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Acute pancreatitis infection: Epidemiology, prevention, clinical characteristics, treatment, and prediction

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Acute pancreatitis infection: Epidemiology, prevention, clinical characteristics, treatment, and prediction

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Editorial: Acute pancreatitis infection: Epidemiology, prevention, clinical characteristics, treatment, and prediction

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Editorial on the Research Topic
Acute pancreatitis infection: Epidemiology, prevention, clinical characteristics, treatment, and prediction

Acute pancreatitis (AP) is a common gastrointestinal disease and has been increasing in recent years worldwide (Hong et al., 2020). This special issue Research Topic highlights advances in the epidemiology, prevention, treatment, and prediction of the severity of AP.

Prevalence and etiology

Gallstones, hypertriglyceridemia, and alcohol are the most common causes of AP (Lee and Papachristou, 2019). However, the etiology composition varies across the globe. He et al. enrolled 5146 adult AP patients from 2011 to 2017 to investigate variations in the disease etiology. They found an upward trend in the diagnosis of alcohol-related AP while a decrease in the incidence of acute biliary pancreatitis (ABP). Moreover,the composition ratios of ABP and hypertriglyceridemic-AP were affected by seasons and festivals, which could be due to an increase in fatty food consumption.

Gallstones accounts for the most common etiology of AP in China, which can progress to severe sepsis or shock if not treated in timely fashion. However, not all patients with gallstones develop the symptomatic disease during their lifetime. The available data is scarce about the development of ABP in patients with symptomatic gallstone diseases such as cholecystitis and choledocholithiasis. Guo et al. conducted a retrospective case-control study and found that age, diabetes, gallbladder wall thickness, gallstone diameter, coexisting choledocholithiasis, direct bilirubin and white blood cell count are

significantly associated with an increased risk of concomitant ABP in patients with symptomatic gallstones. They developed a nomogram consisting of these indices with good discrimination predicting the ABP occurrence.

Chronic pancreatitis (CP) is a fibroinflammatory disorder with irreversible scarring to pancreatic parenchyma (Kothari et al., 2023). Its' common symptoms include abdominal pain, nausea, weight loss, steatorrhea, and diabetes (Kothari et al., 2023). It is well known that both alcohol and tobacco are the common risk factors for CP development. Hao et al. compared the clinical characteristics of smoking and alcohol-related chronic pancreatitis. They found that the development of diabetes and pseudocyst was significantly more common and earlier in smokers than in alcoholic patients. In addition, Steatorrhea was found to be significantly more common in smokers. The findings of this study indicate that smoking-related CP develop early and has higher risk of developing complications than idiopathic CP. The smoking related-CP should be considered as a new independent subtype of CP an these patients should be managed aggressively to prevent the development of complications.

Pathophysiology

Aseptic inflammation is the initial manifestation of injury in AP. However, the pathogenesis of AP has not been fully understood although trypsin-centered theory of AP has been proposed for more than a century. Many additional studies have been conducted to understand the pathogenesis and mechanism of AP development. Besides pathological calcium signaling and endoplasmic reticulum stress, the role of damage-associated molecular patterns (DAMPs) and neutrophil extracellular traps (NETs) was found to play a significant role in activating, signaling and recruiting inflammatory cells and the adaptive immune response giving rise to the sterile inflammation. In order to offer a better understanding of the pathophysiological mechanism and new insights for future investigational AP treatment options, the review by Zhou et al. describes the role of DAMPs and NETs, their interplay in the pathological progress of AP and potential targeted therapeutic modalities against DAMPs and NETs. They suggested that DAMPs could encompass the activation and recruitment of innate immune cells, mediate the formation of NLR Family Pyrin Domain Containing 3 (NLRP3) inflammasome, participating in forming NETs by activated neutrophils. Targeted therapeutic modalities against DAMPs and NETs, such as blocking DAMPs signaling, decreased expression of extracellular DAMPs, increased expression of intracellular DAMPs, and blockage of NETs formation may be helpful for severe AP.

AP is an inflammatory disease often accompanied by the occurrence of systemic inflammatory response syndrome (SIRS) and compensatory anti-inflammatory response syndrome (CARS). However, it is a topic of debate whether SIRS and CARS occur in succession or in parallel when AP arises. Through bioinformatics analysis, the study by Liu et al. on experimental model found that SIRS and CARS occur in parallel. They also found that toll-like receptor 2 (TLR2) could mediate the dysregulation of inflammatory response in AP and can be a novel therapeutic target to manage these patients.

Prediction of disease severity

While most patients with AP have a milder course and the disease is self-limiting, but about 20% of the AP patients progress to severe disease (Hong et al., 2020). Patients with severe acute pancreatitis (SAP) often need to be transferred to the intensive care unit after developingorgan failure. Therefore, it is essential to recognize predictors for severe disease in the early phase of AP, to select those patients who would benefit most from early interventions (Hong et al., 2019; Hong et al., 2020). The study by Li et al. compared the performance of interleukin-6 (IL-6) and C-reactive protein (CRP) as a potential predictor of SAP, organ failure, pancreatic necrosis, infected pancreatic necrosis, and mortality. Their study revealed that IL-6 is a better predictor of mortality and infected pancreatic necrosis in AP (AUC 0.75 vs. 0.70 and 0.81 vs. 0.65, respectively). Multiple past studies have reported that repeating serum amylase levels has no value in assessing the clinical progression and prognosis of AP. However, the study by Hong et al. found a a non-linear association between the amylase day 2/amylase day 1 ratio and incidence of SAP by logistic regression with restricted cubic spline analysis. Integration of Day 2/Day 1 amylase ratio ≥0.3 as 1 point to the bedside index for severity in acute pancreatitis (BISAP) score (BISAP-A score) significantly improved its diagnostic utility compared to the original BISAP score (AUC, 0.86 versus 0.83).

Artificial intelligence is increasing being used in the clinical setting for disease prediction or aiding in making decisions Hong et al. However, implementation of such data remains challenging because of the low interpretability of machine learning results. The study by Hong et al., proposed an interpretable random forest model with a sensitivity of 93.8%, specificity of 82.8%, and a diagnostic accuracy of 83.9%. The local interpretable model-agnostic explanations (LIME) plot was used to explain the individualized prediction. Simultaneously, a logistic regression model with a nomogram consisted of albumin, serum creatinine, glucose, and pleural effusion was also developed as a comparison. The study by Yin et al. developed an automated machine learning model based on the gradient boost machine algorithm to predict the AP severity achieving a sensitivity of 0.583 though its' specificity and accuracy were >0.95.

Management

It is well-known that early enteral nutrition (within 24-72h of admission) in AP patients is associated with decreased rates of complications and mortality (Arvanitakis et al., 2020). Jin et al. performed a retrospective study on 98 patients with predicted

severe acute gallstone pancreatitis who received early therapeutic endoscopic retrograde cholangiopancreatography (ERCP). They found that starting early enteral nutrition (within 48 h) was associated with a decrease in in-hospital mortality, length of stay, need for intensive care, and hospital care costs.

Post-inflammatory pancreaticopleural fistula (PPF) is a rare but serious complication of acute and chronic pancreatitis. The primary pathophysiology is disruption of the main pancreatic duct (MPD) or smaller pancreatic ducts, leading to leakage of pancreatic juice into the pleural cavity. In a prospective study by Jagielski et al. on 22 PPF patients found that ERCP with MPD stent placement for passive transpapillary drainage is effective with procedure success rate of 95.45%. The one-year success rate of endoscopic therapy was achieved in 86.36% patients.The results of this study shows that PPF can be treated effectively with preferential MPD drainage.

The data about antibiotic prophylaxis forendoscopic drainage of post-inflammatory pancreatic and peripancreatic fluid collections is scarce. Jagielski et al. performed a randomized, double-blinded, and placebo-controlled trial to investigate the role of periprocedural antibiotic prophylaxis in endoscopic transmural drainage in 62 patients with symptomatic pancreatic and peripancreatic fluid collections. Their results revealed that prophylacticantibiotics are not needed for pancreatic and peripancreatic fluid collections if the endoscopic transmural drainage was achieved successfully.

Infected pancreatic necrosis (IPN) is one of the primary determinant of severity in AP due to the high mortality of up to 32% (Petrov et al., 2010). Concomitant multi-drug resistance Gram negative bacteremia (MDR-GNB) can occur in AP with IPN in clinical practice. Only a few studies have examined the impact of MDR-GNB bacteremia in IPN patients. In a case–control Wu et al. found that IPN patients with MDR-GNB bacteremia was associated with a higher mortality rate than that without MDR-GNB bacteremia (OR 8.976, 95% CI 1.805 –44.620, p=0.007).

The importance of fungal infection in patients with IPN and pseudocysts is increasing being recognized. Patients with SAP and intra-abdominal fungal infection suffer higher in-hospital mortality than patients with intra-abdominal bacterial infection alone (Trikudanathan et al., 2011). Protracted broad-spectrum antibiotic therapy and a prolonged ICU stay are associated with an increased fungal infection risk (De Waele et al., 2019). However, the clinical presentation of fungal infection in acute necrotizing pancreatitis is somewhat variable and nonspecific (Trikudanathan et al., 2011). The review by Otsuka et al. summarizes recent advances in the clinical influence and molecular mechanisms of pancreatic fungal infection on the development of SAP as well as the efficacy of anti-fungal therapy. Their review summarizes that nucleotide-binding oligomerization domain 1 (NOD1) and toll-like receptor 4 (TLR4) sense the bacteria leading to a robust production of pro-inflammatory cytokines causing intestinal barrier dysfunction and translocation of gut bacteria into the pancreas. In the other hand, recognition of β-d-glucans by Dectin-1 play a important role in translocation of gut fungi into the pancreas due to leakiness of the barrier because Candida cell walls are strong stimulators for Dectin-1 expressed in macrophages and dendritic cells (Netea et al., 2015).

Further large scale *in-vivo* and *in-vitro* studies are needed to elucidate the underlying mechanism of AP and its local complications such as infected pancreatic necrosis. Furthermore, studies with high-quality evidence are needed to develop AP prognostication models using simple laboratory markers and clinical scoring systems. High-quality multicenter randomized controlled trials are required to determine whether prophylactic antibiotics have a role in a specific group of SAP, necrotizing pancreatitis, or endoscopic drainage of post-inflammatory pancreatic and peripancreatic fluid collections (Crockett et al., 2018). More studies are also needed to determine the optimal management of IPN (Working Group, 2013).

Author contributions

WH wrote the first article draft and JP, HG, MZ helped to revise the article. All authors contributed to the article and agreed with the last version to be accountable for the content of the work.

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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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Automated Machine Learning for the Early Prediction of the Severity of Acute Pancreatitis in Hospitals

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Background: Machine learning (ML) algorithms are widely applied in building models of medicine due to their powerful studying and generalizing ability. This study aims to explore different ML models for early identification of severe acute pancreatitis (SAP) among patients hospitalized for acute pancreatitis.

Methods: This retrospective study enrolled patients with acute pancreatitis (AP) from multiple centers. Data from the First Affiliated Hospital and Changshu No. 1 Hospital of Soochow University were adopted for training and internal validation, and data from the Second Affiliated Hospital of Soochow University were adopted for external validation from January 2017 to December 2021. The diagnosis of AP and SAP was based on the 2012 revised Atlanta classification of acute pancreatitis. Models were built using traditional logistic regression (LR) and automated machine learning (AutoML) analysis with five types of algorithms. The performance of models was evaluated by the receiver operating characteristic (ROC) curve, the calibration curve, and the decision curve analysis (DCA) based on LR and feature importance, SHapley Additive exPlanation (SHAP) Plot, and Local Interpretable Model Agnostic Explanation (LIME) based on AutoML.

Results: A total of 1,012 patients were included in this study to develop the AutoML models in the training/validation dataset. An independent dataset of 212 patients was used to test the models. The model developed by the gradient boost machine (GBM) outperformed other models with an area under the ROC curve (AUC) of 0.937 in the validation set and an AUC of 0.945 in the test set. Furthermore, the GBM model achieved the highest sensitivity value of 0.583 among these AutoML models. The model developed by eXtreme Gradient Boosting (XGBoost) achieved the highest specificity value of 0.980 and the highest accuracy of 0.958 in the test set.

Conclusions: The AutoML model based on the GBM algorithm for early prediction of SAP showed evident clinical practicability.

Keywords: automated machine learning, logistic regression analysis, severe acute pancreatitis, predictive models, artificial intelligence

INTRODUCTION

Acute pancreatitis (AP) is a common cause of gastroenterology-related hospitalizations, with a morbidity rate of 34 per 100,000 individuals globally (Zhou et al., 2022). Although AP is inclined to be self-limiting, around 20% of patients will progress to severe AP (SAP), with persistent organ failure (POF) and poor prognosis (Wu et al., 2021). Therefore, early detection of SAP and early treatment such as fluid resuscitation are dispensable for reducing the morbidity and mortality of SAP.

Conventional scoring systems such as the RANSON score, bedside index of severity in acute pancreatitis (BISAP), modified computed tomography severity index (MCTSI), and Acute Physiology and Chronic Health Evaluation (APACHE) II have been generally applied to assess the severity of AP (Bollen et al., 2012; Mounzer et al., 2012). Some novel point systems, such as SABP (Hong et al., 2019), the pancreatic activity scoring system (PASS), and the Chinese simple scoring system (CSSS) (Wu et al., 2021), have been proposed in recent years. However, the traditional scores are relatively complicated for clinical use, and the novel scores are not generalized, whose ability to predict SAP varies and accuracy ranges from 0.70 to 0.95 (Hong et al., 2019; Paragomi et al., 2021; Wu et al., 2021).

Machine learning (ML) applied in medicine, both supervised and unsupervised, is becoming increasingly popular based on its efficient computing algorithms to learn from massive clinical data (Deo, 2015). Previous studies (Han et al., 2022; Liu et al., 2022; Xu et al., 2021; Yang et al., 2021; Zhang et al., 2021; Zhao et al., 2021; Zhou et al., 2021) have confirmed that ML has great potential in building models for disease diagnosis, prognosis prediction, survival analysis, etc. Traditional ML includes logistic regression (LR), support vector machine (SVM), random forest, etc. A novel ML called automated machine learning (AutoML) intelligently selects from various algorithms and hyperparameters to create models customized to target data. It takes less time to develop more accurate models using intelligent early stopping, cross validation, regularization, and hyperparameter optimization when compared to traditional ML.

Our study aims to train, validate, and test a series of ML models for early prediction of SAP within 72-h hospitalization using the H2O AutoML platform in multiple centers. Additionally, traditional logistic regression (LR) analysis is also developed, as well as four existing scoring systems.

METHODS

Participants

A retrospective study was conducted in the three hospitals (the First Affiliated Hospital (First AFF), the Second Affiliated (Second AFF) Hospital, and Changshu No. 1 (Changshu) Hospital) of Soochow University from January 2017 to December 2021. Three hospitals are large-scale and fully equipped tertiary teaching hospitals in Suzhou, Jiangsu, China, There are 1,320 beds in Changshu Hospital, 2,050 beds in Second AFF, and more than 3,000 beds in First AFF. Changshu Hospital, as a county hospital, successfully established five major centers, including chest pain center, stroke center, atrial fibrillation center, etc. Second AFF is a tertiary level-A hospital integrating medicine, teaching, scientific research, prevention, and emergency care, with four research institutes and ten municipal key laboratories. First AFF, as one of the first grade-A hospitals under the Ministry of Health, ranked 32nd in the ranking of top hospitals in China in 2020. Data from two hospitals (First AFF and Changshu) were for development and internal validation, and data from another hospital (Second AFF) were for external testing.

Adult patients (≥18 years old) who were diagnosed with AP based on the 2012 revised Atlanta classification of acute pancreatitis were enrolled. The diagnosis must meet at least two of the following criteria: (1) typical abdominal pain; (2) serum amylase beyond three times the upper limit of normal; and (3) images of characteristic findings of AP (Banks et al., 2013). Severe AP (SAP) was defined as AP with persistent organ failure (POF >48 h). Patients were divided into two groups: SAP and non-SAP. The exclusion criteria were patients who had chronic liver disease, chronic renal disease, hematological diseases, recurrent/chronic/traumatic/idiopathic pancreatitis, pancreatic cancer, and history of pancreatic resection; patients who experienced chemoradiotherapy; and patients who were pregnant. All patients were treated in accordance with the guidelines for the management of AP. This study was approved by the ethics committee of the First Affiliated Hospital of Soochow University (**Figure 1**).

Data Collection

Demographic characteristics such as age, sex, smoke history, and clinical information such as etiology (biliary, hyperlipidemia, alcohol, and others) and concomitant diseases (hypertension and diabetes) were extracted from electronic medical records. Laboratory data within 24-h of admission were collected, including blood routine examination, coagulation tests, and serum biochemical tests. The presence of pleural effusion (PE) was recorded according to the computed tomography (CT) scan within 72-h of admission. Finally, a total of 41 variables were extracted for analysis. Details are listed in **Supplementary Table S2**. Missing variables, which were recognized as missing data at random, were multiple imputed using a random forest algorithm by the "mice" package of R software (Blazek et al., 2021). Other scoring systems such as systemic inflammatory response syndrome (SIRS), RANSON, MCTSI, BISAP, and SABP were calculated as described as Bollen et al. (2012), Hong et al. (2019) and Mounzer et al. (2012), if data were available. The flowchart of this study is shown in **Figure 1**.

Logistic Regression

Univariate analysis was performed by the least absolute shrinkage and selection operator (LASSO) regression model with the "λ_1se" criterion in order to solve such multiple colinear relationships among the explanatory variables. A binary logistic backward stepwise regression analysis was used for model specification. The receiver operating characteristic (ROC) curve, the calibration curve, and the decision curve

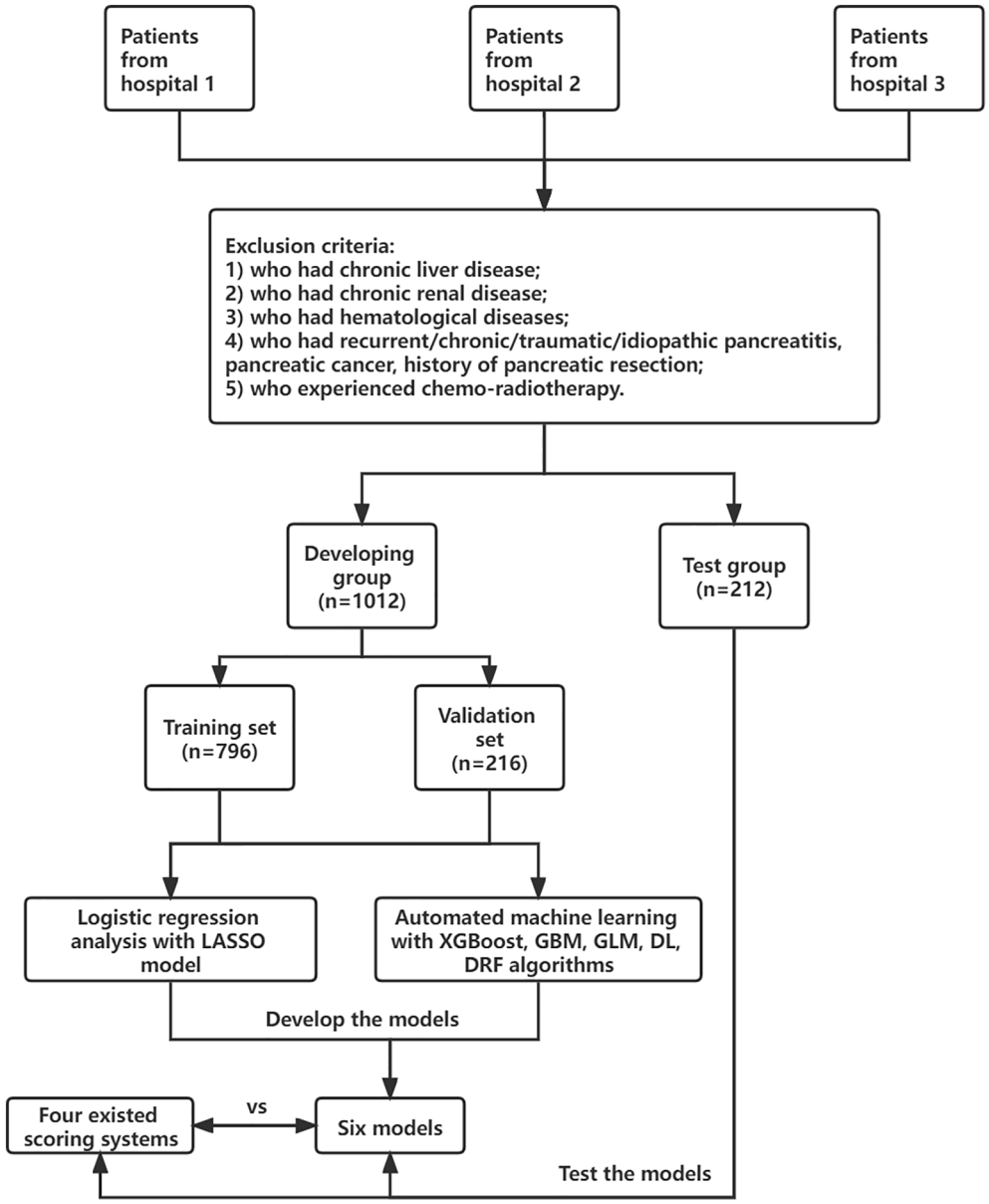

FIGURE 1 | The flow chart of this study.

analysis (DCA) were applied to evaluate the predictive performance of our proposed model. A nomogram was constructed based on the independent risk factors identified in the multivariate analysis.

Automated Machine Learning

The H2O package installed from the H2O.ai platform (www.h2o.ai) was applied to implement AutoML analysis, which automatically selects applicable algorithms and integrates them into multiple ensemble models. Algorithms include a default Random Forest (DRF), an Extremely Randomized Forest (XRF), a random grid of Gradient Boosting Machines (GBMs), a random grid of Deep Neural Nets (DLs), a fixed grid of Generalized Linear Models (GLMs), and a random grid of eXtreme Gradient Boosting (XGBoost). A 5-fold cross-validation grid search was performed on the training set for hyperparameter optimization, which was confirmed by evaluating AUCs for different combinations of hyperparameters included in the grid search. The confusion matrix, consisting of true positives (TP), true negatives (TF), false positives (FP), and false negatives (FN), was established to calculate sensitivity, specificity, positive predictive value (PPV),

negative predictive value (NPV), positive likelihood ration (LR+), negative likelihood ration (LR−), accuracy (ACC), and areas under the ROC curve (AUCs) for evaluating discrimination performance of models. Formulas were as follows: ACC = (TP + TN)/(TP + FP + FN + TN); PPV = TP/(TP + NP); NPV = TN/(TN + FN); LR+ = sensitivity/(1-specificity); LR− = (1-sensitivity)/specificity. The visualization of AutoML was exhibited in the form of feature importance, SHapley Additive exPlanation (SHAP), and Local Interpretable Model Agnostic Explanation (LIME). SHAP analysis explained which features were most important for creating model predictions and how much they contributed to the overall model performance for a particular prediction (Bang et al., 2021). LIME analysis demonstrated how much each feature contributed to predicting the outcome by randomly giving examples from the test set.

Statistical Analysis

Continuous variables were presented as mean ± standard deviation (SD) if fitting a normal distribution and as median (interquartile range) if not. Categorical variables were shown as frequencies. We compared the two groups by the Pearson Chi-square test or Fisher's exact tests for categorical variables and the Student's *t*-test or nonparametric Mann–Whitney *U* test for continuous variables. A two-sided $p < 0.05$ was considered statistically significant. The results were recorded as the odds ratio (OR) with corresponding 95% confidence intervals (CIs). R software (version 4.1.0) was used to implement all statistical analysis, including the H2O package (version 3.36.0.2), tableone package (version 0.12.0), tidyverse package (version 1.3.0), tidyquant package (version 1.0.2), and lime package (version 0.5.1).

RESULTS

Demographic and Clinical Characteristics

A total of 1,224 patients were included in our study. SAP occurred in 136 cases (11.1%) in the whole cohort. Among all patients, 1,012 patients from two hospitals (First AFF and Changshu) were included in the developing dataset, and they were randomly split into the training and validation sets at a ratio of 8 to 2 ($n = 796$ in the training set and $n = 216$ in the validation set). In total, 212 patients from one hospital (Second AFF) were selected as a test dataset to evaluate model performance. In the developing dataset, 58.7% (594/1,012) were men and 41.3% (418/1012) were women. The median age was 52 years (IQR = 38–65 years) in the non-SAP group and 45.5 (IQR = 35–61.75 years) in the SAP group. In the test dataset, the onset of AP and SAP were also more commonly seen in male than in female patients and the median age ranged from 44 to 47 years. Consistent with what Xu et al. (2021) reported, biliary sludge or gallstones (39.49%) was the most frequent etiology of AP in our cohorts, followed by hypertriglyceridemia (17.87%). No statistical differences were observed in sex, age, smoke, history of hypertension, and diabetes in two groups of three datasets ($p > 0.05$). Details are listed in **Table 1**.

Univariate and Multivariate Logistic Regression Analysis

Nine variables of the 41 variables were selected and later reserved as independent risk factors using the LASSO regression model with the "λ_1se (0.03)" criterion, which was achieved by 5-fold cross validation, to solve such multiple colinear relationships among the explanatory variables (**Supplementary Figure S1**). The final logistic model, including nine variables (neutrophil, creatinine, lactic dehydrogenase (LDH), total triglycerides (TGs), INR, ratio of red cell distribution width (RDW) to Ca^{2+} (RCR), ratio of CRP/albumin (CAR), SIRS, and PE), was developed as a nomogram and a score system for clinical use (**Figure 2**). The calibration curves of the training set, validation set, and test set are plotted in **Supplementary Figure S2**, and the mean absolute errors were 0.006, 0.033, and 0.036, respectively, demonstrating that the estimated risk using the LASSO model was close to the observed risk, indicating a high degree of reliability. The DCA plots of the training set, validation set, and test set are presented in **Supplementary Figure S3**, demonstrating that when the threshold probability of SAP predicted by the LASSO model was between 10% and 100%, an intervention might add more benefit (6%–10%). When a clinician considered the patient had a 10% chance of developing SAP, the patient might gain 4% of the benefit from an early intervention, according to the DCA of the test set, which is equivalent to detecting 4 SAP patients and suggesting zero unnecessary treatment per 100 patients. This is a direct comparison with treat none (the horizontal line in **Supplementary Figure S3**), which has zero true positives and zero false positives by default (Van Calster et al., 2018). The net benefit declares that the use of the LASSO model would improve patient outcome irrespective of patient or doctor preference. The ROC curve of the test set is presented in **Supplementary Figure S4**, and its AUC was 0.884 as shown in **Table 2**.

Automated Machine Learning Analysis

A total of 67 models were developed based on five machine learning algorithms (XGBoost, DL, GBM, GLM, and DRF), and stacked ensemble models were removed because of poor interpretability. The GBM model was the best among these models due to its highest value of AUC, which was a comprehensive evaluation for imbalanced samples. As shown in **Figure 3**, albumin was the most important feature, followed by PE, SIRS, TGs, LDH, RCR, Ca^{2+}, neutrophil count, TyG, and prothrombin time (PT). Additionally, PE, neutrophil count, LDH, TGs, RCR, and SIRS were the important variables in common between the GBM model and the LASSO model. SHAP contribution plots based on GBM algorithms are presented in **Figure 4**, including ten important variables (PE, ALB, SIRS, LDH, TG, PT, neutrophil count, ratio of albumin to globulin (AGR), ALT, and Ca^{2+}). The closer the values of the variables were to 1, the more likely patients were to progress to SAP. For example, the red part of PE which was concentrated on the right of axis = 0, revealed that the AP patient with PE would be more likely to develop SAP. **Table 2** demonstrates that GBM algorithm achieved the higher value of AUC than XGBoost, DRF, GLM, and DL algorithms (0.945, 0.898, 0.871, 0.868, and 0.860, respectively). The accuracy was 0.953, 0.958, 0.950, 0.925, and

TABLE 1 | Demographic and clinical characteristics of patients in training, validation and test groups.

Variables		The developing dataset (*n* = 1,012)			The test dataset (*n* = 212)		
	Group	Non-SAP (*n* = 888)	SAP (*n* = 124)	*p*-value	Non-SAP (*n* = 200)	SAP (*n* = 12)	*p*-value
Sex (%)	Male	518 (58.3)	76 (61.3)	0.597	133 (66.5)	9 (75.0)	0.770
	Female	370 (41.7)	48 (38.7)		67 (33.5)	3 (25.0)	
Age (year) (median [IQR])		52.00 [38.00, 65.00]	45.50 [35.00, 61.75]	0.141	47.00 [35.75, 65.25]	44.00 [33.75, 58.75]	0.810
Etiology (%)	Biliary	402 (45.3)	42 (33.9)	<0.001	88 (44.0)	5 (41.7)	0.461
	Hyperlipidemia	158 (17.8)	44 (35.5)		37 (18.5)	4 (33.3)	
	Alcoholic	48 (5.4)	5 (4.0)		21 (10.5)	0 (0.0)	
	Others	280 (31.5)	33 (26.6)		54 (27.0)	3 (25.0)	
Smoke (%)	No	767 (86.4)	108 (87.1)	0.936	161 (80.5)	9 (75.0)	0.927
	Yes	121 (13.6)	16 (12.9)		39 (19.5)	3 (25.0)	
Hypertension (%)	No	592 (66.7)	76 (61.3)	0.279	145 (72.5)	9 (75.0)	1.000
	Yes	296 (33.3)	48 (38.7)		55 (27.5)	3 (25.0)	
Diabetes (%)	No	773 (87.0)	102 (82.3)	0.187	170 (85.0)	8 (66.7)	0.202
	Yes	115 (13.0)	22 (17.7)		30 (15.0)	4 (33.3)	
MAP (mean (SD))		97.12 (11.95)	98.85 (15.57)	0.147	94.95 (12.59)	94.00 (14.20)	0.801
PLT (*10^9/L) (mean (SD))		199.27 (66.43)	212.48 (79.94)	0.040	215.68 (66.31)	225.83 (84.44)	0.612
WBC (*10^9/L) (median [IQR])		12.00 [9.16, 15.30]	16.07 [11.72, 20.64]	<0.001	11.90 [9.07, 14.83]	12.05 [10.50, 19.28]	0.216
N (*10^9/L) (mean (SD))		10.35 (4.61)	14.45 (6.12)	<0.001	10.29 (4.68)	13.27 (6.26)	0.037
L (*10^9/L) (median [IQR])		1.20 [0.80, 1.80]	0.91 [0.66, 1.50]	<0.001	1.30 [0.80, 1.80]	1.00 [0.75, 1.15]	0.174
NLR (median [IQR])		7.75 [4.32, 13.46]	13.71 [8.96, 23.31]	<0.001	6.87 [4.58, 13.60]	11.75 [7.25, 19.68]	0.060
HCT (L/L) (mean (SD))		0.47 (1.52)	0.83 (4.48)	0.097	0.86 (4.24)	0.46 (0.06)	0.741
RDW (%) (mean (SD))		13.00 (1.01)	13.27 (1.58)	0.009	12.92 (1.08)	12.76 (0.51)	0.602
Lr (%) (median [IQR])		10.85 [6.60, 17.30]	6.55 [3.98, 9.60]	<0.001	11.55 [6.47, 16.60]	7.85 [4.90, 11.43]	0.067
PCT (%) (mean (SD))		0.21 (0.11)	0.22 (0.08)	0.291	0.22 (0.06)	0.22 (0.07)	0.695
Cr (μmol/L) (median [IQR])		63.40 [53.90, 75.23]	61.50 [49.85, 85.85]	0.982	64.50 [54.00, 75.00]	66.50 [57.25, 106.75]	0.271
TB (μmol/L) (median [IQR])		21.00 [14.88, 32.23]	19.60 [12.83, 30.40]	0.165	17.15 [12.33, 26.15]	19.70 [14.20, 24.53]	0.666
DB (μmol/L) (median [IQR])		7.20 [4.50, 13.40]	7.35 [4.07, 12.95]	0.613	7.90 [5.47, 13.12]	10.20 [7.30, 16.95]	0.226
DTR (median [IQR])		0.36 [0.28, 0.48]	0.41 [0.30, 0.52]	0.042	0.47 [0.39, 0.60]	0.58 [0.48, 0.67]	0.058
Urea (mmol/L) (median [IQR])		4.90 [3.80, 6.20]	5.70 [4.27, 8.53]	<0.001	4.20 [3.38, 5.90]	6.35 [3.80, 13.40]	0.036
LDH (U/L) (median [IQR])		217.10 [178.00, 289.65]	341.00 [244.70, 498.88]	<0.001	199.00 [165.00, 250.25]	376.50 [211.75, 575.50]	0.001
Ca^{2+} (mmol/L) (mean (SD))		2.18 (0.19)	2.03 (0.28)	<0.001	2.10 (0.15)	1.73 (0.46)	<0.001
TG (mmol/L) (median [IQR])		1.42 [0.88, 3.30]	2.41 [1.27, 9.31]	<0.001	1.12 [0.66, 2.90]	3.42 [0.86, 9.17]	0.154
GLU (mmol/L) (median [IQR])		7.03 [5.84, 9.16]	8.18 [6.62, 11.79]	<0.001	6.98 [5.48, 9.27]	13.08 [9.50, 13.88]	0.002
TyG (median [IQR])		8.98 [8.41, 9.92]	9.79 [8.95, 11.11]	<0.001	8.79 [8.11, 9.75]	10.06 [9.05, 11.46]	0.031
ALT (U/L) (median [IQR])		39.10 [18.90, 141.00]	23.80 [14.20, 53.48]	<0.001	40.00 [17.75, 137.25]	24.00 [13.00, 55.75]	0.225
AST (U/L) (median [IQR])		30.00 [18.60, 86.25]	27.10 [18.95, 52.05]	0.129	28.00 [17.00, 68.75]	30.50 [20.00, 53.75]	0.919
GGT (U/L) (median [IQR])		86.40 [35.92, 267.00]	64.70 [27.75, 195.28]	0.09	102.50 [40.00, 266.00]	106.00 [61.75, 157.75]	0.959
ALP (U/L) (median [IQR])		90.00 [67.57, 131.85]	74.80 [55.80, 101.75]	<0.001	86.00 [67.00, 133.25]	75.50 [64.00, 84.50]	0.095
ALB (g/L) (mean (SD))		37.19 (4.99)	33.45 (6.47)	<0.001	38.17 (5.01)	33.46 (5.55)	0.002
K^+ (mmol/L) (mean (SD))		4.01 (0.45)	3.99 (0.63)	0.256	4.12 (0.53)	4.32 (0.49)	0.205
AGR (median [IQR])		1.40 [1.20, 1.60]	1.20 [1.03, 1.40]	<0.001	1.50 [1.36, 1.69]	1.49 [1.20, 1.56]	0.266
PT (s) (mean (SD))		13.38 (2.19)	14.82 (3.05)	<0.001	13.95 (1.18)	14.80 (1.48)	0.018
INR (mean (SD))		1.10 (0.18)	1.22 (0.29)	<0.001	1.08 (0.11)	1.17 (0.12)	0.011
APTT (s) (mean (SD))		32.58 (6.82)	38.34 (18.43)	<0.001	37.74 (6.00)	40.48 (11.85)	0.154
CRP (median [IQR])		26.05 [3.49, 111.51]	149.02 [15.67, 265.12]	<0.001	76.95 [25.85, 143.78]	244.75 [103.62, 303.18]	0.003
CAR (median [IQR])		0.63 [0.09, 3.19]	4.09 [0.34, 8.99]	<0.001	2.04 [0.60, 4.06]	8.58 [3.07, 9.75]	0.001
RCR (median [IQR])		5.94 [5.53, 6.36]	6.57 [5.89, 7.24]	<0.001	6.06 [5.76, 6.54]	6.78 [6.01, 9.58]	0.019
SIRS (%)	No	640 (72.1)	30 (24.2)	<0.001	163 (81.5)	2 (16.7)	<0.001
	Yes	248 (27.9)	94 (75.8)		37 (18.5)	10 (83.3)	
PE (%)	No	609 (68.6)	15 (12.1)	<0.001	138 (69.0)	2 (16.7)	0.001
	Yes	279 (31.4)	109 (87.9)		62 (31.0)	10 (83.3)	
MCTSI (median [IQR])		2.00 [2.00, 4.00]	4.00 [4.00, 4.00]	<0.001	2.00 [2.00, 4.00]	5.00 [4.00, 6.00]	<0.001
RANSON (median [IQR])		1.00 [0.00, 2.00]	2.00 [1.00, 2.00]	<0.001	1.00 [0.00, 1.00]	2.00 [1.00, 2.25]	0.001
BISAP (median [IQR])		1.00 [0.00, 2.00]	2.00 [2.00, 3.00]	<0.001	1.00 [0.00, 1.00]	2.00 [1.25, 3.00]	0.002
SABP (median [IQR])		3.08 [-2.94, 10.83]	9.10 [1.43, 22.56]	<0.001	2.02 [-2.82, 7.33]	16.89 [-2.09, 28.61]	0.073

*MAP, mean artery pressure; N, neutrophil; L, lymphocyte; NLR, neutrophil/lymphocyte; Lr, percentage of lymphocytes; Cr, creatinine; TB, total bilirubin; DB, direct bilirubin; DTR, direct bilirubin/total bilirubin; TG, total triglycerides; GLU, glucose; TyG, TG/GLU; AGR, albumin/globulin; CAR, CRP/albumin; RCR, RDW/Ca^{2+}; ALB, albumin; SABP, acute biliary pancreatitis (0.55 + SIRS * 1.02 – 0.63 * ALB + 1.76 * BUN/0.356 + 1.66 * PE); PE, pleural effusion.*

0.920 according to the confusion matrix of GBM, XGBoost, DRF, GLM, and DL models on the test set. A LIME plot of the GBM model on the test set exhibited how several important variables contributed to the progress of SAP. As shown in **Figure 5**, for example, the case 4 had a high probability of 0.84 for progressing to SAP as predicted by the GBM model. PE was the most significant feature contributing to the prediction, followed by SIRS, TG, and LDH, while albumin had the opposite effect. The

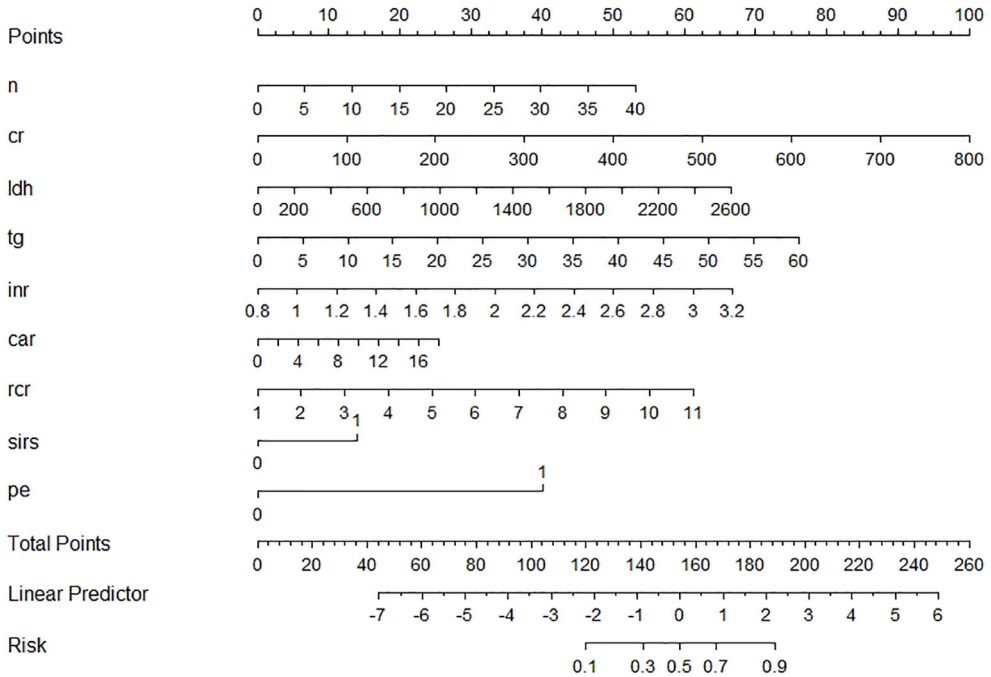

FIGURE 2 | Nomogram of the LASSO model for the early prediction of severe acute pancreatitis.

model can be deployed online, where clinicians can fill in the data in the table and then the predictive outcome will appear. Details can be seen on the official website (clouderizer.com).

