writing – review & editing. WZ: Project administration, Writing – review & editing.

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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/fendo.2024.1415474/full#supplementary-material

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EDITED BY
Ravi Kumar Sharma,
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REVIEWED BY
Mary Phillips,
Royal Surrey County Hospital,
United Kingdom
Alexandra Demcsak,
University of California, Los Angeles,
United States

*CORRESPONDENCE
Ali A. Aghdassi
✉ ali.aghdassi@med.uni-greifswald.de

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An intensified trans-sectoral nutritional intervention in malnourished patients with chronic pancreatitis improves diseases prognosis and identifies potential biomarkers of nutritional status

Mats L. Wiese[1], Fabian Frost[1], Fatuma Meyer[2], Josefine Müller[2], Luzia Valentini[2], Karen Rischmüller[3], Georg Lamprecht[3], Antje Steveling[1], Markus M. Lerch[4] and Ali A. Aghdassi[1]*

[1]Department of Medicine A, University Medicine Greifswald, Greifswald, Germany, [2]Institute of Evidence-based Dietetics (NIED), University of Applied Sciences Neubrandenburg, Neubrandenburg, Germany, [3]Department of Medicine II, Division of Gastroenterology, Rostock University Medical Center, Rostock, Germany, [4]LMU University Hospital, Ludwig Maximilian Universität München, Munich, Germany

Background: Malnutrition is a common complication in chronic pancreatitis and associated with reduced quality of life and life expectancy. Nutritional support is considered mandatory in malnourished patients with chronic pancreatitis but there is only scarce evidence on optimal treatment modalities and the efficacy of nutrition therapy. Here, we investigated the feasibility and efficacy of an intensified nutritional intervention in malnourished patients with chronic pancreatitis and aimed to identify suitable indicators for monitoring nutritional status.

Methods: We performed a single-arm feasibility study, in which malnourished patients with chronic pancreatitis received an intensified trans-sectoral nutritional intervention for 6 months. Multimodal treatment comprised face-to-face dietary counseling, oral nutritional supplementation, and a complementary telephone-based nutrition and exercise coaching. Patients underwent follow-up examinations after 28, 90, and 180 days, when we assessed changes in anthropometric and body composition measures, muscle function, Chronic Pancreatitis Prognosis Score (COPPS), as well as blood parameters and intestinal microbiota composition.

Results: Eleven out of 73 patients initially screened for study participation were enrolled in the trial of which 9 subjects (age (mean ± SD): 56.2 (±14.8) years; male: 67%; alcoholic etiology: 44%) underwent the complete intervention. Patients gained a median of 5.3 kg (8.6%) body weight, including 1.6 kg skeletal muscle mass, and significantly increased gait speed ($p < 0.001$). Ameliorated nutritional status and muscle function were associated with increased blood levels of IGF-1 and cholinesterase as well as altered gut microbiota composition on the phyla and genera level. Moreover, significant improvements in COPPS indicated reduced disease severity after 90 and 180 days.

Conclusion: Malnourished patients with chronic pancreatitis benefit from intensified nutritional therapy. Besides ameliorated nutritional status, a multimodal intervention can improve muscle function as well disease prognosis. Future studies are needed to prove superiority to standard-of-care and to validate potential biomarkers for prospective monitoring of nutritional status.

Clinical trial registration: https://clinicaltrials.gov/study/NCT04476056, NCT04476056.

KEYWORDS

malnutrition, chronic pancreatitis, nutrition therapy, diet, supplementation

1 Introduction

Chronic pancreatitis is a progressive fibro-inflammatory disease with an annual incidence of approximately 10 cases per 100,000 inhabitants (1–3). Chronic pancreatitis causes pain and eventually leads to the irreversible loss of exocrine and endocrine organ function. Due to these pathophysiological changes, patients with chronic pancreatitis have a high risk of malnutrition, which exacerbates a decline in quality of life and life expectancy (4, 5). Recent data suggest that half or more patients with chronic pancreatitis may be affected by malnutrition and a characteristic loss of skeletal muscle mass (6, 7). There is consensus that nutritional therapy is indicated in malnourished patients with chronic pancreatitis but little is known about the optimal modalities of treatment (8, 9). Well-designed, prospective studies showing the efficacy of nutritional therapy are scarce. Therefore, most nutritional recommendations are based on low evidence, which ultimately hampers implementation in clinical practice. Moreover, there is still uncertainty regarding adequate, easily accessible parameters to monitor nutritional status of malnourished patients with chronic pancreatitis (10).

To address these gaps in knowledge, we investigated the feasibility and efficacy of an intensified nutritional intervention in malnourished patients with chronic pancreatitis and studied anthropometric and biochemical changes to identify suitable parameters for monitoring nutritional status.

2 Materials and methods

2.1 Study design and population

This trial was designed as a multicenter, single-arm feasibility study conducted between May 2019 and September 2021 in the state of Mecklenburg-Vorpommern, located in northeast Germany. At University Medicine Greifswald, a local tertiary referral center, we enrolled in- and out-patients 18 years or older with confirmed diagnosis of chronic pancreatitis and concomitant malnutrition for an intensified trans-sectoral nutritional intervention carried out in collaboration with University of Applied Sciences Neubrandenburg. Diagnosis of chronic pancreatitis was based on characteristic findings in imaging studies, including endoscopic ultrasound, computed tomography, or magnetic resonance imaging with magnetic resonance cholangiopancreatography, and/or histology. The international consensus criteria by the Global Leadership Initiative on Malnutrition (GLIM) were applied to diagnose malnutrition (11). Details on the diagnostic approach including methods and cut-offs employed for patients with chronic pancreatitis have been reported previously (6). Exclusion criteria were as follows: (1) diagnosis of any malignant disease within the past 3 years, (2) pregnancy or lactation, (3) concomitant other severe chronic gastrointestinal disease, including liver cirrhosis, or (4) relevant cognitive and/or physical restraints.

The study was approved by the Institutional Review Board at the University Medicine Greifswald (internal registration number: BB 069/19) and registered at clinicaltrials.gov (NCT04476056). The intervention and all other study-related procedures were conducted in accordance with the ethical principles related to the Declaration of Helsinki.

2.2 Intervention

The trans-sectoral nutritional intervention lasted over 6 months and was based on a multimodal approach comprising face-to-face dietary counseling, supplementation with oral nutritional supplements (ONS), as well as a complementary telephone-based nutrition and exercise coaching (Figure 1). Dietary counseling and complementary coaching were carried out in a standardized way according to the German Nutrition Care Process (12) by trained dietitians or nutritionists. Both in- and outpatients received their first session of dietary counseling upon study enrollment, followed by two additional sessions in an outpatient setting after 28 and 90 days, respectively. During these sessions patients were advised on an energy adequate, high protein diet (> 1.5 g/kg bodyweight) respecting individual food tolerance as well as appropriate pancreatic enzyme replacement therapy in case of exocrine pancreatic insufficiency. In addition, alcohol and smoking abstinence were promoted during the counseling sessions. Implementation of these recommendations was supported during the complementary telephone-based nutrition coaching, which took place during the first 90 days of the intervention and was administered by qualified health care professionals at University of Applied Sciences Neubrandenburg. The exercise component of coaching promoted increased everyday physical activity and exercises appropriate to the patients' performance status, e.g., light resistance

Abbreviations: COPPS, Chronic Pancreatitis Prognosis Score; GLIM, Global Leadership Initiative on Malnutrition; ONS, Oral nutritional supplements.

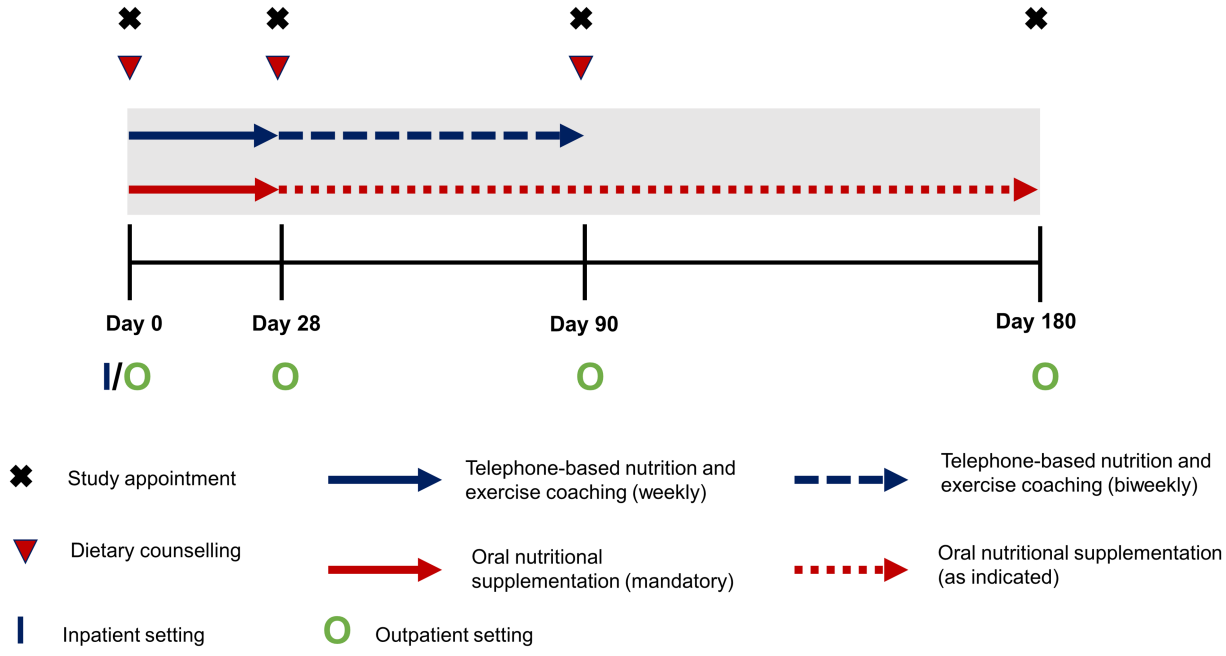


FIGURE 1
Schematic outline of the intensified trans-sectoral nutritional intervention for the treatment of malnourished patients with chronic pancreatitis.

band training. During the first 28 days, phone calls were scheduled weekly and then biweekly thereafter. Regarding supplementation, for the first 28 days patients were instructed to consume daily at least two bottles of a commercial ONS (Fortimel Compact 2.4, Nutricia) containing 300 kcal and 12 g of protein per serving and to record their actual intake. After that, patients were free to continue supplementation, as indicated to achieve adequate energy and nutrient intake, throughout the intervention but without any preset targets regarding quantity of ONS.

2.3 Clinical and patient data

We collected personal and disease-related characteristics by standardized interview or from the patient files. Chronic Pancreatitis Prognosis Score (COPPS), a validated scoring system to predict short-term prognosis in patients with chronic pancreatitis, was used to evaluate disease severity (13). Exocrine pancreatic function was assessed by measurement of fecal elastase using a monospecific enzyme-linked immunosorbent assay (R-Biopharm AG, Darmstadt, Germany) with values of 200 µg/g or less defining exocrine insufficiency. Diagnosis of pancreatogenic diabetes was made based on the presence of major and minor criteria as suggested by Ewald and Bretzel (14). We inquired patients' dietary intake, excluding ONS, by a validated semi-quantitative food frequency questionnaire (15) and employed the short form of the International Physical Activity Questionnaire to assess physical activity.

2.4 Physical examination and blood testing

At all study visits, we performed the following anthropometric measurements in patients using a standardized protocol including repeated measurements: body weight and height, waist, hip and mid-upper arm circumference as well as triceps skinfold thickness. To minimize intra- and inter-operator error, all investigators received comprehensive training based on this protocol. Adequate operator performance was ascertained before the beginning of the study. We further analyzed patients' body composition with an eight-electrode, phase-sensitive, segmental bioelectrical impedance analysis device (mBCA 515, seca, Hamburg, Germany). For assessment of muscle strength, we tested handgrip strength of patients with the Jamar Plus+ Digital Hand Dynamometer (Patterson Medical, Warrenville, IL, United States) and carried out the 4-m gait speed test to assess muscle performance. Blood of patients was drawn to measure routine laboratory parameters markers associated with inflammation or nutritional status.

2.5 Microbiome analyses

We performed 16S rRNA gene sequencing of DNA extracted from fecal samples provided by the patients. Sample material was collected by the patients at home or in hospital and preserved in a test tube, which contained stabilizing DNA buffer. Following extraction (PSP Spin Stool DNA Kit; Stratec Biomedical AG, Birkenfeld, Germany), the DNA was stored at −20°C until analysis by 16S rRNA gene sequencing of the V1–V2 region, which was performed on a MiSeq platform (Illumina, San Diego, California, USA). For data processing and taxonomy assignment, we employed the open-source software package DADA2 (v.1.10) (16). For the analysis, samples were normalized to 10,000 16S rRNA gene read counts.