Comparisons Between Existing Scoring Systems and Models Developed by LR and AutoML

In general, the GBM and XGBoost models achieved the highest accuracy among these models, both beyond 0.950. The LASSO, DRF, and GLM models also obtained relatively high accuracy of 0.943, 0.920, and 0.901. The AUC values obtained by the ten models were 0.945 for GBM, 0.898 for XGBoost, 0.860 for DL, 0.868 for GLM, 0.871 for DRF, 0.898 for LR, 0.764 for RANSON, 0.787 for BISAP, 0.869 for MCTSI, and 0.673 for SABP. MCTSI achieved the highest sensitivity value of 1.000 and the lowest specificity of 0.588. XGBoost achieved the highest specificity value of 0.980 and the highest LR+ of 29.167.

DISCUSSION

In this study, we developed and tested several AutoML models to early identify who would progress to SAP. These models were all

TABLE 2 | Comparison of LR and AutoML models for early prediction of SAP in the test cohort.

	AUC	Sensitivity	Specificity	Accuracy	PPV	NPV	LR+	LR–
AutoML								
GBM	0.945	0.583	0.975	0.953	0.583	0.975	23.333	0.427
XGBoost	0.898	0.583	0.980	0.958	0.636	0.975	29.167	0.425
DRF	0.871	0.417	0.950	0.920	0.333	0.964	8.333	0.614
GLM	0.868	0.500	0.925	0.901	0.286	0.969	6.667	0.541
DL	0.860	0.500	0.920	0.896	0.273	0.968	6.250	0.543
Logistic regression								
LASSO	0.898	0.500	0.965	0.943	0.417	0.975	3.821	0.109
Existed scoring systems								
RANSON	0.764	0.667	0.800	0.896	0.188	0.954	3.335	0.416
MCTSI	0.869	1.000	0.588	0.611	0.128	1.000	2.427	0
BISAP	0.787	0.700	0.796	0.854	0.5	0.884	3.431	0.377
SABP	0.673	0.600	0.871	0.752	0.333	0.825	4.651	0.459

LR, logistic regression; AutoML, automated machine learning; SAP, severe acute pancreatitis; PPV, positive predictive value; NPV, negative predictive value; LR+, positive likelihood ration; LR–, negative likelihood ratio.

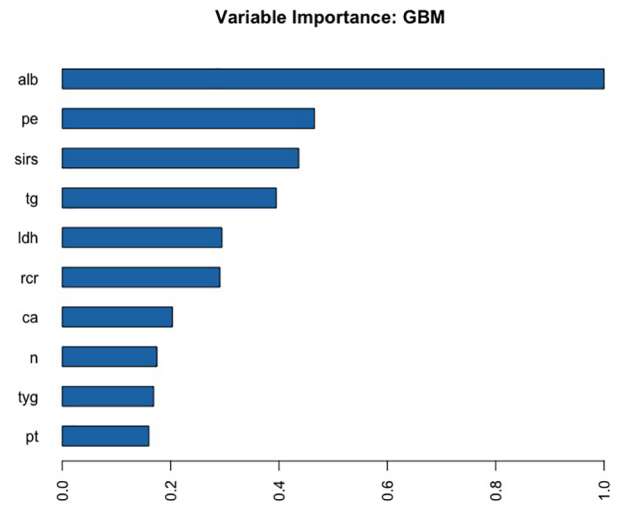

FIGURE 3 | Variable importance of the GBM model in the training set, showing that albumin was the most important feature, followed by PE, SIRS, TGs, LDH, etc. ALB, albumin; PE, pleural effusion; SIRS, systemic inflammatory response syndrome; TG, triglyceride; LDH, lactic dehydrogenase; RCR, ratio of RDW to Ca^{2+}; Ca, Ca^{2+}; *n*, neutrophil count; TyG, ratio of triglyceride to glucose; PT, prothrombin time.

superior to existing scoring systems such as BISAP, RANSON, and MCTSI. Additionally, the GBM model obtained the highest value of AUC above 0.90, with specificity and accuracy all above 0.95.

Early prediction of SAP patients is essential for determining which patients require appropriate management such as intensive care, rapid fluid resuscitation, and early enteral nutrition (Gliem et al., 2021). Up to now, various scoring systems have been developed for early risk stratification of AP patients. Hong et al. (2019) built a prediction score called SABP, consisting of SIRS, albumin, blood urea nitrogen (BUN), and PE, which was trained and validated on 700 and 194 patients from two hospitals. The AUC of SABP on the external validation cohort in their study was superior to that in our study (0.873 vs. 0.673). This difference may be partly explained by the fact that BUN was not a routine examination within 24 h of admission in our hospital. Data deficiency may decrease the efficacy of the SABP score due to the reduction of sample size. Typical models such as RANSON, MCTSI, and BISAP in our study achieved inferior accuracy to the models we built. Besides the probability mentioned above, the other possible explanation may be that relevant indicators of those scores were categorical variables while were converted into continuous variables in our study, which may increase the probability of false-positive results due to the decrease in threshold value.

Compared with traditional univariate and sequent multivariate analyses, AutoML greatly improved work efficiency due to its less time consumption and higher accuracy. Additionally, ensemble models combined various machine-learning algorithms, utilizing multiclassifiers to predict the target outcome *via* taking a vote of individual predictions, which could enhance the overall performance (Goh et al., 2021). In this study, we selected four models built

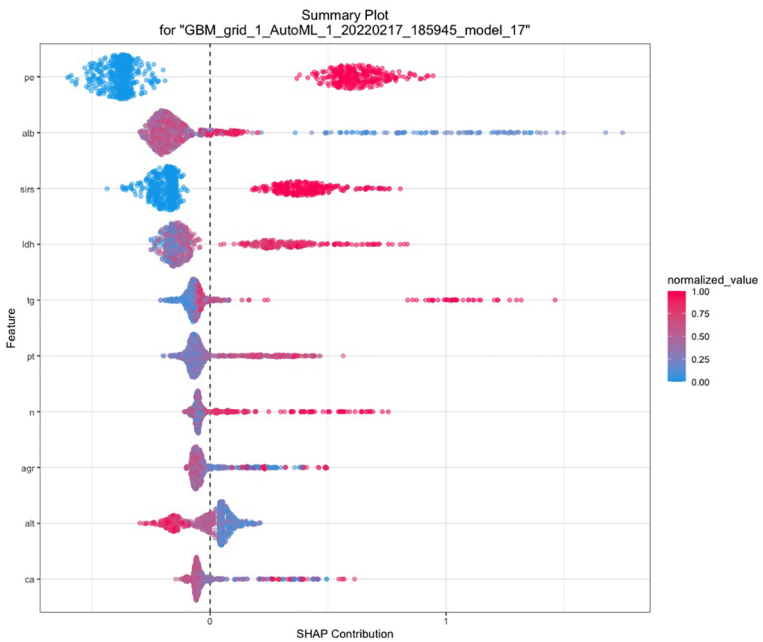

FIGURE 4 | SHAP of the GBM model in the training set. The closer the values of the variables were to 1, the more likely patients were to progress to severity acute pancreatitis. SHAP, SHapley additive explanation; PE, pleural effusion; ALB, albumin; SIRS, systemic inflammatory response syndrome; LDH, lactic dehydrogenase; TG, triglyceride; PT, prothrombin time; n, neutrophil count; AGR, ratio of albumin to globulin; ALT, glutamic-pyruvic transaminase; Ca, Ca^{2+}.

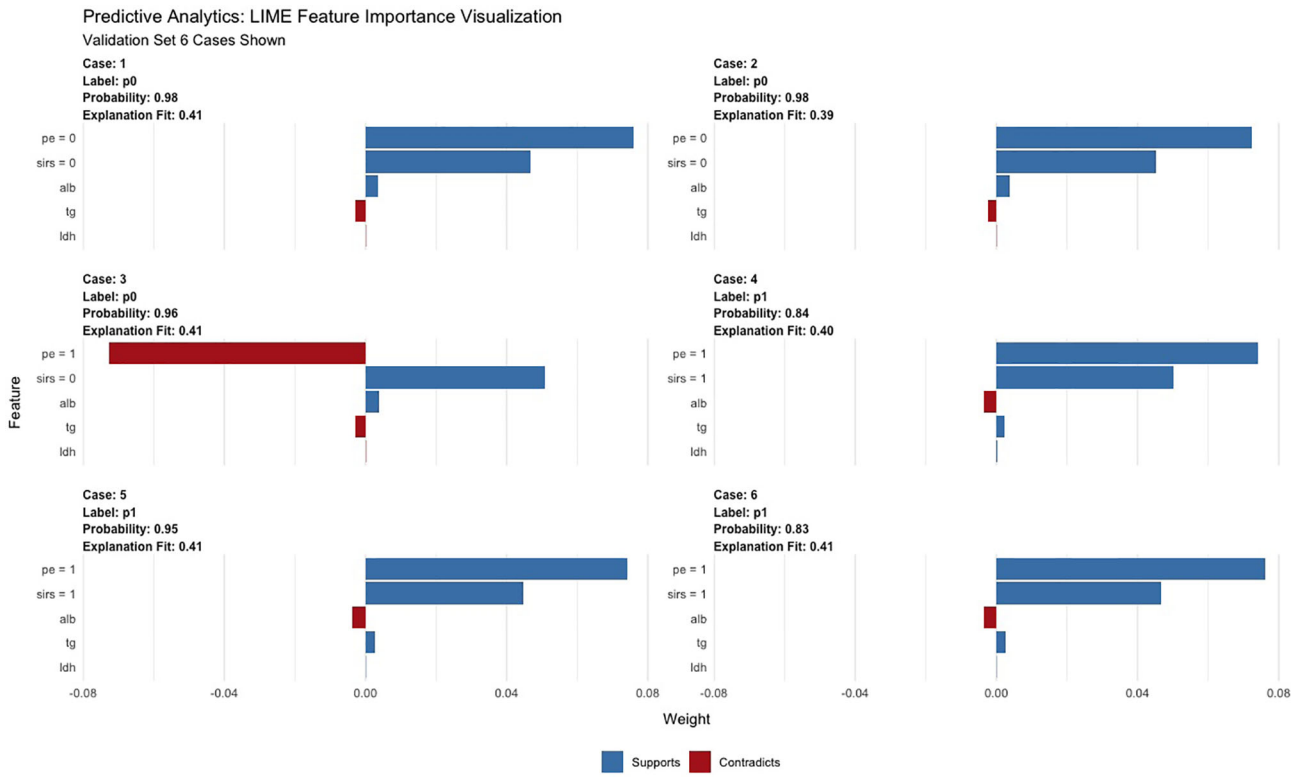

FIGURE 5 | LIME of the GBM model in the test set. LIME, Local Interpretable Model Agnostic Explanation.

by five types of AutoML algorithms (GBM, XGBoost, DRF, GLM, and DL) for predicting the risk of SAP. All models, among which the GBM model ranking first in AUC and XGBoost and in accuracy on the test dataset, yielded satisfactory results. AUC gives a more feasible method to settle the problem of unbalanced data by putting the same weight on both classes in contrast to accuracy (Janitza et al., 2013). Additionally, since our aim is to early detect high-risk AP patients who would progress to SAP, sensitivity is a better choice, which is calculated as the ratio of subjects predicted positive with our proposed models to patients who are actually positive. Therefore, the GBM model was the best one in our study.

The SHAP analysis demonstrated that the occurrence of PE at admission was the most important feature for the GBM model. Yan et al. (2021) conducted research on pleural effusion volume (PEV) for predicting the severity of acute pancreatitis, with an AUC of 0.8158. Similarly, a study from Peng et al. in 2019 (Peng et al., 2020) also revealed that PEV holds a high accuracy (AUC = 0.839) for predicting the occurrence of SAP. In our study, PE was a common important feature selected not only by GBM but also by LASSO, BISAP, MCTSI, and SABP, indicating that PE and PEV were indeed reliable radiologic biomarkers in the prediction of SAP. Albumin has been proven as an independent risk factor for SAP according to previous studies (Hong et al., 2017; Hong et al., 2019; Wu et al., 2020; Xu et al., 2020). Hong et al. (2017) concluded that hypoalbuminemia within 24 h of hospital admission was greatly associated with increased probability of occurrence of POF and death in AP patients. A large-scale retrospective study analyzed the two open-access ICU databases to reveal the predictive significance of serum albumin in patients with AP (Xu et al., 2020). Chen and colleagues (Chen et al., 2021) carried out a subanalysis in hypertriglyceridemia pancreatitis populations for exploring the association between albumin and severity of AP. It was generally believed that elevated level of TG would drive the occurrence of SAP due to toxic effects on pancreatic acinar cell (Chen et al., 2021). The free fatty acids, hydrolyzed by pancreatic lipase from TGs, can bind to albumin in the serum and thus stimulate the inflammatory process. Therefore, Chen's study effectively ruled out the confounding effect of TG and demonstrated that decrease of albumin was indeed an independent predictive factor. In our GBM model, albumin contributed the most to the predictive model, and TG ranked the fourth.

Zhang et al. (Peng et al., 2017) reported a significant correlation between the decrease of serum Ca^{2+} and the incidence of POF by triggering the SIRS process that recruits neutrophils and leads to further release of reactive oxygen species and organ damage. Another study from Gravito-Soares et al. (2018) proposed that RDW, a marker reflecting inflammation status, showed great predictive performance of AP severity with AUC of >0.810 and mortality with AUC of >0.842. Additionally, this study further

suggested that RCR was an excellent predictor of AP severity with AUC value of 0.973. Han et al. (2021) also discovered a positive correlation between a high level of RCR and a poor prognosis for patients with AP. Consistent with the aforesaid studies, our study illustrated that Ca^{2+}, RCR, neutrophil count, and SIRS were among the top 10 important variables in the GBM model.

A new scoring system for predicting organ failure in AP was proposed by Wu et al. (2020) in 2020, consisting of LDH, creatinine, albumin and Ca^{2+}. LDH, also included in the typical RANSON score, is not commonly seen in recent proposed models. In 2008, Gurda-Duda et al. (2008) recommended LDH activity (within 12 h from disease onset) as a biomarker for early predicting prognosis of AP, with sensitivity of 63.6% and a specificity of 89.6%. It is well known that hypertriglyceridemia and hyperglycemia are correlated with severity of AP and organ failure (Gurda-Duda et al., 2008; Park et al., 2020; Chen et al., 2021). Gurda-Duda et al. (2008) suggested that blood glucose concentration (within 36 h from disease onset) could be a complementary measurement, with sensitivity of 72.7% and a specificity of 75.8%. Park et al. (2020) investigated the association between the TyG index (=ln [fasting TG (mg/dl) × fasting plasma glucose (mg/dl)]/2) and the severity of AP in 373 patients. The results showed that the TyG index not only accurately predicted SAP but also increased the predictive value of traditional models. The underlying mechanism might be explained by insulin resistance, which activated proinflammatory molecules accelerating the progression of SAP. PT, a parameter of coagulation state, was among the top 10 important features in our GBM model. However, a multivariate logistic regression analysis performed by Radenkovic et al. (2009) and three machine learning algorithms performed by Qiu et al. (2019) did not include these parameters into the final models.

Here, we built six predictive models using traditional logistic regression and AutoML, with high AUC of >0.860 and high accuracy of >0.896. Furthermore, it is more convenient and efficient to get the predictive probability for SAP using AutoML. Additionally, our study was a multicenter hospital-based research, which is a common way of efficiently evaluating a new technique and may provide a better foundation for the subsequent generalization of our models. However, there are some limitations in our study. Firstly, we divided AP patients into non-SAP and SAP instead of mild AP, moderate SAP, and SAP, which might decrease the sensitivity of our models. Secondly, our study is a retrospective study which might affect the performance of our models in a prospective clinical study. More prospective research needs to be conducted for external validation of our models. Thirdly, the online deployment website needs maintenance, and more data need to be inputted to improve the generalizability and performance of our models.

CONCLUSION

We developed a series of effective models for early prediction of SAP based on AutoML platform, and these models outperformed the existing scoring systems, which might offer insights into AutoML applications in future medical studies. Additionally, the GBM model demonstrated practicable performance in early prediction better than LR and existing scoring systems.

DATA AVAILABILITY STATEMENT

The data presented in this study are available on request from the corresponding authors.

ETHICS STATEMENT

This study was approved by the ethics committee of the first affiliated hospital of Soochow University. Written informed consent from the patients/ participants was not required to participate in this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

MY and RZ contributed to data collection and writing. MY was responsible for statistical analysis. ZZ assisted in data collection and statistical analysis. LL, JG, and CY contributed to data cleaning and creating charts. JL and WX assisted in computer programming. XL and JZ contributed to revising this dissertation. JZ and CX managed this project and provided the funding. All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fcimb.2022.886935/full#supplementary-material

Supplementary Table 1 | Comparison of LR and AutoML models for early predicting SAP in the validation cohort. LR, logistic regression; AutoML, automated machine learning; SAP, severe acute pancreatitis.

Supplementary Figure 1 | Penalty chart of predictive factors for severe acute pancreatitis based on LASSO regression analysis. Left: Regression coefficients. With the value of λ increasing, the absolute values of coefficients decrease. Right: Identification of the optimal λ value in the LASSO regression analysis was achieved by 5-fold cross-validation. (The left vertical line is drawn using the minimum criterion and the right vertical line is drawn using the 1_se criterion. In our study, LASSO regression model with 'λ_1se' criterion was used in the univariate analysis in order to solve such multiple co-linear relationships among the explanatory variables. LASSO, least absolute shrinkage and selection operator.

Supplementary Figure 2 | Calibration curve of the LASSO model in the training, validation and test set, with the mean absolute errors being 0.006, 0.033 and 0.036, respectively. The calibration curves demonstrated that the estimated risk using LASSO model was close to the observed risk, indicating a high degree of reliability.

Supplementary Figure 3 | Decision curve analysis of the LASSO model in the training, validation and test set. The DCA plots demonstrated that when the threshold probability of SAP predicted by the LASSO model was between 10% and 100%, an intervention might add more benefit (6-10%).

Supplementary Figure 4 | ROC curves of all proposed models (GBM and LASSO models) and traditional scoring systems (BISAP, MCTSI, RANSON and SABP). GBM, Gradient Boost Machine; LASSO, the Least Absolute Shrinkage and Selection Operator; BISAP, bedside index of severity in acute pancreatitis; MCTSI, modified computed tomography severity index; RANSON, RANSON score; SABP[5].

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Usefulness of Random Forest Algorithm in Predicting Severe Acute Pancreatitis

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Background and Aims: This study aimed to develop an interpretable random forest model for predicting severe acute pancreatitis (SAP).

Methods: Clinical and laboratory data of 648 patients with acute pancreatitis were retrospectively reviewed and randomly assigned to the training set and test set in a 3:1 ratio. Univariate analysis was used to select candidate predictors for the SAP. Random forest (RF) and logistic regression (LR) models were developed on the training sample. The prediction models were then applied to the test sample. The performance of the risk models was measured by calculating the area under the receiver operating characteristic (ROC) curves (AUC) and area under precision recall curve. We provide visualized interpretation by using local interpretable model-agnostic explanations (LIME).

Results: The LR model was developed to predict SAP as the following function: -1.10-0.13×albumin (g/L) + 0.016 × serum creatinine (μmol/L) + 0.14 × glucose (mmol/L) + 1.63 × pleural effusion (0/1)(No/Yes). The coefficients of this formula were utilized to build a nomogram. The RF model consists of 16 variables identified by univariate analysis. It was developed and validated by a tenfold cross-validation on the training sample. Variables importance analysis suggested that blood urea nitrogen, serum creatinine, albumin, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, calcium, and glucose were the most important seven predictors of SAP. The AUCs of RF model in tenfold cross-validation of the training set and the test set was 0.89 and 0.96, respectively. Both the area under precision recall curve and the diagnostic accuracy of the RF model were higher than

that of both the LR model and the BISAP score. LIME plots were used to explain individualized prediction of the RF model.

Conclusions: An interpretable RF model exhibited the highest discriminatory performance in predicting SAP. Interpretation with LIME plots could be useful for individualized prediction in a clinical setting. A nomogram consisting of albumin, serum creatinine, glucose, and pleural effusion was useful for prediction of SAP.

Keywords: random forest, nomogram, acute pancreatitis, predictor, artificial intelligence, LIME plot

HIGHLIGHTS

1. An interpretable random forest model exhibited the highest discriminatory performance in SAP prediction.
2. Interpretation with LIME plots could be useful for individualized prediction in a clinical setting.
3. A nomogram comprising albumin, serum creatinine, glucose, and pleural effusion is a useful predictor of SAP.

INTRODUCTION

Acute pancreatitis (AP) is one of the most common gastrointestinal problems for hospital admission globally (Hong et al., 2020). While most patients with AP will recover within a week of a mild course and are often self-limiting, 20% of patients progress to severe disease with a historical mortality risk as high as 30% (Trikudanathan et al., 2019). In the absence of specific treatment in the early phase, initial management of severe acute pancreatitis (SAP) focuses on supportive care such as fluid resuscitation, pain control, and nutritional support, aimed to minimize the impact of systemic inflammatory response syndrome (Lee and Papachristou, 2019). Patients with SAP often need to be transferred to the intensive care unit once organ failure occurs. Therefore, it is important to recognize predictors for severe disease in the early phase of AP, to select those patients who would benefit most from enhanced surveillance or early interventions. Early case identification and classification of disease severity could improve the clinical outcomes (Hong et al., 2021).

Many clinical scoring systems have been developed for the prediction of disease severity, such as the Ranson, chronic health evaluation (APACHE-II) score, Pancreatitis Outcome Prediction (POP) Score (Harrison et al., 2007), and Bedside Index Of Severity In Acute Pancreatitis (BISAP) (Wu et al., 2008). However, the existing scoring systems have moderate accuracy in predicting the severity of AP (Mounzer et al., 2012). Recently, Langmead et al. reported that a 5-cytokine panel consisting of angiopoietin-2, hepatocyte growth factor, interleukin-8, resistin, and soluble tumor necrosis factor receptor 1A accurately predicts persistent organ failure early in the disease process and significantly outperforms the prognostic accuracy of existing laboratory tests and clinical scores (Langmead et al., 2021). However, the test of cytokine is not routinely available, resulting in limited use in clinical practice. Several laboratory indexes such as total cholesterol (Hong et al., 2020), low-density lipoprotein cholesterol (Hong et al., 2018), albumin (Hong et al., 2017a), and blood urea nitrogen (BUN) (Lin et al., 2017) have been proposed as single predictors of severity of AP. Recently, Takeda et al. reported that fluid sequestration is a useful parameter in the early identification of SAP (Takeda et al., 2019). Yan et al. described that pleural effusion volume could be a reliable radiologic biomarker in the prediction of severity and clinical outcomes of AP (Yan et al., 2021). Gibor et al. reported that circulating cell-free DNA in patients with acute biliary pancreatitis is associated with disease markers and prolonged hospitalization time (Gibor et al., 2020). However, these single prediction markers are easy to use in practice but lack high accuracy.

Recently, artificial intelligence methods are also being used in the clinical setting for disease prediction or aiding in making decisions. Among several methods, a random forest (RF) is a group of many decision trees, each of which is characterized by a tree-like structure (Genuer and Poggi, 2020). It will randomly choose features and make observations, build a forest of decision trees, and then average out the results (James et al., 2013). RF allows considering qualitative and quantitative explanatory variables together, without preprocessing (Genuer and Poggi, 2020). Random forests are adapted to both supervised classification problems and regression problems (Genuer and Poggi, 2020). In addition, RF can handle datasets with many predictor variables, while also performing very well. Additionally, it can obtain variable importance ranking when used for prediction modeling (Speiser et al., 2019). RF, as a traditional machine learning method, has been shown to outperform other techniques for sets of features in a variety of different settings. RF has recently demonstrated high performance in risk classification and disease prediction (Yu et al., 2021). Lo et al. developed RF model for forecasting allergenic pollen in North America (Lo et al., 2021). Lin et al. reported that using the RF model could predict environmental risk factors in relation to health outcomes among school children from Romania (Lin et al., 2021b). Roguet et al. reported that RF classification with 16S rRNA gene amplicons offers an accurate solution for identifying host microbial signatures (useful in detecting human and animal fecal contamination in environmental samples) (Roguet et al., 2018). Yang et al. provided an RF prediction model for 3-year risk assessment of cardiovascular disease (Yang et al., 2020).

However, to the best of our knowledge, the use of RF model in predicting disease severity in patients with AP has not been performed yet. The aim of this study was to develop an RF model and compare it with a traditional logistic regression (LR) model for prediction of SAP.

PATIENTS AND METHODS

Inclusion and Exclusion Criteria

We conducted a *post-hoc* analysis of a previously reported retrospective cohort study in the First Affiliated Hospital of Wenzhou Medical University in mainland China (Hong et al., 2018). Patients with AP admitted to the First Affiliated Hospital of Wenzhou Medical University within 72 h of symptom onset (from April 1, 2012 to December 31, 2015) were consecutively enrolled in the study (Hong et al., 2020). The diagnosis of AP was based on the presence of two of the following three features: (1) abdominal pain consistent with AP; (2) serum amylase and/or lipase more than three times that of the normal; (3) abdominal imaging findings (Hong et al., 2020). As previously described (Hong et al., 2020), exclusion criteria included endoscopic or trauma related pancreatitis, chronic pancreatitis, pancreatic tumor, history of surgery operation/taking hypolipidemia drugs, malnutrition, and chronic liver or renal disease.

Data Collection

The clinical and laboratory data on admission were obtained with data collection forms from electronic medical records. These data included blood chemica+l analysis, liver, and renal function testing, glucose, lipids, coagulation testing, serum calcium, C-reaction protein, and pleural effusion (Hong et al., 2020).

Definition of Severity and Study Endpoint

SAP is defined as a persistent organ failure (>48 h) in patients. Organ failure for this study was defined according to a Marshall score ≥2, meaning that at least one organ system (respiratory, cardiovascular, renal) must be affected (Hong et al., 2018). The primary study endpoint was the occurrence of SAP during hospitalization.

Sample Size and Missing Values

The calculation of the sample size of this study was according to our previous study (Hong et al., 2020). There were missing values in serum calcium and C-reactive protein data. To handle this issue, missing values were imputed using Multiple Imputations by Chained Equations (MICE) when performing LR and RF analysis (Royston, 2005). The MICE has emerged as one of the principal statistical approaches for dealing with missing data. The missing values were replaced by the estimated plausible values to create a "complete" dataset (Royston, 2005).

Statistical Analysis

Categorical values were described by count and proportions and compared by the $\chi 2$ test or Fisher's exact test. According to the results of the Shapiro-Wilk test, continuous values were expressed by mean ± SD or median and Inter Quartile Range (IQR) and compared using Student's t-test or the Wilcoxon's non-parametric test. The discriminative power of the predictor was assessed by calculating the area under the receiver operating characteristic (ROC) curves (AUC) (Hong et al., 2019). A variable with an AUC above 0.7 was considered useful (Hong et al., 2017b).

The data samples (of 648 patients) were randomly split into training and test sets according to a division of 3:1 (487 *vs.* 161 patients). The RF model was developed on the training set and independently validated on the test set by using "randomForest" (Liaw and Wiener, 2002) and "caret" package (Kuhn, 2008). When we built and tuned the RF model on a training set, we used tenfold cross-validation as the resampling method to avoid overfitting of the model in new data (Kuhn, 2008). The training set was divided into 10 equal-sized sub-samples in which 9 sub-samples were for the training and the remaining ones for testing over all possible permutations. Analysis was repeated 10 times (folds) (Hong et al., 2019). The AUC was calculated for each of the 10 analyses, using only the respective test data (Hong et al., 2019). Then this iteration process was repeated 10 times. At last, the mean AUC with 95%CI, as well as area under precision recall curve was calculated and compared (Saito and Rehmsmeier, 2015; Hong et al., 2019).

After training the RF model, a general approach of interpretability is to identify important variables (features) in the model (Staniak and Biecek, 2018). The RF algorithm estimates the importance of a variable by looking at how much prediction error increases when Out-Of-Bag (OOB) data for that variable are permuted, while all others are left unchanged (Liaw and Wiener, 2002; Genuer and Poggi, 2020). The variable importance is a global explanation of relative importance of each feature in the RF model (Kuhn, 2008). Variables having high importance are drivers of the outcome and their values have a significant impact on the result values.

To overcome the black box problem of the RF model output and improve its interpretability, the local interpretable model-agnostic explanations (LIME) plot was used to explain the individualized prediction (Deshmukh and Merchant, 2020). LIME is a novel explanation technique that explains the predictions of any classifier in an interpretable and faithful manner, by learning an interpretable model locally around the prediction (Ribeiro et al., 2016). The training of the local interpretable model involves giving weight to the disturbance input, followed by the observation of the general (black box) model output, which gives a basis for interpretation of the prediction results (Pan et al., 2020). This feature is deemed important if perturbations at the local level produce a change in the general model while the value of the target feature is determined by the level of change it determines (Bramhall et al., 2020). Local explanation detects variables' contribution at the local level. In other words, LIME could provide easily understood explanations of clinical factors in the RF models, which contribute to each prediction for the individual patient (Petch et al., 2022). LIME was performed by using the "lime" package ("Lime: Local Interpretable Model-Agnostic Explanations, 2021", http://cran.itam.mx/web/packages/lime/index.html), in which two types of inputs (tabular and text) are supported (Pedersen and Benesty, 2021).

A forward-conditional stepwise LR analysis was also applied on the training set. The conditional probabilities for stepwise entry and removal of a factor were 0.05 and 0.06, respectively (Hong et al., 2020). Based on the results of LR, a nomogram was developed to predict SAP. Model calibration, reflecting the link between predicted and observed risk, was evaluated by the Hosmer-Lomeshow goodness of fit test, as well as plotting the predicted *vs*. observed deciles of predicted risk (Hong et al., 2017b). Odds ratios (OR) were calculated, with 95%CI.

We selected the best cut-off point, where the number of true positives was the highest possible (sensitivity >90%). This was done by selecting a threshold value at a point where the longest increase in the specificity of the slope declines for all models and scores. The sensitivity, specificity, and accuracy were calculated and compared (Saito and Rehmsmeier, 2015).

A two-tailed P-value of less than 0.05 was considered statistically significant. All statistical analysis were performed in the R and STATA software. A data flow diagram of our study is shown in **Supplementary Figure S1**.

RESULTS

Baseline Characteristics

Of all the patients, the hospital mortality was 1.54%. There were 247 (58.8%) men and their median age was 53 (42.0–64.5) years. The most common etiology of AP was biliary (42.4%). The median time interval between onset and admission was 2 (IQR 1-2) days. Of these patients 10% developed SAP during hospitalization. The median length of the hospital stay was 10 (IQR 7-14) days. The baseline characteristics of the patients in the training and test sets are shown in **Table 1**.

Univariate Analysis on the Training Sample

As shown in **Table 2**, 16 variables, namely, systemic inflammatory response syndrome (SIRS), hematocrit, platelets, prothrombin time, albumin, aspartate aminotransferase (AST), glucose, serum creatinine, blood urea nitrogen (BUN), total cholesterol, high-density lipoprotein cholesterol (HDL), low-density lipoprotein cholesterol (LDL), triglyceride, serum calcium, C-reactive protein (CRP), and pleural effusion were significantly associated with the development of SAP, as inferred by univariate analysis.

Models Development, Calibration, Tenfold Cross-Validation on the Training Sample

Variables significantly linked to the development of SAP in the univariate analysis were assessed by stepwise LR analysis. LR identified the following four independent variables as predictive of SAP: albumin (OR 0.88, 95%CI 0.81-0.95, P=0.002), serum creatinine (OR 1.02, 95%CI 1.01-1.03, P=0.002), glucose (OR 1.15, 95%CI 1.07-1.24, P<0.001), and pleural effusion (OR 5.11, 95%CI

TABLE 1 | Comparison of clinical and laboratory findings among patients, with and without SAP (training sample set).

Variable	Training set (n = 487)	Test set (n = 161)	P-value
Age, years (IQR)	47 (37,61)	49 (36,64)	0.501
Male sex, N (%)	301 (61.81)	103 (63.98)	0.623
Duration of symptoms (days, IQR)	1.83 ± 0.80	1.78 ± 0.78	0.515
BMI, kg/m^2 (IQR)	23.5 (21.1-26.3)	23.9 (21.5-21.5)	0.573
SIRS, N (%)	191 (39.22)	65 (40.37)	0.795
Biliary etiology, N (%)	207 (42.51)	68 (42.24)	0.584
Laboratory findings			
Hematocrit (l/l)	0.42 (0.38-0.46)	0.42 (0.38-0.46)	0.693
Platelets (109/L)	199 (161-233)	195 (157-233)	0.472
Prothrombin time, s (IQR)	13.8 (13.1-14.6)	13.7 (13.0-14.5)	0.278
Albumin, g/L (IQR)	36.3 (32.6-39.9)	36.4 (34.0-39.8)	0.191
Total bilirubin, mmol/L (IQR)	20 (14-31)	20 (13-32)	0.916
ALT, U/L (IQR)	43 (19-119)	31 (19-82)	0.055
AST, U/L (IQR)	39 (22-88)	28 (19-71)	0.012
Glucose, mmol/L (IQR)	7.9 (6.3-10.5)	8.4 (6.7-11.3)	0.128
Serum creatinine, μmol/L (IQR)	64 (54-77)	64 (55-76)	0.882
BUN, mmol/L (IQR)	4.8 (3.7-6.1)	4.9 (4.0-6.2)	0.346
Total cholesterol, mmol/L (IQR)	4.79 (3.8-6.2)	4.8 (3.8-6.1)	0.970
HDL, mmol/L (IQR)	1.0 (0.7-1.3)	1.0 (0.8-1.3)	0.461
LDL, mmol/L (IQR)	2.5 (1.9-3.2)	2.2 (1.8-3.0)	0.100
Triglyceride (mg/dL), mmol/L (IQR)	1.3 (0.8-3.4)	1.3 (0.8-3.6)	0.995
Serum calcium, mmol/L (IQR)	2.7 (2.1-2.3)	2.2 (2.1-2.3)	0.051
C-reactive protein, mg/L (IQR)	35.0 (11.7-90.0)	29.4 (8.7-85.3)	0.415
Pleural effusion, N (%)	89 (18.28)	35 (21.74)	0.333
Patients with SAP, N (%)	49 (10.1)	16 (9.9)	0.0092
Length of hospital stay, days (IQR)	10 (7-13)	11 (7-15)	0.964
Hospital mortality, N (%)	9 (1.85)	1 (0.62)	0.274

Data were mean ± standard deviation, or numbers and percentages, or median (25th–75th percentile), as appropriate. N, number; IQR, interquartile range; BMI, body mass index; SIRS, systemic inflammatory response syndrome; ALT, alanine aminotransferase; AST, aspartate aminotransferase; HDL, high-density lipoprotein cholesterol; LDL, low-density lipoprotein cholesterol.

TABLE 2 | Comparison of clinical and laboratory findings between patients, with and without SAP in the training sample (487 patients).

Variable	No-SAP (n = 438)	SAP (n = 49)	P-value
Age, years (IQR)	46 (37-60)	51 (38-66)	0.115
Male sex, N (%)	270 (61.6)	31 (63.3)	0.825
Duration of symptoms (days, IQR)	1.8 ± 0.8	1.9 ± 0.8	0.799
BMI, kg/m^2 (IQR)	23.4 (20.9-26.3)	24.4 (22.1-26.6)	0.083
SIRS, N (%)	157 (35.8)	34 (69.4)	<0.001
Biliary etiology, N (%)	190 (43.4)	17 (34.7)	0.243
Laboratory findings			
Hematocrit (l/l)	0.42 (0.38-0.45)	0.44 (0.41-0.49)	0.007
Platelets (10^9/L)	202 (167-234)	184 (135-208)	0.005
Prothrombin time, s (IQR)	13.8 (13.1-14.6)	14.6 (13.6-15.3)	0.004
Albumin, g/L (IQR)	37.1 (33.3-39.3)	30.4 (27.5-33.9)	<0.001
Total bilirubin, mmol/L (IQR)	20 (14-31)	20 (15-28)	0.631
ALT, U/L (IQR)	43 (18-121)	48 (24-77)	0.868
AST, U/L (IQR)	36 (21-88)	60 (41-85)	0.005
Glucose, mmol/L (IQR)	7.7 (6.2-10.0)	10.2 (8.2-14.4)	<0.001
Serum creatinine, μmol/L (IQR)	63 (54-76)	81 (59-154)	<0.001
BUN, mmol/L (IQR)	4.6 (3.6-5.9)	7.3 (5.1-11.4)	<0.001
Total cholesterol, N			0.001
<160 mmol/L	203 (95.75)	9 (9.45)	
160-240 mmol/L	131 (83.97)	25 (16.03)	
>240 mmol/L	104 (87.39)	15 (12.61)	
HDL, mmol/L (IQR)	1.0 (0.8-1.3)	0.6 (0.4-1.0)	<0.001
LDL, mmol/L (IQR)	2.6 (2.0-3.3)	1.6 (1.3-2.4)	<0.001
Triglyceride (mg/dL), mmol/L (IQR)	1.3 (0.8-3.3)	2.4 (1.3-7.2)	<0.001
Serum calcium, mmol/L (IQR)	2.2 (2.1-2.3)	1.9 (1.6-2.1)	<0.001
C-reactive protein, mg/L (IQR)	30.5 (10.4-87.8)	80.0 (28.4-90.0)	0.003
Pleural effusion, N (%)	59 (13.47)	30 (61.22)	<0.001

Data were mean ± standard deviation, or numbers and percentages, or median (25th–75th percentile), as appropriate. N, number; IQR, interquartile range; BMI, body mass index; SIRS, systemic inflammatory response syndrome; ALT, alanine aminotransferase; AST, aspartate aminotransferase; HDL, high-density lipoprotein cholesterol; LDL, low-density lipoprotein cholesterol.

2.38-10.94, P<0.001). The LR model was developed to predict SAP as the following function: -1.10-0.13×albumin (g/L) + 0.016× serum creatinine (μmol/L) +0.14 × glucose(mmol/L) + 1.63 × pleural effusion (0/1)(No/Yes). The coefficients of this formula were utilized to build a nomogram for the prediction of SAP (**Figure 1**). The Hosmer-Lemeshow goodness-of-fit test was significant (P=0.87), suggesting that our prediction model fit the actual data well.

The same 16 variables (SIRS, hematocrit, platelets, prothrombin time, albumin, AST, glucose, serum creatinine, BUN, cholesterol,

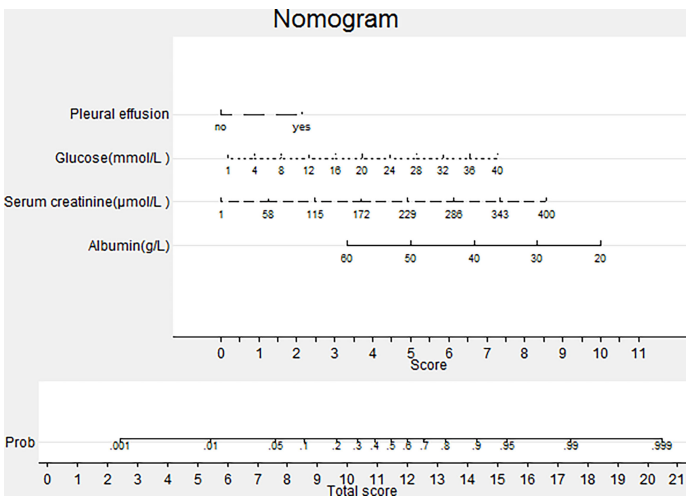

FIGURE 1 | Nomogram predicting the probability of SAP. To obtain the nomogram-predicted probability, patient values on each axis were located and a vertical line was drawn to the point axis to determine how many points were attributed for each variable value. Points for all variables were summed and accessed on the point line to find SAP probability.

HDL, LDL, triglyceride, serum calcium, C-reactive protein, and pleural effusion) were used for the RF model. As shown in **Figure 2**, based on variable important analysis of the RF model, serum creatinine, albumin, blood urea nitrogen, HDL, LDL, calcium, and glucose were the most important 7 predictors of SAP. **Figure 3** depicts the results of tenfold cross-validation. It indicated that the RF model achieved a higher mean AUC (AUC=0.89[95% CI, 0.83-0.95]) than that of the LR model (mean AUC =0.85[95% CI, 0.78-0.92]) (p=0.026). The area under the precision recall curve of the RF model (0.58) was also higher than that of the LR model (0.55) **(Figure 4)**. The calibration plots indicate adequate predicted probabilities against observed proportions of SAP for both RF and LR models **(Figure 5)**.

Validation and Comparison of Prediction Models on the Test Samples

The ROC curves for the RF model, the LR model, and the BISAP score for the prediction of SAP are shown in **Figure 6**. The RF model achieved the highest AUC (AUC=0.96[95% CI, 0.93-0.99]), followed by the LR model (AUC =0.92[95% CI, 0.87-0.97]) and the BISAP score (AUC=0.84[95% CI, 0.73-0.93]) (P=0.03). The area under precision recall curve of the RF model (0.67) was higher than that of both the LR model (0.57) and the BISAP score (0.576) **(Figure 7)**.