2.6 Statistical analysis

Continuous variables are presented as mean (±SD) or median (IQR) for normally and non-normally distributed data, respectively.

Categorical variables are given as absolute and relative frequency. Significance of changes in parameters during the intervention were tested by Friedman test, given non-normal distribution of the data. In case of significant differences by Friedman test, we performed post-hoc analysis using the Conover test with false detection rate correction. Gut microbiome changes were analyzed between composition at Day 0 and Day 180 at the phyla and genera level. For this, we compared all phyla and genera present in more than 50% of samples using the Wilcoxon signed-rank test. Correlations between changes in taxonomic units and parameters of body composition and muscle function were then tested by calculation of Spearmen correlation coefficients. All microbiome analyses were performed on data of 7 individuals as two patients did not provide stool samples at Day 180. All results are presented on per protocol basis. Intention-to-treat analyses were performed, where applicable, and yielded comparable results. A p-value <0.05 was defined as statistically significant. All statistical analyses and graphical visualization were performed using R software (R Core Team, Vienna, Austria) for statistical computing (version 4.1.0). In figures in which we report data for single individuals, colored dots represent the same patient throughout the manuscript.

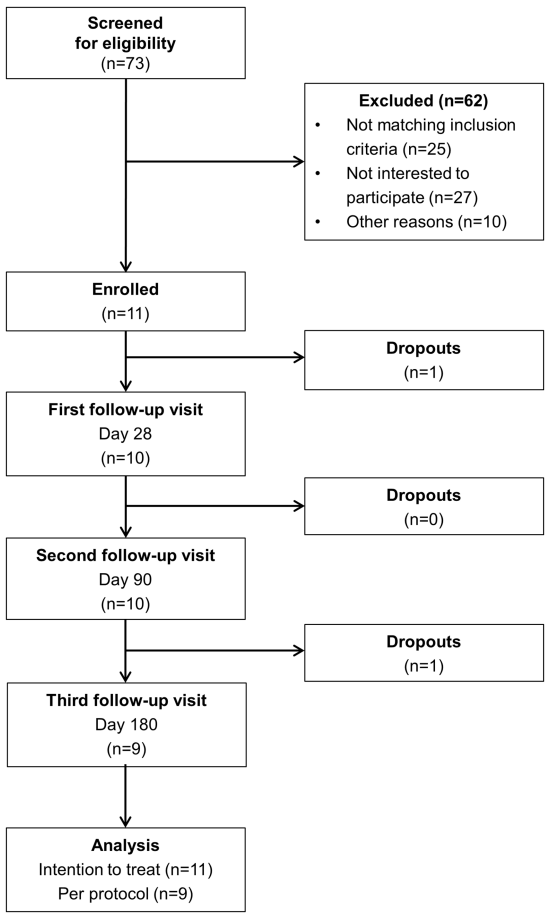


FIGURE 2
Flowchart illustrating the process of patient selection.

3 Results

3.1 Patient selection and characteristics

The process of patient selection is illustrated in Figure 2. Out of 73 initially screened patients with chronic pancreatitis, 11 individuals were eventually enrolled in the study. Among the 62 excluded patients, lack of interest to participate ($n = 27$) and non-fulfillment of inclusion criteria ($n = 25$) were the most common reasons for exclusion. Nine subjects completed the entire 6-months intervention. One patient dropped out before the first scheduled follow-up visit and another one after the second visit.

Relevant demographic and clinical patient characteristics are summarized in Table 1. Mean age of patients was 56.2 years and two thirds were male. Most patients (78%) were recruited as outpatients. The etiology of chronic pancreatitis was either alcoholic (56%) or idiopathic (44%). At study enrollment, alcohol abuse persisted in 22% of individuals and 67% continued smoking. Two thirds of patients had exocrine pancreatic insufficiency based on fecal elastase test. Yet, all but one subject already received pancreatic enzyme replacement therapy at baseline. Pancreatogenic diabetes was present in 33% of patients, who all had all undergone pancreatic surgery before. Time after surgery ranged from 2 to 31 months. Around half of the subjects had received diagnosis of chronic pancreatitis within the last 12 months and 22% required permanent opioid treatment, which was continued unchanged throughout the intervention. Disease severity was equally distributed among grades of COPPS. All patients reported unintentional weight loss, with a median body weight decrease of 7.7 and 19.9% in the last 6 and 24 months, respectively. With one exception all subjects were diagnosed as severely malnourished according to the GLIM criteria.

3.2 Compliance

Most patients showed good adherence to all individual intervention components during the entire study period (Figure 3A). Smoking abstinence was the only recommendation to which less than half of patients (44%) adhered. Notably, 33% of subjects had already ceased smoking before study enrollment and smoking behavior did not change during the intervention in any individual. In contrast, compliance to ONS improved over the study course. In the first 28 days, 56% of patients showed compliance, which was defined as consuming on overage at least two bottles on 6 out of 7 days a week. Based on a consumption record conducted by the patients, ONS provided a median energy intake of 514 kcal and 21 g of protein (Figure 3B). For the remaining study period, when ONS quantity was based on individual requirements, compliance increased to 78 and 89% at days 90 and 180, respectively. All subjects required ONS at least until Day 90 and 56% continued consumption during the entire intervention. Although patients showed high adherence to dietary counseling, intakes of energy and macronutrients from oral food remained unchanged during the intervention (Supplementary Table S1). Moreover, patients were highly compliant to the exercise coaching resulting in increased physical activity which was maintained throughout the entire study (Supplementary Table S2).

TABLE 1 Demographic and clinical characteristics of malnourished patients with chronic pancreatitis participating in the intensified trans-sectoral nutritional intervention ($n = 9$).

Age, yrs[a]	56.2 (±14.8)
Male sex, *n* (%)	6 (67)
Outpatients, *n* (%)	7 (78)
Etiology, *n* (%)	
Alcohol	5 (56)
Idiopathic	4 (44)
Continued substance abuse, *n* (%)	
Alcohol	2 (22)
Smoking	6 (67)
Exocrine pancreatic insufficiency, *n* (%)	6 (67)
Pancreatic enzyme replacement therapy, *n* (%)	8 (89)
Endocrine pancreatic insufficiency, *n* (%)	3 (33)
Pancreatic surgery, *n* (%)	3 (33)
Opioid treatment, *n* (%)	2 (22)
NRS of pain (0–10)[b]	2 (6)
Diagnosis of chronic pancreatitis within past 12 months, *n* (%)	55 (56)
COPPS, *n* (%)	
A	3 (33)
B	3 (33)
C	3 (33)
Weight loss, %[b]	
Past 6 months	7.7 (15.5)
Past 24 months	19.9 (16.8)
Grade of malnutrition[c], *n* (%)	
Moderate	1 (11)
Severe	8 (89)

COPPS, Chronic Pancreatitis Prognosis Score; NRS of pain, Numeric Rating Scale of Pain: 0 = no pain, 10 = worst possible pain (past 7 days). [a]Value is presented as mean (±SD). [b]Values are presented as median (IQR). [c]Diagnosis based on the Global Leadership Initiative on Malnutrition criteria.

3.3 Changes in anthropometric, body composition, and muscle function parameters

The 6-months intervention led to significant changes in most anthropometric and body composition parameters (Table 2). All but one patient, who had edema and ascites at study enrollment, gained body weight during the intervention. Body weight increased by a median of 5.3 kg (8.6%) of which 3.5 kg were fat mass and 1.6 kg skeletal muscle mass. Post-hoc analyses revealed that no significant changes could be observed after 28 days but only after 90 and 180 days of intervention.

Besides the gains in skeletal muscles mass, there were also changes in muscle function (Figure 4). While maximum handgrip strength did not improve significantly during the intervention ($p = 0.152$), there was a continuous increase in gait speed until the study end examination ($p = 0.001$).

3.4 Changes in disease severity

During the intervention we observed a significant amelioration of disease severity as indicated by COPPS ($p = 0.006$) (Figure 5). While at enrollment there was an equal distribution between the three grades of COPPS, at the end of intervention two-thirds of patients had an A score. Changes in disease severity could not be detected before Day 90. In the following 3 months improved COPPS status was maintained until the end of the study. Regarding individual parameters of COPPS, especially body mass index improved whereas pain scores remained unchanged (Supplementary Table S3). However, even when omitting body mass index from the calculation there was still a significant improvement in the total score ($p = 0.049$).

3.5 Changes in blood parameters and gut microbiota composition

Most of the blood parameters that were analyzed remained unaltered during the intervention period (Table 3). However, we detected significant changes in three parameters, i.e., cholinesterase, IGF-1, and HDL cholesterol. For each of these markers, there was a significant rise after 90 days compared to the baseline measurement. Notably, cholinesterase was the only parameter that continuously increased over 6 months whereas IGF-1 remained stable after 90 days and HDL cholesterol levels declined.

Regarding the intestinal microbiome, we observed no major changes in gut microbiota composition following the intervention (Figures 6A,B). However, there were significant changes in relative abundance of two single taxonomic units, i.e., the phylum *Synergistetes* and the genus *Sporobacter*. Both taxa showed low initial relative abundance of 1.06% (*Synergistetes*) and 0.57% (*Sporobacter*), which significantly decreased even further during the intervention ($p = 0.046$ for each taxa, respectively). Notably, the relative decline in abundance of *Synergistetes* was significantly correlated with gains in skeletal muscle (rho = −0.852, $p = 0.015$) but not weight, fat mass, or parameters of muscle function (Figure 6C). On the other hand, reduction in *Sporobacter* was exclusively associated with improved gait speed (rho = −0.778, $p = 0.039$) but not handgrip strength, body weight or composition (Figure 6C).

4 Discussion

Here, we show that a 6-months intensified trans-sectoral nutritional intervention in malnourished patients with chronic pancreatitis not only successfully improves nutritional status but also parameters of disease severity. Moreover, we identify biochemical indicators of ameliorated nutritional status that could serve as future biomarkers.

Our work provides novel insights regarding feasibility of an intensified nutritional invention in patients with chronic pancreatitis. In that regard, there are two particularly noteworthy observations. On the one hand, it must be noted that a substantial fraction of the patients initially screened declined to participate despite being eligible. Although this a common phenomenon in clinical trials, known as Lasagna's Law (17), in the context of a feasibility study this finding raises questions regarding the reasons for such low participation rate. Because patients