The RF model achieved a sensitivity of 93.8%, specificity of 82.8%, and a diagnostic accuracy of 83.9%. As a comparison, the LR model achieved a similar sensitivity of 93.8%, a lower

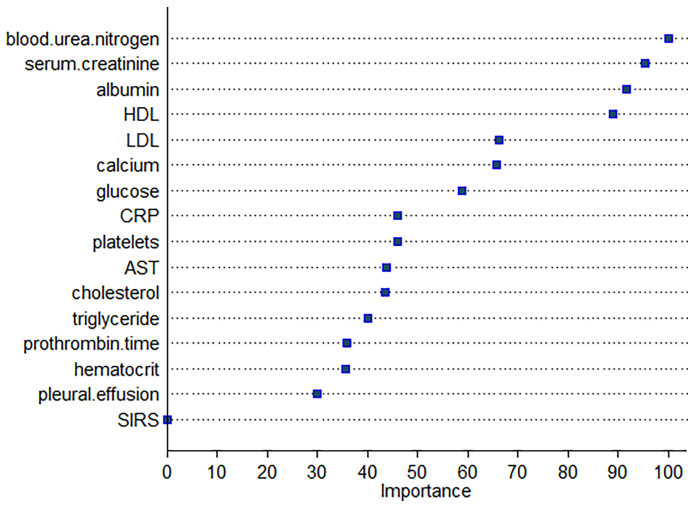

FIGURE 2 | Variable importance plot of the RF for SAP.

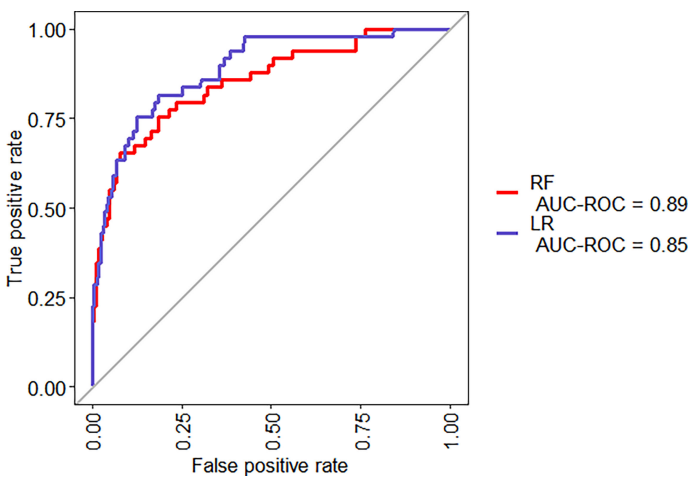

FIGURE 3 | ROC curves for the RF and LR models, for a tenfold cross-validation on the training set.

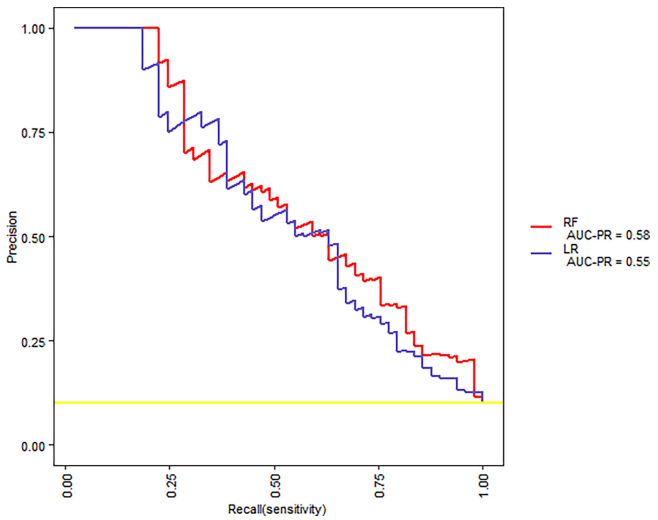

FIGURE 4 | The precision-recall curves for RF and LR models for tenfold cross-validation on the training set.

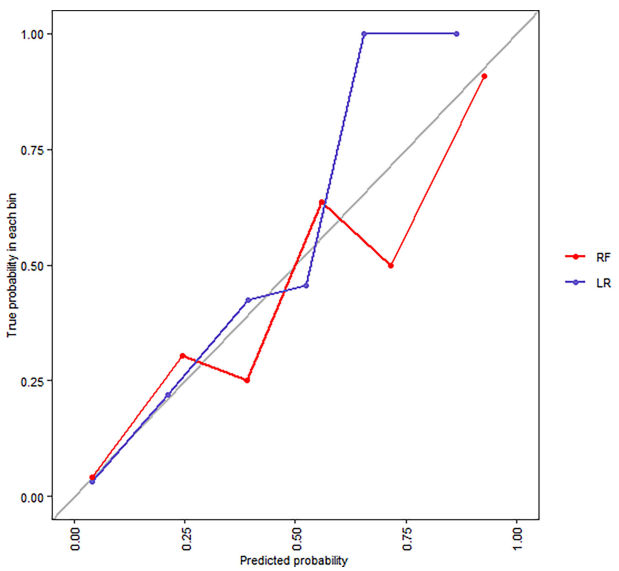

FIGURE 5 | Calibration plots for RF and LR models for tenfold cross-validation on the training set.

specificity of 79.3%, and 80.8% diagnostic accuracy. Both diagnostic performance of the RF and LR models was better than that of the BISAP score (**Table 3**).

Explanation: Individualized Prediction on The Test Sample

To clarify the model prediction for individual patients, the LIME plot was generated. It shows two typical predictions made by the RF model, in which one was for non-SAP and the other was for SAP patients (**Figure 8**). The bar charts represent the influence that individual covariates have on the overall prediction (Chan et al., 2022). The length of the bar indicates the magnitude (absolute value), while the color indicates the sign (red for negative, blue for positive) of the estimated coefficient (Biecek and Burzykowski, 2021). In other words, the length of the bar for each feature indicates the importance (weight) of that feature in making the prediction. A longer bar, therefore, indicates a feature that contributes more toward or against the prediction (Lin et al., 2021a).

As shown in **Figure 8**, the first case (case 49) is a non-SAP patient, who was correctly classified based on the RF model, with a predicted probability of 0.97 as non-SAP. The second case (case 51) is an SAP patient, who was correctly classified based on the

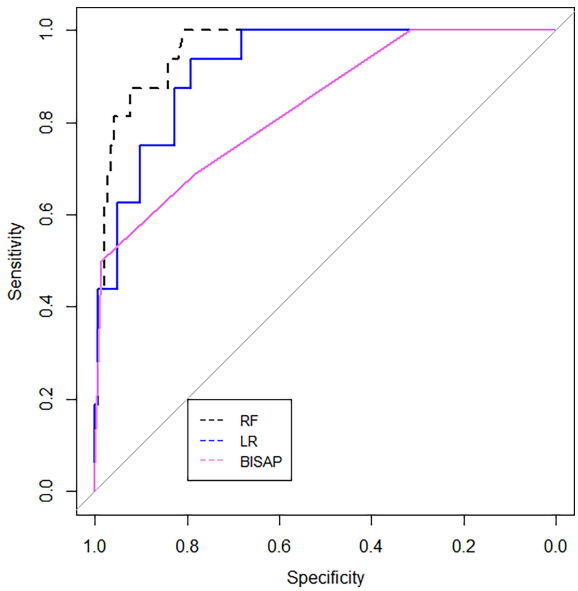

FIGURE 6 | ROC curves for the RF and LR models and BISAP scores, applied on the test set.

TABLE 3 | Diagnostic values of various models of SAP.

Variable	Cut-off value	Sensitivity	Specificity	LR+	LR-	Accuracy
RF model	0.13	93.8%	82.8%	5.44	0.08	83.9%
LR model	0.08	93.8%	79.3%	4.53	0.08	80.8%
BISAP score	2	68.8%	78.6%	3.22	0.40	77.64%

LR+, Positive likelihood ratio; LR-, negative likelihood ratio.

RF model, with a predicted probability of 0.82 as SAP. The levels of creatinine, BUN, glucose, triglyceride, and total cholesterol were positively correlated with the development of SAP. Patients with SAP had lower levels of HDL, albumin, and calcium than that of non-SAP people.

DISCUSSION

Albumin is one of the most important proteins in plasma and plays a role in maintaining osmotic pressure, antioxidants, and scavenging free radicals (Viasus et al., 2013). Albumin has also long been considered a negative acute-phase protein, with reduced production in inflammation, paving the way for inflammatory cytokines (Charlie-Silva et al., 2019). Serum albumin levels undoubtedly decrease in inflammatory states, which may result in shorter half-life and a larger interstitial pool (Barle et al., 2006) as well as capillary leak (Soeters et al., 2019), during the inflammatory process. Excessive oxidative stress is associated with damage to acinar cells which has been observed in cerulein-induced mouse models of AP (Shen et al., 2018). In addition, clinical evidence suggests that oxidative stress is common in the early phase of AP (Hackert and Werner, 2011). Therefore, it was suggested that decreased albumin may reduce the ability to counterwork oxidative stress-induced acinar damage by binding reactive oxygen species in AP (Xu et al., 2020; Belinskaia et al., 2021). Xu et al. has reported that albumin is an independent predictor for SAP and in-hospital mortality in AP patients (Xu et al., 2020). Our previous study also indicated that hypoalbuminemia within 24 h of admission is independently associated with the development of persistent organ failure and mortality in AP (Hong et al., 2017a). Ocskay et al. (Ocskay et al., 2021) reported that the incidence of hypoalbuminemia was 35.7% during hospitalization and it was dose-dependent, associated with severity and mortality in AP. In our current study, the LR analysis indicated that albumin ($p<0.001$) is an independent predictor of SAP **(Figure 1)**. Based on the RF model, albumin is also an important predictor of SAP, based on variable importance analysis **(Figure 2)**. These results are consistent with previous reports.

Creatinine is primarily generated by muscle mass and dietary intake. It is eliminated from the glomerular filtration membrane (Stevens et al., 2006; Earley et al., 2012) and serves as the most widely used functional biomarker of the kidney, which can reflect renal injury in AP (Earley et al., 2012). Apart from renal injury, it also has been reported that the level of serum creatinine is associated with pancreatic necrosis (Muddana et al., 2009; Papachristou et al., 2010; Lipinski et al., 2013). The possible

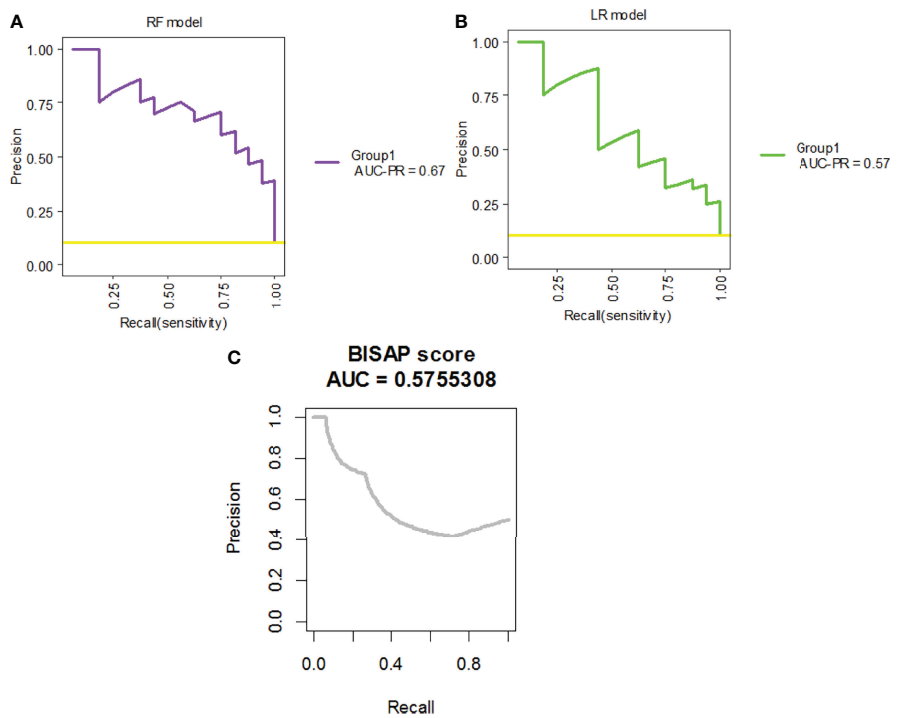

FIGURE 7 | The precision-recall curves for the **(A)** RF model, **(B)** LR model, and **(C)** BISAP score applied on the test set.

explanation is that necrotic cells release a large number of toxic substances and pro-inflammatory factors to cause renal injury, manifesting the elevation of serum creatinine. Therefore, the rise of creatinine may be attributed to the renal injury and pancreatic necrosis along with SAP. Wilkman et al. reported that increased creatinine levels are independently associated with 90-day mortality in AP patients (Wilkman et al., 2013). Wan et al. suggested that serum creatinine levels within 24 h of admission are effective for predicting persistent organ failure in AP (Wan et al., 2019). In addition, several scoring systems, which take creatinine as an index, are widely used in the clinical settings, including the Acute Physiology and Chronic Health Evaluation (APACHE) II, Sequential Organ Failure Assessment (SOFA) score for predicting the severity of pancreatitis, and modified Marshall scoring system for assessing organ dysfunction occurrence in SAP (Mederos et al., 2021). Our study indicated serum creatinine could be a useful predictor in both the RF and the LR model for predicting SAP (**Figures 1**, **2**).

The lipoprotein profile, especially HDL, is markedly decreased in inflammation and the accompanying acute-phase (Jahangiri, 2010). The mechanisms causing low serum HDL and LDL levels in the acute phase of AP remain largely unknown (Hong et al., 2017b; Hong et al., 2018). Jahangiri et al. (Jahangiri, 2010) suggested that it was related to a decreased rate of lipoprotein synthesis in the liver, general catabolism, and activation of the inflammatory system in the acute phase of the disease. Another explanation for the low serum HDL levels is that it may be due to increased expression of the Toll-like receptors (TLRs), especially TLR-4 expression (Zhang et al., 2010). It was reported that stimulated TLR-4 expression suppresses HDL levels (Liao et al., 1999). Khan et al. found that serum lipid concentrations such as HDL cholesterol and LDL cholesterol were associated with patients of SAP in all etiologies (Khan et al., 2013). However, Bugdaci et al. (2011) found a significant association between decreased HDL level and severity of the disease only in alcoholic and hypotriglyceridemic pancreatitis. In hypertriglyceridemic status, it is demonstrated that free fatty acids (FFAs) damage acinar cells and cause pancreatitis attack due to premature activation of trypsinogen, by creating an acidic environment (Okura et al., 2004; Guo et al., 2019). It has been reported that HDL takes part in FFA clearance (Asztalos et al., 2007) so that decreased HDL in hypertriglyceridemic AP cases may lead to an increase in FFA, and further damage acinar cells. Therefore, it has been suggested that an increase in HDL may be helpful for recovery from the disease by contributing to antioxidants (Bugdaci et al., 2011) and anti-inflammatory effect (Murphy and Woollard, 2010). On the other hand, in comparison, few studies are available about the pathophysiological mechanism of decreased LDL in SAP. Our study indicated both HDL and LDL were useful predictors for SAP (**Figure 2**).

Pleural effusion occurs in 3–50% patients with AP, based on a previous study (Basran et al., 1987; Kumar et al., 2019; Peng et al., 2020). The effusion can be asymptomatic and often hemorrhagic, usually resolving as pancreatitis subsides (Basran et al., 1987). Several mechanisms of pleural effusion in pancreatitis have been proposed, such as the trans-diaphragmatic lymphatic blockage, the pancreatic pleural fistula caused by the rupture of the pancreatic duct, and the fluid exudation from the sub-pleural

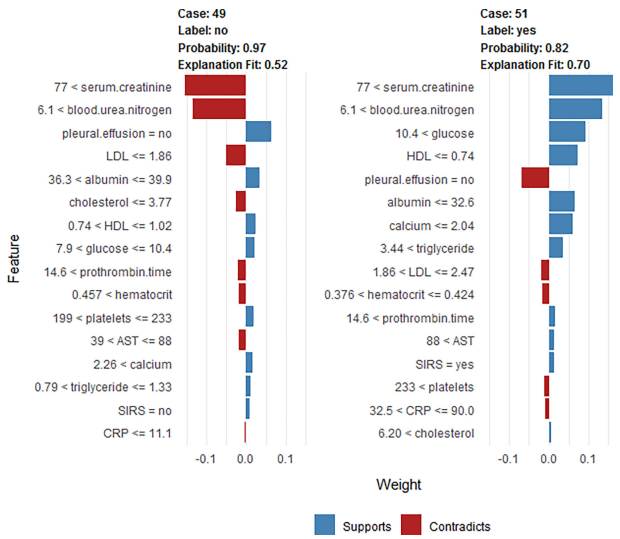

FIGURE 8 | LIME plot for the individualized likelihood of two typical predictions. This shows the main contributing features behind the model prediction. The length of the color bar represents the amount of contribution. The first case (case 49) is a non-SAP patient who was correctly classified, with a prediction probability of 0.97 as non-SAP based on the RF model. The first case (case 49) had a creatinine value of 86 μmol/L, BUN=7.1 mmol/L, no pleural effusion, LDL=1.82 mmol/L, albumin=36.5 mg/dl, total cholesterol=3.24 mmol/L, HDL=0.79 mmol/L, glucose=8.4 mmol/L, prothrombin time=15.2 s, hematocrit=0.465, platelets=206×10^9/L, AST=76 U/L, calcium=2.43 mmol/L, triglyceride=0.96 mmol/L, no SIRS, and CRP=5 mg/L. The second case (case 51) is an SAP patient who was correctly classified, with a prediction probability of 0.82 (SAP based on RF model). The second case (case 51) had a creatinine value of 260 μmol/L, BUN=16.6 mmol/L, glucose =23.2 mmol/L, HDL=0.47 mmol/L, no pleural effusion, albumin =26.5 mg/dl, calcium=0.83 mmol/L, triglyceride=25.6 mmol/L, LDL=1.87 mmol/L, hematocrit=0.39, prothrombin time=15.7 s, AST=155 U/L, SIRS, platelets=243×10^9/L, CRP =76.1 mg/L, and total cholesterol=10.54 mmol/L.

diaphragmatic vessels into the pleural cavity (Kumar et al., 2019). Pleural effusion is reported to be associated with a severe course for initial risk assessment severity in AP and a sign of SAP (Heller et al., 1997; Tenner et al., 2013; Lankisch et al., 2015). Yan et al. reported that pleural effusion volume quantified on chest CT was positively associated with the duration of hospitalization (Yan et al., 2021). As a prognostic factor, pleural effusion has been incorporated in SAP severity predictive systems such as the Bedside Index for Severity in Acute Pancreatitis (BISAP) score (Gao et al., 2015), the Panc 3 score (Brown et al., 2007), and the Extra Pancreatic Inflammation on CT (EPIC) score (De Waele et al., 2007). Following the above outcomes, the present study suggested that pleural effusion (OR 5.11, 95%CI 2.38-10.94) was an independent risk factor for SAP (**Figure 1**).

The mechanism of BUN elevation in AP is thought to be based on the loss of intravascular volume, caused by interstitial extravasations owing to the systemic inflammatory response syndrome and an AP promoted direct renal injury mechanism. It has been reported that BUN, as a single predictor, had moderate accuracy in predicting persistent organ failure in AP (Mounzer et al., 2012). Koutroumpakis et al. reported that the rise in BUN at 24 h was the most accurate in predicting persistent organ failure and pancreatic necrosis (Koutroumpakis et al., 2015). Li et al. suggested that BUN was an independent risk factor to predict in-hospital mortality (Li et al., 2020). Valverde-Lopez et al. indicated that BUN was the best predictor of SAP after 48 h (Valverde-Lopez et al., 2017). BUN is also included in many scoring systems for AP, such as BISAP, JSS, and Glasgow score (Mounzer et al., 2012). Consistent with these reports, our study shows that BUN is the most important predictor of the RF model based on variable important analysis (**Figure 2**).

Decreased levels of serum calcium are commonly seen in critical illness, and hypocalcemia is significantly more frequent in patients with SAP (Peng et al., 2017). The mechanisms of hypocalcemia in SAP may be multi-factorial, such as abnormalities of parathyroid hormone secretion and action as well as vitamin D deficiency, binding of calcium in areas of fat necrosis, likely to contribute to the medication side effects (Weir et al., 1975; Steele et al., 2013). Serum calcium levels are closely related to the severity of the disease and its complications in AP. It has been incorporated in several clinical scoring systems as such as Pancreatitis Outcome Prediction (POP) Score, and Simple Prognostic Score (Harrison et al., 2007; Gonzálvez-Gasch et al., 2009). Mentula et al. suggested that serum calcium was the best single marker in predicting organ failure in AP after 24 h of symptom onset (Mentula et al., 2005). He et al. indicated that serum calcium was one of the independent predictors of the severity of AP in elderly patients (He et al., 2021). Serum calcium was also considered a significant factor in predicting early death in SAP (Shinzeki et al., 2008). As expected, our study indicated that calcium could be a useful predictor of SAP in the RF model (**Figure 2**).

Clinical evidence shows hyperglycemia is the common early feature of AP and abnormal glucose metabolism is present in almost 40% of AP patients (Banks et al., 2013; Chen et al., 2021). According to the traditional view, the mechanism is that the damage of organisms caused by AP activate the neuroendocrine system and

lead to the secretion of many stress hormones (Binker and Cosen-Binker, 2014; Sun et al., 2019; Lu et al., 2021). Meanwhile, it is also related to the damage of the endocrine pancreas caused by SAP attacks. The association between hyperglycemia and adverse clinical outcomes in critically ill patients has been demonstrated in several observational studies, which suggest that high levels of glucose during the progression of AP can promote the release of inflammatory cytokines. These, in turn, influence disease progression (Sun et al., 2019; Chen et al., 2021). Sun et al. has suggested that the level of glucose in serum is positively correlated with the APACHE II scores, TNF-α, and CRP in AP (Sun et al., 2019). However, transient stress hyperglycemia in critically ill patients is considered harmless in some studies, indicating that the body has normal immune regulation ability (Lu et al., 2021), the subsequent derangement of glucose homeostasis could cause damage to the body (Pendharkar et al., 2016). Blood glucose-related indicators are associated with in-hospital mortality in critically ill patients with AP (Lu et al., 2021). Our LR model also shows that glucose is a useful predictor of SAP (**Figure 1**).

Machine learning has been extensively used for the prediction of severity or complication of AP (Zhou et al., 2022). Thapa et al. has reported that an XGBoost model could predict which patients would require treatment for SAP (Thapa et al., 2022). Early prediction of SAP using machine learning has also been attempted (Thapa et al., 2022). Jin et al. reported that the multilayer perception-artificial neural network (MPL-ANN) model based on routine blood and serum biochemical indexes could reliably predict disease severity in patients with AP (Jin et al., 2021). Choi et al. combined clinical (i.e., APACHE-II and BISAP scores) and radiologic (i.e., Balthazar grade and EPIC score) scoring systems by classification tree analysis for predicting SAP (Choi et al., 2018). Xu et al. reported that adaptive boosting algorithm (AdaBoost) could predict development of multiple organ failure, complicated by moderately severe or severe AP (Xu et al., 2021). However, the above models were limited due to lack of individualized prediction on the test sample. Implementation on such data remains challenging because of the low interpretability of results of machine learning (Yu et al., 2021). Our study indicated that, compared to the LR model and BISAP score, RF exhibited the highest discriminatory performance for the prediction of SAP on both training and test samples (**Figures 3**, **4**, **6**, **7**). Using the RF model, we could illustrate key features and establish a prediction model, with high accuracy in patients with SAP. The LIME plot could provide a visual illustration of the individualized interpretation of the importance of different features, which might help clinical doctors to understand results of the RF model (**Figure 8**). The LR model (nomogram) achieved a sensitivity of 93.8%, acceptable specificity of 79.3%, and diagnostic accuracy of 80.8% (**Table 3**). Though the diagnostic performance of the LR model (nomogram) is inferior to the RF model, it is simple and intuitive to calculate the prediction probability of a result, which makes it valuable in predicting SAP (**Figure 1**).

To the best of our knowledge, this is the first study to develop an interpretable RF model for SAP prediction. The strength of this study is a large sample size, which enables a strong statistical power. Both patients in ICU and the general ward were enrolled in this study, thus reducing selection bias. However, our study has some limitations, even if it has been internally validated by tenfold cross-validation technique and test set, testing the performance of our RF model in an external/other independent data set is necessary. In addition, even if effective, RF models are sophisticated and difficult to understand, and thus, comparable to a 'black box'. We have, therefore, demonstrated that by utilizing Lime plots, the results could be more easily interpreted (Al'Aref et al., 2020). At last, we did not evaluate the RF model and single predictors for other clinical outcomes such as patient survival and organ failure occurrence, intensive care unit (ICU) admission, and SAP recurrent rate. It would be interesting to carry out a large-sample prospective study to determine whether our model and other variables such as serum creatinine, albumin, BUN, HDL, LDL, calcium, and glucose play a significant role in predicting these clinical outcomes.

In conclusion, an interpretable RF model exhibited the highest discriminatory performance to predict SAP. Interpretation with LIME plots could be useful for individualized prediction in the clinical setting. A nomogram consisting of albumin, serum creatinine, glucose, and pleural effusion is also useful for the prediction of SAP.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Ethics Committee of the First Affiliated Hospital of Wenzhou Medical University. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

WH conceived the study and carried out the majority of the work. WH participated in data collection and conducted data analysis. WH, YL, XZ, SJ, JP, QL, and SY drafted the manuscript. ZB, MZ, and HG helped to finalize the manuscript. All the authors read and approved the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fcimb.2022.893294/full#supplementary-material

Supplementary Figure 1 | Data flow diagram of this study.

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The Role of Antibiotics in Endoscopic Transmural Drainage of Post-Inflammatory Pancreatic and Peripancreatic Fluid Collections

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Background: Although endoscopic treatment of symptomatic post-inflammatory pancreatic and peripancreatic fluid collections (PPPFCs) is an established treatment method, some aspects of endotherapy and periprocedural management remain controversial. The role of antibiotics is one of the most controversial issues in interventional endoscopic management of local complications of pancreatitis.

Methods: This study was a randomized, non-inferiority, placebo-controlled, and double-blinded clinical trial to investigate the role of antibiotic prophylaxis in endoscopic transmural drainage in patients with symptomatic non-infected PPPFCs and assess the influence of antibiotic treatment on the results of endotherapy in patients with symptomatic infected PPPFCs.This trial included 62 patients treated endoscopically for PPPFCs in 2020 at our medical center. Patients were divided into two groups; group 1 comprised patients who had received empirical intravenous antibiotic therapy during endotherapy and group 2 comprised patients who did not receive antibiotic therapy during endoscopic drainage of PPPFCs. The end points were clinical success and long-term success of endoscopic treatment.

Results: Thirty-one patients were included in group 1 (walled-off pancreatic necrosis [WOPN, 51.6%; pseudocyst, 48.4%) and 31 patients in group 2 (WOPN, 58.1%; pseudocyst, 41.9%) (p=0.6098/nonsignificant statistical [NS]). Infection with PPPFCs was observed in 15/31 (48.39%) patients in group 1 and in 15/31 (48.39%) patients in group 2 (p=1.0/NS). The average time of active (with flushing through nasocystic drainage) drainage in group 1 was 13.0 (6 – 21) days and was 14.0 (7 – 25) days in group 2 (p=0.405/NS). The average total number endoscopic procedures on one patient was 3.3 (2 – 5) in group 1 and 3.4 (2 – 7) in group 2 (p=0.899/NS). Clinical success of PPPFCs was observed in 29/31 (93.5%) patients from group 1 and in 30/31 (96.8%) patients from group 2 (p=0.5540/NS). Complications of endotherapy were noted in 8/31 (25.8%) patients in group 1 and in 10/31 (32.3%) patients in group 2 (p=0.576/NS). Long-term success in group 1 and 2 was reported in 26/31 (83.9%) and 24/31 (77.4%) patients, respectively (p=0.520/NS).

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Conclusions: The effective endoscopic drainage of sterile PPPFCs requires no preventive or prophylactic use of antibiotics. In infected PPPFCs, antibiotic therapy is not required for effective endoscopic transmural drainage.

Keywords: antibiotics, antibiotic therapy, pancreatic fluid collection, pancreatitis, antibiotic prophylaxis, endoscopic drainage, endoscopy

INTRODUCTION

The history of pancreatitis may involve the development of four types of post-inflammatory pancreatic and peripancreatic fluid collections (PPPFCs) as local complications of acute inflammation, including acute peripancreatic fluid collection, pancreatic pseudocysts, acute necrotic collection, and walled-off pancreatic necrosis (WOPN) (Thoeni, 2012; Banks et al., 2013; Sarr et al., 2013). Each collection may be sterile (non-infected) or infected (Thoeni, 2012; Banks et al., 2013; Sarr et al., 2013; Sarathi Patra et al., 2014; Manrai et al., 2018). For many years, the traditional management of local complications of acute pancreatitis consisted of surgical treatment combined with intravenous antibiotic therapy, particularly in cases of suspected tissue infection (Loveday et al., 2008; da Costa et al., 2014). In recent decades, advances have been made in minimally invasive methods for the treatment of post-inflammatory PPFCs, such as endoscopic techniques, which lead to radically shortened recovery times and lower complication and mortality rates (Loveday et al., 2008; Freeman et al., 2012; da Costa et al., 2014; Szeliga and Jackowski, 2014; Jagielski et al., 2018; Jagielski et al., 2020).

Endoscopic transmural drainage involves creating a fistula between the PPPFC cavity and the lumen of the gastrointestinal tract to facilitate free drainage of the collection contents into the digestive tract (Loveday et al., 2008; Freeman et al., 2012; da Costa et al., 2014; Jagielski et al., 2018; Jagielski et al., 2020). During endoscopic transmural drainage of post-inflammatory PPFCs, transmural puncture of the PPPFC is performed under endoscopic ultrasound (EUS) guidance (Loveday et al., 2008; Freeman et al., 2012; da Costa et al., 2014; Jagielski et al., 2018; Jagielski et al., 2020). Next, the puncture site was dilated using a cystostome to form a transmural fistula, a connection between the upper gastrointestinal tract (stomach or duodenum) and PPPFC (Loveday et al., 2008; Freeman et al., 2012; Jagielski et al., 2018; Jagielski et al., 2020). The next stage of the endoscopic procedure consists of enlarging the pancreatogastric or pancreatoduodenal fistula (Loveday et al., 2008; Freeman et al., 2012; da Costa et al., 2014; Jagielski et al., 2018; Jagielski et al., 2020). After enlargement, a self-expanding stent (lumen-apposing metal stent [LAMS]) or plastic endoprosthesis(-ses) is introduced *via* the fistula to enable free passive transmural drainage of the collection contents into the lumen of the gastrointestinal tract (Arvanitakis et al., 2018; Jagielski et al., 2018; Jagielski et al., 2020; Jagielski and Jackowski, 2021). Passive transmural drainage (without flushing through nasocystic drainage) is an effective method for endoscopic treatment of sterile pancreatic pseudocysts with liquid serous content alone (Arvanitakis et al., 2018; Jagielski et al., 2018; Jagielski et al., 2020; Jagielski and Jackowski, 2021). With regards to infected pseudocysts and sterile or infected necrotic collections that contain necrotic tissue in addition to liquefied necrotic contents, active (with flushing through nasocystic drainage) transmural drainage is required, consisting of additional saline irrigation introduced *via* the fistula to rinse the collection cavity in the postoperative period (Arvanitakis et al., 2018; Jagielski et al., 2018; Jagielski et al., 2020; Jagielski and Jackowski, 2021).

Although endoscopic treatment of symptomatic PPPFCs due to pancreatitis is an established treatment method, some aspects of endotherapy and periprocedural management remain contentious (Arvanitakis et al., 2018; Jagielski et al., 2018; Jagielski et al., 2020; Jagielski and Jackowski, 2021);. The role of antibiotics is one of the most controversial issues in interventional endoscopic management of local complications of pancreatitis.

Antibiotic therapy is an important element in the conservative treatment of acute pancreatitis (Tenner et al., 2013; Working Group IAP/APA, 2013; Arvanitakis et al., 2018). The primary indication for the initiation of antibiotic therapy in patients with acute pancreatitis is confirmed pancreatic or extrapancreatic infection (Tenner et al., 2013; Working Group IAP/APA, 2013; Arvanitakis et al., 2018); other indications remain unclear. Prophylactic antibiotics, to prevent infection of necrotic areas, are not recommended in patients with acute pancreatitis (Tenner et al., 2013; Working Group IAP/APA, 2013; Arvanitakis et al., 2018; Crockett et al., 2018) and their overall use in acute pancreatitis remains controversial despite numerous publications. Furthermore, the optimum duration of antibiotic therapy in acute pancreatitis is unknown.

Similar controversies have been raised regarding the use of antibiotics in interventional gastrointestinal endoscopy, which remain the subject of numerous studies. According to available guidelines, the use of antibiotic prophylaxis for gastrointestinal endoscopy should be determined mainly by risk evaluation of bacteremia associated with endoscopic procedures (ASGE Standards of Practice Committee, 2015). Bacterial translocation of endogenous microbial flora into the bloodstream (bacteremia) may occur during endoscopy as consequence of gastrointestinal wall's trauma related to the procedure (ASGE Standards of Practice Committee, 2015). The highest rates of bacteremia have been reported with esophageal dilation, sclerotherapy of varices in the upper gastrointestinal tract, percutaneous endoscopic feeding tube placement and endoscopic instrumentation of obstructed bile ducts (ASGE Standards of Practice Committee, 2015). In these cases antibiotic prophylaxis is indicated (ASGE Standards of

Practice Committee, 2015). In case of EUS-guided fine-needle aspiration (EUS-FNA) the use of antibiotic prophylaxis depends on type of punctured lesion (Barkay et al., 2009; ASGE Standards of Practice Committee, 2015; Polkowski et al., 2017; Facciorusso et al., 2019; Colán-Hernández et al., 2020). Prophylactic antibiotics are not recommended prior to EUS-FNA of solid lesions (ASGE Standards of Practice Committee, 2015; Polkowski et al., 2017). On the other hand, administration of antibiotics has been recommended before EUS-FNA of cystic lesions (Barkay et al., 2009; ASGE Standards of Practice Committee, 2015; Polkowski et al., 2017; Facciorusso et al., 2019; Colán-Hernández et al., 2020). It is also worth to pay attention to use of antibiotic prophylaxis during endoscopic retrograde cholangiopancreatography (ERCP). It is recommended in patients with bile duct obstruction in absence of cholangitis during ERCP with incomplete biliary drainage in order to prevention of cholangitis (ASGE Standards of Practice Committee, 2015). In case of patients with bile duct obstruction in absence of cholangitis during ERCP with complete biliary drainage antibiotic prophylaxis is not required (ASGE Standards of Practice Committee, 2015). Our study based on similar assumption: effective (complete) endoscopic drainage of PPPFCs does not require antibiotic prophylaxis in order to prevention of infection or superinfection of PPPFCs.

The importance of prophylactic antibiotics in invasive endoscopic procedures in the pancreatic field remains unknown. Currently, no clear guidelines are available regarding the need for periprocedural antibiotic prophylaxis or its duration. This study attempted to define the role of antibiotics in the endoscopic treatment of PPPFC.

MATERIALS AND METHODS

This study was conducted in accordance with the Consolidated Standards of Reporting Trials (CONSORT) (Turner et al., 2012). This study was a randomized, non-inferiority, placebo-controlled, and double-blinded clinical trial to investigate the role of antibiotic prophylaxis in endoscopic transmural drainage in patients with symptomatic non-infected PPPFCs and assess the influence of antibiotic treatment on the results of endotherapy in patients with symptomatic infected PPPFCs.

It has been hypothesized that the efficiency of endoscopic PPPFC drainage is the basic criterion for therapeutic success, regardless of the infectious agent in PPPFC. The results of endoscopic treatment were based on effective drainage, regardless of antibiotic prophylaxis, and whether antibiotic therapy was used. This hypothesis was verified by examining the effect of antibiotic prophylaxis in patients with sterile (non-infected) PPPFC and antibiotic therapy in patients with infected PPPFC on the efficacy and safety of endoscopic transmural drainage.

Herein, the primary objective was to investigate the influence of antibiotic prophylaxis on the efficiency and safety of endoscopic transmural drainage in patients with sterile (non-infected) PPPFCs and antibiotic treatment in patients with infected PPPFCs.

The secondary objectives were to assess the influence of antibiotic prophylaxis and treatment on the duration of endotherapy and the number of endoscopic procedures (aggressiveness of endotherapy) in patients with non-infected and infected PPPFCs.

This study was conducted at the Department of General, Gastroenterological, and Oncological Surgery, Ludwik Rydygier Collegium Medicum in Bydgoszcz, Nicolaus Copernicus University in Toruń in 2020.

The study was approved by the Ethics Committee at the Collegium Medicum of Nicolaus Copernicus University (Approval Number KB 294/2020) and was conducted in accordance with the Declaration of Helsinki. All patients provided oral and written informed consent before inclusion in the study. All patients received detailed information regarding the study.

The recruitment period was from 01/01/2020 to 31/12/2020. The observation lasted 12 months from the end of endotherapy (until December 31, 2021). The entire study period was from 01/01/2020 to 31/12/2021.

The diagnosis of pancreatitis, criteria of clinical and morphological categorization, and all definitions were based on the 2012 revised Atlanta classification (Thoeni, 2012; Banks et al., 2013; Sarr et al., 2013). The standards for conservative treatment of pancreatitis are based on commonly available international guidelines (Tenner et al., 2013; Working Group IAP/APA, 2013);. Additional treatment methods were used depending on the concomitant organ impairment and the patient's overall clinical condition. Each individual case of a patient with pancreatitis was thoroughly discussed during interdisciplinary meetings of senior staff. Decisions were made regarding further management of the patient and the potential rationale for interventional treatment.

Study Inclusion Criteria

All consecutive patients with symptomatic post-inflammatory PPFCs in the late stage of pancreatitis (> four weeks from the onset of the disease) were included in this study. All patients aged 18 years were included in this study. All patients with clinical symptoms related to PPPFCs due to acute or chronic pancreatitis were enrolled. All patients who underwent endoscopic drainage for symptomatic PPPFCs in the late stage of pancreatitis were also included. Qualification for endoscopic intervention was based on clinical picture and imaging results, primarily abdominal contrast-enhanced computed tomography (CECT).

All patients were clinically assessed using the Sequential Organ Failure Assessment (SOFA) score/quick Sequential Organ Failure Assessment (qSOFA) score (Vincent et al., 1996; Vincent et al., 1998; Barbara et al., 2018).

Patients were included irrespective of existing suspicion of infection of collection's content (both patients with infected PPPFCs and with sterile/non-infected PPPFCs) if the dynamics of change in SOFA and qSOFA scores during the endoscopic treatment did not exceed two (≤2) points in infected PPPFCs.

Study Exclusion Criteria

Patients aged < 18 years and pregnant women were excluded from the study. Patients with PPPFCs that were not a consequence of pancreatic inflammatory diseases were excluded from the study (1 patient). Patients with post-inflammatory PPFCs without clinical symptoms were excluded (24 patients). Patients who had undergone interventional endoscopic treatment in the early phase of pancreatitis (< four weeks from disease onset) were also excluded (6 patients). Infected PPPFCs patients with SOFA/qSOFA scores that exceeded two (>2) points were excluded from the study (13 patients).

Additional exclusion criteria were as follows: antibiotic therapy for any other indication 7 days before the endoscopic procedure (11 patients) and allergy to antibiotics (piperacillin or tazobactam) (1 patient).

After meeting the exclusion criteria, 56 patients were excluded from the study.

Study Group

After meeting the inclusion and exclusion criteria, the study group consisted of patients who underwent endoscopic transmural drainage of symptomatic post-inflammatory PPFCs. Patients in the study group were randomly assigned to the antibiotic group (group 1) or placebo group (group 2). The mechanism of random allocation of patients to each group was based on the randomness of the allocation resulting from the order of clinically necessary endotherapeutic interventions. Simple randomization was used, assigning consecutive patients undergoing endoscopic treatment to group 1 or group 2. Allocation to the antibiotic or placebo groups was made by the ward clinician on a simple randomization basis. This was the only person with access to the blind data and was not involved in the processing of the results. Patients and clinical staff were not allocated to the end of the study.

Group 1 consisted of patients who were receiving broad-spectrum antibiotic therapy (piperacillin with tazobactam 4.5 g administered intravenously every 6 hours [6.00 am, 12.00 am, 6.00 pm and 12.00 pm]) during endotherapy (from the onset of endoscopic treatment, for the 7 days following drainage, or 14 days in case of prolonged endoscopic drainage due to the large size of the collection). The first dose of antibiotics was administered on the day of the initial endoscopic transmural drainage procedure. In patients with renal dysfunction, the dose of antibiotics was modified depending on the renal parameters in laboratory blood tests.