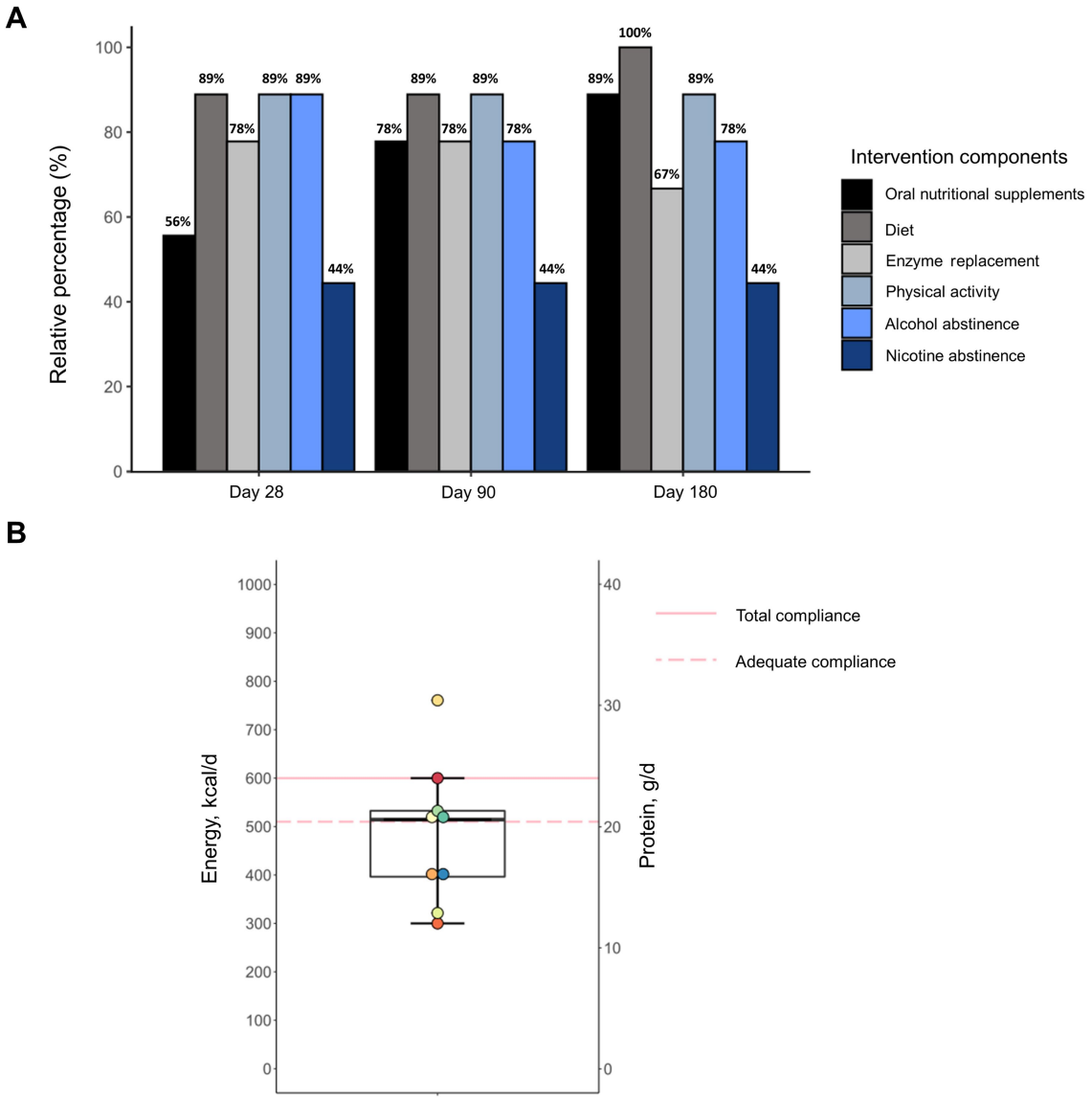


FIGURE 3
Adherence of malnourished patients with chronic pancreatitis to individual components of the intensified trans-sectoral nutritional intervention **(A)** and consumption of oral nutritional supplements in the first 28 days **(B)** ($n = 9$).

by study design were not required to provide reasons for their decision to participate or not, we cannot definitely answer these questions. However, our data shows that especially subjects with severe malnutrition and ongoing pancreatic enzyme replacement therapy participated in this intervention. This could reflect patients' hopes for improved nutritional status in those, where other modes of therapy have been unsuccessful so far. By contrast, persons with moderate malnutrition may be less motivated to participate because they perceive less impairment due to their compromised nutritional status. Notably, alcoholic etiology or continued substance abuse do not seem to present general barriers for enrollment based on our results. This finding is relevant because alcohol and nicotine abuse are commonly seen in persons with chronic pancreatitis and previous studies have suggested that this could hinder enrollment or retention of patients (18, 19). The second important observation on feasibility is that overall patient adherence was good and the drop-out rate during the intervention was low. An earlier study by Singh et al. (20), testing nutritional interventions for the treatment of malnutrition in chronic pancreatitis, reported similar drop-out rates and adherence to oral nutritional supplementation as well as dietary counseling. Our own previous works suggest that malnourished patients with chronic pancreatitis are susceptible to dietary counseling but supplementation with ONS may be required to reverse malnutrition as shown in the current study (21). Further, it should be considered that the study by Singh and colleagues (20) only lasted 3 months and primarily focused on a dietary intervention, as opposed to the multimodal approach followed in our own investigation. In that respect, it should be noted that behavior regarding alcohol consumption and smoking remained unchanged in most patients. Although abstinence should undisputably be promoted as both alcohol and nicotine abuse are known drivers of disease progression, our results suggest that smoking cessation may not be necessary for improvement of nutritional status. By contrast, inclusion of exercise in the multimodal

TABLE 2 Changes in anthropometric and body composition parameters of malnourished patients with chronic pancreatitis in the course of the intensified trans-sectoral nutritional intervention ($n = 9$).

	Day 0	Day 28	Day 90	Day 180	p-value[1]
Body mass index, kg/m^2	19.9 (4.0)	20.1 (4.4)	21.6 (3.97)$^{\#,\dagger}$	22.1 (6.1)$^{\#,\dagger,\ddagger}$	**0.001**
Waist circumference, cm	79.4 (15.0)	83.4 (11.6)	84.9 (7.8)$^{\#}$	88.7 (12.0)$^{\#,\dagger,\ddagger}$	**0.007**
Hip circumference, cm	86.8 (8.2)	88.8 (8.3)	92.4 (11.6)$^{\#,\dagger}$	91.8 (13.8)$^{\#,\dagger,\ddagger}$	**0.003**
Waist-to-Hip ratio	0.90 (0.11)	0.92 (0.15)	0.92 (0.11)	0.93 (0.12)	0.177
Mid upper arm circumference, cm	24.7 (5.5)	24.2 (5.3)	25.4 (4.4)$^{\#,\dagger}$	25.4 (5.0)$^{\#,\dagger,\ddagger}$	**<0.001**
Triceps skinfold thickness, mm	13.4 (8.4)	14.6 (13.0)	13.9 (6.7)	14.0 (7.8)	0.072
Fat mass index, kg/m^2	5.1 (2.7)	5.5 (2.5)	5.7 (2.8)$^{\#,\dagger}$	6.6 (4.1)$^{\#,\dagger,\ddagger}$	**0.002**
Fat free mass index, kg/m^2	16.6 (4.3)	16.1 (3.6)	16.6 (3.7)	16.3 (3.3)	0.057
Skeletal muscle mass index, kg/m^2	6.2 (1.8)	6.8 (1.5)	7.5 (1.8)$^{\#,\dagger}$	7.4 (1.9)$^{\#,\dagger}$	**0.001**
Skeletal muscle to fat mass ratio	1.31 (0.56)	1.26 (0.75)	1.19 (0.79)	1.10 (0.070)	0.131
Phase angle, °	4.2 (1.1)	4.3 (0.4)	4.2 (1.0)	4.4 (0.9)$^{\#,\dagger,\ddagger}$	**0.011**
Total body water, L	34.5 (7.7)	33.8 (7.0)	35.0 (7.1)$^{\#}$	35.2 (6.9)$^{\#,\dagger}$	**0.015**
Extracellular body water, L	14.9 (4.0)	15.1 (3.2)	14.9 (2.7)	14.9 (2.4)	0.725
Extracellular to total body water ratio	0.466 (0.057)	0.458 (0.029)	0.458 (0.043)	0.456 (0.047)	0.095

All data is presented as median (IQR).
[1]Changes over time were tested using Friedman test.
$^{\#}$Indicates significant difference from Day 0 based on Conover post-hoc test with correction for false detection rate, $p < 0.05$.
†Indicates significant difference from Day 28 based on Conover post-hoc test with correction for false detection rate, $p < 0.05$.
‡Indicates significant difference from Day 90 based on Conover post-hoc test with correction for false detection rate, $p < 0.05$.
Bold values denote statistical significance at the $p < 0.05$ level.

approach may be a decisive factor in terms of improving muscle mass and function, which in recent years has become a primary objective in the treatment of disease-related malnutrition (22, 23). In our investigation, we observed that patients were highly compliant regarding the exercise component of the intervention. High adherence was likely facilitated by the individual approach targeted to the patients' performance status. Currently, there is no evidence that supports any specific intervention in terms of physical activity in patients with chronic pancreatitis (24). Although it can be assumed that malnourished patients with chronic pancreatitis will especially benefit from resistance training in terms of reversing muscle loss and inducing protein synthesis (22), more complex and standardized interventions could compromise feasibility. This aspect should be considered as our results show that in general multimodal nutrition therapy in malnourished patients with chronic pancreatitis for up to 6 months is feasible.

In addition to feasibility, we show that this intensified nutritional intervention was highly effective in improving patients' nutrition status. Previous evidence supporting the efficacy of nutrition therapy in malnourished patients with chronic pancreatitis has been limited to less than a handful of studies (20, 25, 26). Despite heterogeneous study designs and populations these previous investigations consistently suggest that malnutrition can be effectively treated. Besides supporting these earlier findings, our study adds to existing knowledge in several respects. First, we showed that patients not only gained body weight but also muscle mass during the intervention. Second, we also found increased gait speed, which suggests improved muscle performance. Interestingly, hand grip strength remained unchanged. However, this does not necessarily confute an amelioration in muscle function as pathologically reduced muscle strength is rarely seen in malnourished patients with chronic pancreatitis despite low muscle mass (6). Finally, for the first time we report data that suggests that nutritional treatment could lower parameters of overall disease severity and thus may enhance prognosis in chronic pancreatitis. These findings are of high clinical relevance because, although malnutrition is known to be a common complication linked to adverse outcome, definite benefits of dietary interventions in that regard have not been demonstrated. Hence, our findings highlight the potential of nutrition therapy in the treatment of an incurable disease.

Last, in our work we identified multiple biochemical parameters that were associated with changes in nutritional status and could therefore potentially serve as future biomarkers. As for blood parameters, cholinesterase and IGF-1 were significantly associated with improved nutritional status. Cholinesterase, a negative acute phase reactant, has previously been suggested as a suitable indicator of nutritional status and to reflect effectiveness of nutritional support (27). In a previous investigation, we already found cholinesterase to be associated with malnutrition in patients with chronic pancreatitis (6). Cholinesterase may have the advantage of a longer half-life of approximately 12 days (27) over other negative acute phase proteins. Albumin, for instance, has repeatedly been shown to inaccurately reflect nutritional status in case of acute inflammation (28). Notably, in our study, in contrast to cholinesterase neither albumin nor prealbumin changed during the intervention, which supports that these parameters are not suitable to monitor nutritional status in patients with chronic pancreatitis. However, as cholinesterase is also synthesized in the liver, its predictive value for nutritional status could be limited in patients with concomitant liver disease, which is common in patients with alcoholic chronic pancreatitis. Thus, further validation of cholinesterase in a larger study population is needed to be established as a biomarker of nutritional status or efficacy of nutritional support in patients with chronic pancreatitis. As a second blood marker, we identified IGF-1 to be associated with improved nutritional status. As IGF-1 is known to be a key factor regulating

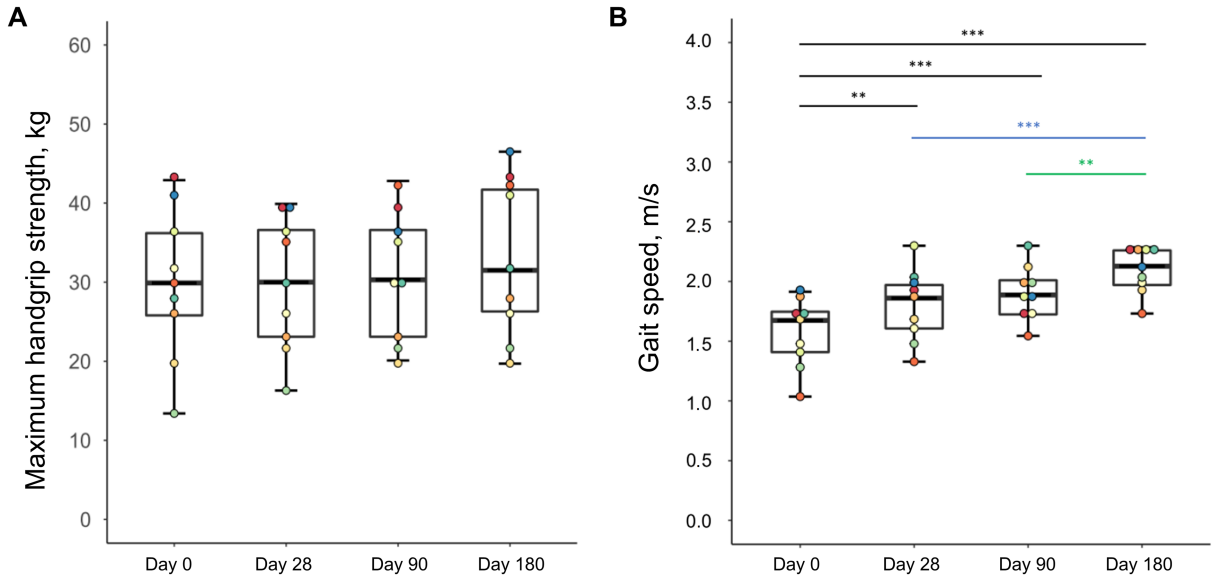
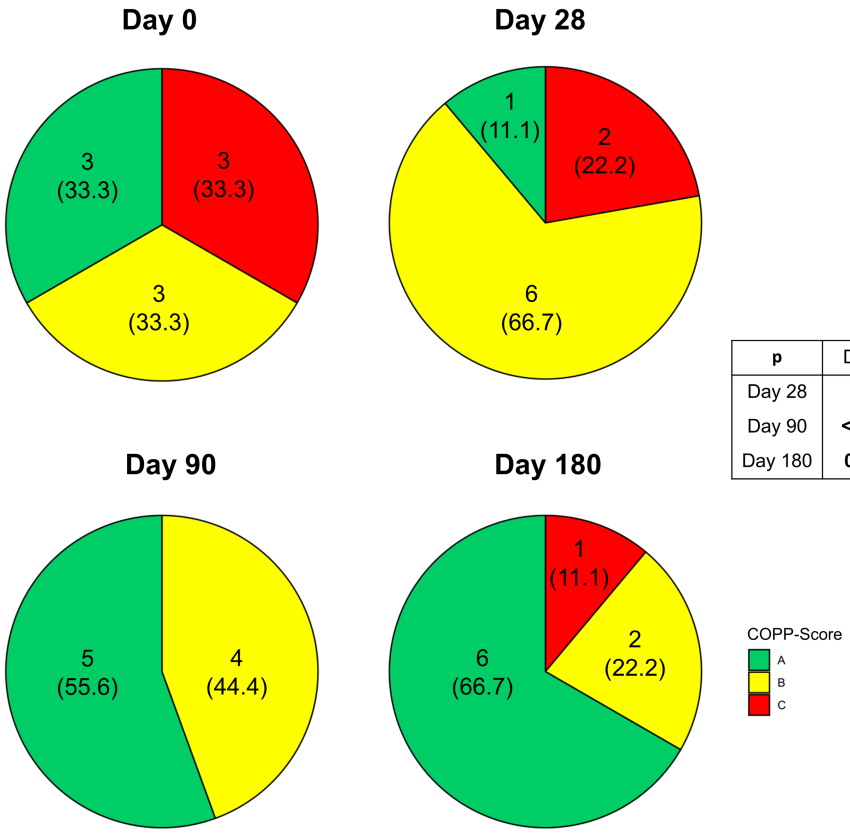


FIGURE 4
Changes in handgrip strength **(A)** and gait speed **(B)** of malnourished patients with chronic pancreatitis in the course of the intensified trans-sectoral nutritional intervention ($n = 9$). $^{**}p < 0.01$; $^{***}p < 0.001$.



p	Day 0	Day 28	Day 90
Day 28	.454	-	-
Day 90	**< .001**	**< .001**	-
Day 180	**0.001**	**< .001**	.658

FIGURE 5
Changes in Chronic Pancreatitis Prognosis Score (COPPS) of malnourished patients with chronic pancreatitis in the course of the intensified trans-sectoral nutritional intervention ($n = 9$).

TABLE 3 Changes in blood parameters of malnourished patients with chronic pancreatitis in the course of the intensified trans-sectoral nutritional intervention ($n = 9$).

	Reference range	Day 0	Day 28	Day 90	Day 180	p-value[1]
Complete blood count						
Hemoglobin, mmol/L	7.4–11.2	7.6 (1.5)	7.8 (1.2)	8.1 (1.7)	8.3 (1.5)	0.087
Hematocrit, L/L	0.350–0.510	0.369 (0.085)	0.381 (0.077)	0.393 (0.074)	0.407 (0.076)	0.091
Mean corpuscular volume, fL	80.0–95.0	88.7 (5.8)	89.9 (2.6)	89.3 (4.6)	90.3 (5.4)	0.577
Mean corpuscular hematocrit, fmol	1.68–2.00	1.85 (0.11)	1.85 (0.10)	1.86 (0.11)	1.86 (0.10)	0.427
Mean corpuscular hemoglobin concentration, mmol/L	18.5–22.5	20.5 (1.4)	20.4 (0.7)	20.4 (1.0)	20.5 (0.7)	0.462
White blood cell count, 10^9/L	4.30–10.00	7.46 (6.80)	6.74 (3.70)	7.43 (4.20)	7.82 (5.90)	0.081
Platelet count, 10^9/L	140–440	219 (150)	267 (215)	254 (115)	249 (118)	0.145
Blood chemistry						
Creatinine, µmol/L	42–97	67 (48)	77 (32)	72 (28)	65 (32)	0.945
Alanine aminotransferase, IU/L	< 46.2	28.2 (17.4)	28.2 (15.3)	31.2 (12.9)	25.8 (5.7)	0.100
Aspartate aminotransferase, IU/L	< 35.4	20.4 (8.7)	19.2 (13.2)	20.4 (10.2)	18.0 (6.0)	0.392
Gamma-glutamyl transferase, IU/L	0.00–57.6	52.2 (100.2)	84.0 (97.5)	84.0 (125.9)	72.0 (63.3)	0.154
Alkaline phosphatase, IU/L	49.8–135.6	78.0 (111.0)	96.0 (57.0)	90.0 (45.0)	102.0 (39.0)	0.653
Cholinesterase, kU/L	5.9–19.0	8.3 (4.1)	11.7 (3.1)[#]	11.5 (4.6)[#]	12.4 (5.0)[#,†,‡]	**< 0.001**
Total bilirubin, µmol/L	0.0–17.0	6.9 (5.6)	7.8 (3.6)	5.0 (2.6)	6.8 (2.7)	0.082
Blood urea nitrogen, mmol/L	2.5–6.4	4.8 (3.4)	4.5 (3.8)	6.2 (3.2)	4.8 (3.3)	0.189
Uric acid, µmol/L	155–428	303 (228)	314 (102)	316 (98)	314 (136)	0.954
Iron, µmol/L	7.0–30.0	14.0 (8.1)	16.0 (5.5)	16.0 (8.1)	15.0 (9.7)	0.105
Albumin, g/L	34–50	37 (15)	37 (7)	38 (4)	37 (3)	0.792
Prealbumin, g/L	0.200–0.400	0.223 (0.173)	0.262 (0.051)	0.268 (0.041)	0.257 (0.062)	0.316
C-reactive protein, mg/L	< 5.0	3.2 (86.3)	3.1 (0.3)	3.1 (0.0)	3.1 (1.9)	0.423
Interleukin 6, pg./mL	< 10.0	2.0 (11.5)	1.5 (2.7)	1.8 (1.7)	1.8 (3.4)	0.362
Interleukin 1 beta, pg./mL	< 5.0	2.0 (2.1)	2.0 (1.5)	3.9 (3.9)	2.4 (2.1)	0.285
Tumor necrosis factor alpha, pg./mL	< 8.1	8.3 (7.8)	8.4 (5.1)	7.8 (4.8)	6.3 (3.4)	0.740
HbA1c, %	< 6.5	6.3 (3.9)	6.1 (2.6)	6.2 (1.3)	6.4 (2.0)	0.581
Insulin, µIU/mL	6.2–26.1	5.9 (5.6)	6.7 (5.3)	6.9 (6.9)	6.1 (5.2)	0.932
Insulin-like growth factor 1, ng/mL	14.0–647.0	101.2 (116.2)	131.7 (35.8)	141.9 (64.1)[#,†]	151.9 (61.3)[#,†]	**0.040**
Triglycerides, mmol/L	0.00–1.90	1.53 (1.49)	1.64 (0.86)	1.44 (1.19)	1.40 (1.06)	0.769
Total cholesterol, mmol/L	< 6.0	4.8 (2.0)	5.0 (0.8)	5.5 (2.1)	5.4 (1.3)	0.460
LDL cholesterol, mmol/L	0.00–3.34	3.09 (1.21)	2.84 (1.05)	2.97 (1.37)	3.01 (1.16)	0.482
HDL cholesterol, mmol/L	> 1.03	1.11 (0.89)	1.82 (1.05)	1.91 (1.17)[#]	1.65 (1.16)[‡]	**0.045**

All data is presented as median (IQR).

[1]Changes over time were tested using Friedman test.

[#]Indicates significant difference from Day 0 based on Conover post-hoc test with correction for false detection rate, $p < 0.05$.

[†]Indicates significant difference from Day 28 based on Conover post-hoc test with correction for false detection rate, $p < 0.05$.

[‡]Indicates significant difference from Day 90 based on Conover post-hoc test with correction for false detection rate, $p < 0.05$.

Bold values denote statistical significance at the $p < 0.05$ level.

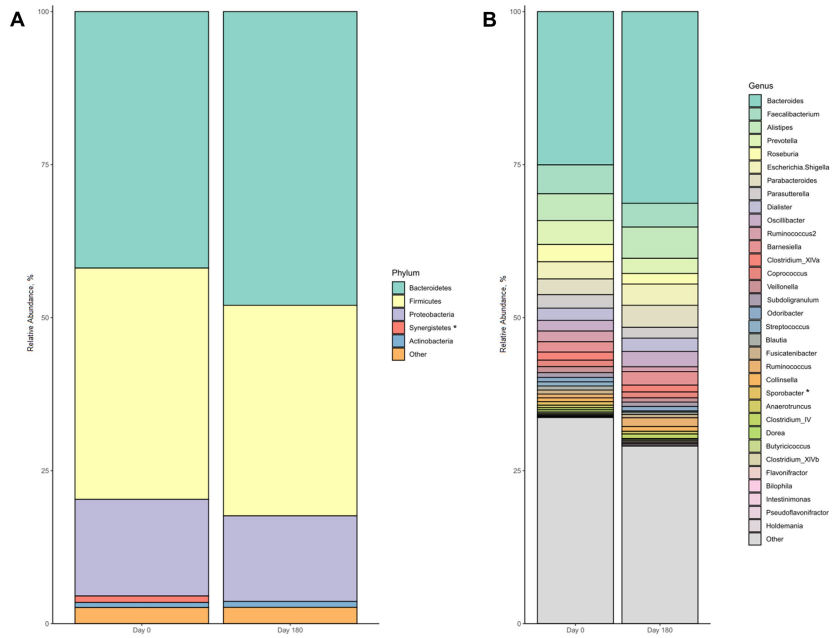


FIGURE 6
Changes in gut microbiota composition of malnourished patients with chronic pancreatitis in the course of the intensified trans-sectoral nutritional intervention at phyla **(A)** and genera **(B)** level. Correlation between changes in altered gut microbiota taxa and parameters of body composition and muscle function **(C)** ($n = 7$). *$p < 0.05$; **$p < 0.01$.

protein synthesis in skeletal muscle (29), the increased concentrations seen during the intervention likely reflect enhanced muscle anabolism in patients. Notably, both IGF-1 and skeletal muscle did not further increase between Day 90 and Day 180, which could imply lowered anabolic competence with longer intervention duration (30–32). These observations further support IGF-1 as a promising indicator of muscle anabolism in malnourished patients with chronic pancreatitis. Yet, also in the case of IGF-1, further validation is warranted as its concentrations are correlated with various other factors, for instance, dietary protein intake and physical activity (33, 34). Besides blood markers, we also detected changes in gut microbiota composition to be associated with improved nutritional status. Stool-based biomarkers offer the advantage of being non-invasive and could overcome some of the shortcomings of blood markers. We found only two taxa that both significantly declined in abundance during the intervention. Both the genus *Sporobacter* and the phylum *Synergistetes* have been linked to pathologically reduced muscle mass and function before (35, 36). Increased *Sporobacter* abundance has been observed in patients with amyotrophic lateral sclerosis, a neurodegenerative disease accompanied by progressive muscular atrophy (35). In line with this, we found a reduction in abundance to be associated with increased skeletal muscle mass in our patients. Further, Peng et al. reported higher abundance of *Synergistetes* in sarcopenic patients with heart failure than in non-sarcopenic controls (36). Interestingly, Peng et al. also considered lowered gait speed as a diagnostic criterion for sarcopenia in their study, which corroborates our observation of an inverse correlation between changes in relative abundance of *Synergistetes* and gait speed. However, our results should be interpreted with caution. For one thing, chronic pancreatitis itself is associated with a distinct gut microbiota composition compared to healthy controls (37) and secondly the multimodal intervention addressed multiple factors, including diet, physical activity, and smoking, which all evidently exert an effect on microbiota composition (38). Therefore, our findings, for now, should be considered explorative with the need for validation in future investigations testing the usability of gut microbiota composition as a nutritional marker in chronic pancreatitis.

In conclusion, we demonstrate that malnourished patients with chronic pancreatitis benefit from an intensified nutritional treatment. Benefits exceed improved nutritional status by also reducing parameters of disease severity and thus potentially prognosis, which highlights that nutrition therapy is a powerful asset in the management of this progressive and incurable disease. Implementation of an intensified trans-sectoral intervention in this patient group seems feasible but superiority to standard-of-care treatment needs to be shown in an adequately powered randomized controlled trial. Likewise, potential biomarkers for assessment of nutritional status and efficacy of nutritional intervention require further validation.