Group 2 comprised patients who did not receive antibiotics during endoscopic drainage (placebo group). Patients in this group did not receive periprocedural antibiotic prophylaxis. The patients in group 2 received an equivalent volume of saline solution administered intravenously every 6 h, as mentioned above, during endotherapy (from the onset of endoscopic treatment, for the 7 days following drainage, or 14 days in case of prolonged endoscopic drainage due to the large size of the collection). The patients, study investigators, and clinical staff were blinded to the allocation until the study was completed.

The Strategy of Interventional Treatment

In patients with symptomatic PPPFCs in the late phase of pancreatitis, transmural drainage was performed if EUS revealed that the distance between the collection wall and the gastrointestinal wall did not exceed 30 mm (Jagielski et al., 2018; Jagielski and Jackowski, 2021).

In patients with pancreatic pseudocysts, passive (without flushing through nasocystic drainage) transmural drainage was the method of choice. In case of ineffective passive transmural drainage of the pseudocyst, active transmural drainage using a nasocystic drain was performed.

In patients with WOPN, the standard endoscopic intervention method is active (with flushing through nasocystic drainage) transmural drainage. In the event that endoscopic drainage of WOPN proved ineffective, the position of the transmural nasocystic drain was changed or another fistula in a new location (multiple transluminal gateway technique [MTGT]) (Jagielski et al., 2018; Jagielski and Jackowski, 2021) was performed during the next endoscopic procedure. If the transmural drainage system did not drain the entire necrotic area or if transmural drainage was unsuccessful for WOPN patients, direct endoscopic necrosectomy was performed. Not draining area of WOPN was defined on the basis of clinical image and additional examinations. Another method of imaging of not drained area was fluoroscopic nasocystic tube-check imaging of an existing drain, where the incomplete drainage of WOPN was stated.

If endoscopic techniques with transmural access were ineffective, additional access to the collection cavity was created using percutaneous drainage (transperitoneal or retroperitoneal) or transpapillary drainage (through the major duodenal papilla).

Endoscopic Procedures

Endoscopic procedures were performed under general anesthesia with tracheal intubation. All patients provided informed consent for the procedure. All procedures were performed by a single endoscopist with no access to the study protocol. Endoscopic procedures included carbon dioxide insufflation and the use of a linear echoendoscope (Pentax EG3870UTK, Pentax Medical, Tokyo, Japan), duodenoscope (Olympus TJF-Q180V, Olympus Corporation, Tokyo, Japan), and gastroscope (Olympus GIF-H185, Olympus Corporation). Samples of the material contained in PPPFC were collected for microbiological, cytological, and laboratory analyses.

Endoscopic Transmural Drainage

Placement of the pancreaticogastric or pancreaticoduodenal anastomosis in the form of a transmural cystostomy was performed under EUS guidance (Jagielski et al., 2018; Jagielski and Jackowski, 2021). The anastomosis between the gastrointestinal lumen and the collection cavity was created using a 10 Fr cystotome (Cystotome CST-10, Cook Endoscopy Inc., North Carolina, USA) and dilated with a high-pressure balloon with a diameter of up to 15 mm (Cook Endoscopy or Boston Scientific). Through the stomy, a transmural metal

endoprosthesis (LAMS) was inserted, measuring 16 mm in diameter and 20, 30, or 40 mm in length (Taewoong Medical or Olympus). For active transmural drainage, a 7 Fr or 8.5 Fr nasal drain (Cook Endoscopy) and 7 Fr or 8 Fr double pigtail stents (Cook Endoscopy) were inserted into the collection cavity through the LAMS. In the case of passive (without flushing through nasocystic drainage) transmural drainage, only 7 Fr or 8.5 Fr double pigtail stents (Cook Endoscopy) were used through LAMS.

Drainage System

When active (with flushing through nasocystic drainage) transmural drainage was used, the PPPFC was flushed with saline (60 – 200 mL) through the nasal drain every 2 h during the first 48 h postoperatively and every 4–6 h on the following days.

Treatment Efficacy Assessment

During active (with flushing through nasocystic drainage) transmural drainage, the size of the fluid collection was measured every seven days *via* abdominal ultrasound. Abdominal CECT was used to confirm complete regression of fluid collection or in cases where the patient's clinical condition deteriorated despite ongoing treatment. Active (with flushing through nasocystic drainage) drainage was discontinued once clinical success was established, while the patients were still on passive (without flushing through nasocystic drainage) transmural drainage. After four weeks, an endoscopic procedure was performed during subsequent hospitalization, and passive transmural drainage was either continued (with transmural endoprostheses replaced) or discontinued (with the transmural endoprostheses removed). The decision to continue passive (without flushing through nasocystic drainage) transmural drainage was dependent on the fluid collection size and the presence of any disruption in the main pancreatic duct, as revealed during ERCP. If the PPPFC persisted in residual form (30 – 40 mm) or recurred (>40 mm), passive endoscopic drainage was continued and the transmural endoprostheses were replaced for another four weeks. If size of the collection was between 30 and 50 mm only the plastic "double pigtail" stents were introduced transmurally. If the size of the collection was over 50mm, the next LAMS was replaced. In cases of complete PPPFC regression, an endoscopic procedure was performed to remove the transmural endoprostheses and passive endoscopic drainage was completed.

In the case of passive (without flushing through nasocystic drainage) transmural drainage of the pancreatic pseudocyst, drainage was used during the following weeks. During the next hospitalization, an endoscopic procedure was performed and passive transmural drainage of the pancreatic pseudocyst was either continued (with transmural endoprostheses replaced according to scheme described above) or discontinued (with the transmural endoprostheses removed) depending on the size of the collection. In the case of pseudocyst regression (<30 mm), passive transmural drainage was discontinued.

After the end of endoscopic treatment the patients were placed under observation, which consisted of additional outpatient care within the surgical or gastroenterological clinic. These patients all underwent imaging control examinations of the abdomen, mostly abdominal CECT, after 3, 6, 12, and 24 months of observation or immediately in cases where patients were suspected of having clinical symptoms related to PPPFCs.

Definitions

Technical success was defined as placement of the transmural stent with its distal flange in the PPPFC cavity and its proximal flange in the lumen of the gastrointestinal tract (stomach or duodenum) under endoscopic and radiologic guidance.

Effective transmural endoscopic drainage was considered successful if the contrast agent administered flowed freely from the PPPFC through the transmural stent without leaking out of the gastrointestinal tract or the stent. In the case of active (with flushing through nasocystic drainage) transmural drainage, the drainage was effective if the contrast agent administered through the nasal drain filled the whole cavity of the PPPFC and subsequently allowed for free outflow of content through the transmural fistula to the gastrointestinal tract.

Complications of endotherapy were defined as consequences of adverse events during endoscopic treatment.

Clinical success was defined as regression of symptoms associated with the presence of PPPFC and regression of the collection (diameter decreased to <40 mm) in imaging examinations.

Long-term success was defined as the absence of symptoms related to PPPFC and complete PPPFC regression (size decreased to <40 mm) during follow-up after endoscopic drainage.

Recurrence of PPPFC was defined as a collection size >40 mm on imaging examinations or the appearance of symptoms associated with the presence of PPPFC during follow-up.

Statistical Analysis

All statistical calculations were conducted using STATISTICA version 12.0 (StatSoft; Tulsa, Oklahoma, United States). Quantitative variables were characterized by arithmetic means, standard deviation, minimal and maximal values (range), and 95% confidence intervals (CIs). Qualitative data were presented as numbers and percentages. To assess whether quantitative variables were normally distributed, the Shapiro-Wilk test was used. Levene's (Brown-Forsythe) test was used to test the hypothesis of equality of variance. The significance of the differences between two groups (independent variables model) was analyzed using the Student's t-test, Welch's t-test (in the case of unequal variances), or Mann-Whitney U test (when Student's t-test was not applicable or for variables measured with an ordinal scale).

The significance of differences between more than two groups was assessed using the F (ANOVA) or Kruskal-Wallis test (in case of failure to meet the applicability conditions of ANOVA). When statistically significant differences were obtained between the groups, *post hoc* tests were used (Tukey's test for F, Dunn's test for the Kruskal-Wallis test). In the case of the model of two related variables, the Student's t-test or the Wilcoxon pair-order test was used (in the case of failure to meet the applicability conditions of the Student's t-test or for variables measured on an

ordinal scale). The significance of differences between more than two variables in the model of related variables was assessed by analysis of variance with repeated measures or Friedman's test (in case of not meeting the applicability conditions of ANOVA with repeated measures or for variables measured on an ordinal scale).

The chi-squared test of independence was used for qualitative variables (with Yates's correction for continuity when the cell number was less than 10, when Cochran's condition was met, Fisher's exact test).

To determine the relationship between the strength and direction of the variables, a correlation analysis was used to calculate the Pearson and/or Spearman correlation coefficients.

Statistical significance was set at P=0.05.

The analysis of the values presented in the manuscript revealed that in all applied statistical tests the power calculation was not smaller than 0.80 for significance level α=0.05.

RESULTS

Patient Characteristics

The study enrolled 62 patients (12 women, 50 men; average age 49.73 [22 – 79] years) with symptomatic post-inflammatory PPFCs who underwent endoscopic transmural drainage.

Group 1 consisted of 31 patients (eight women, 23 men; mean age 51.5 [25 – 76] years) who were receiving broad-spectrum antibiotic therapy (piperacillin with tazobactam) during endotherapy.

Group 2 (placebo group) consisted of 31 patients (four women, 27 men; mean age 48.0 [22 – 79] years) without antibiotics administered during endoscopic drainage.

Detailed patients' characteristics are presented in **Table 1**. Parameters in laboratory blood test in patients with PPPFCs before the endoscopic procedure was presented in **Table 2**.

PPPFCs Characteristics

In group 1, 16 (51.6%) patients were diagnosed with WOPN and 15 (48.4%) patients were diagnosed with pancreatic pseudocysts. In group 2, 18 (58.1%) patients were diagnosed with WOPN and 13 (41.9%) were diagnosed with pancreatic pseudocysts (p= 0.6098).

The average size of the PPPFC was 137.3 (68 – 247) mm in group 1 and 156.6 (70 – 320) mm in group 2 (p= 0.2370). Infections of PPPFC (**Figures 1A,B**) diagnosed on the basis of positive microbial culture content were present in 15 (48.4%) patients in group 1 and 2, respectively (p=1.0). In both groups, the most common bacterial pathogens isolated from the fluid sample were *Escherichia coli*, *Klebsiella pneumoniae*, *Enterococcus faecalis*, and *Staphylococcus epidermidis*.

The remaining indications (apart from infection with PPPFC) for endotherapy are listed in **Table 3**. In 13 (41.94%) patients in group 1 and 13 (41.94%) patients in group 2, more than one indication for endotherapy was present (p=1.0).

The mean time from the onset of pancreatitis to the start of endotherapy was 78.6 (30 – 240) days in group 1 and 88.0 (33 – 252) days in group 2 (p=0.4102).

Chronic pancreatitis was diagnosed in eight (25.8%) patients in group 2 and in six (19.4%) patients in group 2 (p=0.5435).

Endoscopic Treatment Technique

Transmural access to the PPPFCs (**Figures 2A-E**) was performed in all 62 patients (transgastric, 58; transduodenal, four). Active (with flushing through nasocystic drainage) transmural drainage was used in 16 (51.6%) patients in group 1 and in 18 (58.1%) patients in group 2 (p= 0.61). MTGT were used in four (12.9%) patients in group 1 and five (16.13%) patients in group 2 (p=0.429). Single transluminal gateway techniques were applied in the remaining 53 patients. Direct endoscopic necrosectomy was performed in 10 (32.3%) patients in group 1 and 11 (35.5%) patients in group 2 (p= 0.7884). Additional transpapillary drainage was used in three patients in group 1 and four patients in group 2. Additional percutaneous drainage was performed in four and three patients from group 1 and 2, respectively.

Duration of Endotherapy

The mean duration of active (with flushing through nasocystic drainage) endoscopic drainage was 13 (6 – 21) days in group 1 and 14 (7 – 25) days in group 2 (P= 0.405). The average duration of passive (without flushing through nasocystic

TABLE 1 | Characteristics of the patients from study group.

	Group 1 (n=31)	Group 2 (n=31)	*p*-value
Age, mean, [range]	51.5 [25-76]	48.0 [22-79]	0.3198
Sex, n, men (%)	23 (74.2%)	27 (87.1%)	0.1985
Etiology of pancreatitis, n, (%)			0.1797
Alcoholic	18 (58.1%)	23 (74.2%)	
Non-alcoholic	13 (41.9%)	8 (25.8%)	
PPFCs size, mm, mean (range)	137.3 (68-247)	156.6 (70-320)	0.2370
Type of PPFCs			0.6098
Pancreatic pseudocyst	15 (48.4%)	13 (41.9%)	
Walled-off pancreatic necrosis	16 (51.6%)	18 (58.1%)	
Time from the pancreatitis to endotherapy (days), mean (range)	78.6 (30-240)	88.0 (33-252)	0.4102
SOFA score, points, n (%)			0.4560
1	20 (64.5%)	16 (51.6%)	
2	11 (35.5%)	15 (48.4%)	
CTSI (computed tomography severity index) (points), mean (range)			0.9140
	9 (5–10)	8 (5–10)	

TABLE 2 | Parameters in laboratory blood test in patients with PPPFCs before the endotherapy.

Parameter in blood test	Group 1	Group 2	*p*-value
Leukocytes, mm^3			0.3542
Mean (SD)	15.2 (6.1)	13.8 (6.3)	
Range	5.5-32.3	4.5-26.5	
Thrombocytes, mm^3			0.0146
Mean (SD)	397.4 (147.9)	314.8 (129.6)	
Range	168.0-723.0	110.0-503.0	
C-reactive protein, mg/L			0.2001
Mean (SD)	142.9 (103.9)	115.7 (109.7)	
Range	1.9-404.5	0.8-344.2	
Procalcitonin, µg/L			0.1748
Mean (SD)	2.1 (3.5)	3.6 (5.2)	
Range	0.0-12.5	0.0-22.5	
Creatinine, mg/dl			0.0001
Mean (SD)	0.8 (0.4)	1.4 (0.6)	
Range	0.3-2.0	0.4-2.5	
Amylase, U/L			0.3789
Mean (SD)	139.7 (126.0)	148.1 (106.3)	
Range	13.0-552.0	34.0-511.0	
Bilirubin, mg/dL			0.1881
Mean (SD)	2.8 (4.1)	2.6 (3.2)	
Range	0.4-17.8	0.2-13.4	
ALT, U/L			0.7957
Mean (SD)	313.3 (259.8)	260.4 (261.6)	
Range	36.0-849.0	49.0-1 008.0	
AST, U/L			0.9646
Mean (SD)	300.1 (254.5)	268.0 (274.4)	
Range	34.0-893.0	71.0-1 107.0	

drainage) transmural drainage was 84 (29 – 265) days in group 1 and 96 (33 – 222) days in group 2 (P= 0.342). The mean number of endoscopic procedures was 3.3 (2 – 5) in group 1 and 3.4 (2 – 7) in group 2 (p=0.899).

Complications of Endotherapy

Complications during endoscopic transmural drainage were observed in eight (25.8%) and 10 (32.3%) patients in group 1 and 2 (p=0.576), respectively. Surgical treatment of endotherapy complications was necessary in two patients from group 1 and 2, respectively. Detailed information regarding the complications is presented in **Table 4**.

The most common complication observed in both groups was gastrointestinal bleeding, it was observed in five patients in group 1 and six patients in group 2 (p= 0.740). In all cases, the cause was bleeding from the PPPFC through the transmural cystostomy into the gastrointestinal lumen. Conservative treatment with packed red blood cell transfusions and blood derivatives proved successful in seven patients with gastrointestinal bleeding during ongoing transmural drainage. Endoscopic treatment with hemostatic powder (*Hemospray*, Cook Endoscopy) sprayed into the collection cavity effectively managed bleeding in two patients. Another two patients required surgical treatment. During laparotomy, the bleeding artery, the gastroduodenal artery in one case and the splenic artery in one case, was ligated using the stick-tie technique.

Efficacy of Endotherapy

Technical success of the transmural drainage procedure was achieved in 30 patients (96.77%) in both groups (p=1.0). In two patients, during first endoscopic procedure (performing of cystogastrostomy) inproper location of transmural stent was stated in form of proximal migration of this stent to the lumen of the collection. In both cases, the correction of the position of the LAMS with use of endoscopic forceps was performed, but there was no technical success.

Effective transmural endoscopic drainage was noted in 30 patients (96.77%) in both groups (p=1.0). In group 1, clinical success was achieved in 29 (93.55%) patients compared to 30 (96.77%) patients in group 2 (p=0.5540).

Mortality

Mortality during endoscopic drainage was observed in two patients (one patient from group 1 and one patient from group 2) and was not associated with ongoing endoscopic treatment. All fatal cases were caused by multiple organ failure during the course of severe acute necrotizing pancreatitis in patients with pancreatic necrosis.

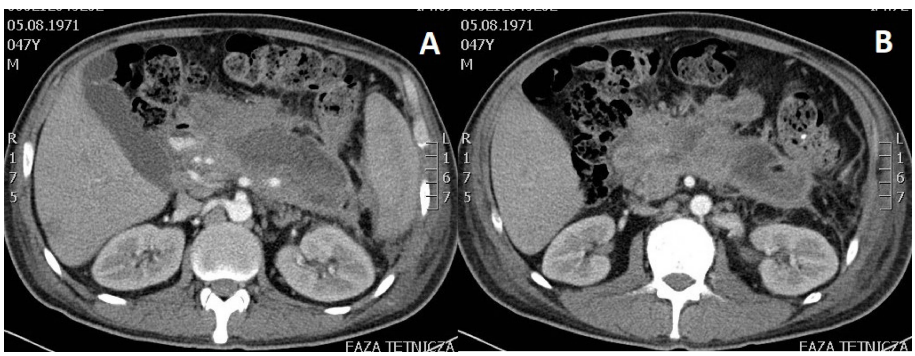

FIGURE 1 | **(A, B).** Abdominal contrast-enhanced computed tomography scan performed in a patient with infected walled-off pancreatic necrosis six weeks after acute necrotic pancreatitis of alcoholic etiology. Gas bubbles seen within the lumen provide an indirect proof of infected fluid collection after excluding spontaneous fistulization of pancreatic necrosis to the gastrointestinal tract.

TABLE 3 | Indications for endoscopic treatment of PPPFCs.

Indication	Group 1Number of patients, n (%)	Group 2Number of patients, n (%)	*p*-value
Infection	15 (48.4%)	15 (48.4%)	1.0
Subileus/ileus	11 (35.5%)	10 (32.3%)	0.7884
Icterus	4 (12.9%)	5 (16.1%)	0.7185
Abdominal pain	6 (19.4%)	8 (25.8%)	0.5435
Weight loss	5 (16.1%)	6 (19.4%)	0.7396

Long-Term Success

During the follow-up period, which lasted an average of 598 (484 – 804) days, long-term success of endotherapy was achieved in 26 (83.9%) patients in group 1 and 24 (77.4%) in group 2 (p=0.520).

PPPFC recurrence during follow-up occurred in three (9.7%) patients in group 1 and five (16.1%) patients in group 2 (p= 0.449). All patients with recurrent PPPFC underwent successful endoscopic treatment.

Laboratory Blood Tests

A comparison of the results of the laboratory blood tests is presented in **Table 5**-**Table 6**.

DISCUSSION

Endoscopic treatment of PPPFCs during the course of acute pancreatitis is based on drainage of the liquid contents of the collection cavity (Loveday et al., 2008; Freeman et al., 2012; da Costa et al., 2014; Szeliga and Jackowski, 2014; Jagielski et al., 2018; Jagielski et al., 2020). Effective transmural drainage consists of free drainage of the contents of the collection *via* the transmural fistula into the gastrointestinal tract (Arvanitakis et al., 2018; Jagielski et al., 2018; Jagielski et al., 2020; Jagielski and Jackowski, 2021). No antibiotic therapy is required for successful endoscopic transmural drainage of either sterile or infected post-inflammatory PPFCs.

In the case of infected PPPFCs, free drainage of contaminated contents is accomplished by efficient active (with flushing through nasocystic drainage) transmural drainage (Jagielski et al., 2018; Jagielski et al., 2020; Jagielski and Jackowski, 2021). Thus, effective endoscopic drainage provides a means of controlling infection. No additional use of antibiotics was required for the treatment of infected PPPFCs *via* endoscopic drainage.

With regards to sterile PPPFCs, secondary contamination occurs as a result of transmural puncture for passive (without flushing through nasocystic drainage) transmural drainage (Loveday et al., 2008; Freeman et al., 2012; da Costa et al., 2014; Jagielski et al., 2018; Jagielski et al., 2020; Jagielski and Jackowski, 2021). However, efficient passive transmural drainage facilitates free outflow of the collected contents into the gastrointestinal tract (i.e., from a high-pressure compartment to a low-pressure compartment) (Loveday et al., 2008; Freeman et al., 2012; da Costa et al., 2014; Jagielski et al., 2018; Jagielski et al., 2020; Jagielski and Jackowski, 2021), thus, preventing infection. No prophylactic or periprocedural use of antibiotics is required for the treatment of primary sterile PPPFCs through efficient endoscopic drainage.

This study showed that effective endoscopic drainage of sterile post-inflammatory PPFCs requires no preventive or prophylactic use of antibiotics. In the case of contaminated PPPFC, the success of treatment depends on infection control. Antibiotic therapy is the basis of conservative treatment for infected post-inflammatory PPFCs and is responsible for controlling the infection. When endoscopic (interventional) treatment of infected PPPFCs is initiated, effective transmural drainage determines the control of infection (Jagielski et al., 2018; Jagielski et al., 2020); thus, antibiotic treatment is no longer required. No antibiotic therapy is required in cases of efficient endoscopic transmural drainage of infected post-inflammatory PPFCs.

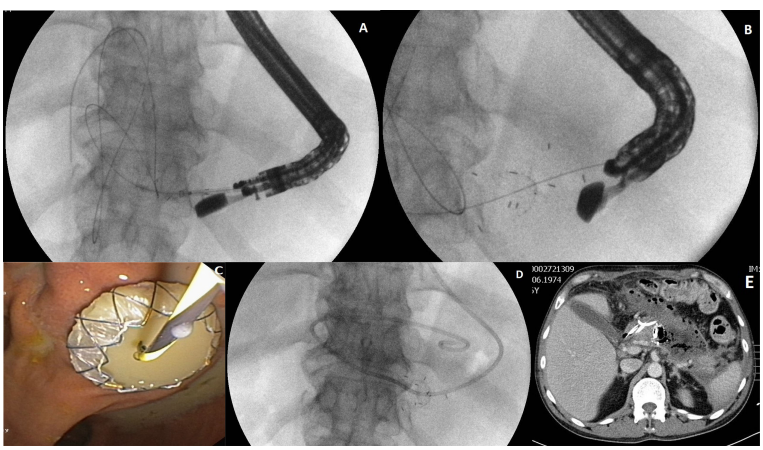

FIGURE 2 | **(A–E)** Active (with flushing through nasocystic drainage) transmural drainage of a walled-off pancreatic necrosis. After the transmural fistula is created and a self-expanding stent (LAMS) is inserted transmurally **(A–C)** through the fistula, a nasal drain **(D)** is introduced along a guidewire into the necrotic area. Active transmural drainage of pancreatic necrosis is visible in computed tomography of abdomen **(E)**.

TABLE 4 | Complications of endoscopic treatment of patients with PPPFCs.

Complication		Group 1Number of patients, n (%)	Group 2Number of patients, n (%)	*p*-value
Total number of complications		8 (25.8%)	10 (32.3%)	0.576
Upper gastrointestinal bleeding		5 (16.13%)	6 (19.35%)	0.740
Kind of treatment	*Conservative*	3	4	
	Endotherapy	1	1	
	Surgical	1	1	
Dislocation of transmural stent		3 (9.68%)	4 (12.9%)	0.519
Kind of treatment	*Endotherapy*	2	3	
	Surgical	1	1	

The risk of pancreatic cystic lesions, such as post-inflammatory PPFCs, becoming infected after EUS-FNA has not been well established (Polkowski et al., 2017). The current guidelines for EUS-FNA of sterile pancreatic cystic lesions recommend the use of periprocedural antibiotic prophylaxis; however, this recommendation supported by low-quality scientific evidence (ASGE Standards of Practice Committee, 2015; Polkowski et al., 2017).

However, recently, an increasing number of published studies have emerged that undermine the validity of periprocedural antibiotic prophylaxis following EUS-FNA of pancreatic cystic lesions (Facciorusso et al., 2019; Colán-Hernández et al., 2020). A multicenter, randomized clinical trial published in 2020 showed that the risk of pancreatic cystic lesions becoming infected as a result of EUS-FNA is low and antibiotic prophylaxis is not required (Colán-Hernández et al., 2020). If periprocedural antibiotic prophylaxis is unnecessary, as the risk of infection following transmural puncture and aspiration of sterile cyst contents without endoscopic drainage is low, preventive or periprocedural use of antibiotics is even less justified in the efficient passive (without flushing through nasocystic drainage) endoscopic drainage of sterile pseudocysts and should not be used.

In relation to endoscopic drainage of post-inflammatory PPFCs, no studies supporting the use of antibiotic therapy during the course of treatment are currently available in the literature. No reference to antibiotic therapy is provided in the available guidelines for the endoscopic treatment of local complications of pancreatitis (Arvanitakis et al., 2018).

Apart from this study, the only study regarding the role of antibiotic therapy in endotherapy of PPPFCs was published in 2018 (Sahar et al., 2018). In their retrospective study, Sahar et al. compared the outcomes of endoscopic treatment of WOPN following either short-term (≤5 days) or long-term (>5 days) antibiotic prophylaxis (Sahar et al., 2018). The study showed that the outcomes of minimally invasive treatment of sterile pancreatic necrosis were comparable between the two groups (Sahar et al., 2018). However, long-term antibiotic prophylaxis has been shown to predispose patients to secondary infections, such as colitis caused by *Clostridium difficile* (Sahar et al., 2018). The authors suggested that further studies are necessary to evaluate the role and duration of antibiotic prophylaxis during drainage of P PPPFCs (Sahar et al., 2018).

Assuming that the treatment of infected PPPFCs is based on an appropriate drainage system (Loveday et al., 2008; Freeman et al., 2012; da Costa et al., 2014; Szeliga and Jackowski, 2014; Jagielski et al., 2018; Jagielski et al., 2020) to ensure infection control, the use of systemic antibiotic therapy is not justified. No systemic antibiotic therapy is required in cases of efficient drainage of infected PPPFCs formed during the course of pancreatitis, as proven in this study.

It is worth noting that in ERCP, the prophylactic use of antibiotics depends on the surgeon's expectations of procedural success (ASGE Standards of Practice Committee, 2015). Effective endoscopic drainage of PPPFCs requires no antibiotic prophylaxis in cases of sterile collections, or antibiotic treatment in cases of infected collections. The decision to use antibiotics during endotherapy for local complications of acute and chronic pancreatitis should also be based on the anticipated effectiveness of the procedure and the efficiency of drainage in the postoperative period.

According to international guidelines, patients with acute pancreatitis should receive antibiotics in either of the following two cases: (1) extrapancreatic infection, most frequently a respiratory infection, urinary tract infection, or biliary tract infection, or (2) infection of pancreatic/peripancreatic necrotic areas, including PPPFCs (Tenner et al., 2013; Working Group IAP/APA, 2013; Arvanitakis et al., 2018; Crockett et al., 2018). PPPFCs generally become infected through translocation of the gut microbiota from the gastrointestinal tract. The most common pathogens include *Escherichia coli*, *Klebsiella pneumoniae*, *Enterococcus faecalis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Proteus mirabilis*,

TABLE 5 | Comparison of the results of C-reactive protein (mg/L) in groups of patients during endotherapy.

Day of endoscopic drainage	Group 1	Group 2	*p*-value
1.			0.6222
Mean (SD)	136.7 (90.0)	125.2 (81.4)	
Range	11.5-450.7	13.3-308.7	
3.			0.6990
Mean (SD)	97.4 (78.2)	83.1 (61.4)	
Range	11.2-315.1	9.4-306.7	
5.			0.5895
Mean (SD)	64.2 (74.5)	47.4 (45.4)	
Range	7.9-333.1	3.0-234.5	
7.			0.2571
Mean (SD)	42.6 (52.8)	27.7 (32.5)	
Range	0.9-232.0	3.4-167.6	
10.			0.0083
Mean (SD)	41.1 (43.7)	19.3 (19.4)	
Range	5.7-196.5	0.9-86.1	
15.			0.2628
Mean (SD)	18.6 (12.6)	16.0 (17.4)	
Range	4.5-45.2	3.3-71.5	

TABLE 6 | Comparison of the results of leukocytes (mm^3) in groups of patients during endotherapy.

Day of endoscopic drainage	Group 1	Group 2	*p*-value
1.			0.4592
Mean (SD)	14.5 (5.0)	13.6 (4.7)	
Range	6.5-28.8	6.6-24.4	
3.			0.8360
Mean (SD)	13.3 (5.5)	12.3 (3.2)	
Range	6.2-30.0	6.8-22.0	
5.			0.6309
Mean (SD)	11.7 (4.8)	10.6 (2.5)	
Range	6.8-31.7	6.1-17.9	
7.			0.1797
Mean (SD)	10.8 (4.3)	9.4 (2.1)	
Range	6.0-30.0	6.1-14.5	
10.			0.0791
Mean (SD)	10.6 (3.1)	9.1 (1.8)	
Range	6.1-22.5	6.6-11.8	
15.			0.9158
Mean (SD)	9.4 (1.1)	9.4 (1.6)	
Range	7.9-11.1	6.6-11.8	

and *Streptococcus* spp. (Olson and Allen, 1989; Stamatakos et al., 2010). Antibiotics for the treatment of infected PPPFCs may include the following agents used in monotherapy: imipenem, meropenem, piperacillin with tazobactam, or combination therapies consisting of metronidazole and one of the following antibiotics: ceftriaxone, cefotaxime, ceftazidime, and ciprofloxacin (Olson and Allen, 1989; Stamatakos et al., 2010; Villatoro et al., 2010; Tenner et al., 2013; Working Group IAP/APA, 2013; Arvanitakis et al., 2018; Crockett et al., 2018). In empirical antibiotic therapy, antibiotic agents should be selected based on good organ penetration (Tenner et al., 2013; Working Group IAP/APA, 2013; Arvanitakis et al., 2018; Crockett et al., 2018). In the case of targeted antibiotic therapy, the antibiotic agent should be selected mainly based on swab culture/antibiogram results (Olson and Allen, 1989; Stamatakos et al., 2010; Villatoro et al., 2010; Tenner et al., 2013; Working Group IAP/APA, 2013; Arvanitakis et al., 2018; Crockett et al., 2018).

However, it should be stressed that the above recommendations concerning antibiotic therapy in acute pancreatitis only apply to patients receiving conservative rather than interventional treatment (Tenner et al., 2013; Working Group IAP/APA, 2013; Arvanitakis et al., 2018; Crockett et al., 2018). No recommendations regarding antibiotic therapy are available for patients with acute pancreatitis undergoing interventional procedures (Arvanitakis et al., 2018).

Irrespective of the technique used in minimally invasive treatment of the sequelae of acute pancreatitis, the essence of interventional treatment consists of drainage of the liquid contents (Loveday et al., 2008; Freeman et al., 2012; da Costa et al., 2014; Szeliga and Jackowski, 2014; Jagielski et al., 2018; Jagielski et al., 2020). Efficient drainage facilitates the free evacuation of liquid content from the collection cavity (Arvanitakis et al., 2018; Jagielski et al., 2018; Jagielski et al., 2020; Jagielski and Jackowski, 2021). The establishment of an appropriate drainage system is the basis for the effective treatment of pancreatitis complications, such as PPPFCs (Loveday et al., 2008; Freeman et al., 2012; da Costa et al., 2014; Szeliga and Jackowski, 2014; Jagielski et al., 2018; Jagielski et al., 2020). Thus, effective drainage prevents proliferation of microorganisms by continuously draining the contents from the collection cavity during treatment; thus, antibiotic prophylaxis and antibiotic treatment are not required for sterile and infected collections, respectively. Effective transmural drainage of PPPFCs does not require antibiotic therapy, and thus should contribute to reducing the abuse of antibiotics.

An abscess is an inflammatory collection formed during an infection that is filled with purulent material (Sartelli et al., 2017; Perrone et al., 2020). For years, drainage has been known to be an effective method of abscess treatment (Sartelli et al., 2017; Perrone et al., 2020). An abdominal abscess is a collection of cellular debris, enzymes, and liquefied remains from an infectious or noninfectious source (Sartelli et al., 2017; Perrone et al., 2020). A separate subgroup of abdominal abscesses consists of retroperitoneal abscesses (Sartelli et al., 2017; Perrone et al., 2020), including contaminated PPPFCs localized in this region resulting from pancreatitis, that is, infected pancreatic pseudocysts and WOPN. Abdominal sepsis control is the recommended treatment for abdominal fluid collection (abscesses), including post-inflammatory peritoneal fluid collection (Sartelli et al., 2017; Perrone et al., 2020). Indeed, antibiotic therapy is recommended and even necessary to control infection until interventional treatment of post-inflammatory PPFCs is initiated (Tenner et al., 2013; Working Group IAP/APA, 2013; Arvanitakis et al., 2018; Crockett et al., 2018). However, when interventional treatment is initiated, that is, effective drainage is accomplished using endoscopic techniques, the infection is controlled by effective transmural drainage; therefore, ongoing antibiotic treatment should not be continued. In the case of infected PPPFCs, interventional management is based on the evacuation of infected necrotic content inside and outside the pancreas, which is key to the successful treatment of the sequelae of acute pancreatitis and the control of infection (Loveday et al., 2008; Freeman et al., 2012; da Costa et al., 2014; Szeliga and Jackowski, 2014; Jagielski et al., 2018; Jagielski et al., 2020).

As stated above, the reduction in antibiotic use in daily clinical practice is consistent with the principles of rational antibiotic therapy. Abuse of antibiotics leads to increased costs of medical procedures and possible allergic reactions to drugs; however, it also increases the risk of drug resistance against different types of microorganisms. Irrational antibiotic therapy, understood as the excessive and unjustified use of antibiotics, can lead to secondary infections, such as colitis caused by *Clostridium difficile* or fungal infections.

This study is the first randomized trial to prove that the effective endoscopic drainage of sterile PPPFCs requires no preventive or prophylactic use of antibiotics. In infected PPPFCs, antibiotic therapy is not required for effective endoscopic transmural drainage.

The main limitation of our study is that it was conducted on a selected group of patients from one medical center. Future, multi-center studies with larger sample sizes are required to validate our results.

In summary, the effective endoscopic drainage of post-inflammatory PPFCs reduces the use of antibiotics in everyday

clinical practice, which is consistent with the principles of rational antibiotic therapy.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Ethics Committee at the Collegium Medicum of Nicolaus Copernicus University (Approval Number KB 294/2020). The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

Conceptualization was performed by MatJ, WK, JP, and MarJ. Formal analysis was performed by MatJ, WK, JP, and MarJ. The methodology was performed by MatJ, WK. Project administration was performed by WK, JP, and MarJ. Endoscopic procedures was performed by MatJ. The writing of the original draft was performed by MatJ, WK and JP. Editing was performed by MatJ, and MarJ. All authors (MatJ, WK, JP, and MarJ) revised and approved the submitted version of the manuscript.

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Early *Versus* Delayed Enteral Feeding in Predicted Severe Acute Gallstone Pancreatitis: A Retrospective Study

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Background: The optimal timing of enteral nutrition (EN) initiation in predicted severe acute gallstone pancreatitis (SAGP) and its influence on disease outcomes are not well known.

Methods: We conducted a retrospective study of patients with predicted SAGP treated with endoscopic retrograde cholangiopancreatography and EN. The patients were classified into two groups according to the timing of EN initiation after admission: within 48 h, and more than 48 h. The primary outcome was in-hospital mortality. The secondary outcomes were length of hospital stay, need for intensive care admission, need for surgical intervention, improvements in blood test results after 7-10 days of EN, incidence of pancreatic necrosis and infection, and hospital care costs. The microbiological profiles of infectious complications were also evaluated.

Results: Of the 98 patients, 31 and 67 started EN within 48 h, and more than 48 h after admission, respectively. Early EN was associated with a decrease in in-hospital mortality (0 vs. 11.9%; p=0.045), length of hospital stay (median:18 *vs*. 27 days; p=0.001), need for intensive care admission (3.2% *vs*. 20.9%; p=0.032), and hospital care costs (median:9,289 *vs*. 13,518 US$; p=0.007), compared to delayed EN. Moreover, early EN for 7-10 days had more beneficial effects on blood test results than delayed EN, including total protein (p=0.03) and CRP (p=0.006) levels. However, the need for surgical intervention and incidence of pancreatic necrosis did not differ between the two groups. In our study, Gram-negative bacteria were the main responsible pathogens (50.5%). Infection with multidrug-resistant organisms (MDRO) was found in 19.4% of the patients. The most common MDRO was MDR *Enterococcus faecium*. Early EN was not superior in reducing incidence of infected pancreatic necrosis, bacteremia, polymicrobial infection, or MDROs.

Conclusions: In patients with predicted SAGP, early EN is associated with a decrease in in-hospital mortality, length of hospital stay, need of intensive care admission, and hospital care costs, compared to delayed EN. There are no significant benefits of early EN in reducing the rate of infection-related complications. Further studies with larger sample sizes are warranted.

Keywords: pancreatitis, enteral nutrition, infection, mortality, multiple drug resistant bacteria

INTRODUCTION

Acute pancreatitis (AP) is the most common pancreatic disease in the world. Severe acute pancreatitis (SAP) develops in approximately 20–30% of patients with AP and is associated with a high mortality rate of up to 30%. (Leppaniemi et al., 2019) Gallstones are the most common cause of AP. (Yadav and Lowenfels, 2013) When accompanying biliary obstruction occurs, patients with gallstone pancreatitis are recommended to undergo endoscopic retrograde cholangiopancreatography (ERCP) to remove obstructing gallstones, in order to ameliorate the disease course. (Arvanitakis et al., 2018) The traditional approach for treating AP patients has been based on bowel rest. However, current guidelines recommend early (generally within 24 h) enteral nutrition (EN) for AP, to maintain the gut mucosal barrier and prevent infected necrosis. (Crockett et al., 2018) Nonetheless, there is still a need to more precisely define the optimal timing for initiating early EN in patients with predicted severe acute gallstone pancreatitis (SAGP). Moreover, knowledge on the effect of early EN on the bacteria/fungi spectrum and antibiotic resistance characteristics of AP is limited.

Therefore, we conducted a single-center retrospective study in patients with SAGP, comparing the efficacy of early EN (within 48 h of admission) with delayed EN (after more than 48 h of admission), and further investigated the microbiological profiles of infectious complications associated with different routes of nutritional support.

MATERIALS AND METHODS

Study Design and Participants

A retrospective analysis was performed on consecutive patients diagnosed with predicted SAGP and treated with ERCP and EN from March 2012 to March 2022 in a tertiary care hospital. The exclusion criteria were as follows: age ≤ 18 years; pregnancy; pancreatitis due to other causes such as hyperlipemia, alcohol abuse, chronic pancreatitis, etc.; patients who did not receive ERCP and/or EN; patients presenting to the hospital more than four days after symptom onset; or no data on any of the primary or secondary outcomes.

We defined EN initiation within 48 h of admission as early EN, and initiation more than 48 h of admission as delayed EN. AP was diagnosed as the presence of at least two of the following criteria: typical abdominal pain, a serum lipase or amylase level that was more than three times the upper limit of normal (ULN), or characteristic findings on imaging. (Banks et al., 2012) Gallstone pancreatitis was diagnosed by either biliary sludge or gallstones on imaging, a dilated common bile duct on imaging, or an alanine aminotransferase concentration of more than twice the ULN. (Tenner et al., 2013) Predicted SAP was defined based on an Acute Physiology and Chronic Health Evaluation (APACHE-II) score of eight or more, a Bedside Index of Severity in AP (BISAP) score of two or more, a computed tomography severity index (CTSI) score of three or more, or serum C-reactive protein concentration higher than 150 mg/L within 24 h of admission. (Papachristou et al., 2010; Cho et al., 2015) System Inflammatory Reaction Syndrome (SIRS) was defined as the presence of at least two of the following parameters for a continuous period of 48 h: body temperature > 38°C or < 36°C heart rate > 90 beats per minute; hyperventilation with a respiratory rate > 20 breath per minute or a $PaCO_2$ < 32 mmHg; and white blood cell count > 12,000/mm^3 or <4000/mm^3. (Bone et al., 1992) Organ failure was defined as a modified Marshall score of 2 or more as proposed in the revised Atlanta classification of acute pancreatitis. (Banks et al., 2012) Cholestasis was defined as a serum bilirubin of more than 2.3 mg/dL (40 μmol/L) or a dilated common bile duct (> 8 mm in patients aged ≤ 75 years, or >10 mm in patients aged > 75 years). (Schepers et al., 2020) Cholangitis was defined as fever in combination with either common bile duct stones, a dilated common bile duct, or (progressive) cholestasis. (Schepers et al., 2020) Isolation of pathogens was defined as a positive culture obtained from blood, and/or the drainage of percutaneous, endoscopic procedure, or surgery.