Data availability statement

The datasets presented in this article are not readily available because of ethical and legal considerations. Requests to access the datasets should be directed to the corresponding author.

Ethics statement

The studies involving humans were approved by the Ethics Committee of University Medicine Greifswald. The studies were conducted in accordance with the local legislation and institutional

requirements. The participants provided their written informed consent to participate in this study.

Author contributions

MW: Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. FF: Writing – review & editing, Methodology, Investigation, Data curation. FM: Writing – review & editing, Methodology, Investigation, Data curation. JM: Writing – review & editing, Investigation, Data curation. LV: Writing – review & editing, Methodology, Funding acquisition, Conceptualization. KR: Writing – review & editing, Methodology, Data curation. GL: Writing – review & editing, Methodology, Funding acquisition. AS: Writing – review & editing, Investigation, Funding acquisition. ML: Writing – review & editing, Supervision, Methodology, Funding acquisition, Conceptualization. AA: Writing – review & editing, Supervision, Methodology, Funding acquisition, Conceptualization.

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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.

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Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2024.1446699/full#supplementary-material

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Ravi Kumar Sharma,
Chandigarh University, India

REVIEWED BY
Juan Xiao,
Affiliated Hospital of Guilin Medical University, China
Hoi Huen Chan,
The Education University of Hong Kong, Hong Kong SAR, China

*CORRESPONDENCE
Xian Li
✉ Lixian_king@163.com
Shixiang Hu
✉ shixiang1968hu@163.com

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

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Efficacy of integrated traditional Chinese and western medicine in managing mild-moderate acute pancreatitis: a real-world clinical perspective analysis

Sailei Jia[1†], Qian Chen[2,3†], Xitong Liu[4†], Yanhong Li[3], Lihui Wang[2], Xian Li[4*] and Shixiang Hu[2*]

[1]Department of Critical Care Medicine, Henan Provincial Hospital of Traditional Chinese Medicine, Henan University of Traditional Chinese Medicine, Zhengzhou, China, [2]Department of Critical Care Medicine, Henan Provincial Hospital of Traditional Chinese Medicine, The Second Affiliated Hospital of Henan University of Traditional Chinese Medicine, Zhengzhou, China, [3]Department of Medical School, Huanghe Science and Technology College, Zhengzhou, China, [4]Department of Gastroenterology, Henan Provincial Hospital of Traditional Chinese Medicine, The Second Affiliated Hospital of Henan University of Traditional Chinese Medicine, Zhengzhou, China

Background: Given the prevalent utilization of integrated traditional Chinese and western medicine (ITCWM) in the management of acute pancreatitis, the majority of studies have concentrated on severe cases, lacking robust evidence-based medical research. Real-world investigations can provide an objective assessment of the clinical effectiveness of combining traditional Chinese medicine with western medicine. Consequently, relying on real-world research, we intend to evaluate the clinical efficacy and safety of the combined approach in treating mild to moderate acute pancreatitis.

Methods: A total of 563 AP patients from Henan Provincial Hospital of Traditional Chinese Medicine were collected from January 2019 to October 2023. A propensity score matching (PSM) analysis was conducted to evaluate the clinical efficacy of traditional Chinese medicine (TCM) in treating mild to moderate acute pancreatitis. Patients were divided into a control group (61 cases) and an integrated traditional Chinese and Western medicine (ITCWM) group (120 cases). To further assess the clinical efficacy of TCM enema in the treatment of mild to moderate acute pancreatitis, PSM analysis was conducted across three groups. The patients were categorized into a control group (n = 49), an oral TCM treatment group (OCM group, n = 274), and an oral TCM plus enema treatment group (OCM+E group, n = 131). Logistic regression was used to analyze factors after treatment in each group, and the Kaplan-Meier method compared symptom duration in each group.

Results: Compared with the control group, the ITCWM group significantly decreased C-reactive protein (CRP, mg/L) (17.8 [1.2–59.5] vs. 8.0 [3.3–33.5], P = 0.022), shortened the duration of abdominal distension, abdominal pain, nausea and bitter taste symptoms ($P < 0.05$), and shortened the length of hospital stay (median 19.0 and 11.5 days, respectively, P = 0.001); Compared with the other two groups, the neutrophil percentage (NEUT%) was lower (74.1 vs. 61.9 vs. 59.5, $P < 0.05$) and serum prealbumin (PA, mg/L) was higher (116.0 vs. 184.4 vs. 220.0, $P < 0.05$), the length of hospitalization (days) was shortened (19.0 vs.12.0 vs.10.0, $P < 0.05$) in the OCM+E group.

Conclusion: The combination of traditional Chinese medicine and modern medicine has been shown to effectively decrease inflammatory indicators in patients with mild to moderate acute pancreatitis, leading to a reduction in symptom duration and hospitalization period, as well as promoting disease recovery. Notably, the use of traditional Chinese medicine in conjunction with enema therapy yields more pronounced benefits.

KEYWORDS

integrated traditional Chinese and western medicine, acute pancreatitis, real-word study, mild-moderate pancreatitis, herbal enema

1 Introduction

Acute Pancreatitis (AP), a common and emergent digestive disease, arises from pancreatic enzyme activation, characterized by pancreatic inflammation and caused by various etiologies. AP often progresses from localized damage to systemic organ dysfunction, known as severe acute pancreatitis (SAP) (1). The annual incidence of AP in high-income countries is approximately 34/100,000. The AP incidence has increased by 62.9% and AP-related mortality by 64.8% since 1990 (2, 3).

Although self-limiting in most patients, moderate even severe AP still occurs in approximately 20% of patients, characterized by (surrounding) pancreatic tissue necrosis and/or (multi-organ) failure and a mortality rate up to 20–40% (4, 5). Early control on mild and moderate AP can effectively reduce disease progression and facilitate patients' recovery, thereby contributing to better outcomes in AP (6).

Oral or external application of Traditional Chinese medicine (TCM), along with enema, has shown significant benefits in treating AP (7–9). TCM demonstrates efficacy in reducing capillary permeability, inhibiting the production of inflammatory cytokines, and suppressing neutrophil activation, thereby mitigating pancreatic injury (7, 10). Furthermore, TCM offers benefits such as improving clinical symptoms, reducing medical costs, and enhancing patient satisfaction, all of which position it as a viable complementary and alternative therapy for AP (11, 12). Despite its primary use in severe AP (13, 14), there lacks robust evidence-based medical research on TCM in treating mild to moderate AP. Real-world studies, as opposed to rigorous randomized controlled trials, provide a more authentic reflection of the advantages of TCM. These studies offer innovative perspectives and methods for evaluating treatment efficacy through evidence-based approaches. Thus, our retrospective cohort study, conducted in real-world and approved by the clinical medical research ethics committee of Henan Province Hospital of Traditional Chinese Medicine (ethics batch number: 1480), investigated the efficacy of TCM in treating mild to moderate AP. The study employed PSM to balance clinical baseline data across the groups.

Abbreviations: AP, Acute Pancreatitis; SAP, Severe Acute Pancreatitis; TCM, Traditional Chinese medicine; HIS, Hospital Information System; ITCWM, Integrated Traditional Chinese and Western Medicine treatment; OCM, Oral Chinese Medicine Treatment; RAC, Revised Atlanta Classification; PSM, Propensity Score Matching; BISAP, Bedside Index for Severity in Acute Pancreatitis; MCTSI, Modified CT Severity Index; CRP, C-reactive protein; Ca, Blood Calcium; PLT, Platelets; PCT, Procalcitonin; ALT, Alanine aminotransferase; AST, Aspartate aminotransferase; ALB, Serum Albumin; UREA, Urea; Cr, Creatinine; TP, Total Protein; PA, Prealbumin; SA, Serum Amylase; SL, Serum Lipase; UA, Urinary Amylase; PA, Serum Prealbumin; NEUT%, neutrophil percentage.

2 Materials and methods

2.1 Data sources

Data of patients diagnosed with AP, and hospitalized within 48 hours of symptom onset, were retrospectively collected from hospital information system (HIS) of Henan Province Hospital of Traditional Chinese Medicine, spanning from January 2019 to October 2023. Uniformity of diagnosis and pathological staging was ensured by the data standardization processing according to the criteria outlined in 'American Gastroenterological Association Institute Guideline on Initial Management of Acute pancreatitis' (6).

2.2 Diagnostic criteria

The diagnostic criteria of AP followed the guidelines published in 'American Gastroenterological Association Institute Guideline on Initial Management of Acute pancreatitis' (6). The severity of acute pancreatitis was graded using the revised RAC score, based on the 'Classification of Acute Pancreatitis—2012: Revision of the Atlanta Classification and Definitions by International Consensus' (1). The diagnostic criteria of TCM symptoms or signs of AP were diagnosed based on the 'The consensus of integrative diagnosis and treatment of acute pancreatitis-2017' (15), which are divided into four syndrome types, namely, liver depression and qi stagnation, liver and gallbladder dampness and heat, chest binding and interior excess, blood stasis (toxin) binding with hot, see Supplementary Table 1 for the specific diagnosis.

2.3 Inclusion criteria

Eligible participants included: (1) admission within 48 hours of symptom onset; (2) age between 18 and 75 years; (3)

informed consent, either from relatives or guardians, for voluntary participation in this study.

2.4 Exclusion criteria

Exclusion criteria included: (1) patients with severe AP requiring surgery or non-internal medicine treatments (abdominal lavage or others); (2) patients with severe hypertension (systolic blood pressure > 180 mmHg and limited response to medicines); (3) patients diagnosed with advanced-stage tumors; (4) patients had received other clinical trials within three months prior to disease onset; (5) pregnant or lactating women.

3 Methods

3.1 Grouping and treatment methods

Firstly, according to the treatment situation, it can be divided into two groups: the Control group and the Integrated Traditional Chinese and Western Medicine treatment group (the ITCWM group). The control group implemented contemporary medical treatment approaches, encompassing etiological treatment, fasting, early fluid resuscitation, analgesia, inhibition of digestive enzyme secretion, and nutritional support, among others. In contrast, the ITCWM group employed contemporary medical treatment methods in conjunction with TCM classification treatment, as outlined in Supplementary Table 1. In order to enhance the understanding of the clinical effectiveness of enema with traditional Chinese medicine, the treatment group consisting of integrated traditional Chinese and western medicine was divided into two subgroups: the simple oral Chinese medicine treatment group (OCM group) and the oral Chinese medicine and enema treatment group (OCM+E group). These two groups were then compared with the control group. Traditional Chinese medicine enema therapy involves making a mixture of 30g of raw rhubarb and 200mL of water. This mixture is then boiled, filtered to remove any residues, and cooled to a temperature of 38–40°C. The enema is administered with an intubation depth of 30–35 cm and should be retained for 1–2 h, twice a day. The study design is briefly described in Figure 1.

3.2 Propensity score matching

To mitigate the impact of potential confounding variables, we employed the MatchIt package in R software (version 4.2.2) to conduct bias matching between the two groups (16). A logistic regression model was conducted with TCM therapy as the dependent variable, and potential confounders as independent variables. The confounders were those that were AP-related and showed imbalances between the ITCWM group and the control group. With a caliper width set at a quarter of the log standard deviation of the propensity score, PSM for both two groups were performed utilizing the nearest neighbor matching (1:2), involving variables such as age, gender, smoking and alcohol use histories, past history, pre-treatment evaluative scores and laboratory. Matching quality was evaluated by comparing baseline characteristics between patients receiving ITCWM group and matched control patients. DECISIONLINNC 1.0 software[1] was used to conduct three groups of propensity matching scores (PSM). The software employs the twang package's mnps function to estimate the average processing effect for the entire population by comparing each sample's trend score. It calculates the average weight of these scores and the sample count per group. The data is then divided into three groups, sorted by weight from largest to smallest. We created a data set by extracting and merging the highest-weighted samples from each group. To assess match quality, we conducted a comparative analysis of the standardized mean differences across the three sets of covariates and observed that the baseline characteristics were approximately balanced following the matching process.

3.3 Observation outcomes

Primary outcomes were shortenings in symptom durations (abdominal distension, abdominal pain, Constipation, nausea, and etc.) and improvements in key laboratory indicators (white blood cell count, neutrophil count, CRP levels, Procalcitonin levels) pre- and post-treatment. Secondary outcomes included various scores (BISAP score, MCTSI score, APACHE II score and etc.) and hospitalization duration in AP patients.