Procedures

In the present study, ERCP was performed within 48 h of admission. Considering that some patients had two or more ERCP procedures during their hospitalization, we only evaluated the first ERCP session. Biliary sphincterotomy was performed when the presence of bile duct stones was confirmed. If the common bile duct could not be cannulated, precut sphincterotomy was performed. Stones or sludge were extracted using baskets and/or balloons, if indicated. Stents or nasobiliary tubes were placed at the discretion of endoscopists. A nasoenteral feeding tube was placed endoscopically. According to the timing of EN initiation, patients were classified into one of two groups: early EN group, in which the nasoenteral feeding tube was placed in the same session with ERCP, and EN was initiated within 48 h of admission; and delayed EN group, in which the timing of both nasoenteral feeding tube placement and EN initiation was more than 48 h of admission. Enteral nutritional suspension Peptison (NUTRICIA, Wuxi, China) was preheated to 100 degrees Fahrenheit and then initiated at a speed of 25 ml/h; the speed was increased by 10 ml/h every 6 h if tolerated, until the desired caloric intake of patients was reached. Oral feeding was reintroduced when the serum amylase level decreased to < 2-fold ULN and abdominal pain had resolved. Along with ERCP and EN, the patients were treated with standard medical therapy for AP, fluid resuscitation, and other supportive therapies for organ failure. Antibiotics were prescribed only if bacterial infection was confirmed. Third generation cephalosporins were initially used as antibiotics. Samples from blood, bile, or peri-pancreatic fluid were gram- and fluorescence -stained to identify pathogens. Isolated pathogens were tested for antimicrobial susceptibility using the VITEK2 compact automated microbiology system

(BioMerienx, France), based on the standards of the Clinical and Laboratory Standards Institute. (Humphries et al., 2018)

Data Collection

The following data were obtained using data collection sheets from electronic medical records: age, sex, body-mass index, evidence of gallstone etiology (such as gallstones or sludge, cholestasis, or cholangitis), and disease severity (such as APACHE-II score, CTSI score, BISAP score, and presence of SIRS or organ failure) within 12 h of admission; the time from pancreatitis onset to admission, the time from admission to ERCP, the time from admission to initiation of EN, and the time from initiation of EN to initiation of oral feeding; laboratory data including serum levels of total bilirubin, total protein, urea, white blood cell, procalcitonin and C-reactive protein (CRP) within 12 h of admission and after 7-10 days of EN; characteristics of pathogens isolated during hospitalization based on a blood culture, bile culture, or local culture; and radiological data including characteristics of contrast enhanced CT scan within 12 h of admission and after 7-10 days of EN, and characteristics of first ERCP after admission.

Outcomes

The primary outcome was in-hospital mortality. Secondary outcomes were the length of hospital stay, need for intensive care admission, need for surgical intervention, improvements in blood test results after 7-10 days of EN (negative value indicated that the value of the result became larger after treatment), incidence of pancreatic necrosis, infected pancreatic necrosis, bacteremia, polymicrobial infection, multidrug-resistant organism (MDRO), and hospital care costs. Pancreatic necrosis was defined as the presence of diffuse or focal areas of pancreatic non-enhancement on contrast enhanced CT. (Banks et al., 2012) Infected pancreatic necrosis was diagnosed when positive culture from peripancreatic fluid collection was demonstrated, or the presence of gas within the collection was seen on CT. (Banks et al., 2012) Bacteremia was defined as an infection with positive blood cultures. Surgical interventions included endoscopic, laparoscopic, or laparotomy for drainage or necrosectomy in infected pancreatic necrosis. MDRO was defined as acquired non-susceptibility to at least one agent in three or more antimicrobial categories. (Magiorakos et al., 2012)

Statistical Analysis

IBM SPSS Statistics version 22 was used for statistical analysis. Categorical variables were expressed as frequencies and percentages. The means and standard deviation were used for quantitative variables with parametric distribution, and the medians and interquartile range (IQR) were used for variables with nonparametric distributions. Dichotomous data were compared using the Pearson's χ^2 test or Fisher's exact test, and continuous data with the Student's t-test for those with parametric distribution and the Mann-Whitney U test were used for those with non-parametric distribution. A two-sided p value of less than 0.05 was considered to indicate statistical significance.

RESULTS

Baseline Characteristics of Patients

A total of 98 patients with a predicted SAGP were finally included, of which 31 (31.6%) were in the early EN group and 67 (68.4%) were in the delayed EN group. Baseline characteristics, presented in **Table 1**, were equally distributed between the two groups, except for the time from admission to ERCP (median [IQR]:13 [3-25] *vs*. 27 [9-64] h; p<0.05), and the time from admission to initiation of EN (median [IQR]:30 [15-33] *vs*. 128 [82-216] h; p=0.035). The time from pancreatitis onset to admission (median [IQR]:2 [2-3] *vs*. 2 [1-3] days; p=0.84) and the time from initiation of EN to initiation of oral feeding (mean [SD]:15 [14] *vs*. 16 [20] days; p=0.92) were similar between the two groups.

Clinical Outcomes

Table 2 reports the clinical outcomes between the two groups. The overall in-hospital mortality was 8.2% (8/98). All deaths occurred in patients with delayed EN, and a significant difference was noted between the two groups (p=0.045). All patients that died were in the intensive care unit because of multiple organ failure. Early EN was associated with a shorter length of hospital stay (median [IQR]: 18 [12-30] *vs*. 27 [18-43] days; p=0.001) and a lower rate of intensive care admission (3.2% *vs*. 20.9%; p=0.032) than delayed EN. The need for surgical intervention (0% *vs*. 7.5%; p=0.18) and pancreatic necrosis incidence (87.0% *vs*. 91.0%; p=0.72) were similar between the two groups. After 7-10 days of EN support, there was a more significant improvement in total protein (median [IQR]: -3.9 [-9.2-1.3] *vs*. 2.1 [-5.4-6.1] g/L; p=0.03) and CRP (median [IQR]: 129.0 [63.8-177.4] *vs*.31.7 [-6.1-123.8] g/L; p=0.006) levels in patients from the early EN group compared to those from the delayed EN group. The improvements of total bilirubin (median [IQR]: 2.9 [-0.5-10.8] *vs*. 11.7 [5.8-25.1] μmol/L; p=0.31) and white blood cell (median [IQR]: 4.0 [0.8-6.6] *vs*. 4.4 [0.1-7.5] 10^3/μL; p=0.72) levels were similar between the two groups. Other blood test results (urea and procalcitonin) were not included in the final analysis because of sparse data. No significant difference was observed between the two groups in the incidence of infected pancreatic necrosis (3.2% *vs*. 16.4%; p=0.096), bacteremia (0 *vs*. 10.4%; p=0.094), polymicrobial infection (16.1 *vs*. 29.8%; p=0.15), or MDRO (16.1 *vs*. 20.9%; p=0.78). The median hospital care costs per patient were $9,289 in the early EN group compared with $13,518 in the delayed EN group, in favor of the early EN group (p=0.007).

ERCP Characteristics

The ERCP characteristics were similar between the two groups (**Table 3**). Intact papilla accounted for 96.8% of the patients in the early EN group compared with 82.1% in the delayed EN group. In the

TABLE 1 | Baseline demographic and clinical characteristics of patients.

	Early EN (n=31)	Delayed EN (n=67)	p Value
Age, yrs	71 (50-79)	65 (57-71)	0.65
Female sex	12 (38.7%)	33 (49.3%)	0.33
Basis of gallstone etiology†			0.58
Gallstones or sludge	24 (77.4%)	53 (79.1%)	
Cholestasis	13 (41.9%)	38 (56.7%)	
Cholangitis	20 (64.5%)	36 (53.7%)	
Body-mass index, kg/m^2	23.7 (4.1)	24.8 (3.4)	0.32
Disease severity			
APACHE-II	7.2 (2.9)	7.2 (2.9)	0.52
CTSI	2.2 (1.1)	3.3 (2.4)	0.11
BISAP	2.2 (0.8)	2.3 (0.8)	0.61
CRP, mg/L	169.0 (104.9-264.4)	141.4 (62.2-202.8)	0.07
SIRS	19 (61.3%)	37 (55.2%)	0.57
Organ failure	7 (22.6%)	16 (23.9%)	0.89
Tests on admission to hospital			
Total bilirubin, μmol/L	28.6 (15.8-40.3)	31.7 (17.8-54.7)	0.27
Total protein, g/L	62.0 (55.5-67.9)	63.2 (59.1-70.1)	0.14
Urea, mmol/L	5.19 (3.6-6.8)	6.5 (5.0-8.2)	0.14
White blood cell, 10^3/μL	15.2 (11.7-17.3)	12.6 (9.7-15.8)	0.17
Procalcitonin, ng/ml	5.33 (17.3)	5.26 (11.4)	0.62
Time from pancreatitis onset to admission, days	2 (2-3)	2 (1-3)	0.84
Time from admission to first ERCP, hrs	13 (3-25)	27 (9-64)	**<0.05**
Time from admission to initiation of EN, hrs	30 (15-33)	128 (82-216)	**0.035**
Time from initiation of EN to initiation of oral feeding, days	15 (14)	16 (20)	0.92

Data expressed as n (%), mean (SD), or median (IQR).
The bold values indicate that there was a significant difference.
APACHE, acute physiology and chronic health evaluation; BISAP, Bedside Index of Severity in acute pancreatitis; CRP, C-reactive protein; CTSI, computed tomography severity index; EN, enteral nutrition; ERCP, endoscopic retrograde cholangiopancreatography; SIRS, systemic inflammatory response syndrome.
†One case may involve one or more gallstone etiology.

TABLE 2 | Clinical Outcomes.

Outcome	Early EN (n=31)	Delayed EN (n=67)	p Value
Mortality	0	8 (11.9%)	**0.045**
Length of hospital stay, days	18 (12-30)	27 (18-43)	**0.001**
Intensive care admission	1 (3.2%)	14 (20.9%)	**0.032**
Surgical intervention	0	5 (7.5%)	0.18
Improvements of blood tests results After 7-10 days of EN			
Total bilirubin, μmol/L	2.9 (-0.5-10.8)	11.7 (5.8-25.1)	0.31
Total protein, g/L	-3.9 (-9.2-1.3)	2.1 (-5.4-6.1)	**0.03**
White blood cell, 10^3/μL	4.0 (0.8-6.6)	4.4 (0.1-7.5)	0.72
CRP, mg/L	129.0 (63.8-177.4)	31.7 (-6.1-123.8)	**0.006**
Pancreatic necrosis	27 (87.0%)	61 (91.0%)	0.72
Infected pancreatic necrosis	1 (3.2%)	11 (16.4%)	0.096
Bacteremia	0	7 (10.4%)	0.094
Polymicrobial infection	5 (16.1%)	20 (29.8%)	0.15
MDRO	5 (16.1%)	14 (20.9%)	0.78
Hospital care costs, US$	9289 (6263-12844)	13518 (10106-20392)	**0.007**

Data expressed as n (%), or median (IQR).
The bold values indicate that there was a significant difference.
CRP, C-reactive protein; EN, enteral nutrition; MDRO, multiple drug resistant organism.

early EN group, successful biliary cannulation was achieved in 29 (93.5%) of the 31 patients, 25 of whom underwent sphincterotomy. In the delayed EN group, successful biliary cannulation was achieved in 59 (88.1%) of the 67 patients, 53 of whom underwent sphincterotomy. In all 10 patients, biliary cannulation failed because of edematous papilla. Precut sphincterotomy was performed in 6 (19.4%) of the 31 patients in the early EN group, and in 15 (22.4%) of the 31 patients in the delayed EN group. ERCP-related complications were observed in only one patient in the delayed EN group (1.5%); this complication was a post-sphincterotomy bleeding incident, and pinpoint hemostasis was achieved using a clip during ERCP.

TABLE 3 | ERCP characteristics.

	Early EN (n=31)	Delayed EN (n=67)	p Value
Intact papilla, n (%)	30 (96.8)	55 (82.1)	0.06
Technical success, n (%)	29 (93.5)	59 (88.1)	0.49
Pancreatic duct cannulation (unintentional), n (%)	6 (19.4)	22 (32.8)	0.23
Sphincterotomy, n (%)	25 (80.6)	53 (79.1)	0.86
Precut sphincterotomy, n (%)	6 (19.4)	15 (22.4)	0.73
Stone extraction, n (%)	14 (45.2)	22 (32.8)	0.24
ENBD, n (%)	25 (80.6)	47 (70.1)	0.27
ERBD, n (%)	3 (9.7)	11 (16.4)	0.54
ERPD, n (%)	10 (32.3)	27 (40.3)	0.45
ERCP-related complication, n (%)	0	1 (1.5)	0.49

EN, enteral nutrition; ENBD, endoscopic nasobiliary drainage; ERBD, endoscopic retrograde biliary drainage; ERCP, endoscopic retrograde cholangiopancreatography; ERPD, endoscopic retrograde pancreatic drainage.

Spectrum and Distribution of Pathogens

A total of 91 pathogenic bacterial strains were isolated from 60 specimens, including 47 transpapillary drainage specimens, 7 blood specimens, and 6 percutaneous drainage specimens (**Table 4**). Forty-six Gram-negative bacterial strains accounted for 50.5% of the isolates, 36 Gram-positive bacterial strains for 39.6%, and 9 fungi for 9.9%. Among the Gram-negative bacteria, the main pathogens were *Klebsiella pneumoniae*, *Stenotrophomonas maltophilia*, *Escherichia coli*, and *Enterobacter cloacae* (in descending order of infection frequency). *Enterococcus faecium*, *Enterococcus faecalis*, and *Staphylococcus* spp. were the main Gram-positive bacteria. *Candida albicans* and *Candida tropicalis* were the main fungi. Some patients had polymicrobial infections; however, no significant difference was found between the two groups (16.1% *vs.* 29.8%; p=0.15). (**Table 2**)

Infection with MDRO was diagnosed in 19.4% (19/98) of patients. It occurred in 5 patients (16.1%) in the early EN group, compared to 14 (20.9%) in the delayed EN group. No significant difference was found between the two groups (p=0.78). (**Table 2**) In total, 25 isolates (27.5%) of MDRO were detected, of which 12 (48%) were MDR Gram-negative and 13 (52%) were MDR Gram-positive bacteria. No obvious drug resistance in fungi was observed. The most common MDRO was MDR *Enterococcus faecium* (n=12). Other common pathogens were carbapenem-resistant *Klebsiella pneumoniae* (n=3) and ESBL-producing *Klebsiella pneumoniae* (n=3). The distributions and proportions of the pathogenic bacteria are shown in detail in **Figure 1**.

DISCUSSION

The present study demonstrated that early EN in predicted SAGP was associated with a decrease in in-hospital mortality, length of hospital stay, need for intensive care admission, and hospital care costs, compared with delayed EN. In addition, early EN had more beneficial effects on blood test results than delayed EN, including total protein and CRP levels.

The fact that early EN is beneficial in AP is supported by experimental and clinical data. (Gupta et al., 2003; Yi et al., 2012) Guidelines have recommended early oral feeding in mild AP. (Tenner et al., 2013) In patients with predicted SAP, hospital stay is typically prolonged and patients are often intolerant to oral feeding. In these latter groups of patients, establishing a definite diagnosis of SAP usually occurs between 3 and 5 days after the initial presentation, a time when nasoenteral feeding is recommended to prevent infected necrosis. (Vege et al., 2018) To date, there have been few studies focusing on direct comparisons of the early and delayed timing of initiation of EN in predicted SAP.

A retrospective analysis (Wereszczynska-Siemiatkowska et al., 2013) has compared EN started within 48 h of admission to EN started 48 h after admission in SAP and revealed that early EN reduced infection of pancreatic necrosis or fluid collection, respiratory failure, mortality, and the need for intensive care admission, compared to delayed EN. However, the etiology of AP in that study was heterogeneous, including alcohol and gallstones. Whether the ERCPs performed in both study arms were comparable is unknown, and this may have caused potential bias. Nakashima et al. noted that EN initiation within 48 h after the diagnosis of SAP was associated with reduced mortality compared with late EN. Moreover, EN within 24 h may not confer more benefits than EN between 24 and 48 h. (Nakashima et al., 2021) However, none of the other clinical outcomes were evaluated.

Our study is the largest observational study to date focusing on patients with predicted SAGP who underwent ERCP. Our results are consistent with some of the data from the above-mentioned studies. These findings seem to corroborate the theory that maintaining EN modulates the acute-phase response and preserves visceral protein metabolism. (Kudsk et al., 1994) Moreover, early EN is thought to help protecting the gut mucosal barrier, thereby reducing bacterial translocation and the risk of infectious complications. (Windsor et al., 1998) However, our study did not show the superiority of early EN in reducing the rate of infection-related complications. There are several possible explanations for this negative result. First, tube feeding in the early EN group should have been started even earlier, for example, within 24 h of

TABLE 4 | Total microorganisms and multidrug resistant microorganisms.

	Isolates, NO. (%)
Total microorganisms	**91**
Total isolated GNB	46 (50.5)
Klebsiella pneumoniae	13 (14.3)
Stenotrophomonas maltophilia	10 (11.0)
Escherichia coli	6 (6.6)
Enterobacter cloacae	4 (4.4)
Acinetobacter baumannii	3 (3.3)
Pseudomonas aeruginosa	2 (2.2)
Enterobacter aerogenes	2 (2.2)
Citrobacter freundii	2 (2.2)
Others	4 (4.4)
Total isolated GPB	36 (39.6)
Enterococcus faecium	24 (26.4)
Enterococcus faecalis	6 (6.6)
Staphylococcus aureus	1 (1.1)
Streptococcus viridans	1 (1.1)
Staphylococcus hominis	1 (1.1)
Staphylococcus haemolyticus	1 (1.1)
Staphylococcus epidermidis	1 (1.1)
Others	1 (1.1)
Fungus	9 (9.9)
Candida albicans	5 (5.5)
Candida tropicalis	2 (2.2)
Candida parapsilosis	1 (1.1)
Other yeast-like fungi	1 (1.1)
Multidrug resistant bacteria	**25**
Total isolated multidrug resistant GNB	12 (48)
Carbapenem-resistant *Klebsiella pneumoniae*	3 (12)
ESBL-producing *Klebsiella pneumoniae*	3 (12)
ESBL-producing *Enterobacter cloacae*	1 (4)
ESBL-producing *Escherichia coli*	1 (4)
MDR *Stenotrophomonas maltophilia*	1 (4)
Carbapenem-resistant *Stenotrophomonas maltophilia*	1 (4)
Carbapenem-resistant *Pseudomonas aeruginosa*	1 (4)
Carbapenem-resistant *Klebsiella oxytoca*	1 (4)
Total isolated multidrug resistant GPB	13 (52)
MDR *Enterococcus faecium*	12 (48)
MDR Other G+ bacilli	1 (4)
Origin of specimen	**60**
Trans-papillary drainage	47 (78.3)
Blood	7 (11.7)
Percutaneous drainage	6 (10.0)

ESBL, extended-spectrum beta-lactamase; GNB, Gram-negative bacteria; GPB, Gram-positive bacteria; MDR, Multidrug resistant. The bold values represent sum total of isolates.

admission. However, in daily practice, patients with SAP are often intolerant to an earlier start of tube feeding. In our cohort, only 11 patients were administered EN within 24 h of admission, which was not sufficient to detect differences between the study arms. Second, our cohort included a large proportion of patients with cholangitis (57.1%), which could have influenced the final results associated with infection. Third, we observed that early EN was associated with a trend toward a decreased incidence of infectious complications, as shown in **Table 2**. The differences between the two groups were not significant, probably because of the small sample size. Additional studies with larger sample sizes are warranted to further investigate this.

In the early EN group in our study, a nasoenteral feeding tube was placed in the same session as the ERCP. This would relieve the suffering of patients by avoiding another step for tube placement, and would reduce the risk of repeated anesthesia, as well as hospital care costs. It's worth noting that nasoenteral tube feeding frequently causes excessive nausea, abdominal distension, or diarrhea. Enteral nutritional fluids preheated and started at a slow rate were effective in decreasing adverse reactions in our study.

The present study indicates that infection with MDRO was found in 19.4% of the patients, which was generally lower than the level reported in previous studies. (Lee et al., 2014; Tian et al., 2020) This could be explained by the restricted use of prophylactic antibiotics in our study. MDR pathogens may be selected by unrestricted usage of broad-spectrum antibiotics. (Behrman et al., 2011) Whereas a high frequency of enteric bacilli might have been anticipated, our results were noteworthy for the high detection rate of Gram-positive bacteria in contrast to the results of a previous study. (Zhu et al., 2021) The most common MDRO in our study was MDR *Enterococcus faecium*. There are several possible explanations for this finding. First, all participants underwent ERCP; hence, duodenoscope-transmitted MDR bacterial infections should also be considered. (Balan et al., 2019) The nosocomial infections after ERCP are more polymicrobial, with a predominance of Gram-positive bacteria. Moreover, the presence of central venous catheters and abdominal drainage may also increase the risk of nosocomial infections. Second, long-term use of broad-spectrum antibiotics would alter the bacteriology of pancreatic infections in SAP from predominantly Gram-negative coliforms to predominantly Gram-positive organisms. (Howard and Temple, 2002)

The findings of this study should be interpreted in the context of its limitations. First, the results of our study were derived from a single-center experience. Therefore, the specific microbiological profiles cannot be generalized to other hospitals. Second, patients with concomitant cholangitis were included in this study; therefore, the incidence of infections due to SAP may have been overestimated, although the basis of gallstone etiology in both study arms is comparable. Third, scoring systems for the prediction of severity of AP are only moderately accurate, which could lead to the inclusion of patients who are initially classified as high risk for SAP, but who eventually develop mild pancreatitis. Fourth, the retrospective nature of the study increases the likelihood of recall and selection biases. Fifth, the sample size in the early EN group was relatively small. Thus, the interpretation of our results is limited by the difference in the sample size between the two groups; additional future comparisons with balanced data are therefore required.

CONCLUSION

In conclusion, the present study demonstrates that in patients with predicted SAGP, early EN started within 48 h of admission is associated with a decrease in in-hospital mortality, length of hospital stay, need for intensive care admission, and hospital care costs, compared to delayed EN started after 48 h. However, the need for

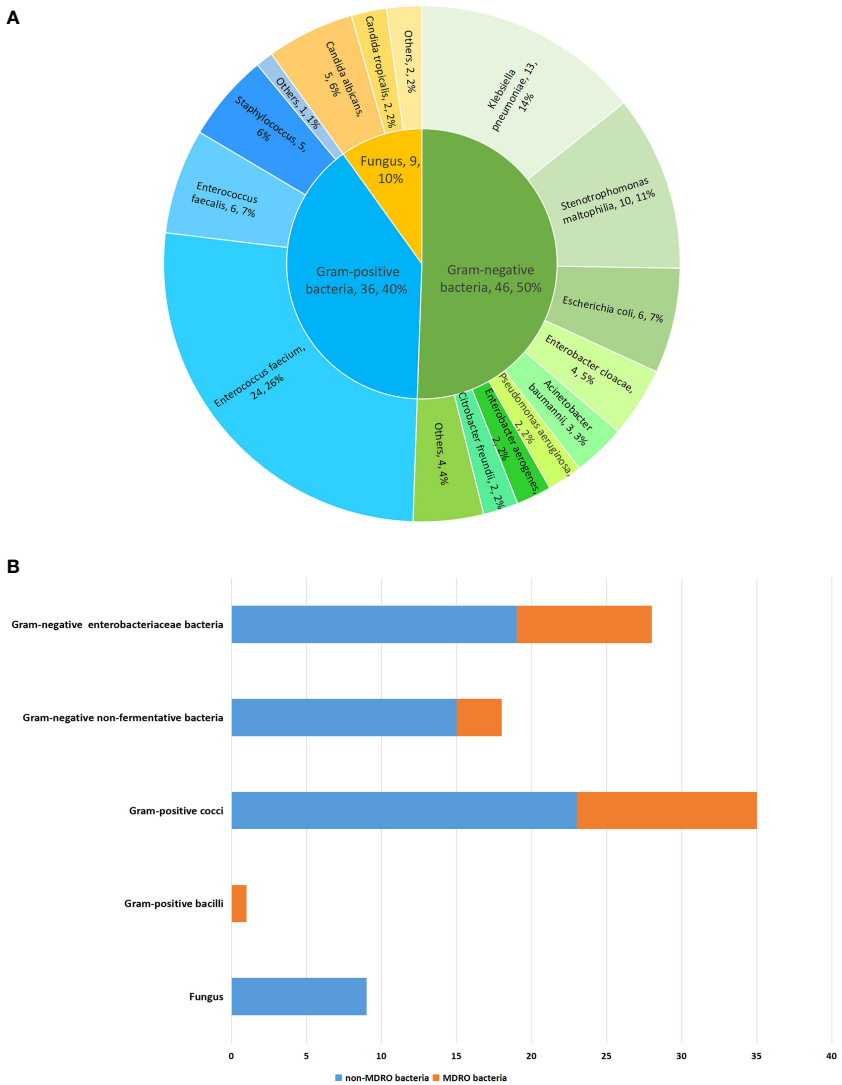

FIGURE 1 | The distribution and proportion of pathogenic bacteria in 98 patients with predicted severe acute gallstone pancreatitis. **(A)** The distribution and proportion of pathogenic bacteria. **(B)** The distribution and proportion of pathogenic bacteria by dividing of MDRO.

surgical intervention, and incidence of pancreatic necrosis do not differ between the two groups. In our study, Gram-negative bacteria are the main responsible pathogens. Infection with MDRO is found in 19.4% of the patients. The most common MDRO is MDR *Enterococcus faecium*. Early EN is not superior in reducing incidence of infected pancreatic necrosis, bacteremia, polymicrobial infection, and MDROs. Further studies with larger sample sizes are required to confirm our results.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by ethics committee of the Hangzhou First People's Hospital. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

HJ and XZ: study design, reviewing, and final approval; ZJ: analysis, manuscript writing, and reviewing; YW and SH: data collection and manuscript reviewing; MS, MF, and HS: data collection; JY: manuscript reviewing; All authors contributed to the article and approved the submitted version.

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Pancreatic colonization of fungi in the development of severe acute pancreatitis

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Acute pancreatitis is a common emergent disorder, a significant population of which develops the life-threatening condition, called severe acute pancreatitis (SAP). It is generally accepted that bacterial infection is associated with the development and persistence of SAP. In addition to bacterial infection, recent clinical studies disclosed a high incidence of fungal infection in patients with SAP. Moreover, SAP patients with fungal infection exhibit a higher mortality rate than those without infection. Although these clinical studies support pathogenic roles played by fungal infection in SAP, beneficial effects of prophylactic anti-fungal therapy on SAP have not been proved. Here we summarize recent clinical findings as to the relationship between fungal infection and the development of SAP. In addition, we discuss molecular mechanisms accounting for the development of SAP in the presence of fungal infection.

KEYWORDS

acute pancreatitis, fungi, intestinal barrier, walled-off necrosis, cytokines

Introduction

Acute pancreatitis (AP) is a sudden-onset gastrointestinal disorder, which usually requires hospitalization. Although most cases with AP are self-limiting, a significant fraction of patients with AP develops a life-threatening condition, called severe acute pancreatitis (SAP). Indeed, the mortality rate of SAP is estimated to be approximately 20% (Boxhoorn et al., 2020). Despite such a high mortality rate of SAP, no curative treatments have been established in AP since the pathogenesis of this emergent disorder has been poorly understood.

Over-activation of pancreatic digestive enzymes, especially trypsinogen, followed by autodigestion of the pancreatic tissues underlies the pathogenesis of AP (Watanabe et al., 2017). In the steady state, conversion of trypsinogen into trypsin occurs in the duodenum upon exposure to enterokinase (Logsdon and Ji, 2013). Excessive drinking of alcohol and/or intake of high fatty foods sometimes initiates ectopic and intrapancreatic activation of trypsinogen followed by autodigestion (Watanabe et al., 2017). This trypsin-centered view regarding the pathogenesis of AP has been supported by the fact that human hereditary

pancreatitis arises from mutations of genes associated with trypsinogen activation (Whitcomb, 2010). It should be noted, however, that the pathogenesis of AP cannot be explained by the trypsin-centered view alone. Recent studies provide evidence that mice deficient in T7 trypsinogen, an isoform equivalent to cationic human trypsinogen, still get experimental acute and chronic pancreatitis (Dawra et al., 2011; Sah et al., 2013). These surprising experimental data strongly support the view that AP is driven by multiple factors and that ectopic activation of trypsinogen is one of pathogenic triggers for acute pancreatic injury.

It is well established that AP is characterized by intestinal barrier dysfunction caused by pro-inflammatory cytokine responses. Leaky gut syndrome induced by AP allows translocation of gut microorganisms into the pancreas and thereby promotes further pro-inflammatory cytokine responses (Watanabe et al., 2017). SAP leads to local and systemic complications such as pancreatic necrosis and sepsis (Frossard et al., 2008; Lankisch et al., 2015). These local and systemic complications have been considered to arise from invasion of the pancreatic tissue by gut bacteria and subsequent dissemination to systemic organs leading to endotoxemia. Furthermore, the presence of multiple organ failure and pancreatic necrosis has been identified as the prognostic factors for SAP as shown by the fact that the mortality rate of SAP is much higher in cases with infection than in those without bacterial infection (Petrov et al., 2010). In addition, recent experimental studies support the view that sensing of gut bacteria followed by pro-inflammatory cytokine responses play pathogenic roles in the development of SAP. Recognition of gut Gram-negative bacteria by toll-like receptor 4 (TLR4) and nucleotide-binding oligomerization domain 1 (NOD1) expressed by pancreatic acinar cells and immune cells causes pro-inflammatory cytokine responses in experimental pancreatitis (Sharif et al., 2009; Tsuji et al., 2012; Watanabe et al., 2016). Therefore, these clinical and experimental studies strongly suggest that pro-inflammatory cytokine responses caused by translocation of gut bacteria into the pancreas act in concert with intrapancreatic activation of trypsin to induce SAP. Thus, pancreatic infection with gut bacteria is one of the most pathogenic events in the development of SAP.

Given the fact that gut microorganism community is composed not only of bacteria but also of fungi, it is rational to assume that translocation and colonization of gut fungi into the pancreas is associated with the development of SAP. Indeed, gastroenterologists often encounter with SAP cases exhibiting candidemia (Rasch et al., 2018). Although pathogenic roles played by pancreatic infection with gut bacteria have been established, those played by pancreatic colonization of gut fungi have not been fully understood. In addition, it remains controversial whether pancreatic colonization of gut fungi is a bystander phenomenon or pathogenic event. In this Minireview article, we summarize recent findings as to the relationship between SAP and fungal infection.

Link between bacterial infection and human SAP

AP is classified into interstitial edematous pancreatitis and necrotizing pancreatitis based on the characteristic findings of dynamic computed tomography (CT). Interstitial edematous pancreatitis can be diagnosed by the presence of pancreas swelling alone whereas appearance of hypo-enhancement areas and fluid collection in the pancreas itself or surrounding the pancreas in the dynamic CT study suggests necrotizing pancreatitis. Pancreatic necrosis develops early in the clinical course of SAP (Mederos et al., 2021). Necrotizing pancreatic tissue is further subclassified into acute necrotic collection (ANC) and walled-off necrosis (WON) by the duration displaying fluid collection and necrotic materials. Pancreatic and/or peripancreatic fluid collection and necrotic areas detected within four weeks from the onset are regarded as immature and non-sequestrated forms of pancreatic necrosis and thus called as ANC. Pancreatic necrosis, which persists more than four weeks after the onset, leads to the generation of matured and encapsulated forms of necrotic tissues, called WON (Banks et al., 2013). Although both ANC and WON are composed of fluid and necrotic materials, the latter form, but not the former form, is a matured and encapsulated form of pancreatic necrosis. Discrimination of WON from ANC is very important since necrosectomy is usually required for the treatment of WON (van Santvoort et al., 2010).

Colonization of gut bacteria into the pancreas plays critical roles in the development of ANC or WON and progression of ANC into WON (Dragonetti et al., 1996; van Minnen et al., 2006). In fact, Gram-negative rods in the intestine are the most frequently isolated and identified species from local or blood cultures of pancreatic infections, with *E. coli* being the most frequently reported (Ignatavicius et al., 2012). Moreover, ANC or WON has been implicated as a reservoir for systemic dissemination of gut bacteria (Bradley et al., 2008). Thus, it is no doubt that translocation of gut bacteria into the pancreas followed by colonization and infection is an indispensable step for the development of SAP displaying ANC or WON. Strong support for this idea comes from a meta-analysis using six randomized controlled trials showing that prophylactic antimicrobial therapy reduced mortality and complication rates associated with pancreatic infection (Ukai et al., 2015).

Link between fungal infection and human SAP

Increased intestinal permeability associated with SAP may permit entry not only of bacteria but also of fungi into the pancreas. Therefore, it is likely that translocation of gut fungi into the pancreas promotes the development of SAP displaying ANC or WON. Indeed, there are several reports showing the association between SAP and fungal infection (Kochhar et al., 2011;

Rasch et al., 2018; Singh et al., 2021). As shown in Table 1, the rates of fungal infection as assessed by isolation of blood or pancreatic tissues or by the presence of β-d-glucan antigenemia are highly variable ranging from 7.6% to 46.3% in patients with AP (Kochhar et al., 2009; Vege et al., 2009; Ignatavicius et al., 2012; Werge et al., 2016; Mandal et al., 2017; Rasch et al., 2018; Ning et al., 2021). Such highly variable results can be explained by the difference of severity in patients with AP; the incidence of pancreatic fungal infection is parallel to the severity of AP. In fact, pancreatic infection with fungi occurs in around 40% of SAP patients bearing WON (Werge et al., 2016; Rasch et al., 2018). Rasch et al. examined the incidence of *Candida* infection in patients with pancreatic necrosis and found that *Candida* species were isolated from the pancreatic necrotic tissues in 54 patients among total 136 patients with pancreatic necrosis (Rasch et al., 2018). More importantly, patients positive for pancreatic *Candida* infection exhibited a higher mortality rate as compared with those negative for *Candida* infection (35.2% vs. 13.4%) (Rasch et al., 2018). Such a high mortality rate observed in patients with pancreatic *Candida* infection might be partially explained by the presence of candidemia since the highest mortality rate was observed in patients with both candidemia and pancreatic *Candida* infection (Rasch et al., 2018). Thus, disseminated *Candida* followed by pancreatic infection with this organism or vice versa determines the prognosis of patients with SAP. Consistent with these data, the latest meta-analysis, in which the incidence of *Candida* infection and its impact on mortality were studied in 22 reports, also verified that pancreatic or systemic *Candida* infection increases the mortality (Singh et al., 2021).

In another report, fungal infection has been shown to be associated with the development of WON (Werge et al., 2016). Werge et al. retrospectively analyzed the incidence of fungal infection in SAP patients bearing WON and found that fungi were isolated in necrotic pancreatic tissues or fluids in 57 patients among total 123 patients (Werge et al., 2016). Such high incidence of fungal infection in SAP patients bearing WON strongly suggests involvement of fungal infection in the maturation and encapsulated process of pancreatic necrosis. Confirmation of this idea requires studies directly comparing the incidence of pancreatic fungal infection in SAP patients with ANC or WON. However, the incidence of pancreatic fungal infection in SAP patients bearing ANC has not been examined since necrosectomy is not usually performed and thus pancreatic necrotic tissues are not obtained for the isolation of fungi in such cases. Another important issue that need to be addressed is which is more critical for the prognosis of SAP, bacterial or fungal infection. In this regard, SAP patients with intraabdominal fungal infection exhibited longer hospital and intensive care unit stays than those with bacterial infection (Werge et al., 2016).

Candida species have been identified as the most common organism associated with SAP (Grewe et al., 1999). Chakrabarti et al. reported that *Candida tropicalis* (43.9%) and *Candida albicans* (36.6%) were the most common isolates from pancreatic tissues of 335 SAP patients with fungal infection (Chakrabarti et al., 2007). Similarly, *Candida albicans* (55%) and *Candida glabrada* (20%) are frequently isolated from WON (Werge et al., 2016). On the other hand, *Candida krusei* (4.5%) is a minor population of pancreatic fungal community in many cases with pancreatic necrosis (Chakrabarti et al., 2007). There are also reports suggesting involvement of infection with *Torulopsis glabrata* or *Saccharomyces* (Grewe et al., 1999; Berzin et al., 2007). In contrast, infection with *Aspergillus* species, prototypical commensal fungi in the respiratory tract, has been rarely reported except in immunosuppressed SAP patients (Bhatt and Cappell, 1990). Thus, *Candida* species have been identified as virulence fungi in the development of SAP.

High detection rates of *Candida* species from the blood and pancreatic tissues in patients with SAP implicate possible translocation pathways of this microorganism (Rasch et al., 2018). Given that *Candida* species is predominant fungi in commensal fungal community of human gastrointestinal (GI) tract (Pérez, 2021), *Candida* species are likely to migrate from the gut to the pancreas upon intestinal barrier injury associated with SAP. However, we need to consider translocation of

TABLE 1 Fungal infection in severe acute pancreatitis.

Author	Patients, n	Fungal infection cases, n (%)	WON cases, n (%)	Antifungal therapy		Mortality, n (%)
				Prophylactic, n (%)	Symptomatic, n (%)	
Ning et al (2021)	78	8 (10.2)	NA	0 (0)	8 (10.2)	NA
Rasch et al (2018)	136	54 (39.7)	136 (100)	0 (0)	36 (26.4)	30 (22.0)
Mandal et al (2017)	76	NA	9 (6.6)	NA	NA	1 (1.3)
Werge et al (2016)	123	57 (46.3)	123 (100)	0 (0)	39 (31.7)	22 (17.8)
Ignatavicius et al (2012)	210	16 (7.6)	31 (14.8)	NA	NA	34 (16.2)
Vege et al (2009)	207	30 (14.4)	107 (51.6)	19 (9.1)	NA	41 (19.8)
Kochhar et al (2009)	50	18 (36.0)	25 (50.0)	0 (0)	13 (26.0)	21 (42.0)

NA, not applicable; WON, walled-off necrosis.

Candida species from the skin to the pancreas since *Candida* species constitute skin fungal community (Brown et al., 2012). It should be noted, however, that previous studies have utilized conventional culture methods for isolation of fungi. Therefore, a comprehensive evaluation of the fungal flora in pancreatic tissues and blood has not been achieved, and it is unknown which types of fungal flora dysbiosis may affect the development of SAP. Next generation sequence (NGS) analyses targeting fungal internal transcribed spacer region of the nuclear ribosomal repeat have been employed to visualize composition of fungal species (Toju et al., 2012). Given that isolation of fungal species was performed by conventional culture methods alone, application of NGS utilizing necrotic pancreatic specimen and/or blood may enable us to understand the fungal community and diversity associated with the development of WON.

Anti-fungal therapy in SAP

It is generally accepted that anti-microbial therapy needs to be started for patients with AP when the infection with bacteria or fungi is highly suspected by the serum detection of microbial components such as endotoxin or β-d-glucan or by the presence of bacteremia or fungemia (King et al., 2005; Kochhar et al., 2009; Vege et al., 2009). In cases with fungal infection, administration of amphotericin B and/or fluconazole is required (King et al., 2005; Kochhar et al., 2009; Vege et al., 2009). Consistent with this idea, prophylactic and early administration of fluconazole significantly reduced the incidence of fungal infection in patients with SAP (De Waele et al., 2003; He et al., 2003). However, prophylactic fluconazole administration failed to improve survival despite a significant reduction in fungal infection (Shorr et al., 2005). Furthermore, another report identified prophylactic fluconazole administration as one of the risk factors for *Candida* infection in the pancreatic necrotic tissues (Chakrabarti et al., 2007). Thus, beneficial effects of prophylactic anti-fungal therapy on SAP have not been demonstrated and prophylactic anti-fungal therapy is not recommended according to current guidelines for AP (Tenner et al., 2013; Crockett et al., 2018; Leppäniemi et al., 2019; Takada et al. 2022). Several studies addressed the efficacy of anti-fungal therapy in relation to the incidence of WON (Table 1). As mentioned above, fungal infection rates in the patients with acute pancreatitis ranged from 7.6 to 46.3%. Notably, fungal infection rates tended to be higher in the patients with WON than in the patients without WON, suggesting pathogenic roles played by fungal infection in the development of WON.