3.4 Statistical methods

Statistical analyses were executed using SPSS (version 26.0). Quantitative data were expressed as mean ± standard deviation, while qualitative data as frequency. The t-test was utilized for normally distributed data with homoscedasticity, whereas the rank sum test for data exhibiting non-normally distributed or heteroscedasticity. Counting data analysis was performed using adjusted Chi-squared test and rank sum test for ranked data. Logistic regression analysis was applied to analyze the clinical efficacy of each group, and Kaplan-Meier (K-M) analysis was used to determine the symptom relief duration. K-M survival curves were plotted using GraphPad Prism 8 software. A p-value of less than 0.05 was considered statistically significant.

4 Results

4.1 Clinical characteristics before and after PSM

From January 2019 to October 2023, we collected medical records of 613 AP patients at Henan Province Hospital of Traditional Chinese Medicine, of which 563 met the inclusion and exclusion criteria. Ultimately, 181 patients were enrolled after PSM, and divided into the control group and the ITCWM group. Analysis of clinical data pre- and post-PSM covered variables

1 https://fast.statsape.com

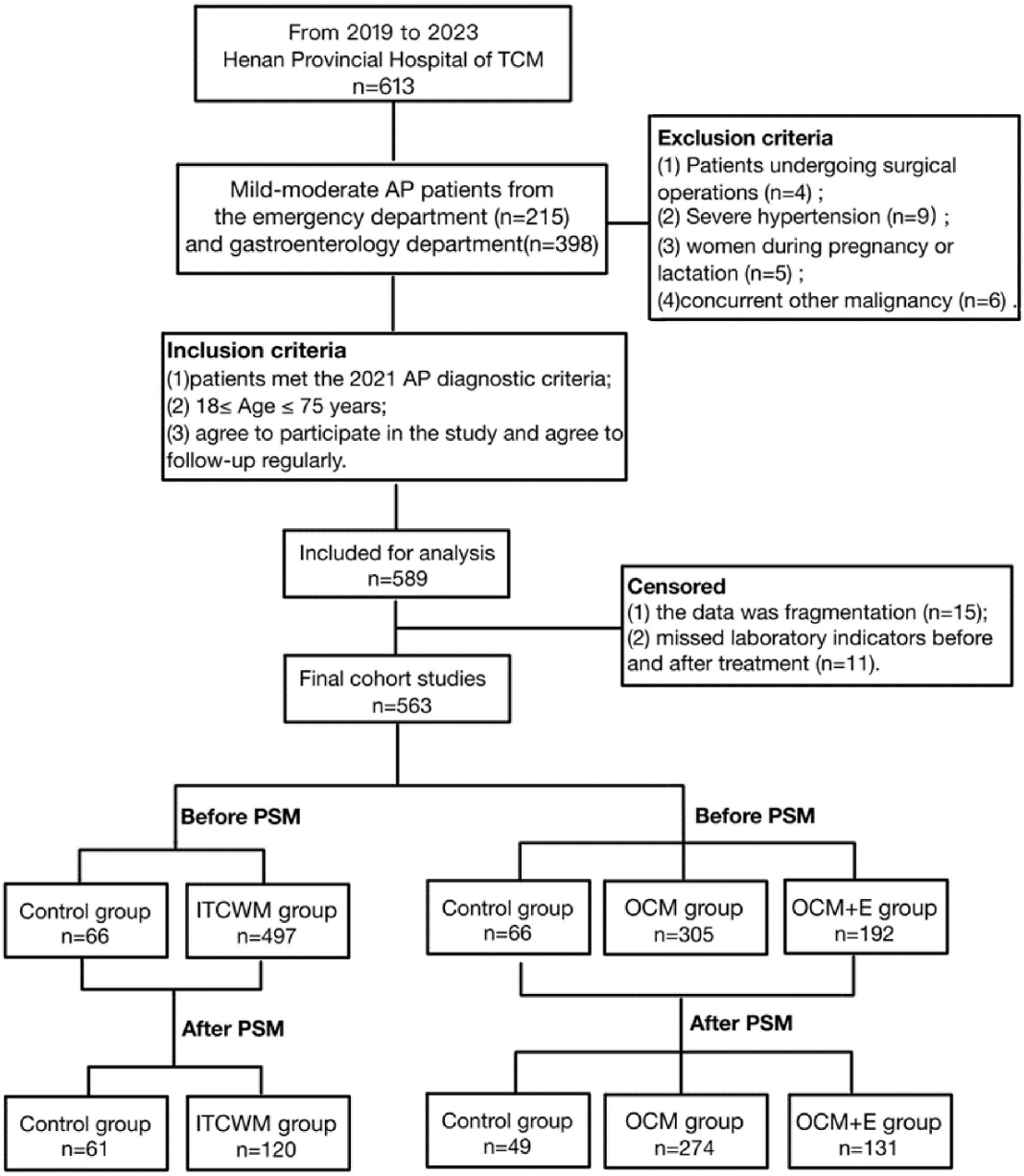

FIGURE 1
Flow diagram of the trial. TCM, Traditional Chinese Medicine; PSM, Propensity Score Matching; ITCWM group, Integrated Traditional Chinese and Western Medicine; OCM group, the Oral Chinese Medicine Treatment group; OCM+E group, the Oral Chinese Medicine and Enema treatment group.

including age, gender, smoking and alcohol use histories, past history, pre-treatment evaluative scores and laboratory indicators. Before PSM comparison, the levels of serum amylase (SA) and serum lipase (SL) in each group were as follows: the SA level (U/L) (133.0 [69.0,349.0] in the ITCWM group vs. 156.5 [88.0,365.0] in the control group, P = 0.478), and SL level (U/L) (499.0 [112.0,1349.0] in the ITCWM group vs. 585.0 [118.0,1347.0] in the control group, P = 0.736). In addition, initial pre-PSM comparison revealed significant differences between two groups in urea (UREA) levels (mmol/L) (4.4 [3.5,5.5] in the ITCWM group vs. 4.6 [4.0,5.9] in the control group, P = 0.041), CRP level (mg/L) (15.0 [5.0,54.0]in the ITCWM group vs. 6.7 [2.0,32.0] in the control group, P = 0.004), and urinary amylase (UA) level (U/L) (744.0 [336.0,1843.0] in the ITCWM group vs. 503.0 [243.0,1066.0] in the control group, P = 0.014). However, after PSM, baseline characteristics were balanced (P > 0.05), and no significant differences were observed in symptom frequency between two groups (Table 1 and Supplementary Table 2).

4.2 Comparative analysis of influencing factors post-treatment

Univariate logistic regression analysis indicated a significant reduction in CRP levels in the ITCWM group post-treatment

TABLE 1 Comparison of basic characteristics between two groups before and after PSM.

Items (pre-treatment)	Before PSM			After PSM		
	Control group (66)	ITCWM group (497)	*P*	Control group (61)	ITCWM group (120)	*P*
Age (years)	40.0 [32.0, 54.0]	40.0 [34.0, 49.0]	0.976	38.0 [30.0, 49.0]	41.0 [35.0, 57.5]	0.326
Gender male [n (%)]	43 (65.2)	357 (71.8)	0.327	39 (63.9)	81 (67.5)	0.754
Weight	71.4 ± 13.1	72.0 ± 13.7	0.129	71.4 ± 13.1	72.0 ± 13.7	0.651
Pathogenesis [n (%)]						
Biliary	20 (30.3)	168 (33.8)	0.669	18 (29.5)	39 (32.5)	0.810
Lipogenic	36 (54.5)	331 (66.6)	0.073	35 (57.4)	70 (58.3)	1.000
Alcoholic	1 (1.5)	5 (1.0)	0.528	1 (1.6)	4 (3.3)	0.664
Smoke	17 (25.8)	126 (25.4)	1.000	15 (24.6)	30 (25.0)	1.000
Drink	19 (28.8)	151 (30.4)	0.903	17 (27.9)	32 (26.7)	1.000
Co-morbidities [n (%)]						
Hypertension	10 (15.2)	108 (21.7)	0.283	8 (13.1)	17 (14.2)	1.000
Diabetes	12 (18.2)	104 (20.9)	0.722	10 (16.4)	21 (17.5)	1.000
RAC (mild) [*n* (%)]	38 (58.5)	354 (71.1)	0.066	38 (58.5)	71 (54.6)	0.637
BISAP (points)	0.0 [0.0, 1.0]	0.0 [0.0, 1.0]	0.889	0.0 [0.0, 1.0]	0.0 [0.0, 1.0]	0.838
MCTSI (points)	2.0 [2.0, 2.0]	2.0 [2.0, 2.0]	0.998	2.0 [2.0, 2.0]	2.0 [2.0, 2.0]	0.752
APACHE II (points)	13.2 ± 2.6	13.1 ± 2.6	0.548	13.2 ± 2.6	13.1 ± 2.6	0.744
SOFA (points)	0.0 [0.0, 1.0]	0.0 [0.0, 1.0]	0.613	0.0 [0.0, 1.0]	0.0 [0.0, 1.0]	0.983
WBC ($\times 10^9$/L)	9.3 [5.7, 13.4]	9.8 [6.5, 13.0]	0.498	9.4 [5.7, 13.4]	9.8 [6.0, 12.7]	0.851
NEUT (%)	77.5 ± 13.1	76.9 ± 12.3	0.752	77.5 ± 13.1	76.9 ± 12.3	0.659
RBC ($\times 10^{12}$ /L)	4.6 ± 0.7	4.7 ± 0.7	0.728	4.6 ± 0.7	4.7 ± 0.7	0.789
HGB (g/L)	142.8 ± 21.9	142.1 ± 24.9	0.342	142.8 ± 21.9	142.1 ± 24.9	0.878
Ca (mmol/L)	2.3 ± 0.2	2.3 ± 0.2	0.052	2.3 ± 0.2	2.3 ± 0.2	0.896
PCT (ng/mL)	0.1 [0.1, 0.2]	0.1 [0.1, 0.2]	0.916	0.1 [0.1, 0.2]	0.1 [0.1, 0.1]	0.571
CRP (mg/L)	6.7 [2.0, 32.0]	15.0 [5.0, 54.0]	0.004	6.0 [1.5, 32.0]	11.0 [5.0, 32.0]	0.067
ALT (U/L)	28.5 [17.0, 75.0]	31.0 [17.0, 65.0]	0.704	28.0 [17.0, 75.0]	26.5 [17.0, 60.5]	0.457
AST (U/L)	24.5 [18.0, 51.0]	27.0 [20.0, 47.0]	0.331	24.0 [18.0, 49.0]	26.0 [19.0, 43.5]	0.987
ALB (g/L)	41.7 ± 5.7	41.2 ± 5.3	0.091	41.7 ± 5.7	41.2 ± 5.3	0.811
UREA (mmol/L)	4.6 [4.0, 5.9]	4.4 [3.5, 5.5]	0.041	4.6 [3.9, 5.5]	4.5 [3.5, 5.5]	0.233
Cr (umol/L)	60.0 [46.0, 73.0]	60.0 [49.0, 71.0]	0.790	58.0 [45.0, 72.0]	61.0 [51.5, 71.5]	0.131
SA (U/L)	156.5 [88.0, 365.0]	133.0 [69.0, 349.0]	0.478	158.0 [88.0, 365.0]	128.0 [60.5, 272.0]	0.471
SL (U/L)	585.0 [118.0, 1347.0]	499.0 [112.0, 1349.0]	0.736	578.0 [118.0, 1347.0]	341.0 [78.0, 949.0]	0.075
UA (U/L)	503.0 [243.0, 1066.0]	744.0 [336.0, 1843.0]	0.014	525.0 [263.0, 1066.0]	685.0 [279.0, 1339.0]	0.250

RAC, Revised Atlanta Classification; BISAP, Bedside Index for Severity in Acute Pancreatitis; MCTSI, Modified CT Severity index; Ca, Blood Calcium; PCT, Procalcitonin; ALT, Alanine aminotransferase; AST, Aspartate aminotransferase; ALB, Serum Albumin; UREA, Urea; Cr, Creatinine; SA, Serum Amylase; SL, Serum Lipase; UA, Urinary Amylase.

(8.0 [3.3–33.5] in the ITCWM group vs. 17.8 [1.2–59.5] in the control group, $P = 0.022$). The median hospitalization duration in the ITCWM group was notably shorter at 11.5 days, in contrast to the 19.0 days in the control group, indicating the significant reduction of total treatment duration in the ITCWM group ($P < 0.001$) (Table 2). Multivariate analysis of demographic and clinical characteristics showed a generalized lower odds ratio (OR) in the ITCWM group, including CRP levels and hospitalization duration (OR for CRP levels = 0.976, 95% CI 0.953–0.999, $P = 0.043$; OR for hospitalization duration = 0.822, 95% CI 0.765–0.884, $P < 0.001$), suggesting the evident efficacy of TCM in reducing inflammation and hospitalization duration in mild to moderate AP (Table 3).