As for the efficacy of anti-fungal therapy consisting of amphotericin B and/or fluconazole for SAP, four studies started anti-fungal therapy after isolation of fungi (Kochhar et al., 2009; Werge et al., 2016; Rasch et al., 2018; Ning et al., 2021). On the other hand, antifungal therapy was initiated prophylactically in one study in which prophylactic antifungal therapy was performed in 19 cases due to β-D-glucan positivity in the serum (Vege et al., 2009) despite without fungal detection in culture tests. As shown in Table 1, the mortality rates are comparable whether anti-fungal therapy is initiated after the isolation of fungi or detection of β-D-glucan. In terms of the mortality rates, administration of anti-fungal agents upon detection of serum β-D-glucan might not be beneficial for patients with SAP. However, Tissot et al. reported superiority of β-D-glucan detection for the diagnosis of intraabdominal *Candida* infection as compared with conventional culture methods (Tissot et al., 2013). Alternatively, the presence of multiple organ failure might be an indication for prophylactic anti-fungal therapy (Ning et al., 2021). Further studies addressing the efficacy and timing of anti-fungal therapy for SAP are absolutely required.

Anti-fungal therapy is sometimes accompanied by side effects such as liver dysfunction, kidney dysfunction, and allergy (Nivoix et al., 2020). We have to be cautious regarding the doses of amphotericin B and/or fluconazole since patients with SAP usually have liver and kidney dysfunction as a result of multiple organ failure. In addition, another concern is an emergence of multidrug-resistant organisms due to the long-term administration of antifungal drugs. Indeed, cases with drug-resistant *Candida glabrata* infection are increasing after anti-fungal therapy (Pfaller et al., 2012).

Duration of antibiotics has been shown to increase the risk of fungal infection in patients with SAP (Kochhar et al., 2011). As in the case of fungal infection, current guidelines do not recommend prophylactic antibiotics therapy for all of the cases with AP (Tenner et al., 2013; Crockett et al., 2018; Leppäniemi et al., 2019; Takada et al., 2022). It is because meta-analysis using seven randomized controlled trials showed no significant improvement with prophylactic anti-microbial therapy in both mortality or infectious complication rates in SAP (Tenner et al., 2013; Crockett et al., 2018; Leppäniemi et al., 2019; Takada et al., 2022). Thus, no consensus has been reached regarding the timing of anti-bacterial or anti-fungal therapy.

Involvement of pathogenic immune responses against fungi has been extensively studied in patients with inflammatory bowel disease (IBD) and thus we can learn management of fungal infection from clinical practice in IBD (Underhill and Braun, 2022). A significant fraction of IBD patients have elevated levels in serum antibodies against *Saccharomyces cerevisiae* and polymorphisms in *CARD9* (*Caspase activation and recruitment domain 9*) encoding CARD9, a critical intracellular molecule for immune responses against fungi, are associated with the development of IBD (Underhill and Braun, 2022). Furthermore, clinical responses in fecal microbiota transplantation (FMT) in ulcerative colitis are associated with reduction of *Candida* species after FMT (Leonardi et al., 2020). Despite these extensive studies in fungal communities in IBD, no definitive anti-fungal therapy has been established. Thus, further basic and clinical studies are required to clarify the molecular mechanisms accounting for the link between fungi infection and IBD or SAP and then to establish anti-fungal therapy for these disorders.

Molecular mechanisms accounting for the development of SAP by colonization of fungi into the pancreas

As mentioned above, clinical studies clearly showed that the incidence of fungal infection is high in SAP patients bearing WON and that fungal infection is one of the prognostic factors. However, beneficial effects of prophylactic anti-fungal therapy have not been demonstrated so far. Elucidation of molecular mechanisms how pancreatic colonization of fungi leads to the development of SAP displaying WON is required to establish new treatment strategies against fungal infection associated with SAP.

SAP is characterized by impaired intestinal barrier function and pro-inflammatory cytokine responses (Wu et al., 2014; Watanabe et al., 2017). Potential triggers such as excessive drinking of alcohol cause pro-inflammatory cytokine responses due to intrapancreatic activation of trypsin and subsequent auto-digestion of the pancreatic tissues (Watanabe et al., 2017). Pro-inflammatory cytokines such as TNF-α, IL-1β, and IL-6 increase the intestinal permeability and then as a result translocation of gut bacteria into the pancreas is promoted (Watanabe et al., 2017). Pancreatic acinar cells, macrophages, and dendritic cells express functional pattern recognition receptors (PRRs) for the detection of bacterial components. NOD1 and TLR4 are the prototypical PRRs for the detection of cell wall components derived from gut bacteria translocated into the pancreas. Recognition of bacterial cell wall components by NOD1 and TLR4 leads to a robust production of pro-inflammatory cytokines by immune cells and acinar cells, which in turn further increases the intestinal permeability and accelerates translocation of gut bacteria. Such positive feedback loop connecting pro-inflammatory cytokines and impaired intestinal barrier function have been considered to underlie the immunopathogenesis of SAP and to contribute to the development of SAP displaying WON (Figure 1) (Watanabe et al., 2017). Based on the inflammatory cascades leading to the development of SAP, one might assume that SAP can be efficiently treated by the neutralization of PRRs-mediated signaling pathways (Watanabe et al., 2017). In fact, mice deficient in TLR4 or NOD1 are highly resistant to induction of experimental pancreatitis (Sharif et al., 2009; Tsuji et al., 2012; Watanabe et al., 2016). Alternatively, SAP can be treated by biologics targetting TNF-α, IL-6, or IL-1β as in the case of autoimmune disorders (Watanabe et al., 2017). Previous trials addressing the efficacy of prophylactic antibiotic treatment for the prevention of SAP were not successful (Takada et al., 2022). Although the reasons for the unsuccessful results remain unknown, overgrowth of antibiotic-resistant bacteria and fungi may be involved. Therefore, inflammatory cascades linking bacterial colonization to pro-inflammatory cytokines can be new treatment targets for SAP.

C-type lectin receptors (CLRs) are the main family of PRRs for the detection of fungal cell wall components (Netea et al., 2015). β-d-glucan antigenemia is widely used for the early detection of candidemia in patients with SAP. β-d-glucans derived from *Candida* cell walls are strong stimulators for Dectin-1 expressed in macrophages and dendritic cells (Netea et al., 2015). Recognition of β-d-glucans by Dectin-1, a prototypical CLRs, leads to an increased production of pro-inflammatory cytokines through activation of downstream signaling molecule, spleen tyrosine kinase (SYK). Therefore, the presence of β-d-glucan antigenemia in patients with SAP may mean enhancement of Dectin-1-mediated pro-inflammatory cytokine responses, which result in translocation of gut fungi into the pancreas due to leakiness of the barrier (Figure 1). Based on this, we propose the following mechanism as to involvement of fungal infection in the development of SAP. Initial pro-inflammatory cytokine responses caused by intrapancreatic activation of trypsinogen and subsequent auto-digestion impairs intestinal barrier function, which allows pancreatic entry of gut fungi. Migrated fungi are recognized by Dectin-1 expressed in pancreatic macrophages and dendritic cells to induce pro-inflammatory cytokine responses and to promote maturation of pancreatic necrotic tissues leading to generation of WON. Thus, we assume that inflammatory cascades linking pancreatic fungal colonization to pro-inflammatory cytokines mediates the development of SAP as in the case of pancreatic bacterial colonization. This scenario explains the fact fungal infection is preferentially detected in patients with SAP displaying WON. Although no studies have addressed this issue in experimental models of AP, fungi colonized in the pancreas have been shown to promote inflammatory as well as oncogenic pathways in pancreatic cancer (Aykut et al., 2019; Alam et al., 2022).

Conclusions

Colonization of fungi into the pancreas is associated with the development of SAP displaying WON and high mortality rates although beneficial effects of prophylactic anti-fungal therapy on SAP have not been confirmed. Elucidation of molecular mechanisms accounting for the relationship between WON and fungi infection is required to establish treatment strategies against fungal infection in AP. As far as we know, few studies addressed involvement of fungi in experimental models of SAP. Moreover, previous studies as to the identification of fungal species utilized conventional culture methods, but not NGS. Comprehensive understanding of involvement of fungi in SAP requires both animal and human studies employing NGS to uncover overlooked fungi

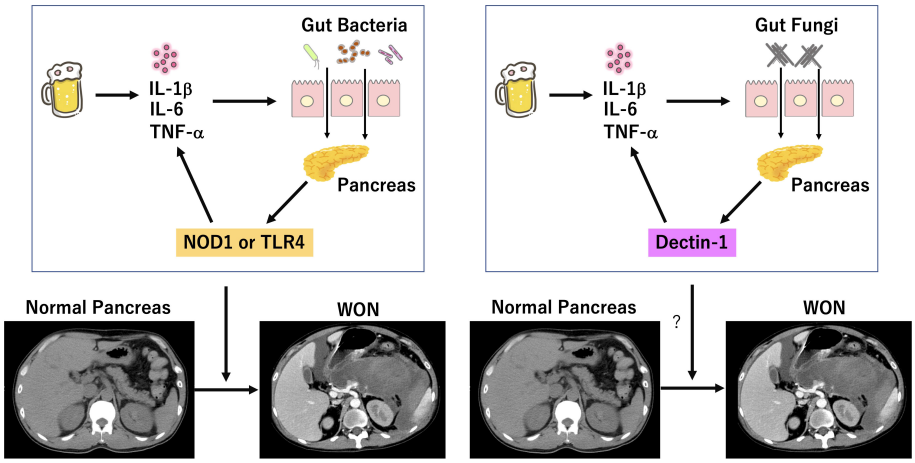

FIGURE 1
Involvement of bacterial and fungal infection in the development of severe acute pancreatitis. Excessive drinking of alcohol induces auto-digestion of pancreatic tissues due to intrapancreatic activation of trypsinogen. Pro-inflammatory cytokine responses induced by initial inflammation dampen intestinal barrier function and allow entry of gut bacteria into the pancreas. Sensing of bacteria by nucleotide-binding oligomerization domain 1 (NOD1) and toll-like receptor 4 (TLR4) leads to a robust production of pro-inflammatory cytokines, which in turn increases intestinal permeability and translocation of gut bacteria. Intestinal barrier dysfunction also allows entry of gut fungi into the pancreas. Sensing of fungi by Dectin-1 may lead to a robust production of pro-inflammatory cytokines, which in turn increases intestinal permeability and translocation of gut fungi. Positive feedback loop between intestinal gut barrier dysfunction and pro-inflammatory cytokine responses induced by colonization of bacteria and/or fungi may be involved in the development of SAP displaying walled-off necrosis (WON). We created the Figure, which consisted of original cartoon pictures and CT pictures from patients who visited our hospital. CT pictures have not been published. This figure is original and based on our proposal and assumption.

species. For this purpose, it is necessary to examine whether fungal dysbiosis is associated with the development of animal and human SAP through visualization of gut and pancreatic fungal community by NGS.

Author contributions

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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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A nomogram for clinical estimation of acute biliary pancreatitis risk among patients with symptomatic gallstones: A retrospective case-control study

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Background/Purpose: Currently, there are no effective tools to accurately assess acute biliary pancreatitis (ABP) risk in patients with gallstones. This study aimed to develop an ABP risk nomogram in patients with symptomatic gallstones.

Methods: We conducted a retrospective nested case-control study and data on 816 conservatively treated patients with symptomatic gallstones admitted to The First Affiliated Hospital of Harbin Medical University between January 6, 2007 and January 22, 2016 were retrospectively collected. We conducted a propensity-score matched (PSM) analysis based on follow-up time in a ratio of 1:4 between ABP group (n=65) and non-ABP group (n=260). These matched patients were randomly divided into study cohort (n=229) and validation cohort (n=96) according to a ratio of 7:3. In the study cohort, independent risk factors for ABP occurrence identified using Cox regression were included in nomogram. Nomogram performance and discrimination were assessed using the concordance index (C-index), area under the curve (AUC), calibration curve, decision curve analysis (DCA) and clinical impact curve (CIC). The model was also validated in the validation cohort.

Results: Nomogram was based on 7 independent risk factors: age, diabetes history, gallbladder wall thickness, gallstone diameter, coexisting common bile duct (CBD) stones, direct bilirubin (DBIL), and white blood cell count (WBC). The C-index of nomogram was 0.888, and the 10-year AUCs of nomogram was 0.955. In the validation cohort, nomogram still had good discrimination (C-index, 0.857; 10-year AUC, 0.814). The calibration curve showed good

homogeneity between the prediction by nomogram and the actual observation. DCA and CIC demonstrated that nomogram was clinically useful.

Conclusions: The ABP risk nomogram incorporating 7 features is useful to predict ABP risk in symptomatic gallstone patients.

KEYWORDS

gallstones, acute biliary pancreatitis, predictors, nomogram, receiver operating characteristic curves

Introduction

Acute pancreatitis (AP), as a common acute abdominal condition, is an inflammatory disorder of the pancreas accompanied by potentially severe local or systemic complications and high mortality (Guo et al., 2019). As a leading cause of admission to the hospital for gastrointestinal disorders worldwide, AP is characterized by the main clinical feature of autodigestion of the pancreas, sometimes accompanied by multiple organ dysfunction. There are various etiological factors capable of inducing an acute attack of AP, such as gallstones, alcohol misuse, smoking, drug use, genetic factors, and tumors (Lowenfels et al., 2000). According to these factors, AP is frequently divided into acute biliary pancreatitis (ABP), acute alcoholic pancreatitis, acute hyperlipidemic pancreatitis, acute idiopathic pancreatitis and so forth (Nauck, 2013).

ABP is recognized as the leading type of AP worldwide, accounting for 35-60% of AP cases, with a reported mortality rate ranging from 5% to 20%. The pathogenesis of ABP might be associated with passage of small gallbladder stones or biliary sludge through the ampulla of Vater and other factors, such as anatomical variations, iatrogenic factors including surgical operation and endoscopic retrograde cholangiopancreatography (ERCP), ampullary carcinoma, and pancreatic head carcinoma (Ridtitid et al., 2019). Of all these risk factors, gallstones are still the main cause of ABP. Studies suggest that ABP is frequently the first symptom of gallstone disease in approximately 40% of patients without a preceding episode of biliary colic (van Erpecum, 2006). Clinical data and experience also show that not all patients with symptomatic gallstones will eventually develop ABP, which results in an arduous challenge and several questions: Which patients with gallstones are more likely to develop ABP? What are the related risk factors for ABP? How can clinicians accurately predict the occurrence of ABP in patients with gallstones and take timely preventive measures?

Some scholars have provided a nomogram combining CT and clinical features for early diagnosis of ABP in admission, however, as for early prevention of ABP, the development of a prediction model for ABP among patients with symptomatic gallstones is still desirable (Zver et al., 2022). Of all the available models, a nomogram can provide an individualized, evidence-based, highly accurate risk estimation. And nomograms are easy to use and can facilitate management-related decision making (Balachandran et al., 2015).

Method

Patients

Between January 6, 2007, and January 22, 2016, data on inpatients were retrospectively collected from the First Affiliated Hospital of Harbin Medical University. The patients who meet inclusion criteria were mainly diagnosed with symptomatic gallstones during hospitalization and most patients were admitted to the hospital for acute abdominal pain. All patients in this study were treated conservatively after admission. The exclusion criteria mainly included (1) incomplete medical record data and serum test and imaging examination results, (2) patients used to undergo any surgical and endoscopic treatment such as cholecystectomy and ERCP (3) ambiguous diagnosis, (4) other concomitant major diseases that would interfere with the study, such as heart failure, renal failure, and multiple organ dysfunction syndrome (MODS), (5) female patients during pregnancy or lactation. These patients were followed from the day of discharge until the last documented follow-up. The study was approved by the Ethics Committee of First Affiliated Hospital of Harbin Medical University (ethics board approval number: ChiCTR1800016492). All the patients' data were used for only research. The study did not affect the treatment of patients. The primary endpoint was ABP excluding other types of AP such as hypertriglyceridemic and alcoholic acute pancreatitis. Non-ABP controls were matched to ABP cases by a ratio of 4:1 using a propensity-score matched (PSM) analysis based on follow-up time. Among ABP cases and non-ABP controls, some patients were randomly divided into the study cohort for nomogram development; the others formed the validation cohort to confirm the model's performance.

ABP diagnostic criteria

The ABP diagnostic criteria were as follows: (1) having gallstones confirmed by abdominal ultrasound, CT, MRCP or other imaging examination; (2) having two or more of the following laboratory examination indicators: ①alkaline phosphatase (AKP)>125 U/L, ②alanine transaminase (ALT) >150 U/L, ③total bilirubin (TBIL)>2.3 mg/dl, and ④gamma-glutamyl transferase (GGT)>40 U/L; (3) conforming to the diagnostic criteria of AP and having at least 2 of the following 3 clinical characteristics: ① abdominal pain consistent with AP; ② serum amylase and/or lipase activity at least 3 times higher than the upper limit of normal; ③ abdominal imaging examination consistent with the imaging changes associated with AP; and (4) no other causes of abnormality of serum amylase and lipase and liver function test (Zhao, 2002; Zhu and Lin, 2012; Coffey et al., 2013; Surlin et al., 2014).

Clinicopathologic variables

The clinicopathologic variables in this study are reported in Table 1, and these variables were recorded before diagnosis of ABP. The imaging data included gallbladder size, thickness of the gallbladder wall, number of gallstones, diameter of gallstones, gallstone morphology, coexisting common bile duct (CBD) stone, and diameter of the bile duct. The normal size of the adult gallbladder was defined as 7-10 cm in length and 3-4 cm in transverse diameter (John et al., 2017). A gallbladder with a size differing from this criteria was seen as abnormal. The gallbladder wall thickness, as an objective marker of cholecystitis severity and laparoscopic cholecystectomy (LC) complexity, was divided into two groups: ≤3 mm (normal) and >3 mm (Kokoroskos et al., 2020). The characteristics of gallstones were represented by the number of gallstones, the diameter of gallstones and the gallstone shapes. The number of gallstones was divided into two groups: solitary gallstones and multiple gallstones (≥2). According to the gallstone shapes shown by imaging examinations, the shapes were classified into spherical stones, sand-like stones and irregular stones. Considering that smaller gallstones were frequently seen in patients with AP, especially stone sizes <3 mm, the diameter of gallstones was separated into three groups: <3 mm, 3~10 mm and >10 mm, and the minimum diameter of gallstones was recorded in case of multiple gallstones (Gonzalez et al., 2012). During the imaging examination, two experienced radiologists independently evaluated all imaging data. Any controversies in imaging findings between radiologists were settled by discussion, and a final standard radiologic report on each patient was generated. At present, although abdominal ultrasound is still the most common method of screening for gallstones and bile duct stones, due to intestinal gas interference, the diagnostic value is always limited (Soyer et al., 2013). CT is clinically more valuable than ultrasound in diagnosing gallstones and bile duct stones, but it is easily affected by stone composition and density, especially low-density stones with cholesterol as the main component (Chan et al., 2006). In contrast, MRCP's sensitivity, specificity, and diagnostic accordance rate in the diagnosis of gallstones are higher than those of ultrasound and CT (Griffin et al., 2012). Therefore, the imaging results of MRCP were recorded preferentially in this study.

Statistical analysis

To minimize bias between the ABP and non-ABP groups, we conducted a propensity-score matched (PSM) analysis in a ratio of 1:4. After samples matching, these samples were randomly divided into study cohort and validation cohort according to the ratio of 7:3. Frequency (ratio) was utilized to describe the characteristics of categorical variables, and comparisons between the two cohorts were performed using chi-square tests. Then the data in the study cohort were used to establish a model and the data in validation set were applied to evaluate the efficacy of the model. Based on the data in the study cohort, univariable Cox proportional hazards analysis was performed for each variable. P-values of the variables were calculated based on the univariable Cox proportional hazards regression model. The variables with p-values less than 0.05 were included in a multivariable Cox proportional hazards regression model. Then, the factors with p-value less than 0.05 were included in the prediction model to establish nomogram. In the nomogram, the sum of these points, plotted on the "total points" line, corresponded to the prediction of 10-year ABP occurrence-free rates in patients with symptomatic gallstones. Receiver operating characteristic curve (ROC) analysis and Harrell's concordance index (C-index) were used to assess the discrimination of the model, and a calibration plot was used for internal verification. Decision curve analysis (DCA) and clinical impact curve (CIC) were utilized to evaluate the clinical application value of the model. Kaplan–Meier cumulative hazard analysis was used to estimate the risk of being diagnosed as ABP during the follow-up period. All analyses were performed using SPSS (22.0 IBM, Armonk, NY, USA) and R (version 4.2.1) software.

Results

Clinicopathologic characteristics and univariate analysis results

In this study, after exclusion, 816 patients with gallstones who met the inclusion criteria were finally enrolled. We conducted a propensity-score matched (PSM) analysis based on follow-up time in a ratio of 1:4 between ABP group (n=65)

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TABLE 1 Characteristics of patients in the study and validation cohorts.

Variables	Study cohort(n=229)	Validation cohort(n=96)	*P*-value
Age (year)			
<50	104 (45.4%)	50 (52.1%)	
≥50	125 (54.6)	46 (47.9%)	0.272
Sex			
Female	148 (64.6%)	52 (54.2%)	
Male	81 (35.4%)	44 (45.8%)	0.77
Alcoholic history			
No	192 (83.8%)	75 (78.1%)	
Yes	37 (16.2%)	21 (21.9%)	0.219
Smoking history			
No	190 (83.0%)	79 (82.3%)	
Yes	39 (17.0%)	17 (17.7%)	0.883
Diabetes history			
No	172 (75.1%)	67 (69.8%)	
Yes	57 (24.9%)	29 (30.2%)	0.321
Gallbladder size			
Normal	167 (72.9%)	62 (64.6%)	
Abnormal	62 (27.1%)	34 (35.4%)	0.133
Gallbladder wall thickness (mm)			
≤3	137 (59.8%)	58 (60.4%)	
>3	92 (40.2%)	38 (39.6%)	0.921
Gallstone number			
1	33	14	
≥2	196	82	0.968
Gallstone diameter (mm)			
<3mm	49 (21.4%)	18 (18.8%)	
3-10mm	112 (48.9%)	54 (56.3%)	
>10mm	68 (29.7%)	24 (24.9%)	0.479
Gallstone shape			
Sphere	121 (52.8%)	66 (68.8%)	
Irregular	53 (23.1%)	17 (17.7%)	
Sand-like	55 (24.1%)	13 (13.5%)	0.240
Bile duct stones			
No	175 (76.4%)	76 (79.2%)	
Yes	54 (23.6%)	20 (20.8%)	0.590
Diameter of CBD (mm)			
≤10	193 (84.3%)	84 (87.5%)	
>10	36 (15.7%)	12 (12.5%)	0.455
ALT (U/L)			
<150U/L	180 (78.6%)	83 (86.5%)	
≥150U/L	49 (21.4%)	13 (13.5%)	0.100
AST (U/L)			
<53.6U/L	161 (70.3%)	72 (75.0%)	
≥53.6U/L	68 (29.7%)	24 (25.0%)	0.391
AST/ALT			
<1.0	145 (63.3%)	50 (52.1%)	
≥1.0	84 (36.7%)	46 (47.9%)	0.059
GGT (U/L)			
<150	139 (60.7%)	66 (68.8%)	

(Continued)

TABLE 1 Continued

Variables	Study cohort(n=229)	Validation cohort(n=96)	*P*-value
≥150	90 (39.3%)	30 (31.2%)	0.170
AKP (U/L)			
<125	162 (70.7%)	65 (67.7%)	
≥125	67 (29.3%)	31 (32.3%)	0.587
TBIL (mg/dL)			
<1.4	151 (65.9%)	67 (69.8%)	
≥1.4	78 (34.1%)	29 (30.2%)	0.500
IBIL (mg/dL)			
<0.8	182 (79.5%)	80 (83.3%)	
≥0.8	47 (20.5%)	16 (16.7%)	0.422
DBIL (mg/dL)			
<1.0	196 (85.6%)	83 (86.5%)	
≥1.0	33 (14.4%)	13 (13.5%)	0.838
WBC ($\times10^9$/L)			
<10	164 (71.6%)	68 (70.8%)	
≥10	65 (28.4%)	28 (29.2%)	0.887
GRAN% (%)			
<80	147 (64.2%)	59 (61.5%)	
≥80	82 (35.8%)	37 (38.5%)	0.641

P<0.05 was statistically significant.
AKP, Alkaline phosphatase; ALT, Alanine transaminase; AST, Aspartate transaminase; CBD, Common bile duct; DBIL, Direct bilirubin; GGT, Gamma-glutamyl transferase; GRAN%, Granulocyte%; IBIL, Indirect bilirubin; TBIL, Total bilirubin; WBC, White blood cell count.

and non-ABP group (n=260). Then matched patients were randomly divided into study cohort (n=229) and validation cohort (n=96) according to a ratio of 7:3 (Figure 1). The median follow-up time after discharge was 89 months [IQR 45.8-130.0 months]. The 10-year cumulative risks of being diagnosed as ABP were 12.59% (95% CI 9.41% to 15.66%) during the follow-up, and this risk appeared to continue in subsequent years (Figure 2).

The clinicopathologic characteristics of the patients are listed in Table 1. The baseline clinicopathologic data were similar

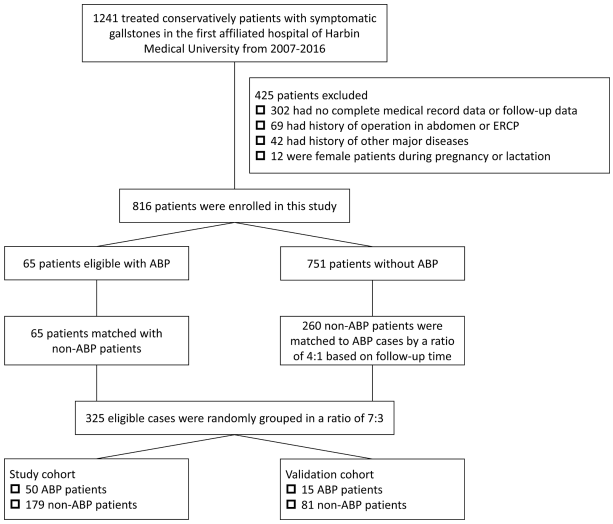

FIGURE 1
Flow chart of the study.

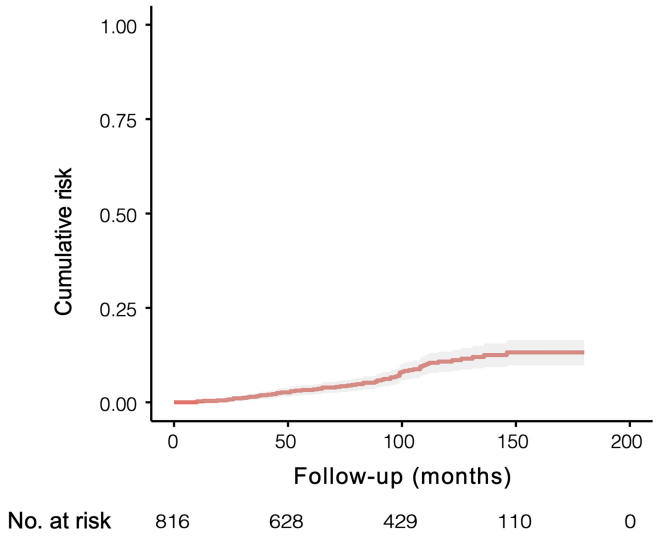

FIGURE 2
Cumulative risk of being diagnosed as ABP in 816 individuals with symptomatic gallstones and followed for a median of 89 months [IQR 45.8-130.0 months].

between the study and validation cohorts. The univariate cox analysis result of the study cohort showed that age, sex, alcoholic history, diabetes history, gallbladder wall thickness, gallstone number, gallstone diameter, gallstone shape, coexisting CBD stone, GGT, AKP, TBIL, direct bilirubin (DBIL), white blood cell count (WBC) and granulocyte% (GRAN%) were significantly different between the ABP group and the non-ABP group ($p < 0.05$) (Table 2).

Multivariate analysis result and establishment of an ABP-predicting nomogram

The significant factors obtained in the univariate cox analysis results were included in the multivariate cox regression model to analyze whether each factor was an independent risk factor for inducing ABP. In the multivariate analysis, with results reported as HRs (95% CIs), age >50 years (3.491 [1.514-8.047]), diabetes history (4.585 [1.926-10.914]), gallbladder wall thickness >3 mm (0.195 [0.079-0.480]), gallstone diameter (3-10 vs <3 mm, 0.311 [0.105-0.924], >10 mm vs <3mm, 0.248 [0.094-0.655]), coexisting CBD stone (2.382 [1.177-4.821]), DBIL >1.0 mg/dL (4.867 [1.734-13.660]) and WBC >10×109 (3.628 [1.397-9.427]) were independently associated with ABP (Table 2; Figure 3). These independently associated risk factors were utilized to make an ABP risk estimation nomogram based on 10-year ABP-occurrence free rate (Figure 4).

Validation and effect evaluation of the nomogram

The resulting model was internally validated based on validation cohort. The performance of the nomogram was measured by ROC curve (Figure 5). The C-index of nomogram was 0.888, and the 10-year AUCs of nomogram were 0.955. In the validation cohort, nomogram still had good discrimination (C-index, 0.857; 10-year AUC, 0.814). Based on cutoff value of ROC in study cohort (1.249), the sensitivity and specificity of prediction model in validation cohort were 73.3% and 79.0% (97.6% and 79.7% in study cohort) (Table 3). The calibration curve showed good homogeneity between the prediction by nomogram and the actual observation (Figure 6). The decision curve analysis for the ABP incidence risk nomogram is also presented in Figure 6. The DCA demonstrated that the prediction model could provide great net benefit and make valuable and profitable judgements. The CIC result showed that the number of patients who were at high risk (the number of ABP patients predicted using the nomogram) was well matched with the number of patients who were at high risk with the event (the number of truly-diagnosed ABP patients) (Figure 6).

Performance of the nomogram

As seen in nomogram, selected predictors were assigned with a score according to the value in the nomogram based on

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TABLE 2 Univariate and multivariate Cox analysis of the study cohort.

Variables	Univariate analysisHR (95% CI)	*P*-value	Multivariate analysisHR (95% CI)	*P*-value
Age (year)				
≥50 vs. <50	5.479 (2.545-11.797)	**<0.001**	3.491 (1.514-8.047)	**0.003**
Sex				
Male vs. Female	2.844 (1.575-5.135)	**0.001**	1.250 (0.537-2.911)	0.604
Alcoholic history				
Yes vs. No	1.955 (1.012-3.778)	**0.046**	0.917 (0.371-2.267)	0.851
Smoking history				
Yes vs. No	1.941 (1.001-3.764)	0.05		
Diabetes history				
Yes vs. No	5.378 (2.978-9.712)	**<0.001**	4.585 (1.926-10.914)	**0.001**
Gallbladder size				
Abnormal vs. Normal	0.732 (0.363-1.476)	0.384		
Gallbladder wall thickness (mm)				
>3 vs. ≤3	0.308 (0.152-0.622)	**0.001**	0.195 (0.079-0.480)	**<0.001**
Gallstone number				
≥2 vs. 1	4.394 (1.065-18.126)	**0.041**	1.559 (0.334-7.270)	0.572
Gallstone diameter (mm)				
3-10mm vs. <3mm	0.267 (0.112-0.636)	**0.003**	0.311 (0.105-0.924)	**0.035**
>10mm vs. <3mm	0.402 (0.216-0.749)	**0.004**	0.248 (0.094-0.655)	**0.005**
Gallstone shape				
Irregular vs. Sphere	0.762 (0.339-1.713)	0.511	0.842 (0.322-2.202)	0.726
Sand-like vs. Sphere	2.086 (1.105-3.937)	**0.023**	1.018 (0.454-2.285)	0.965
Coexisting CBD stones				
Yes vs. No	3.522 (1.981-6.262)	**<0.001**	2.382 (1.177-4.821)	**0.016**
Diameter of CBD (mm)				
>10 vs. ≤10	0.397 (.292-1.630)	0.397		
ALT (U/L)				
≥150U/L vs. <150U/L	1.809 (0.988-3.312)	0.550		
AST (U/L)				
≥53.6U/L vs. <53.6U/L	1.528 (0.857-2.725)	0.151		
AST/ALT				
≥1.0 vs. <1.0	0.979 (0.540-1.776)	0.944		
GGT (U/L)				
≥150 vs. <150	2.428 (1.323-4.458)	**0.004**	0.436 (0.143-1.335)	0.146
AKP (U/L)				

(Continued)

TABLE 2 Continued

Variables	Univariate analysisHR (95% CI)	*P*-value	Multivariate analysisHR (95% CI)	*P*-value
≥125 vs. <125	1.695 (0.949-3.029)	0.075		
TBIL (mg/dL)				
≥1.4 vs. <1.4	2.273 (1.267-4.078)	**0.006**	1.049 (0.380-2.893)	0.926
IBIL (mg/dL)				
≥0.8 vs. <0.8	0.810 (0.378-1.735)	0.588		
DBIL (mg/dL)				
≥1.0 vs. <1.0	2.214 (1.095-4.121)	**0.026**	4.867 (1.734-13.660)	**0.003**
WBC ($\times10^9$/L)				
≥10 vs. <10	5.494 (2.998-10.066)	**<0.001**	3.628 (1.397-9.427)	**0.008**
GRAN% (%)				
≥80 vs. <80	4.033 (2.198-7.398)	**<0.001**	1.717 (0.728-4.051)	0.217

P<0.05 was statistically significant.
AKP, Alkaline phosphatase; ALT, Alanine transaminase; AST, Aspartate transaminase; CBD, Common bile duct; DBIL, Direct bilirubin; GGT, Gamma-glutamyl transferase; GRAN%, Granulocyte%; IBIL, Indirect bilirubin; TBIL, Total bilirubin; WBC, White blood cell count.
'bold values' means statistical significant values (<0.05).

the established prediction model. Then a vertical line perpendicular to the point axis was drawn from this point. The intersection point on the point axis represented the score under the determined value of the predictor. For example, for a 55-year-old patient with gallstones and a history of diabetes, abdominal ultrasound suggested that the thickness of gallbladder wall was 3 mm, the diameter of gallstones was 3~5mm and there was no CBD stone. The laboratory examination results were as follows: DBIL 1.0 mg/dL; WBC 9×109/L. The 10-year occurrence free rate of ABP for this patient can be calculated as 30%. Considering the worse prognosis of elderly patients with acute pancreatitis, therefore, we recommend that the patient receive medical check-ups regularly and even undergo cholecystectomy if necessary to prevent occurrence of ABP.

Discussion

AP is characterized by acute onset, rapid progression and a high likelihood of developing severe acute pancreatitis (SAP), with severe complications and a high mortality rate of 30% (Guo et al., 2019). Gallstones are still one of the key causative factors of AP, and in the present study, approximately 20% of gallstone patients in hospitals were diagnosed with ABP

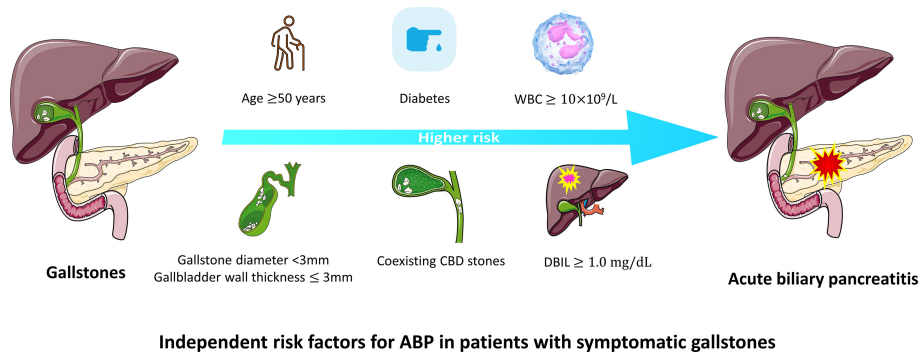

FIGURE 3
Independent risk factors for ABP in patients with gallstones.

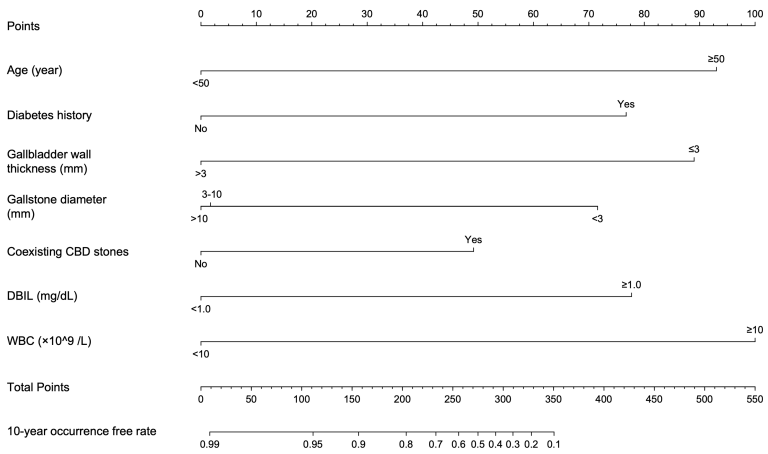

FIGURE 4
Nomogram conducted by Cox regression, including age, diabetes history, gallbladder wall thickness, gallstone diameter, coexisting CBD stones, DBIL and WBC in patients with symptomatic gallstones.

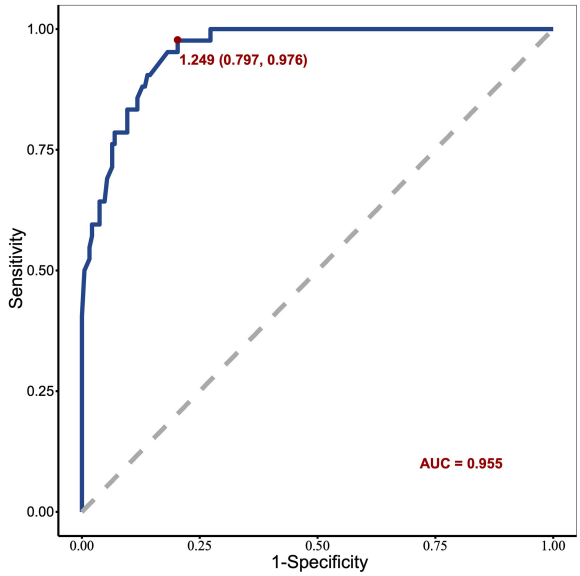

FIGURE 5
Receiver operating characteristic curve of the nomogram in the study cohort.

(Zhu and Lin, 2012). Therefore, it is clinically important for clinicians to prevent gallstone patients from developing AP and SAP. Our study also suggests that risk factors, including age, diabetes history, gallbladder wall thickness, gallstone diameter, coexisting CBD stone, DBIL and WBC, are significantly associated with the incidence of ABP.

In line with the current literature, our data regarding incidence of ABP indicate that elderly patients with gallstones had higher risk of ABP during follow-up (HR: 3.491, 95% CI: 1.514~8.047). The relation between advanced age and incidence of AP is not surprising. For instance, most studies indicate that the mean age of the first AP attack is 60 years and with increasing age, the incidence and mortality of AP are also increasing, and there have been studies indicating a correlation between age and mortality as an independent risk factor (Spanier BW and Bruno, 2008; Beard et al., 2016). Relevant

TABLE 3 Accuracy of prediction model in validation cohorts.

Prediction	Actual observation	
	ABP	Non-ABP
ABP	11	17
Non-ABP	4	64

Sensitivity of model: 73.3% (11/15).
Specificity of model: 79.0% (64/81)/

studies have demonstrated that although some controversy exists, diabetic patients are generally thought to have a twofold to threefold increased risk of cholesterol gallstones. Moreover, owing to poor anti-infection ability and immunity, when patients with diabetes suffer cholecystitis, they are prone to serious biliary tract infection and even other severe complications, such as gallbladder abscess, gangrene, and perforation (Ransohoff et al., 1987). In this study, diabetes history is an important risk factor for ABP (HR: 4.585, 95% CI: 1.926~10.914). We speculate that diabetes is more likely to be associated with biliary tract infection and that biliary tract function is worse in patients with diabetes than in the normal population. Therefore, gallstone incarceration is more likely to occur during the downward movement of gallstones, thus inducing ABP.