TABLE 2 Univariate comparison between the two groups.

Parameters (post-treatment)	Control group (61)	ITCWM group (120)	OR	95%CI	*P*
BISAP (points)	1.0 [0.3, 1.8]	0.0 [0.0, 1.0]	1.275	0.737–2.208	0.385
MCTSI (points)	1.0 [0.0, 2.0]	0.0 [0.0, 2.0]	1.118	0.779–1.604	0.546
APACHE II (points)	10.7 ± 2.1	10.9 ± 2.0	1.050	0.899–1.226	0.538
SOFA (points)	0.0 [0.0, 0.8]	0.0 [0.0, 1.0]	0.978	0.510–1.875	0.946
WBC ($\times 10^9$/L)	6.0 [5.0, 7.4]	6.5 [5.2, 7.5]	0.955	0.851–1.071	0.427
NEUT (%)	64.2 ± 13.7	59.8 ± 12.7	0.975	0.949–1.001	0.059
RBC ($\times 10^{12}$/L)	4.3 ± 0.7	4.4 ± 0.6	1.180	0.683–2.040	0.552
HGB (g/L)	131.5 ± 20.9	132.8 ± 18.3	1.004	0.986–1.022	0.695
Ca (mmol/L)	2.3 [1.9, 2.5]	2.3 [2.1, 2.4]	0.960	0.715–1.288	0.784
PLT ($\times 10^9$/L)	181.5 [100.3, 328.8]	193.0 [180.0, 280.0]	1.002	0.997–1.006	0.439
PCT (ng/mL)	0.1 [0.0, 2.3]	0.1 [0.0, 0.2]	0.548	0.222–1.352	0.192
CRP (mg/L)	17.8 [1.2, 59.5]	8.0 [3.3, 33.5]	0.974	0.944–1.005	0.022
ALT (U/L)	26.5 [16.3, 72.8]	23.0 [12.5, 37.5]	0.995	0.989–1.001	0.106
AST (U/L)	40.0 [21.5, 129.0]	26.0 [19.5, 29.5]	0.997	0.991–1.002	0.254
ALB (g/L)	36.4 ± 5.4	37.8 ± 4.3	1.066	0.971–1.171	0.177
SA (U/L)	78.0 [71.0, 83.5]	54.0 [34.5, 113.0]	1.011	0.998–1.004	0.649
SL (U/L)	139.0 [91.8, 1194.8]	94.0 [70.5, 927.5]	0.976	0.999–1.001	0.723
UA (U/L)	136.0 [44.8, 428.3]	381.0 [167.3, 607.6]	1.120	1.000–1.101	0.369
Hospital stays (days)	19.0 [18.1, 21.3]	11.5 [10.5, 14.0]	0.855	0.806–0.907	0.000

BISAP, Bedside Index for Severity in Acute Pancreatitis; MCTSI, Modified CT Severity Index; Ca, Blood Calcium; PLT, Platelets; PCT, Procalcitonin; ALT, Alanine aminotransferase; AST, Aspartate aminotransferase; ALB, Serum Albumin; SA, Serum Amylase; SL, Serum Lipase; UA, Urinary Amylase.

TABLE 3 Multivariate comparison between the two groups.

Parameters (post-treatment)	Control group (61)	ITCWM group (120)	OR	95%CI		*P*
	M (P_{25}, P_{75})	M (P_{25}, P_{75})		Lower	Upper	
CRP (mg/L)	17.8 [1.2, 59.5]	8.0 [3.3, 33.5]	0.976	0.953	0.999	0.043
Hospital stays (days)	19.0 [18.1, 21.3]	11.5 [10.5, 14.0]	0.822	0.765	0.884	0.000

4.3 Comparison of K-M survival curves for symptom relief duration

The K-M survival analysis highlighted longer-term relief in symptoms like abdominal distension, abdominal pain, nausea and bitter taste in the ITCWM group (P = 0.000–0.021). However, the analysis found no significant difference in the relief of vomiting and constipation between the two groups ($P > 0.05$), as detailed in Figure 2.

4.4 Comparative analysis of post-treatment outcomes across the three groups

The study categorized 563 patients into three groups based on the inclusion of various TCM therapies in their treatment: control group (66 patients), OCM group (305 patients), and OCM+E group (192 patients). Following PSM, the groups were reorganized as control group (49), OCM group (274), and OCM+E group (131). Prior to matching, the baseline characteristics exhibited significant disparities among the three groups. For instance, the incidence of lipogenic pancreatitis was 35 (53.8%) in the control group, 193 (63.3%) in the OCM group, and 139 (72.0%) in the OCM+E group. The proportion of patients with mild acute pancreatitis (AP) was 38 (58.5%) in the control group, 241 (79.0%) in the OCM group, and 113 (58.5%) in the OCM+E group. Procalcitonin (PCT) levels (ng/mL) were 0.1 (0.1,0.2) in the control group, 0.1 (0.1, 0.2) in the OCM group, and 0.1 (0.1, 0.3) in the OCM+E group. Additionally, SA and SL levels were similarly imbalanced. Specifically, SA levels (U/L) were 158.0 (88.0,346.5) in the control group, 107.0 (61.5,236.5) in the OCM group, and 254.0 (108.0,571.5) in the OCM+E group. Similarly, SL levels (U/L) in the control group, OCM group, and OCM+E group were 592.0 (140.0,1251.6), 345.0 (91.0,870.5), and 943.0 (243.0,1924.0), respectively. Following matching, the baseline characteristics of the three groups were essentially balanced, as detailed in Supplementary Tables 3–4.

One-way ANOVA analysis revealed statistically significant superior therapeutic outcomes in the OCM+E group compared to

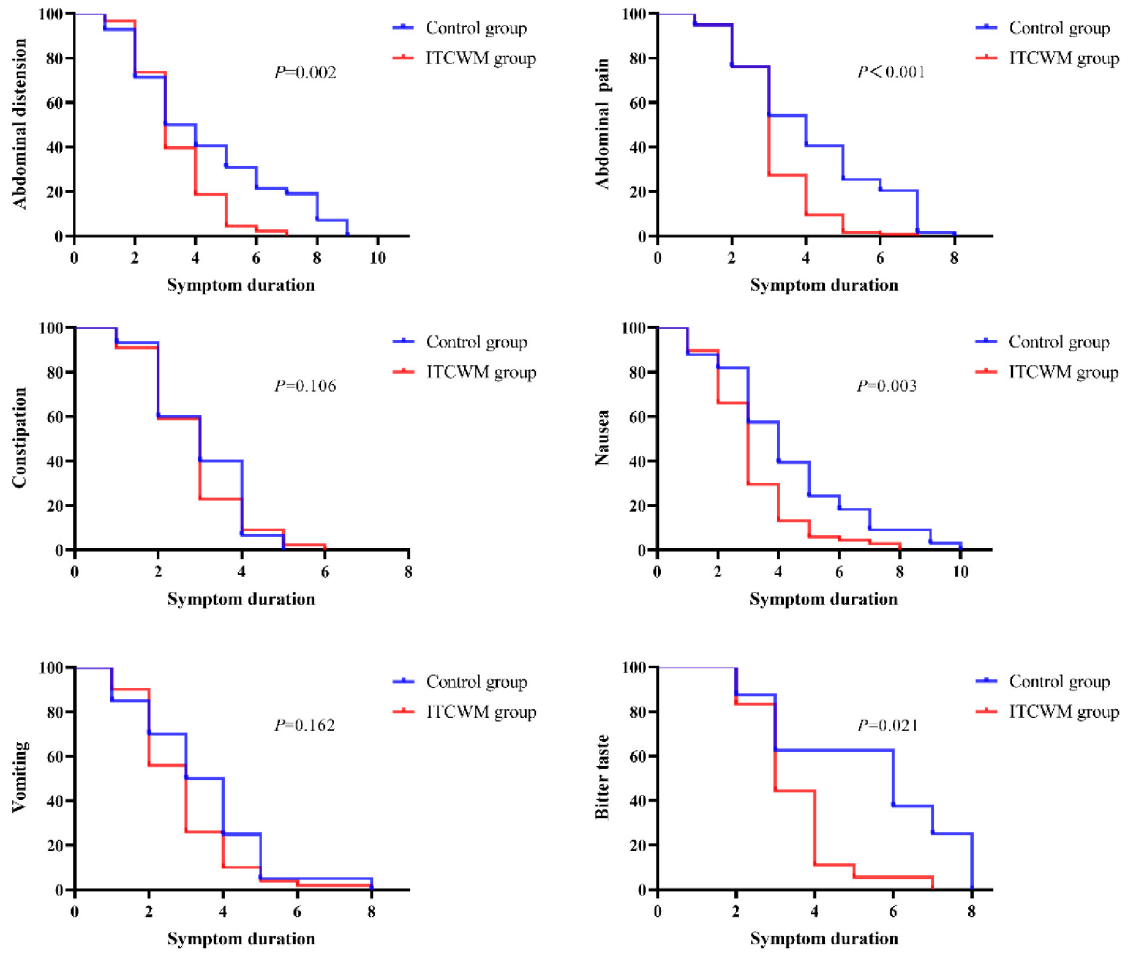

FIGURE 2
Comparison of K-M survival curves for symptom relief duration in the two groups.

the control group. Notably, neutrophil percentage (NEUT%) (59.5 vs. 74.1) was lower in the OCM+E group ($P < 0.05$), and blood calcium (Ca) (2.3 vs. 2.0), serum prealbumin (PA) (220.0 vs. 116.0) were notably higer in the OCM+E group, compared to the control group ($P < 0.05$). Additionally, the OCM+E group experienced a significantly reduced hospitalization duration compared to the control group (10.0 days vs. 19.0 days, $P < 0.05$), underscoring the efficacy of the combined treatment approach. Compared to the OCM group, AP patients in the OCM+E group had a significantly lower NEUT% (59.5 vs. 61.9, P < 0.05), and the reduction in hospitalization duration was more pronounced in the OCM+E group (Table 4).

Upon incorporating demographic indicators, clinical characteristics, and variables with $P < 0.05$ into the multivariate logistic regression analysis, we identified NEUT%, PA levels (mg/L), and hospitalization duration as significant factors across the three groups. The OCM+E group had the shortest hospital duration (10.0 [OCM+E] vs. 12.0 [OCM] vs. 19.0 [control] d, $P < 0.05$). The NEUT% was lower and the PA level (mg/L) was higher in the OCM+E group compared to the other two groups ($P < 0.05$), as detailed in Table 5. These findings indicated the enhanced efficacy of TCM combined with Enema in mitigating inflammatory indicators, boosting immunity and shortening hospital duration, compared to Western medicine or TCM alone.

4.5 Comparative analysis of K-M survival curves for symptom relief duration across the three groups

The comparative analysis revealed that the OCM+E group exhibited a significantly longer-term relief in abdominal distension, abdominal pain, and constipation, compared to the other two groups ($P < 0.001$). Conversely, the duration of symptoms, such as nausea, vomiting, and bitter taste, did not demonstrate statistically significant difference across the groups ($P > 0.05$) (Figure 3). This suggested that the integration of enema with TCM therapy might notably reduce the duration of certain symptoms in mild to moderate AP patients.

5 Discussion

TCM has demonstrated its potential in managing acute pancreatitis and individualized therapy, particularly in cases of mild

TABLE 4 Univariate logistic regression analysis across the three groups.