Among the factors related to gallstones and the biliary tract, this study showed that the thickness of the gallbladder wall, gallstone diameter, and coexisting CBD stone were all significant risk factors for ABP in patients with gallstones. The risk of ABP in the normal gallbladder group was significantly higher than that in the abnormal gallbladder group. In addition, gallbladder wall thickness ≤ 3 mm was a risk factor for ABP (in comparison, the HR of the thickness of > 3 mm was 0.195, 95% CI: 0.079~0.480); that is, the risk of ABP in patients with a thickness of the gallbladder wall ≤ 3 mm was 5.13 times as high as that of patients with a thickness of > 3 mm. The reason might be that when the gallbladder wall thickness is normal, the gallbladder's contraction function is relatively good (Yamada and Yamada, 2001). Therefore, when the gallbladder contracts, the gallbladder tube is able to normally expand, thus making gallstones easily discharged into the CBD and inducing ABP. In contrast, long-term inflammation frequently leads to a

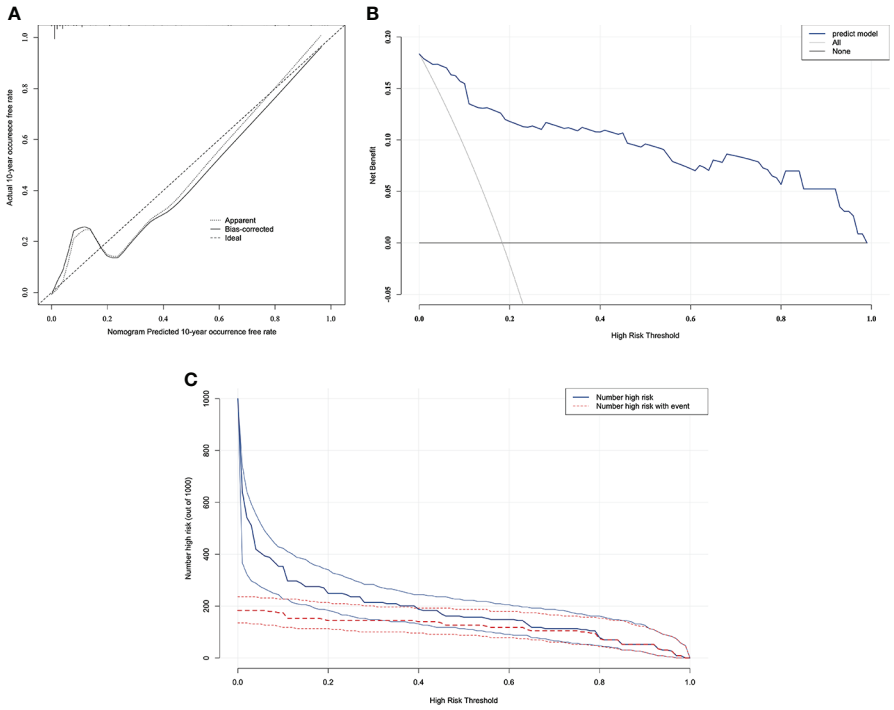

FIGURE 6
The calibration curve and results of the DCA and the CIC analysis of the nomogram in the validation cohort. **(A)** Calibration curves represent the difference between the actual prediction and the ideal perfect prediction (45∘ line). **(B)** The DCA curve of the nomogram for predicting ABP. It revealed that the nomogram could obtain a greater net benefit than either the "treat all" or the "treat none" strategy. **(C)** The CIC curve of the nomogram for predicting ABP. The solid blue line (Number high risk) represents the number of ABP patients predicted using the nomogram at each threshold probability; the dotted red line (number high risk with event) represents the number of true-positive ABP patients at each threshold probability.

thicker gallbladder wall and relatively poor gallbladder contraction function, and the gallbladder duct has difficulty expanding (Stads et al., 2007). Therefore, gallstones are difficult to discharge and are likely to remain in the gallbladder, making the risk of ABP relatively lower. In the gallstone size analysis, patients were divided into three groups according to stone diameter: < 3 mm, 3-10 mm and >10 mm, and patients with gallstone diameters < 3 mm were 3.22 times more likely to develop ABP than those with diameters 3-10 mm and 4.03 times more likely to develop ABP than those with diameters >10 mm. The reason may be that when the gallbladder contracts, stones with smaller diameters are more likely to enter the common channel, and they easily cause channel blockage and finally induce ABP. In contrast, larger diameter stones tend to be incarcerated in the gallbladder neck and do not easily enter the common channel. Therefore, the risk of inducing ABP is relatively reduced (van Geenen et al., 2010). Coexisting CBD stone were also an essential risk factor for ABP in gallstone patients (HR: 2.382, 95% CI: 1.177~4.821). Our analysis showed that the risk of ABP in patients with gallstones and bile duct stones was about twice as high as that in patients with only gallstones. The reason is possibly that compared with gallstones, bile duct stones are more likely to cause duodenal papillary edema or stenosis, especially in the ampullary segment and the lower CBD, which are more likely to result in ABP than the upper CBD (Lee et al., 2018).

In terms of biochemical indicators commonly used in clinical practice, combined with multivariate cox regression analysis, we demonstrated that DBIL and WBC were all important and independent risk factors for ABP development in patients with gallstones. Among them, the abnormal level of DBIL reflects the degree of bile duct obstruction during the pathogenesis of ABP and the degree of hepatocyte injury caused by bile reflux (Surlin et al., 2014). Moreover, the level of WBC is able to reflect the severity of biliary tract infection during ABP (Lankisch et al., 2015). In general, when patients with gallstones suffer severe obstruction and infection of the biliary tract, their risk of concurrent ABP is obviously higher, and these biochemical indicators have good prediction value for ABP.

There are no guidelines that indicate which patient to offer a cholecystectomy or conservative treatment. Therefore, the indication to perform a cholecystectomy always lies within the surgeons' preference leading to variations in practice and consequently unnecessary cholecystectomies. Some findings show that asymptomatic gallstone patients should not undergo prophylactic cholecystectomy. In a study by Gracie et al., only 18% of asymptomatic gallstone carriers developed biliary pain or a gallstone complication during 15-year follow-up (Gracie and Ransohoff, 1982). Furthermore, symptomatic complicated gallstone patients, especially ones with mild-to-moderate acute cholecystitis, common bile duct stones, or mild biliary pancreatitis, are now recommended for same admission cholecystectomy as opposed to delayed cholecystectomy in previous guidelines (MP., 2018).

However, for symptomatic uncomplicated gallstone patients, who are primarily involved in this study, whether and when cholecystectomy should be performed remains controversial. Most studies recommend that cholecystectomy is the therapy of the first choice for patients with uncomplicated symptomatic gallstone disease. Many patients have had an unnecessary cholecystectomy with associated risks of complications and unnecessary healthcare expenses, and some studies indicated that up to 33% of patients do not experience relief of their abdominal symptoms, despite cholecystectomy (Lamberts et al., 2013). Moreover, some patients with suggested uncomplicated symptomatic gallstone disease should be treated conservatively because of a high risk of persistent symptoms or suboptimal benefit of cholecystectomy. However, from the perspective of ABP prevention, there are still no uniform guidelines for choosing the optimal timing of cholecystectomy. Although some patients are at high risk of ABP in clinical practice, they may still choose conservative treatment and refuse effective surgical treatment for some reasons. All these above may increase the potential risk of ABP in patients with gallstones. In this study, most of the samples are asymptomatic uncomplicated patients with gallstones. Therefore, the nomogram can be used to identify these patients' potential risk for ABP through commonly used clinical indicators and to help clinicians make better clinical decisions on the optimal timing of cholecystectomy. Moreover, it is also beneficial to encourage patients to avoid risk factors for ABP and receive a medical check-up regularly. Finally, it is worth noting that in this study, decision curves indicate that when the risk of ABP in patients is greater than 10%, carrying effective intervention will bring the population positive overall benefit. However, the intervention may include regular follow-up, regular medical check-ups, endoscopic treatment and surgical treatment. According to patients' different risks for ABP, which intervention effectively prevents the occurrence of ABP remains to be further studied.

Our study had some limitations. First, this analysis was based on data from a single institution; it is necessary to validate the results in other centers. Second, there are still many risk factors affecting the incidence of ABP in patients with gallstones. Due to limited data, the risk factors selected in this study were not complete. Some potential risk factors of patients, such as body mass index (BMI) and blood lipids, were not included in the study, and more risk factors should be included in this study to further improve the accuracy of the prediction model. Finally, although the nomogram is more convenient than the traditional statistical model, it is no denying that there are still some limitations in the actual application. In the future, we will put the scoring system on a website or an app for use on a smart phone for surgeons in

the hospital, and the score could automatically calculate results online.

Conclusion

By combining seven clinical risk factors for ABP in symptomatic gallstone patients, a nomogram was constructed. The model provides an accurate and optimal estimation of ABP risk in patients with symptomatic gallstones. The nomogram provides an effective tool for quantitative clinical assessment of risks and benefits, which is conducive to the early prevention and treatment of ABP in patients with symptomatic gallstones. This model could also help clinicians and patients make scientific clinical decisions to maximize the clinical benefits of patients.

Data availability statement

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

Ethics statement

The studies involving human participants were reviewed and approved by the Ethics Committee of First Affiliated Hospital of Harbin Medical University (ethics board approval number: ChiCTR1800016492). The patients/participants provided their written informed consent to participate in this study.

Author contributions

XG, YL and HL contributed equally to this article. XG, YL and HL participated in the design of the study and drafted the manuscript. LC, ZH, ZL and NM participated in patients follow-up and data collection. BS, GW and QT conceived of the study, and participated in design and coordination and helped to draft the manuscript. All authors read and approved the final manuscript.

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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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Damage associated molecular patterns and neutrophil extracellular traps in acute pancreatitis

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Previous researches have emphasized a trypsin-centered theory of acute pancreatitis (AP) for more than a century. With additional studies into the pathogenesis of AP, new mechanisms have been explored. Among them, the role of immune response bears great importance. Pro-inflammatory substances, especially damage-associated molecular patterns (DAMPs), play an essential role in activating, signaling, and steering inflammation. Meanwhile, activated neutrophils attach great importance to the immune defense by forming neutrophil extracellular traps (NETs), which cause ductal obstruction, premature trypsinogen activation, and modulate inflammation. In this review, we discuss the latest advances in understanding the pathological role of DAMPs and NETs in AP and shed light on the flexible crosstalk between these vital inflammatory mediators. We, then highlight the potentially promising treatment for AP targeting DAMPs and NETs, with a focus on novel insights into the mechanism, diagnosis, and management of AP.

KEYWORDS

DAMPs (damage-associated molecular patterns), NETs (neutrophil extracellular traps), acute pancreatitis (AP), HMGB1 (high mobility group box 1), HSP (heat shock protein), histone

Introduction

Acute pancreatitis (AP) is an acute inflammation of the exocrine pancreas and represents one of the most common gastrointestinal diseases, leading to acute admission to the hospital. The annual incidence in high-income countries reaches around 34 per 100,000 persons/year (Xiao et al., 2016). In the USA alone, the annual cost of treatment reaches $9.3 billion and the average hospitalization cost reaches $6,240 per patient (Mederos et al., 2021; Peery et al., 2021). AP is a complex disease that varies in severity and clinical course, from a self-limited course that resolves in a few days/weeks to severe conditions which result in systemic inflammatory response syndrome–associated extrapancreatic organ failure and even death (Habtezion et al., 2019). The past decades have witnessed more significant advances in understanding the pathology and mechanism of AP and some potentially promising therapeutic approaches to reduce morbidity and treatment costs (Peery et al., 2021). Nevertheless, still, no effective therapeutic agents currently exist to treat or prevent AP (Lee and Papachristou, 2019). Therefore, deeper and more evidential insights, as well as investigation into the mechanisms of AP, are urgently required.

Since Chiari observed autolysis of the pancreas in an autopsy study in 1896, a trypsin-centered theory of AP has been proposed and investigated for more than a century (Saluja et al., 2019). However, further in-depth exploration and the use of more effective research tools and models have enabled the discovery of other mechanisms underlying AP, including pathological calcium signaling and endoplasmic reticulum (ER) stress (Lee and Papachristou, 2019). Pathological calcium signaling is historically linked with early-stage pancreatitis and, over the years, it has been more widely studied and become universally acknowledged for its inducing both pro-cell death and pro-inflammatory pathways in AP. This is typical for premature trypsinogen activation, activation of nuclear factor-κb (NF- κB), mitochondrial dysfunction and necrosis (Lee and Papachristou, 2019). Inositol trisphosphate receptor (IP3R) and ryanodine receptor (RyR) Ca^{2+} channel induction by the toxin, thus enhances the release of Ca^{2+} from the ER, causing the continued elevation of Ca^{2+} in the cytosol ($[Ca^{2+}]i$) and mitochondria ($[Ca^{2+}]m$) (Saluja et al., 2019). On the other hand, the rising concentration of $[Ca^{2+}]m$ induces the mitochondrial permeability transition pore (MPTP) and leads to the dysfunction of mitochondria (in producing ATP and reducing the cytosolic calcium), altogether resulting in the elevated concentration of calcium (Habtezion et al., 2019).

When it comes to the role of the immune system, "damage associated molecular patterns" (DAMPs), which are produced or released by injured, dying, or dead cells to activate signaling and sterile inflammation, have a significant role (Saluja et al., 2019). In 1994, Polly Matzinger proposed the "danger theory", which claimed that distressed or damaged cells could release endogenous danger signals (Matzinger, 1994). Later, these danger signal substances were named "damage-associated molecular patterns" (DAMPs) by Land in 2003 (Land, 2003). In the next two decades, the significant role of DAMPs in the pathology of AP, which links the local tissue damage to systemic inflammation reaction syndrome (SIRS), became increasingly clear (Kang et al., 2014a). Aseptic inflammation is the initial manifestation of injury in AP, and numerous studies have shown that DAMP-mediated aseptic inflammation is the essential event mediating further pancreatic injury, downstream organ injury, and disease resolution, suggesting that DAMPs are an important factor in the initiation and perpetuation of AP (Hoque et al., 2012). Considering the vital roles of linking local tissue damage to systemic inflammation, DAMPs could be moieties of concern in the pathogenesis of AP, which function by recruiting inflammatory cells and activating adaptive immune responses (Kang et al., 2014b).

Neutrophils are primarily thought to be the first immune cells to be recruited to inflammatory tissues in the event of acute inflammation (Maas et al., 2018). *In vivo* evidence suggests that neutrophils are the first line of defense against bacterial and fungal infections (Wan et al., 2020). If activated, they perform various antimicrobial functions and tasks, including phagocytosis, cytokine secretion, and degranulation (Land, 2003). These cells kill invading pathogens with a large number of antibacterial agents, including reactive oxygen species (ROS) and hydrolases (Wan et al., 2020). Recently, the formation of so-called neutrophil extracellular traps (NETs) has been described as a new defense mechanism. NETs were first described in 1996 as a pathway of cellular death designated NETosis, different from apoptosis and necrosis (Takei et al., 1996), further detailed by Brinkmann et al. They demonstrated that after stimulation of isolated neutrophils with interleukin-8 (IL-8), a major neutrophil chemoattractant, bacterial lipopolysaccharide (LPS, a component of gram-negative bacteria) or phorbol 12-myristate 13-acetate (PMA, a potent activator of protein kinase C, PKC), NETs could be generated *in vitro* to create a physical barrier that prevents the spread of pathogens and facilitates killing microbes *via* high concentrations of antimicrobial proteins and phagocytosis (Brinkmann et al., 2004). Further studies, on the contrary, demonstrated the essential role of NETs in tissue damage, vaso-occlusion promotion, and sterile inflammation promotion (Papayannopoulos, 2018). Research has revealed the involvement of NETs in the pathogenesis of sepsis, connective tissue diseases, cardiovascular diseases, autoimmune diseases, and cancer (Döring et al., 2017; Lee et al., 2017; Bonaventura et al., 2020; Masucci et al., 2020; Klopf et al., 2021; Cristinziano et al., 2022). Its double-edged function is uncovered for its protective role in mediating host defense by trapping and killing microorganisms as well as the detrimental effect, when excessive NETs lead to tissue injury by facilitating thrombus formation, causing a "no-reflow" phenomenon and exacerbating hepatic ischemia reperfusion injury (Cahilog et al., 2020). Given these novel findings, a comprehensive investigation into the

complex interaction between NETs and APs might be of special interest.

In this review, we introduce the pathology of DAMPs and NETs, and their interplay in the pathological progress of AP. Given the close relationship between DAMPs and NETs, the new approach targeting DAMPs and NETs could be promising and worth further investigation. Here we also discuss the existing potential therapeutic interventions targeting both DAMPs and NETs in AP and shed a light on the future strategy in the hope of more effective interventions at the early stage of AP.

DAMPs are endogenous danger signals in AP

DAMPs are endogenous substances that are usually sequestered inside cells by a cell membrane or organelle membrane to play an intracellular physiological role through precise regulation. They are localized within the nucleus and cytoplasm (HMGB1), cytoplasm alone (S100 proteins), exosomes (exRNAs), oncosomes (HSPs), secretory lysosomes (ATP) and in plasma components, such as complement cascade elements C3a, C4a, and C5a (Tang et al., 2012). Once released (either passively or actively) by dead or dying cells, they can serve as endogenous danger signals to alert the innate immune system to unscheduled cell death, stimulate anti-microbial defense, and respond to stress (Lotze et al., 2007; Tang et al., 2012). Despite their essential role in tissue healing after inflammation, excessive exposure to DAMPs could lead to uncontrolled sterile inflammation and various diseases, like sepsis, diabetes mellitus, and chronic neurodegenerative disorders (Shin et al., 2015; Thundyil and Lim, 2015; Venereau et al., 2015; Denning et al., 2019a).

Former studies have shed light on the close association between DAMPs and AP, emphasizing their role as endogenous danger signals in AP (Liu et al., 2017; Wu et al., 2018b; Biberci Keskin et al., 2019). DAMPs initiate immune responses through the receptors which are divided into two types: pattern recognition receptors (PRR), which include Toll-like receptors, c-type lectin receptors, nod like receptor (NLR)-family pyrin domain-containing 3, Retinoic acid-inducible gene I (RIG-I)-like receptors, cytoplasmic DNA sensors as well as non-PRR DAMP receptors, which include receptors for advanced glycation end (RAGE) products and trigger receptors expressed on myeloid cells, G-protein-coupled receptors and ion channels (Gong et al., 2020). The detailed functions of those receptors and the corresponding DAMPs in AP are outlined in Table 1 and further discussed below.

DAMPs and AP

DAMPs released or exposed from dying or dead cells play an important role in the pathogenesis of AP by linking local tissue damage to systemic inflammation. With inflammatory response as the central link, we divided the pathological mechanism of DAMPs in AP into the following five aspects, which will be expounded later.

1. Inducing production and release of inflammatory cytokines, interferons, chemokines and cell adhesion molecules (West and Shadel, 2017; Gong et al., 2020).

2. Mediating the formation of NLR Family Pyrin Domain Containing 3 (NLRP3) inflammasome (Liu et al., 2017).

3. Encompassing the activation and recruitment of innate immune cells, such as macrophages, dendritic cells and neutrophils (Kang et al., 2014a; Zhao et al., 2018; Habtezion et al., 2019)

4. Participating in the formation of NETs by activated neutrophils (Merza et al., 2015; Hu et al., 2020; Linders et al., 2020a).

5. Affecting the inflammatory process by participating in autophagy, necrosis, apoptosis and other pathways (Zhang et al., 2013; Yu et al., 2016; Gukovskaya et al., 2017).

HMGB1 and AP

High mobility group box-1 (HMGB1) is one of the most prototypical DAMPs that has been well-studied for almost half a century. HMGB1, DNA chaperone, is an abundant and highly conserved nuclear protein that acts as a key determinant in reverse chromosomal DNA binding and bending to facilitate nucleosome formation and regulate gene events. A meta-analysis suggested that HMGB1 is an useful indicator of the degree of pancreatic inflammatory response from the healthy control, MAP patients to SAP patients (Li et al., 2018). In experimental AP models, HMGB1 levels decrease when inhibitors (such as pyrrolidine, dithiocarbamate) and neutralizing antibodies are used (Yasuda et al., 2007; Yang et al., 2008; Zhang et al., 2010b). Targeting the 3' untranslated region of HMGB1, micro-RNA (miR)-340-5p downregulates HMGB1 expression and restrains the activation of Toll-like receptor 4 and enhanced protein kinase B (AKT) signaling, leading to subsequent inhibition of inflammation and apoptosis (Gao et al., 2022a). Taken together, these studies have established extracellular HMGB1 as a critical mediator in AP.

HMGB1 can be passively released by somatic cells in the process of the necrosis, when disrupting membrane integrity or actively secreted from regulated cell death processes, such as necroptosis, pyroptosis, ferroptosis, or apoptosis (Scaffidi et al., 2002; Jiang et al., 2007; Kaczmarek et al., 2013; Lu et al., 2014; Hou et al., 2018; Murao et al., 2021a). Similarly, it can be actively excreted by either immune cells (such as monocytes) or non-immune cells (such as epithelial cells) (Shen and Li, 2015). HMGB1 signals through RAGE and *via* distinct toll-like receptors (TLR), e.g. TLR2 and TLR4 (Gong et al., 2020). MiR-181a-5p/HMGB1/TLR4EV, a new signaling pathway, was

TABLE 1 DAMPs receptors, associated DAMPs, expression pattern and their effects related to AP.

DAMP receptors	DAMPs	Expression pattern	Main effect	Refs
TLR		Ubiquitous, high in immune cells	Promote the expression of pro-inflammatory genes, thus upregulate the production of cytokines, chemokines, and adhesion molecules.	(Yu et al., 2010; Lin et al., 2011; Vidya et al., 2018; Gong et al., 2020)
TLR2	HMGB1, HSP60, HSP70, histone			
TLR4	HMGB1, HSP22, HSP60, HSP70, HSP72, histone			
TLR9	DNA, HMGB1			
NLRP3	ATP	DCs, neutrophils, monocytes and macrophages	Promote the activation of caspase-1. Increase the secretion of IL-1β and IL-18. Initiate pyroptosis.	(Sutterwala et al., 2006; Jin and Flavell, 2010; Mangan et al., 2018; Gong et al., 2020)
RAGE	HMGB1	Ubiquitous, high in T cells, B cells, and macrophages	Promote the expression of pro-inflammatory genes. Mediate cell migrationand apoptosis.	(Chuah et al., 2013; Hudson and Lippman, 2018; Gong et al., 2020)
P2X7R (G protein-coupled receptor)	ATP	Ubiquitous	Promote the release of cytokine and chemokine, the activation of NLRP3 inflammasome, transcription factor and T cells.	(Di Virgilio et al., 2017b; Adinolfi et al., 2018; Martínez-García et al., 2019; Gong et al., 2020)
P2Y2R (ion channel)	ATP	Ubiquitous, high in immune cells, epithelial and endothelial cells	Promote the migration, and activation of immune cells. Control iron channels.	(Xu et al., 2018; Gong et al., 2020)

proposed by (Liu et al., 2021), suggesting that the encapsulated MALAT1 (Metastasis associated lung adenocarcinoma transcript 1) competitively binds to miR-181a-5p, thus inhibiting HMGB1 induced TLR4 signaling pathway, inducing M1 polarization of macrophages in AP, thereby promoting the release of IL-6 and tumor necrosis factor (TNF)-α (Liu et al., 2021).

The action of HMGB1 appears to differ from localization. The release of extracellular pancreatic HMGB1 could be a central event in early stage of pancreatitis (Figure 1), potentially by inducing autophagy resulting in necrosis when widely participate in numerous biological processes, including the formation of the chromosomal protein glycyl lysine isopeptide cross-link, and the positive regulation of phosphorylation, protein acid phosphorylation, the phosphate metabolic process and the phosphorus metabolic process (Gao et al., 2017; Gukovskaya et al., 2017). Likewise, HMGB1 might also serve as a late inflammatory factor to stimulate NF-κB nuclear translocation thereby enhancing inflammatory cells (such as the monocytes, neutrophils and dendritic cells) positive regulating the release of inflammatory cytokines such as IL-1α, IL-1β, TNF-α, etc., thus, act as an instrumental mediator in amplifying and maintaining the inflammatory cascade (Yu et al., 2015). Recent evidence indicates that HMGB1 participates in pancreatic, intestinal and lung injury during AP (Luan et al., 2013; Chen et al., 2017; Huang et al., 2019). Based on these effects, some new therapeutic approaches, such as calycosin and mesenteric lymph duct ligation to alleviate acute lung injury and euphorbia kansui to restore intestinal mucosa in severe AP were recently studied (Huang et al., 2021; Qiu et al., 2021; Tang et al., 2021; Zhu et al., 2021). In an experimental AP model, macrophage-derived HMGB1 served as a pain mediator in the early stage of AP (Irie et al., 2017). A recent study claims HMGB1 might induce AP through activation of NET and subsequent production of IL-1β, which may offer therapeutic targets for inflammation suppression (Irie et al., 2017). On the contrary, endogenous pancreatic HMGB1 may have an anti-inflammatory effect for its action in enhancing cell survival by increasing autophagic flux (Tang et al., 2010). This thought was supported by a study in which the observation of endogenous pancreatic HMGB1 deficient mice by the knockout gene of HMGB1 resulted in accelerated tissue injury and high mortality in AP (Kang et al., 2014b).

Heat shock proteins (HSPs) and AP

Heat shock proteins (HSPs) are conserved proteins, mainly involved in protein folding and maturation (Wu et al., 2017). The major groups of HSPs are classified based on the weights of molecules, including HSP27, HSP40, HSP60, HSP70, HSP72, HSP90, HSP100 and HSP110 (Zininga et al., 2018). As molecular chaperones, HSPs play an important cytoprotective role and their expressions are induced under stressful conditions such as heat shock (Wu et al., 2017; Narayanankutty et al., 2019). HSPs were initially found intracellular, although extracellular HSPs, also representing DAMPs, were discovered later, which have been found in extracellular vehicles such as exosomes and

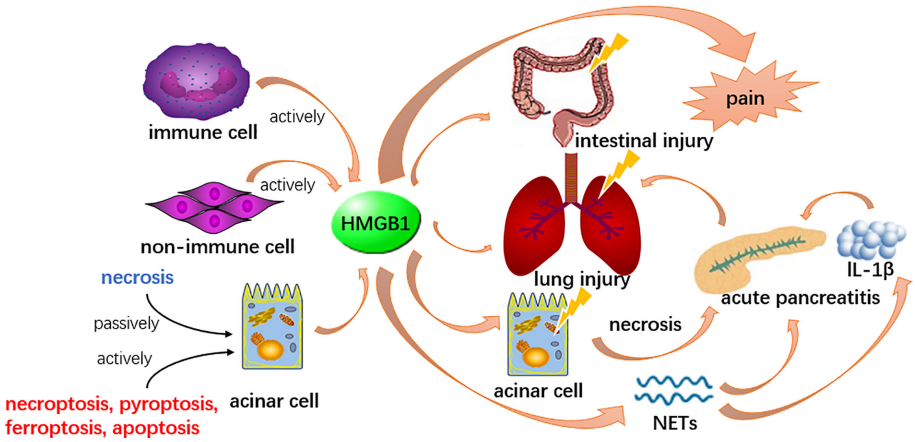

FIGURE 1
HMGB1 could be released by acinar cells and other cells in both active and passive ways. It participates in the formation of NETs and induce other acinar cell necrosis to boost the inflammation, therefore enhance the pathological process of AP. Meanwhile, HMGB1 is involved in lung and intestinal injury secondary to AP and serves as a pain mediator.

oncosomes, on membrane surfaces or acting as free HSPs under various pathological conditions (Taha et al., 2019).

It has been reported that intracellular HSPs are upregulated in AP models, including the arginine, dibutyltin dichloride, and cerulein-induced AP models, and the high level of HSPs may have a protective effect against tissue damage (Feng and Li, 2010). In the same direction, heat shock factor protein 1 (HSF-1) knockout mice with defects in HSPs synthesis were reported to be more severely affected by cerulein-induced pancreatitis (Bhagat et al., 2002).

Intracellular HSP27, HSP60, and HSP70 are direct effectors in mediating a protective effect against AP (Kang et al., 2014a), by inhibiting trypsinogen activation (Lee et al., 2000; Giri et al., 2017), modulating NF-κB signaling (Giri et al., 2017), preventing proinflammatory cytokines (Meng et al., 2013), reducing autophagy (Kim et al., 2011), preserving the actin cytoskeleton (Kubisch et al., 2004), aiding in cytoskeletal recovery and limiting oxidative damage (Ethridge et al., 2000). Many studies have also found that enhancing the function of those three types of intracellular HSPs can protect against AP in mice by using co-inducers, that promote the expression or introduction of HSPs (Rakonczay et al., 2002; Szabolcs et al., 2009; Li et al., 2021a). This could be a future research subject and a potential treatment target for AP in humans.

Although the transgenic overexpression or specific pre-induction of Hsp72 failed to protect against cerulein-induced pancreatitis, it did accelerate tissue recovery, possibly through an attenuated NF-κB signaling (Rakonczay et al., 2003; Lunova et al., 2012). This might also represent a potential future treatment strategy.

While intracellular HSPs function as chaperones to assist with biosynthetic pathways, extracellular HSPs released from damaged cells that are generally dying, following apoptosis, necrosis, and cellular stress can function as alarm signals to induce inflammation through activation of TLR2, TLR4, and cluster of differentiation (CD)91 (Schaefer, 2014; Roh and Sohn, 2018; Taha et al., 2019).

Song et al. found out that extracellular HSP70 may induce SIRS-like reactions to aggravate cerulein-induced pancreatitis through TLR-4 in mice by the administration of recombinant HSP70 (Song et al., 2008). In addition, low serum HSP70 levels are associated with poor prognosis in AP patients (Arriaga-Pizano et al., 2018). Budvytyte et al. discussed these two findings above, as they found out the serum HSP90 levels increased linearly with increasing AP severity while the relationship was not linear in the case of HSP70 (Budvytyte et al., 2021). The mechanisms of extracellular HSP in AP are not yet conclusively elucidated, so further investigations are still required.

DNA and AP

The study from Gornik et al. showed that serum free DNA was relevant to AP and its severity (Gornik et al., 2009). DNA methylation patterns based on plasma DNA have been developed as a new model to predict the severity of AP (Sun et al., 2021). During cell damage, nuclear and mitochondrial DNA leaks into the blood and activates the immune system, leading to a variety of diseases, including multiple organ dysfunction, failure to respond to sepsis or trauma, neurodegenerative diseases, etc. (Zhang et al., 2013).

The main source of free serum DNA in AP may be cell death (apoptosis or necrosis) or necrosis of pancreatic tissue (Gornik et al., 2009). The mechanism of DNA release into the circulation

has not been elucidated, but the release of DNA from neutrophils *via* NETosis may be one way in which it is released into the circulation (Gould et al., 2015). Neutrophils release DNA by forming NETs which could aggravate inflammation of AP and promote pancreatic duct obstruction (Murthy et al., 2019; Goggs et al., 2020; Hu et al., 2020; Shi et al., 2020). The DNA released by dead and necrotic cells is also a typical DAMP (Habtezion et al., 2019), and it has been proved that DNA released from necrotic cells could be an effective activator of the innate immune system, including dendritic cells and macrophages (Zhao et al., 2018; Habtezion et al., 2019).

STING (stimulator of the interferon gene) and apoptotic and necrotic DNA fragments are recognized by STING's unique receptor function (Song et al., 2008). When acute pancreatitis occurs, the expression of STING protein can activate downstream signaling pathways and promote inflammation (Sundar et al., 2021). Studies have found that STING is associated with AP, in which the DNA released by the dying acinar cells activates STING in macrophages, and STING drives the formation of pro-inflammatory cytokines and type I interferon to worsen AP-associated inflammation (Palmai-Pallag and Bachrati, 2014; West and Shadel, 2017; Picca et al., 2018; Zhao et al., 2018; Sundar et al., 2021) (Figure 2). Preventing the accumulation of their own DNA and blocking the over-activation of STING signals to reduce the generation of pro-inflammatory factors provides an attractive direction for future drug development (Ahn and Barber, 2014).

Toll-like receptors, such as TLR9 could recognize DNA to exacerbate inflammation in AP (Palmai-Pallag and Bachrati, 2014; Patel, 2018; Habtezion et al., 2019). The mechanism of double-stranded DNA could be toll-like receptor activation of the NF-kB signaling pathway (Lee and Papachristou, 2019). TLR9 recognition signals activate mitogen-activated protein kinases (MAPKs) and NF-κB to induce inflammatory downstreaming passways (Palmai-Pallag and Bachrati, 2014; West and Shadel, 2017; Barrera et al., 2021). The signaling pathway promotes gene expression of pro-inflammatory cytokines, chemokines, and adhesion molecules, as well as triggers inflammation through adaptor myeloid cell differentiation for major reactive protein 88 (MYD88) (Palmai-Pallag and Bachrati, 2014; West and Shadel, 2017; Lee and Papachristou, 2019; Barrera et al., 2021) (Figure 2). Animal experiments carried out by Hoque et al. demonstrated that TLR-9 receptor antagonists can significantly reduce pancreatic edema and inflammatory cell infiltration, highlighting new therapeutic avenues for AP (Hoque et al., 2011).

Mitochondrial DNA (mtDNA) is one of the mitochondrial DAMPs (West and Shadel, 2017; Patel, 2018; Barrera et al., 2021), which is released when mitochondria are dysfunctional or damaged (Barrera et al., 2021). Research by Kocsis et al. supports signaling *via* plasma DNA, and increased plasma DNA concentration is correlated to AP and its severity (Kocsis et al., 2009). The elevation of plasma mtDNA in AP patients could serve as a precise early predictive index of pancreatic necrosis (Wu et al., 2018b). MtDNA can cause inflammation through hypomethylated CpG motifs similar to bacterial DNA and bacteria-like non-methylated CPG-rich motifs (Palmai-Pallag and Bachrati, 2014; Picca et al., 2018). MtDNA is released systemically by T/HS system and activates p38 MAPK in neutrophils, probably *via* TLR-9, which results in the development of an inflammatory phenotype (Zhang et al., 2010a) MtDNA can be activated by TLR9, NLRs, cyclic GMP–AMP (cGAS), and other innate sensors that lead to inflammatory responses (Palmai-Pallag and Bachrati, 2014; West and Shadel, 2017; Picca et al., 2018). MtDNA is an effective DAMPs due to its specific characteristics, take its unique structure, relative hypomethylation and its enhancive sensitivity to oxidative damage for example (West and Shadel, 2017).

Histones and AP

Histones are the basic structural constituents of cellular chromatin. Eukaryotic DNA is surrounded by an octahedron of H2A, H2B, H3 and H4. The linker histone H1 facilitates further organization of chromatin. Except for their structural effects on chromosomes, the role and dynamics of chromosomes are also influenced by histone variants and the process of post-translational modifications (Campos and Reinberg, 2009; Biterge and Schneider, 2014). In addition to the physiological functions described above, when histones spill out of the nucleus, it can also serve as endogenous danger signs and DAMPs, playing an important role in the production and development of inflammation (Lu et al., 2014).

Histones can be released passively, for example, necrosis, necroptosis, apoptosis, and NETosis (Murao et al., 2021a). In particular, citrullinated histones are an important component of NETs and may be the mechanism by which the innate immune system exacerbates pancreatic injury (Thiam et al., 2020). In addition to passive released by cell death, histones can also be actively secreted through the exosomal exocytosis of living cells (Murao et al., 2021a).

Extracellular histones mediate sterile inflammatory responses, distant multiorgan damage and even death through activation of TLRs and the NLRP3 inflammasome signaling pathway (Semeraro et al., 2011; Allam et al., 2012; Li et al., 2021b). In terms of TLRs, histones specifically bind to and activate TLR2 and TLR4, which trigger MyD88 signaling followed by NF-κB and MAPK expression (Semeraro et al., 2011; Allam et al., 2014). Histones activate NLRP3 primarily through induction of intracellular oxidative stress (Allam et al., 2014). The NLRP3 inflammasome is likely a downstream component that integrates various indirect stimuli of IL-1β and IL-18 proteolysis (Allam et al., 2013) (Figure 3). Apart from the above two pathways, histones also have the pathogenic

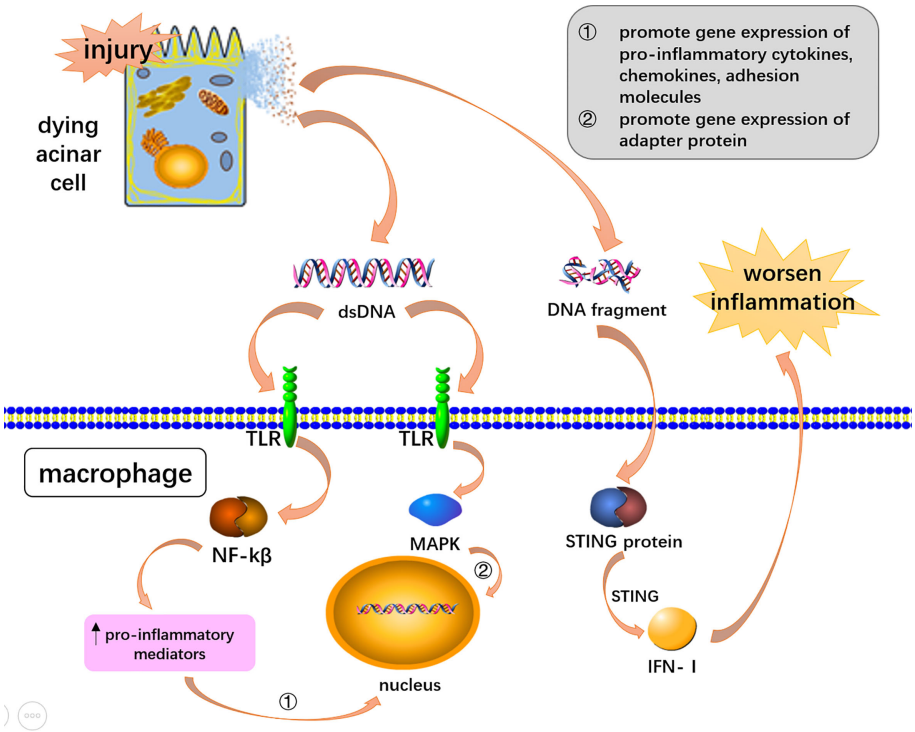

FIGURE 2
DNA mediated inflammation *via* DAMPs receptors and STING signaling. Double-stranded DNA and DNA fragments were released from dying acinar cells. DsDNA activated the NF-KB and MAPK pathway through toll-like receptors to induce AP-associated inflammation. DNA fragments worsen AP-associated inflammation through STING signaling pathway in macrophages.

effects of bactericidal ability, cell toxicity, platelet activation, and protecting DNA degradation (Allam et al., 2014). In experiments where individual components of histones induced inflammatory cytokines in BMDC (bone marrow-derived dendritic cells), it was concluded that all histones had the ability to induce the production of TNF and IL-6 (Allam et al., 2012).

Researches showed that histones in the blood serve as DAMP and cause sterile inflammation leading to SIRS and organ failure in the process of AP (Kang et al., 2014a). At the cellular level, platelets, endothelial cells and leukocytes are also exposed to the toxic effects of extracellular histones. (Allam et al., 2014). Liu et al. further indicate that elevated levels of circulating histones are strongly associated with AP's disease severity and mortality (Liu et al., 2017). Circulating histones accumulate in the inflamed pancreas and actively contribute to pancreatic acinar cell necrosis by destroying the plasma membrane in a burden- and dose-dependent manner (Liu et al., 2017; Szatmary et al., 2017; Biberci Keskin et al., 2019). In a previous experimental model of AP, we found a good correlation between elevated extracellular histones and pancreatic necrosis events. This is in addition to the concomitant damage to distant multiple organs, such as the heart, liver, lungs and kidneys, which are the most commonly affected organs (Guo et al., 2014; Ou et al., 2015). Circulating concentrations of nucleolytic degradation products reflect the extent of tissue damage and cell death. As the most abundant protein in the nucleus, histones can be used as a better biomarker to stratify the severity of a disease. (Ou et al., 2015).

Now that we know histone represents key mediators of AP, we might explore histone as a therapeutic target for treatment in the future. Negatively charged surface molecules (e.g., phosphatidylserine) enhance histone membrane interactions, so neutralizing the charge on circulating histones could be a promising therapeutic strategy (Szatmary et al., 2017). In mouse models, we found that anti-histone antibodies could be used to rescue mice from multi-organ failure due to histone injections (Xu et al., 2009; Xu et al., 2011; Li et al., 2021b). In subsequent experiments, the histone neutralizing antibody BWA3 halted histone related lesions, but it remains unclear exactly how it can block the physiological effects of circulating histones. (Monestier et al., 1993). Therefore, a potential therapeutic approach could be the development of anti-histone therapies to delay the second attack early in the process of AP (Kang et al., 2014a; Hartman et al., 2015; Liu et al., 2017). In addition to this, activated protein C serves as a serum protease, it can destroys histones that spill over into the extracellular space, thereby blocking the

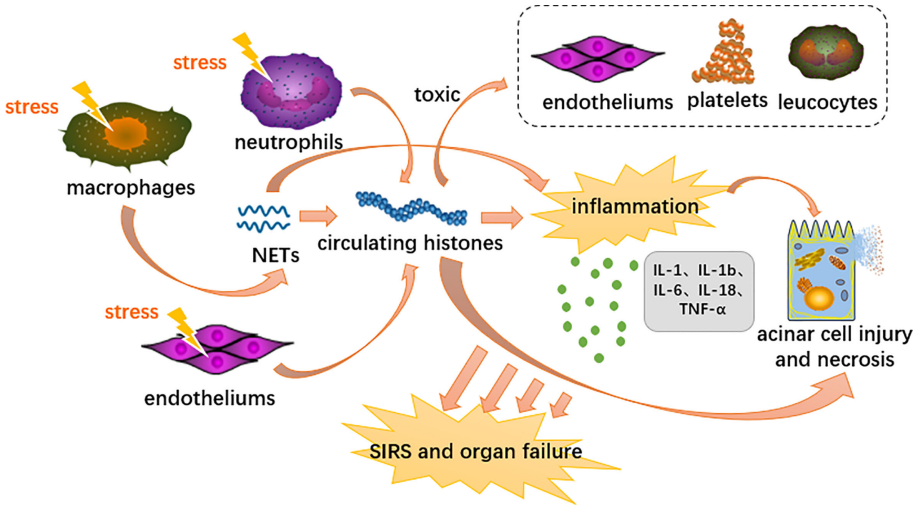

FIGURE 3
Proposed immunopathological roles of circulating histones in acute pancreatitis. DAMPs activate innate immune cells (neutrophils and macrophages) and endothelial cells through PRRs, triggering highly inflammatory programmed cell death such as neutrophil extracellular traps, necroptosis and necrosis. Extracellular histones mediate inflammation response, organ injury and death through TLR and NLRP3 inflammasome pathways.

pathological processes associated with histones (Ammollo et al., 2011).