Parameters (post-treatment)	Control group (49)	OCM group (274)	OCM+E group (131)	*P*
BISAP (points)	1.5 [0.0, 1.6]	0 [0.0, 0.0]	0 [0.0, 1.0]	0.068
MCTSI (points)	2.0 [2.0, 2.0]	0 [0.1, 1.5]	0 [0.3, 0.7]	0.150
APACHE II (points)	14.0 ± 1.4	10.4 ± 2.1	11.1 ± 2.6	0.242
SOFA(points)	0.0 [0.0, 1.0]	0 [0.0, 1.0]	0 [0.0, 0.0]	0.067
WBC ($\times 10^9$/L)	6.9 [5.9, 9.6]	6.8 [5.3, 8.8]	6.5 [5.7, 8.2]	0.096
NEUT%	74.1 ± 5.2	61.9 ± 15.0*	59.5 ± 12.3*▲	0.001
RBC ($\times 10^{12}$ /L)	4.9 [4.1, 13.9]	4.3 [4.0, 4.6]	4.3 [3.9, 4.5]	0.415
HGB (g/L)	95.5 ± 27.6	134.2 ± 8.8	121.0 ± 19.2	0.391
Ca (mmol/L)	2.0 ± 0.2	2.2 ± 0.1*	2.3 ± 0.1*	0.009
PLT ($\times 10^9$ /L)	258.0 [112.0, 343.0]	314.0 [150.8, 400.0]	233.0 [194.6, 306.6]	0.646
PCT (ng/mL)	0.7 [0.0, 1.3]	0.2 [0.1, 0.3]	0.1 [0.0, 0.2]	0.483
CRP (mg/L)	21.0 [2.0, 50.0]	19.5 [1.3, 52.1]	18.7 [1.4, 42.7]	0.146
ALT (U/L)	18.5 [8.7, 75.7]	20.0 [18.0, 30.4] *	16.0 [3.3, 53.3] *	0.041
AST (U/L)	24.0 [19.5, 87.5]	22.0 [18.9, 25.9]	26.0 [19.8, 39.6]	0.330
TP (g/L)	54.2 ± 0.2	61.0 ± 8.3	66.0 ± 3.8	0.992
ALB (g/L)	28.0 ± 0.8	35.5 ± 4.6	37.3 ± 3.0	0.282
TBIL (umol/L)	16.9 [17.0, 22.8]	16.3 [12.9, 21.3]	11.1 [9.4, 17.0]	0.095
DBIL (umol/L)	11.0 [5.6, 21.5]	8.0 [5.7, 10.5]	3.7 [3.1, 7.4]	0.380
PA (mg/L)	116.0 ± 24.0	184.4 ± 58.0*	220.0 ± 39.7*▲	0.001
UREA (mmol/L)	3.6 [2.4, 4.8]	4.4 [2.6, 5.1]	3.6 [2.8, 4.9]	0.684
Cr (umol/L)	53.0 ± 17.0	58.1 ± 15.0	61.7 ± 18.8	0.310
SA (U/L)	82.0 [79.0, 85.0]	78.0 [54.0, 83.0]	70.0 [62.0, 106.0]	0.795
SL (U/L)	157.5 [111.0, 204.0]	66.5 [56.0, 135.0]	97.0 [74.0, 120.0]	0.808
UA (U/L)	78.5 [16.0, 141.0]	561.5 [256.0, 1298.0]	367.0 [221.0,6 12.0]	0.777
Hospital duration (days)	19.0 [16.5, 22.0]	12.0 [8.0, 17.0] *	10.0 [7.0, 14.0]*▲	0.000

Comparison between the OCM+E group and the control group, *$P < 0.05$; comparison between the OCM+E group and the OCM group, ▲$P < 0.05$; BISAP, Bedside Index for Severity in Acute Pancreatitis; MCTSI, Modified CT Severity Index; Ca, Blood Calcium; PLT, Platelets; PCT, Procalcitonin; ALT, Alanine aminotransferase; AST, Aspartate aminotransferase; TP, Total Protein; ALB,Serum Albumin; PA, Prealbumin; UREA, Urea; Cr, Creatinine; SA, Serum Amylase; SL, Serum Lipase; UA, Urine Amylase.

TABLE 5 Multivariate logistic regression analysis across the three groups.

Parameters (post-treatment)	Control group (49)	OCM group (274)	OCM+E group (131)	OR	95%CI		*P*
					Lower	Upper	
PA (mg/L)	116.0 ± 24.0	184.4 ± 58.0*	220.0 ± 39.7*▲	1.018	1.004	1.032	0.013
NEUT%	74.1 ± 5.2	61.9 ± 15.0*	59.5 ± 12.3*▲	0.857	0.811	0.945	0.001
Hospital duration (days)	19.0 [16.5, 22.0]	12.0 [8.0, 17.0] *	10.0 [7.0, 14.0]*▲	0.860	0.775	0.954	0.004

Comparison between the OCM+E group and the control group, *$P < 0.05$; comparison between the OCM+E group and the OCM group, ▲$P < 0.05$; PA, Serum Prealbumin.

to moderate AP. In TCM, AP is categorized into "stomach duct pain" and "spleen and heart pain", originating from the spleen and involving the gallbladder, liver, and stomach. TCM attributes the mechanism of AP to "organ congestion and toxin accumulation" (15). Current TCM therapies for AP center on alleviating liver congestion, reducing heat and dampness, and ensuring smooth functioning of organs (15), all of which reflect the TCM principles of holistic adjustment and syndrome differentiation. Evidence has exhibited the ability of TCM to modulate the production of pro-inflammatory and anti-inflammatory cytokines in inflammatory responses, thus achieving such favorable outcomes as reduced hospitalization duration (7, 17). Due to its complex and capricious mechanism, AP can be effectively managed by adjusting the ratios of herbal components in a TCM formulation (18). TCM has shown its effectiveness in reducing both the incidence and recurrence of AP, thus enhancing the quality of life for patients (10). Prealbumin, a liver-synthesized serum biomarker, sensitively reflects the protein

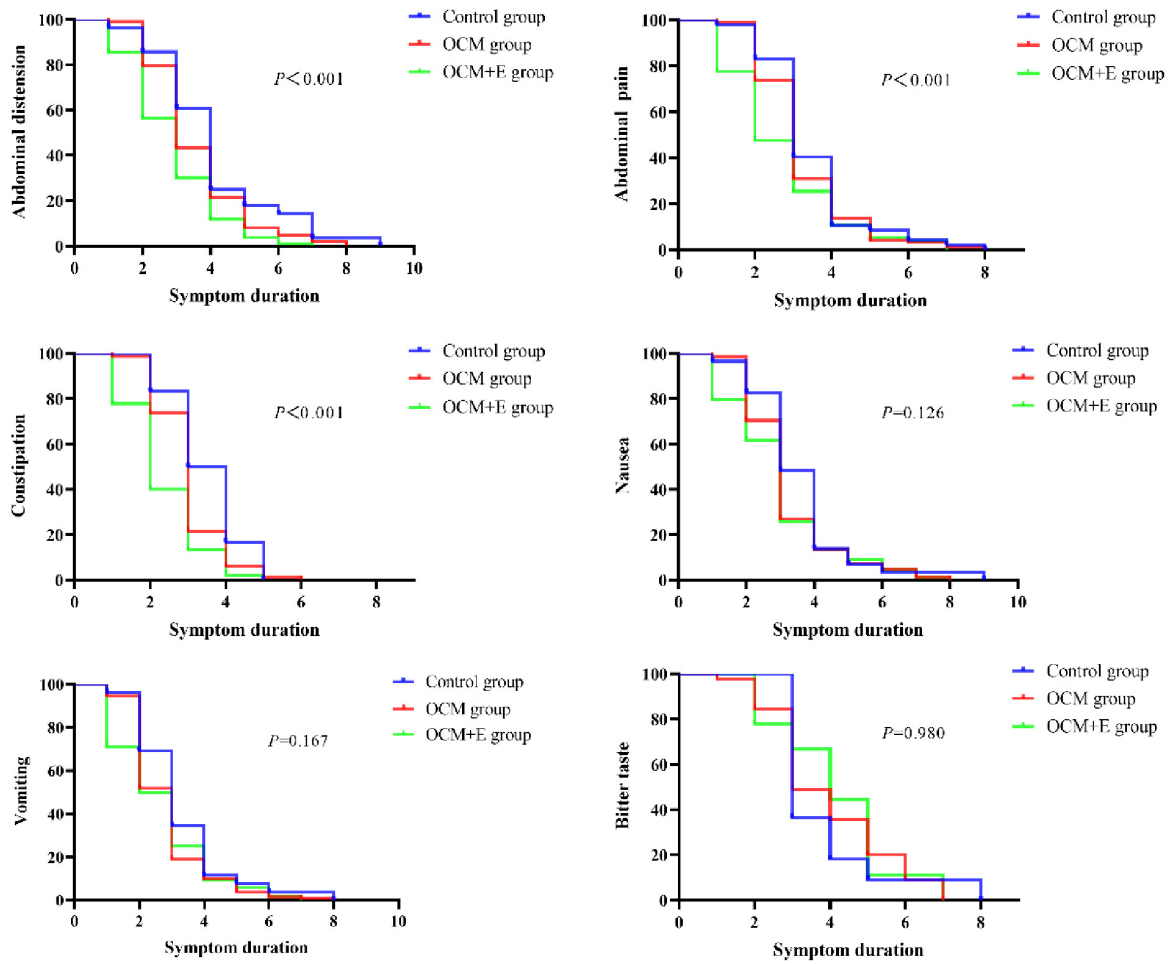

FIGURE 3
Comparison of K-M survival curves for the symptom relief duration across the three groups.

turnover and indicates the nutritional status in the body. A lower level of prealbumin represent a decline in overall health. TCM has been shown to elevate serum prealbumin (PA) levels, thereby promoting the recovery from AP (19). However, previous studies are primarily limited by small sample sizes, indicating a lower level of evidence-based medical support. Therefore, based on the real world, this study with a larger sample size, provides a stronger evidence of the reliable effectiveness of TCM in treating mild to moderate AP.

This study verifies that TCM posed positive impacts on inflammatory indicators, symptom and hospitalization duration in mild to moderate AP. Here, PSM was employed to ensure covariate balance between groups. After PSM, TCM exhibited significant effectiveness in improving inflammatory indicators, curtailing hospitalization duration, and diminishing symptom duration in mild to moderate AP. These results, aligning with those in prior research, further prove the therapeutic potential of TCM in coping with AP. Additionally, the study highlighted the efficacy of herbal enema, a characteristic TCM therapy, in enhancing laboratory indicators and clinical symptoms in AP. Notably, the safety of combining TCM and enema therapy was commendable (8, 9, 20). Extensive clinical research has demonstrated that rhubarb enema therapy is effective in treating acute pancreatitis (AP) (9, 21–23). Especially, using a 200 ml solution of 30 g raw rhubarb infusion for jejunal irrigation has shown excellent therapeutic results for AP (9, 21). Other studies indicate that raw rhubarb enema can reduce serum inflammatory cytokines, C-reactive protein (CRP), and endotoxin levels in AP patients, thereby alleviating systemic inflammatory stress responses (21, 24, 25). It has been proven beneficial in protecting pancreatic function and delaying disease progression (9, 10, 18). Furthermore, in patients suffering from severe abdominal symptoms, such as distension, pain, and constipation, herbal enema showed efficacy in stimulating gastrointestinal motility and boosting mucosal absorption. Additionally, the herbal medicine could serve as lubricants to aid bowel movements and thus reduce intra-abdominal pressure due to accumulated intestinal content (26–28). Moreover, herbal enema avoids gastrointestinal mucosal irritation and liver first-pass effects. To further assess the effectiveness of TCM combined with enema for AP, we subdivided the ITCWM group into TCM+ enema and TCM alone. Our findings indicated a notable superiority of the TCM+ enema over the other two therapies in mitigating inflammatory indicators, shortening symptom and hospitalization durations in AP patients. Additionally, this combination therapy raised the prealbumin level, consequently enhancing immune response.

Nevertheless, there remain limitations in the present study, such as the sample size inadequate, particularly within the control group, which might have limited the robustness of our statistical conclusions. Additionally, the lack of endpoint events impeded precise statistical analysis. Moreover, our patients mainly hailed from Henan, China, potentially limiting the generalizability of our findings. Despite using PSM to balance covariates between the groups, the retrospective nature of this study limited its validity due to non-randomization and other potential biases, including selection, informational, and recall biases. Consequently, the evidence level provided by our study was insufficiently persuasive. Prospective studies, particularly rigorously homogenized randomized controlled trials, are recommended to provide more robust substantiation.

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 the Clinical Medical Research Ethics Committee of Henan Province Hospital of Traditional Chinese Medicine (ethics batch number: 1480). The need for written informed consent was waived by the Clinical Medical Research Ethics Committee of Henan Province Hospital of Traditional Chinese Medicine due to retrospective nature of the study.

Author contributions

SJ: Data curation, Writing – original draft, Writing – review & editing. QC: Methodology, Writing – original draft, Writing – review & editing. XLiu: Visualization, Writing – original draft, Writing – review & editing. YL: Data curation, Formal analysis, Writing – review & editing. LW: Supervision, Investigation, Funding acquisition, Writing – review & editing. XLi: Funding acquisition, Project administration, Writing – review & editing. SH: Funding acquisition, Project administration, Writing – original draft, Writing – review & editing.

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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/fmed.2024.1429546/full#supplementary-material

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