ATP and AP

The pancreatic acinar cells are typical excitable exocrine cells featured by its high secretory turnover which is mainly supplied and closely dependent by mitochondrial production of ATP (Petersen and Tepikin, 2008). Given that ATP mainly kept in reserve at acinar cells with high concentration, the elevating extracellular concentration of ATP is thought to attribute to the release of injured cells (Yegutkin et al., 2006). Necrotic Acinar cells undergoing cytoplasmic membrane destruction are likely to be the main source of extracellular ATP (eATP) (Dixit et al., 2019b). Other sources of high eATP levels may include activated immune cells, duct cells, endothelial cells, or even cells undergoing apoptosis (Bodin and Burnstock, 1996; Elliott et al., 2009; Junger, 2011; Kowal et al., 2015).

The decrease of mitochondrial ATP production is the typical event at the early stage of AP. In normal physiological condition, Ca^{2+} is released from the ER and maintain a stable concentration to promote steady function as part of a signaling mechanism that stimulates the production of ATP in mitochondria (Criddle et al., 2007). The cellular Ca^{2+} concentration overload or other toxins (like bile acid, alcohol) cause mitochondrial permeability transition pores continue to open in a high-conductance state, resulting in the disorder of the membrane potential needed to generate ATP (Mukherjee et al., 2016). ATP depletion deteriorates and perpetuates the toxic overload of Ca^{2+} by destroying ATP-dependent Ca^{2+} channels in the smooth ER (SERCAs) and plasma membrane from clearing excessive cytosolic calcium. This results in the impairment of cytoprotective mechanisms such as autophagy and the unfolded protein response (UPR) that needs ATP (Biczo et al., 2018). Hence, this interaction enhances cell damage. Nevertheless, there still could be an abundant amount of ATP, which, if released, can play an essential role as DAMPs by activating purinergic signaling (Dixit et al., 2019b).

Once released, the massive accumulation of extracellular nucleotide could be regulated in the way of phosphorylation and dephosphorylation by triggering the activation of extracellular enzymes (ecto-enzymes). CD-39 mediates phosphohydrolysis of eATP (from ATP/ADP to AMP), which is subsequently converted to adenosine by CD73 (from AMP to adenosine) (Minor et al., 2019). CD73 has key role in maintaining homeostasis and regulates pathophysiological responses related to immune tolerance, inflammation, infection and cancer (Linden et al., 2019; Schneider et al., 2019; Vuerich et al., 2019). One study suggested that soluble CD73 is a biomarker of persistent organ failure and the severity of AP is better than C-reactive protein or creatinine (Maksimow et al., 2014). Nevertheless, there are still controversial attitudes toward this study (Sun and Messaris, 2014; Jiang et al., 2015). CD39 expression is regulated by several pro-inflammatory cytokines (such as IL-2, TGF-β), tissue damage, oxidative stress, tissue remodeling and hypoxia leading to the accumulation of eATP (Antonioli et al., 2013; Timperi and Barnaba, 2021). Künzli et al.

found that CD39 deletion decreases fibrogenesis and alleviates inflammation in experimental pancreatitis (Kunzli et al., 2008). Another group of enzymes participated in the conversion eATP includes adenylate kinases (AKs) and the nucleoside diphosphate kinase (NDPK/NME) family, which *via* phosphohydrolysis to accelerates the transition from ATP to ADP (Zimmermann et al., 2012; Yegutkin, 2014).

eATP has been shown to be involved in guiding neutrophils to the site of injury, activating T cells, platelets, mast cells and monocytes, promoting inflammasome activation in macrophages, and releasing cytokine (Cekic and Linden, 2016). One study has shown that the release of eATP promotes systemic injury in severe acute pancreatitis (Dixit et al., 2019b). With the increasing concentration of eATP, purinergic signaling is triggered by nucleotides' interaction in P2 receptors, such as transcell membrane cationic channels (P2XR) and G-protein coupled receptors (P2YR), thereby initiating autocrine/paracrine signaling, (Beavis et al., 2012; Zimmermann et al., 2012). P2X7 is a major stimulant of the intensity and duration of inflammation and immunity and is involved in the pathologies of cancer, nervous and cardiovascular diseases (Di Virgilio et al., 2017a; Adinolfi et al., 2018). By activating intracellular signal transduction pathways including NF-κB, nuclear factor of activated T cells (NFAT), PI3K-AKT-GSK-3b and hypoxia-inducible factor 1α (HIF1α), it promotes the release of various cytokines (such as IL-6 from human fibroblasts and TNF from human dendritic cells) and chemokines in different immune cells (Gong et al., 2020). Considered as one of the most potent activators of the NLRP3 inflammasome, the role of P2X7R for establishing an inflammatory response is widely studied in the activated innate myeloid cells (Martínez-García et al., 2019). Targeting P2X7/NLRP3 signaling pathway, a new study demonstrated that effectively alleviates ATP–induced pancreatic ductal cell injury to avoid ductal occlusion in AP by downregulating the protein levels of P2X7 (Zhang et al., 2019). Therefore, inhibition of the P2X7/NLRP3 signaling pathway could be a novel therapeutic target in AP.

eCIRP and AP

Cold-inducible RNA-binding protein (CIRP) was discovered in the early twenty-first century during the research of mechanism of cold stress adaptation in mammals. Since then, the role of intracellular CIRP (iCIRP) as a stress-response protein has been extensively studied. In contrast, extracellular CIRP (eCIRP), an 172-amino acid RNA chaperone protein, was recently discovered to also act as DAMP. It plays an important role in pathobiology of inflammatory diseases (Aziz et al., 2019; Denning et al., 2019b). By drawing plasma at 24~48h after admission from patients with AP, the study found that in both mild and moderate severe/severe AP, the plasma levels of eCIRP were increased remarkably, and the results also suggest that eCIRP promotes systemic inflammatory responses to AP (Linders et al., 2020b).

The release of eCIRP can be divided into passive and active release. The eCIRP can be passively released by necrotic cells, but an *in vitro* study using macrophages suggests that passive release is not a major source of eCIRP during hypoxia or endotoxemia, and remains to be studied in AP (Aziz et al., 2019). Active release means that CIRP can migrate from the nucleus to the cytoplasmic emergency particles and then be released into the extracellular space through the lysosomal extracellular pathway during periods of cellular stress, such as hypothermia, hypoxia and hyperradiation (Denning et al., 2019b; Murao et al., 2021b).

As DAMPs, eCIRP induces endothelial cell activation, macrophage secretion of pro-inflammatory mediators and NETs formation (Qiang et al., 2013; Ode et al., 2019; Linders et al., 2020b). Among them, the function of inducing endothelial cell activation has been fully proved in the studies related to acute lung injury, but there is no relevant study in AP (Ode et al., 2019). It can activate the GSDMD (Gasdermin D) pathway of macrophages to promote MET formation, where GSDMDS is also a reforming protein involved in NET formation (Lee et al., 2021; Tan et al., 2022). Moreover, by recognizing TREM-1, an innate immune receptor expressed primarily in myeloid cells, it can activate macrophages (Denning et al., 2020; Murao et al., 2020; Lee et al., 2021; Murao et al., 2021b). eCIRP also binds to the TLR4-MD2 receptor complex, which activates the TLR4/MyD88/NF-κB pathway and induces macrophages to release pro-inflammatory cytokines (TNF-α, IL-6 and IL-1b) and chemokines (keratinocyte chemoattractant and MIP-2), as well as HMGB1 (Figure 4) (Linders et al., 2020b; Murao et al., 2021b). This pathway also promotes mitochondrial DNA damage and degradation, causing STING activation, which leads to the production of type I IFNs and pro-inflammatory cytokines, and has also been shown to activate NETs (Denning et al., 2019b; Gurien et al., 2020; Chen et al., 2021a; Chen et al., 2021b). In promoting the formation of NETs, eCIRP can induce the production of a different type of neutrophils, ICAM-1+ neutrophils, which can produce more NETs (Murao et al., 2020). Recent studies have also shown that eCIRP can also increase NET formation *in vivo* and *in vitro* by activating PAD4 (Denning et al., 2019b)

Inhibition of eCIRP has been shown to reduce NET formation, pro-inflammatory mediators and tissue damage in AP (Linders et al., 2020b). Qiang et al. developed a CIRP antagonist, C23, with a high affinity for the TLR4/MD2complex, where it reduces inflammation and tissue damage in sepsis, shock, and ischemia-reperfusion (Murao et al., 2021b). Moreover, C23-targeted eCIRP inhibits inflammation and tissue damage in AP (Linders et al., 2020b). Microrna130b-3p has been shown to inhibit ECIRP-mediated aseptic inflammation in the treatment of sepsis and acute lung injury, but its effect in AP has not been studied (Ode et al., 2019).

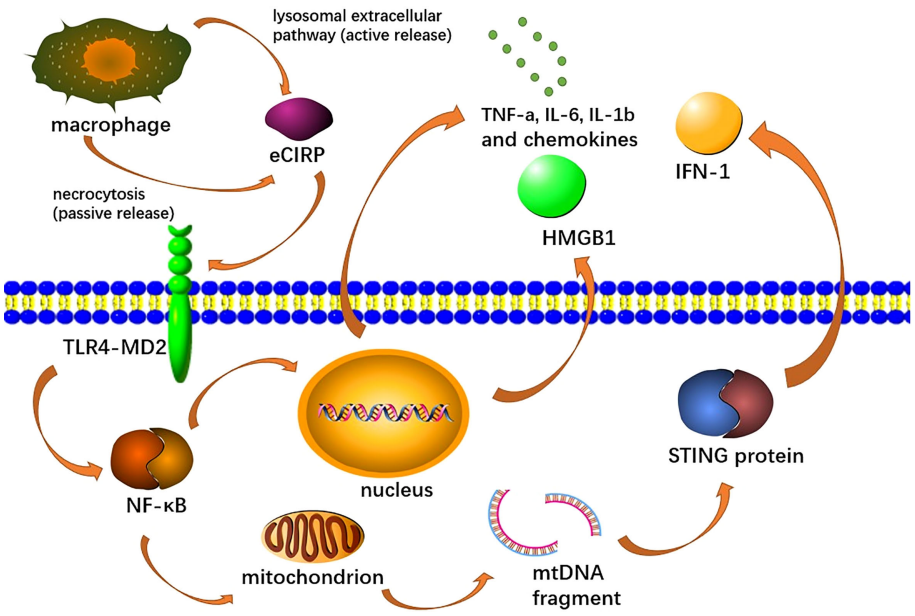

FIGURE 4
Extracellular CIRP (eCIRP) is released to the extracellular space *via* necrosis or lysozyme extracellular pathway, afterward binds to the TLR4-MD2 receptor complex on macrophages, activates the TLR4/MyD88/NF-κB pathway and induces macrophages to release pro-inflammatory cytokines (TNF-A, IL-6, Il-1b), chemokines (keratinocyte chemical attractor and MIP-2), and HMGB1. This pathway also promotes mitochondrial DNA damage and degradation, leading to STING activation, which leads to the production of type I IFN and pro-inflammatory cytokines.

NETs as extracellular fibrillar DNA networks

As the most abundant innate immune cells in human body, neutrophils play a key role in fighting bacterial infections and function mainly *via* three mechanisms, i.e. phagocytosis, degranulation and NET formation (Papayannopoulos, 2018; Wan et al., 2020). NETs are extracellular networks consisting of decondensed chromatin fibers studded with granular and cytoplasmic proteins and peptides (Brinkmann, 2018). NETs often contribute to pathogen clearance, but in excessive NETs can also lead to inflammation and tissue damage (Tan et al., 2021). DNA in NETs is mainly originated from the nucleus, but also mitochondria (Lood et al., 2016; Papayannopoulos, 2018). The proteins of NETs are mainly composed of histones as well as granular enzymes and peptides, among which are neutrophil elastase (NE), S100A8, lactoferrin, azurocidin, cathepsin G (CG), S100A9, myeloperoxidase (MPO), proteinase 3 (PR3), pentraxin 3, gelatinase, actin, lysozyme C, calprotectin, defensin, cathelicidin and catalase (Urban et al., 2009; Brinkmann, 2018). The release of NETs occurs primarily through a cellular death process termed NETosis (Papayannopoulos, 2018). There is a variety of inducing factors for NETosis such as infectious agents (viruses, bacteria, bacterial components, parasites, fungi), physiological stimuli (activated platelets, complement-derived peptides, antibodies, cytokines, microcrystals) and chemicals (hydrogen peroxide, tobacco smoke) (Brinkmann, 2018). There are two types of NETosis: suicidal NETosis, in which cells die, and vital NETosis, in which cells maintain certain viability and effector functions (Vorobjeva and Chernyak, 2020).

Suicidal NETosis, which lasts 2–4 h, begins with the activation of neutrophils upon the recognition of stimuli, mostly PMA, which induces the activation of the NADPH oxidase (NOX) complex and subsequent production of ROS through protein kinase C (PKC)/Raf/MERK/ERK (Masuda et al., 2016; Delgado-Rizo et al., 2017). The NADPH-dependent ROS production not only leads to chromatin decondensation by boosting the activation of calcium influx and peptidyl arginine deaminase 4 (PAD4) but also promotes the progressive nuclear membrane separation and loss (Delgado-Rizo et al., 2017; Denning et al., 2019b). The transport of elastase and myeloperoxidase from granules to nuclei also plays a key role in NET formation (Papayannopoulos et al., 2010). Cytoplasm and karyoplasm are mixed and finally released outside the cell through membrane pores and cellular lysis (Delgado-Rizo et al., 2017; Brinkmann, 2018).

Vital NETosis has been found out following specific microbe-associated molecular patterns (MAMP) recognized by host pattern recognition receptors, such as toll-like receptors, independent of ROS and the Raf/MERK/ERK pathway (Masuda et al., 2016; Delgado-Rizo et al., 2017). The induction of vital

NETosis normally takes 30 minutes after PMN stimulation, as opposed to several hours for suicidal NETosis (Denning et al., 2019b; Zhou et al., 2020a). After the activation of vital NETosis, the nuclear envelopes dilates hugely and vesicles are formed (Brinkmann, 2018). Those DNA-containing vesicles concentrate near the plasma membrane and eventually fuse with it, releasing contents without cell lysis (de Buhr and von Köckritz-Blickwede, 2016; Brinkmann, 2018). Another type of vital NETosis independent of cell death while dependent of ROS has also been described, in which the mitochondrial DNA was released by neutrophils instead of the nuclear (Yousefi et al., 2009).

NETs and AP

The important role of neutrophils in AP is well known, and Merza et al. demonstrated for the first time that neutrophils-derived NET is a core part of the pathophysiological process in severe AP (Merza et al., 2015). It has shown that the biomarker NET is not only present in AP but also associated with the severity of AP (Murthy et al., 2019). In septic AP, NETs fight infection by trapping and killing invading microorganisms (Pan et al., 2021). Neutrophils could cause partial and distal organ injury due to the release of NETs in severe AP (Korhonen et al., 2015).

NETs could participate in the pathogenesis of AP by induction of trypsin activation, damage of tissue and accelerating systemic inflammatory responses (Pan et al., 2021). In AP, premature activation of trypsinogen is considered to be a key factor in triggering the disease induction (Gui et al., 2020). Korhonen et al. proposed a convincing mechanism by which neutrophils activate intra-acinar trypsin (Korhonen et al., 2015). The study has shown that NETs are effective stimulators of macrophage-1 antigen (MAC-1) expression and ROS formation in neutrophils, and NETs can directly activate neutrophils (Merza et al., 2015). Inhibition of NETs resulted in a 93% reduction in matrix metallopeptidase-9 (MMP-9) circulating levels in taurocholic acid-exposed animals (Merza et al., 2015). Neutrophil-derived MMP-9 is important in AP as it is an effective trypsinogen activator and regulates trypsinogen activation and tissue damage (Awla et al., 2012). Signal transducer and activator of transcription-3 (STAT-3) is also a vital signal molecule of acinar cells (Madhi et al., 2019b). It has been found that NETs modulate STAT-3 activity and trypsin activation in acinar cells (Merza et al., 2015), and another study showed that the stimulation of NET could significantly enhance the liveness of STAT-3 (Madhi et al., 2019b). Therefore, NETs could activate trypsinogen through STAT-3, MMP-9, and other mechanisms, thus magnifying the extent of pancreatic damage in AP (Wan et al., 2020).

The initial injury in AP is sterile (Hoque et al., 2012), and Yang et al. firstly proposed an interesting new role for NETs in pancreatitis without microbiological infection (Yang et al., 2015). NETs have been shown to be key mediators of innate immune responses in aseptic inflammatory diseases (Murthy et al., 2019). Systemic inflammatory cascade activation is characteristic of severe AP and could result in systemic inflammatory response syndrome in pathological progression of disease (Hu et al., 2020). NETs not only cause damage to the pancreas, but also lead to severer damage to other involved organs, such as lung, blood vessel, kidney and heart damage (Hu et al., 2020). NETs mediate inflammation and thrombosis and play a role in the transitional period of hypercoagulability and severe inflammation in the incipient stage of acute pancreatitis (Wan et al., 2020). Formation of NET in inflammation has been shown to be highly pro-coagulant, due to exposure to cell free DNA (cfDNA), histones and neutrophil proteases, which leads to coagulation activation and inactivation of antithrombotic proteins, predisposed to formation of disseminated intravascular coagulation (DIC) (Liaw et al., 2016).

Neutrophils could cause acinar cell damage by activating trypsinogen and could directly cause acinar cell damage by secretion of ROS and MMP-9 (Saffarzadeh et al., 2012). NETs have cytotoxic effects (Saffarzadeh et al., 2012), and histones from NETs can cause acinar cytoplasmic leakage and cell death by disrupting the pancreatic acinar cell serosa (Hu et al., 2020). NETs on internal body surfaces are useful for monitoring potential hazards, but excessive formation of NETs may lead to epithelial cell damage (Leppkes et al., 2019). It has been reported that NET-derived histones could lead to damage and death of both epithelial and endothelial cells (Saffarzadeh et al., 2012).

Condensed layers of aggregated NETs can spatially screen and insulate the necrotic site, creating a temporary barrier to limit the spread of necrosis-related proinflammatory mediators (Bilyy et al., 2016). However, aggregated NETs could block secretion flow, driving focal pancreatitis and parenchymal remodeling (Leppkes et al., 2016). The production of intraductal aggregated NETs results in occlusion of the pancreatic duct and persistence of inflammation, leading to pancreatitis (Leppkes et al., 2016). NET is a double-edged sword, coordinating the innate immune response while it also carries the danger of precipitating autoimmunity and epithelial injury (Leppkes et al., 2019).

Interaction between DAMPs and NETs

Both NETs and DAMPs are closely associated with the development of AP, and there is growing support for a potential link between the two as well (Figure 5). NETs are covered by proteins such as histones, granulins and cytosolic proteins, and these major components are identified as DAMPs (Pires et al., 2016; Chapman et al., 2019). Some DAMPs acted as

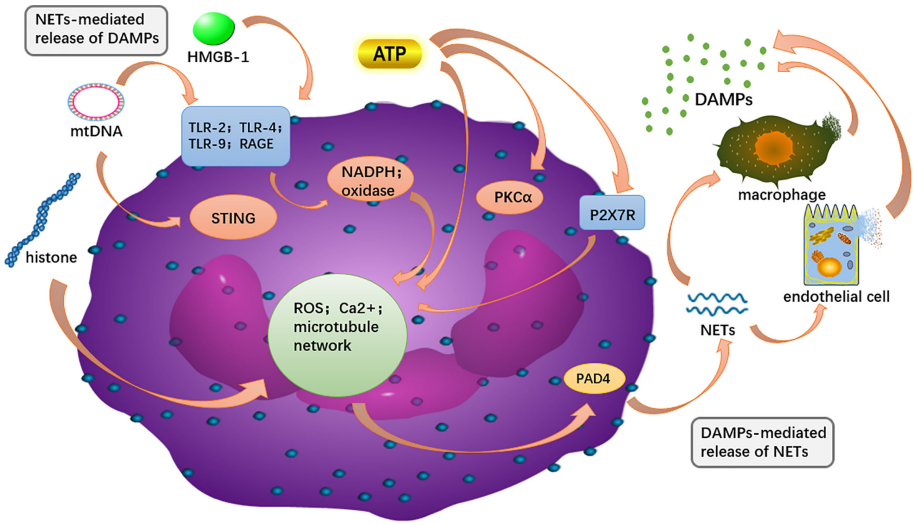

FIGURE 5
The intracellular DAMPs is released to the outside of the cell and can recognize PRR on surrounding neutrophils, and in turn, activate PAD4 to promote NET formation. NETs components, such as DNA and H3 histones, can further activate macrophages and endothelial cells, releasing more DAMPs out of the cell to amplify the inflammatory cascade.

components of the NET enhance the inflammatory cascade by further stimulating immune cells as well as endothelial cells to release more DAMPs (Denning et al., 2019a). Correspondingly, various DAMPs have been shown to induce NETs.

HMGB-1 contributes to NET formation by attaching to receptors such as TLR2/4 and RAGE, which was reliant on NADPH oxidase (Ma et al., 2016; Wu et al., 2018a). NADPH oxidase, as an important source of ROS production, was engaged in HMGB-1-mediated activation of neutrophils (Xiang et al., 2011). Oxidative stress is thought to contribute to the formation of NETs, with HMGB-1 serving as a mediator for it (Wu et al., 2016). HMGB-1 induces NETosis, as reported in previous studies, including in hepatic I/R and LPS-injected animal models (Tadie et al., 2012; Huang et al., 2015). In liver I/R animal model, the authors found that the histone H3 of TLR4-KO neutrophils in response to HMGB1 and histones was reduced, with a more significant decrease observed in HMGB1-stimulated neutrophils. The result suggests that TLR4 is the predominant receptor for HMGB1 to form NETs (Huang et al., 2015).

In addition to its structural role as a component of NETs, mitochondrial DNA (mtDNA) activates neutrophils to release more NETs and participates in the inflammatory response (Yousefi et al., 2009; Balogh et al., 2013; McIlroy et al., 2014). Neutrophils releasing mtDNA is ROS-dependent and independent of cell death, and mtDNA was shown to markedly induce extracellular DNA release (Yousefi et al., 2009; Liu et al., 2019). Research shows that mtDNA-induced NET formation is dependent on the both TLR9 and STING pathways (Liu et al., 2019). Liu et al. found that NET formation by mtDNA was suppressed in Sting-/- and TLR9-/-mice, indicating that the TLR9 and STING signal ways may exert its effects on the mtDNA-induced NET formation. In subsequent experiments, Sting-/-and TLR9-/- neutrophils exhibited decreased phosphorylation of extracellular signal-regulated kinases (ERK1/2) and p38 MAPK, and reduced levels of PAD4 and Ras-related C3 botulinum toxin substrate 2 (Rac2) in response to mtDNA. ERK1/2 and p38 MAPK are located downstream of the STING and TLR9 pathways. Therefore, when these pathways are inhibited, it results in a remarkable decrease in expression of PAD4 and Rac2 induced by mtDNA (Liu et al., 2019). Tumburu et al. suggested that a cytosolic pathway is responsible for mtDNA-induced NET formation and that serine/threonine TBK1 is involved in the process (Tumburu et al., 2021).

Citrullinated histones are key mediators in NET genesis and their co-localization with cytoplasmic components of neutrophils suggests the formation of NETs (Nakazawa et al., 2017a). Histone citrullination weakens the link between DNA and histones, ultimately leading to chromatin depolymerization and NET release. (Remijsen et al., 2011; Nakazawa et al., 2017b). Previous studies have demonstrated that histone H4 triggers NET formation *via* calcium and PAD4. Neutrophil membrane permeability and intracellular calcium content are continuously increased due to histone H4, it is a necessary condition for PAD4 activation (Shi et al., 2021). PAD4 promotes histone hypercitrullination, a primary precondition for NET formation, which in turn mediates chromatin

depolymerization and facilitates the release of NETs (Li et al., 2010). Additionally, Hamam et al. showed that histone acetylation promoted NET formation by facilitating chromatin decondensation (Hamam et al., 2019).

The formation of NETs needs the genesis of ATP in the glycolytic system, reorganization of the cytoskeleton and ejective release of cytoplasmic granules along with mtDNA (Stojkov et al., 2017; Amini et al., 2018). NETosis mediated by ATP in blood polymorphonuclear leukocytes and bone marrow-derived PMN has been identified to occur by a mechanism involving Ca2 + influx by P2X7R, activation of PKC-α, elevated ROS genesis and subsequent increase of PAD4 (Kim et al., 2020). As is the case for HMGB1, DNA and histone, ATP is also an intracellular constituent released in the course of NETosis. During NETosis, numerous ectonucleotides (e.g. ATP, ADP, UDP, UTP and adenosine) are released, and it is speculated that there may be a vicious circle between NETosis and purinergic receptor-mediated autocrine responses to ectonucleotides (Sil et al., 2017; Kim et al., 2020).

Application of DAMPs and NETs in AP therapy

Nowadays targeted therapeutic modalities against DAMPs are discussed mostly in four categories (Table 2):

1. Decreased expression of extracellular DAMPs
2. Increased expression of intracellular DAMPs
3. Enhancing DAMPs elimination by receptor antagonists, antibody neutralization, competitive antagonism, etc.
4. Blocking DAMPs signaling

Although research has uncovered the involvement of NETs in the pathogenesis of sepsis, connective tissue diseases, cardiovascular diseases, autoimmune diseases and cancer, the investigation into the pathologies and promising therapeutic approaches of NETs in AP are still limited (Döring et al., 2017; Lee et al., 2017; Bonaventura et al., 2020; Masucci et al., 2020; Klopf et al., 2021; Cristinziano et al., 2022). Nowadays targeted therapeutic modalities against NETs mostly focusing on the intervention by the blockage of NETs formation (Madhi et al.,

TABLE 2 Intervention of DAMPs in AP therapy, with intervention type, targeted DAMPs and associated mechanism.

Intervention type	Substance	Targeted DAMPs	Mechanism	Reference
Decrease expression of extracellular DAMPs	LincRNA-EPS; Dexamethasone; Midazolam combined with sufentanil; pRNA-U6.1/Neo; Calycosin; Sodium Butyrate;microRNA-141; miR-340-5p; Danaparoid sodium	HMGB1	Suppress the HMGB1-NF-κB-dependent inflammation gene expression	(Hagiwara et al., 2009a; Hagiwara et al., 2009b; Zhang et al., 2010b; Luan et al., 2013; Zhang et al., 2015; Zhu et al., 2016; Zhao et al., 2018; Zhou et al., 2020b; Chen et al., 2021c; Zhu et al., 2021; Gao et al., 2022b)
Increase the expression of intracellular DAMPs	Hydrogen-rich gases	HSP	Increase the expression of HSP60 and resist the oxidation	(Li et al., 2021a)
	BRX-220	HSP	Increase the production of HSPs incluing HSP60 and HSP72	(Rakonczay et al., 2002)
	Bortezomib		Induce the pancreatic HSP72 and inhibit the proteosome	(Szabolcs et al., 2009)
	Insulin and insulin-mimetics	ATP	Upregulate glycolysis, prevent POA-induced ATP depletion, and inhibit Ca2+ overload	(Bruce et al., 2021)
	Cyclosporin A derivative (NIM811)		Serve as a cyclophilin D inhibitor to inhibit the opening of the mitochondrial	(Petersen et al., 2021)
Enhancing DAMPs elimination	HMGB1 neutralizing antibody	HMGB1	Bock high-mobility group box 1 and reduce the TLR4 and TLR9 expression	(Sawa et al., 2006; Chen et al., 2017)
	Heparin, Activated protein C, Thrombomodulin	Histones	Bind and inactivate histones	(Ammollo et al., 2011; Nakahara et al., 2013; Wildhagen et al., 2014)
	IRS954		Block TLR to reduce pancreatic edema and inflammatory	(Hoque et al., 2011)
Blocking DAMPs signaling	Protocatechuic acid; ALR;Abdominal paracentesis drainage;	HMGB1	Target HMGB1/TLR4/NF-κB signaling pathway	(Hagiwara et al., 2009a; Pan et al., 2018; Abdelmageed et al., 2021; Huang et al., 2021)
	Suramin	eATP	Block the P2 receptors and lead to reduced levels of plasma IL-6 and TNF-α	(Dixit et al., 2019a)

2019a; Madhi et al., 2019b; Madhi et al., 2019c; Murthy et al., 2019; Linders et al., 2020a; Wu et al., 2021). It could be worthy to explore more potential treatments such as facilitating the elimination of DAMPs (Table 3).

Given the close relationship between DAMPs and NETs, the new approach targeting DAMPs and NETs could be promising and worth further investigation. However, until now, only one research from Wu focused on the close crosstalk between DAMPs and NETs in AP, revealing that HMGB1 could induce AP through activation of NETs and subsequent production of IL-1β, therefore a new therapeutic intervention could be attempted by targeting HMGB1 and NETs. Considering the rather limited research, further studies providing insights into the DAMPs and NETs in AP could be useful and are required.

Conclusion

In this review, we gave an overview about the role of DAMPs and NETs in AP with concentrations on their mutual effects and therapeutic methods, in order to offer a better understanding of the pathophysiological mechanism and new insights for future investigational AP treatment options.

DAMPs are essential mediators in AP that may assume different roles depending on their location (intracellular vs. extracellular). While intracellular DAMPs may have protective value with regard to inducing autophagy that inhibiting the activation of the inflammasome, extracellular DAMPs may act as inflammatory factors through a variety of complex mechanisms. NETs could fight infection but also involved in the exacerbation of AP (Wan et al., 2020). The formation of NETs have been shown to be induced by variety of DAMPs. Both DAMPs and NETs have already been treatment targets for AP in many animal experiments.

However, new DAMPs are still being discovered and proposed definition and categories of DAMPs remain controversial. In addition, due to the complexity of the DAMPs pathways and the controversy of the NET formation, there are still many possible mechanisms and effects of DAMPs, NETs and their interactions that remain to be explored. With more precise mechanisms and newer DAMPs being proposed, further targeted and effective therapies can be established to minimize pancreatic injury and systemic inflammation, based on the information. They could also minimize damage to patient immunity. Although further discoveries are required, it is time to increase the development of the therapeutics/pharmaceutics and to put some fundamental findings into practice.

In conclusion, we performed a literature review on the role of DAMPs, NETs, and their interactions in AP. This could be useful for understanding of mechanism and prove noteworthy for people working in this area of research.

TABLE 3 Intervention in NETs, and their mechanisms in AP therapy.

Substance	Mechanism	Reference
Chloroquine	Reduce serum cell-free DNA and citrullinated histone H3 in murine models of pancreatitis to regulate NET formation	(Murthy et al., 2019)
C3 gene-deficient	Disrupt complement cascades, reduce chemokine secretion, as well as decrease early infiltration of neutrophils into the pancreas and neutrophil extracellular traps formation through PAD4.	(Linders et al., 2020a)
GZD824	Abolish activation of c-Abl kinase to regulate NET formation, as well as decrease levels of citrullinated histone 3 in the pancreas and DNA-histone complexes in the plasma.	(Madhi et al., 2019a)
Cl-amidine and GSK484	Reduce taurocholate-induced increase of histone 3 citrullination in the pancreas and DNA-histone complexes in the plasma to regulate NET formation	(Madhi et al., 2019c)
Platelet IP6K1 gene-deficient	Induce exogenous PolyP to regulate NET formation	(Madhi et al., 2019b)
PD1	Decrease early infiltration of neutrophils into the pancreas and neutrophil extracellular traps formation through PAD4.	(Wu et al., 2021)

Author contributions

XZ, SJ, JP, QL, SY, and WH conceptualized the study, searched and analyzed the literature, and wrote the draft of the manuscript. PA, ZB, VZ, and WH helped to finalize the manuscript. All authors listed have made a substantial, direct, and intellectual contribution to the work, and approved it for publication.

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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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Glossary

DAMPs	Damage associated molecular patterns
NETs	neutrophil extracellular traps
AP	Acute Pancreatitis
ER	endoscoplasmatic retiuculum
NF- κb	nuclear factor-κb
RyR	ryanodine receptor
MPTP	mitochondrial permeability transition pore
SIRS	systemic inflammation reaction syndrome
LPS	bacterial lipopolysaccharide
PMA	phorbol 12-myristate 13-acetate
PKC	protein kinase C
PRR	pattern recognition receptors
NLR	nod like receptor
RIG-I	retinoic acid-inducible gene I
RAGE	receptors for advanced glycation end
HMGB1	high mobility group box-1
TLR	toll-like receptors
MALAT1	metastasis associated lung adenocarcinoma transcript-1
HSPs	heat shock proteins
HSF-1	heat shock factor protein 1
CD	cluster of differentiation
STING	(stimulator of the interferon gene)
MAPKs	mitogen-activated protein kinases
MYD88	major reactive protein 88
cGAS	cyclic GMP–AMP
BMDCs	bone marrow-derived dendritic cells
SERCAs	channels in the smooth ER
UPR	unfolded protein response
AKs	adenylate kinases
NDPK/NME	nucleoside diphosphate kinase
NFAT	nuclear factor of activated T cells
HIF1α	hypoxia-inducible factor 1α
CIRP	cold-inducible RNA-binding protein
NE	neutrophil elastase
MPO	myeloperoxidase
PR3	leukocyte proteinase 3
NOX	NADPH oxidase
PAD4	peptidyl arginine deaminase 4
ROS	reactive oxygene species
MAC-1	macrophage-1 antigen
MMP-9	matrix metallopeptidase-9
cfDNA	cell free DNA
DIC	disseminated intravascular coagation
Rac2	Ras-related C3 botulinum toxin substrate 2
CitH3	citrullinated histone 3
PMNs	polymorphonuclear leukocytes
BM-PMNs	bone marrow-derived PMN

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Clinical characteristics of smoking-related chronic pancreatitis

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Objective: The pathogenesis of chronic pancreatitis (CP) is not completely clear. With further studies, smoking is toxic to the pancreas. This study classified smoking-related CP as a new etiology of CP and defined the cutoff of smoking.

Design: Patients with CP admitted from January 2000 to December 2013 were included in the study. The characteristics were compared between smoking patients, drinking patients, and a group of patients who never smoke or drink (control group). The cumulative rates of steatorrhea, diabetes mellitus (DM), pancreatic pseudocyst (PPC), pancreatic stone, and biliary stricture after the onset of CP were calculated, respectively.

Results: A total of 1,324 patients were included. Among them, 55 were smoking patients, 80 were drinking patients, and 1,189 were controls. The characteristics of smokers are different from the other two groups, especially in age at the onset and diagnosis of CP, initial manifestation, and type of pain. The development of DM (P = 0.011) and PPC (P = 0.033) was significantly more common and earlier in the smokers than in the other two groups. Steatorrhea also developed significantly more in the smokers than in the controls (P = 0.029). Smokers tend to delay the formation of pancreatic stones and steatorrhea.

Conclusion: The clinical characteristics of smoking-related CP is different from CP of other etiologies. A new type of CP, smoking-related CP, was put forward.

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Smoking-related CP should be separated from idiopathic CP and defined as a new independent subtype of CP different from alcoholic CP or idiopathic CP.

KEYWORDS

chronic pancreatitis, smoking, drinking, etiology, natural course

Introduction

The characteristic of chronic pancreatitis (CP) is gradual and irreversible damage of the pancreatic structure. Calcification of the pancreas, ductal calculus, stenosis and dilation of the pancreatic duct, and parenchymal atrophy were the morphologic changes of CP. Acute and chronic pancreatitis were considered as distinct entities as late as the Marseilles conference in 1984 (Singer et al., 1985). The criteria of alcoholic CP were defined by Lankisch PG et al. until 1995 (Lankisch et al., 1995). A recent study reported that 51.6% of CP patients were smokers (Han et al., 2018) who have a worse quality of life.

Because of its harmful role in CP, smoking continues to attract attention. It was reported as a clear risk factor for CP development in a dose-dependent manner (Talamini et al., 1999; Maisonneuve et al., 2005; Maisonneuve et al., 2006; Tolstrup et al., 2009; Yadav et al., 2009; Law et al., 2010; Yadav et al., 2010; Yadav and Whitcomb, 2010; Rebours et al., 2012; Sadr-Azodi et al., 2012) and has a toxic effect similar to alcohol. Smoking has a synergistic effect with alcohol, which accelerates the development of CP. Furthermore, once CP develops, smoking can promote the formation of complications such as exocrine insufficiency, pancreatic calcifications, and pseudocysts, suggesting that smoking may accelerate the progression of CP (Bernard et al., 1992; Cavallini et al., 1994; Hartwig et al., 2000; Cote et al., 2011; Luaces-Regueira et al., 2014; Greer et al., 2015; Sankaran et al., 2015; Ahmed Ali et al., 2016; Lee et al., 2016; Setiawan et al., 2016).

Wittel *et al.* demonstrated that high-dose tobacco exposure in rats led to the damage of the pancreas with CP features at the molecular levels. Chowdhury *et al.* showed that, in the pancreas of rats, nicotine was significantly accumulated, suggesting that nicotine may play an active role in pancreatic inflammation (Chowdhury et al., 1993; Chowdhury et al., 2002; Wittel et al., 2006; Wassef et al., 2014; Han et al., 2016). With the in-depth research and evidence during the past decades, we assume that smoking-related CP may be an independent subgroup of CP.

This study was based on a retrospective prospective cohort of 2,153 CP patients, who were followed up after the onset with a long duration. We aimed to assess the epidemiological features, natural course, and complications as well as compare them between smokers, alcoholics, and without tobacco or alcohol users.

Abbreviations: AIP, autoimmune pancreatitis; CP, chronic pancreatitis; CT, computed tomography; DM, diabetes mellitus; ERCP, endoscopic retrograde cholangiopancreatography; ESWL, extracorporeal shockwave lithotripsy; GP, groove pancreatitis; MPD, main pancreatic duct; MRI, magnetic resonance imaging; PPC, pancreatic pseudocyst; SD, standard deviation.

Materials and methods

Patients and database

The database of CP in our hospital (version 2.1, YINMA Information Technology Inc., Shanghai, China) was established in 2005, which has been reported in several studies on CP (Li et al., 2014; Xin et al., 2014; Sun et al., 2015; Li et al., 2016; Pan et al., 2016; Hao et al., 2017; Hao et al., 2017; Hao et al., 2017). Information including tobacco and alcohol consumption, course of CP, demographic data (age, sex, birthplace, *etc.*), history of other diseases, medical history, laboratory and imaging findings, family history of pancreatic diseases and diabetes mellitus (DM), and treatment strategy were documented in detail.

Patients in the database system were called for clinical checkups. In addition to follow-up due to discomfort associated with CP, each patient is recalled regularly (at least annually) for clinical examinations. Computed tomography, ultrasound, or magnetic resonance imaging was chosen as the assessment method during each visit. An assessment of the patients who have not returned to our center for each revisit or by telephone enquiry has been added to the database. In December 2013, we contacted all patients included in the database for a final assessment, except for patients who died or were lost to follow-up. The follow-up period was defined as the time from the onset of CP to death, the last personal contact, or end of follow-up (December 2013), whichever comes first.

The study was approved by the Ethics Committee of Changhai Hospital, The Second Military Medical University, Shanghai, China. All participating patients received written informed consent. All diagnosis and treatment methods were carried out in accordance with existing guidelines (Maydeo et al.,

2007; Schreyer et al., 2014; Ito et al., 2016; Dumonceau et al., 2019).

Definitions

CP can be diagnosed when one of the following conditions is established: (1) pancreatic ductal changes (according to the Cambridge classification system, moderate or marked disease), (2) pancreatic calcification appearance, (3) characteristics of CP in endoscopic ultrasound, (4) abnormal results of pancreatic endocrine or exocrine function, or (5) histological proof of CP as described by the Asia-Pacific consensus (Tandon et al., 2002). The onset of CP was considered when the first symptom relevant to CP occurred, such as acute pancreatitis attack, recurrent pancreatic pain, DM, steatorrhea, chronic pancreatic pain, or asymptomatic patients diagnosed of CP in the course of physical examinations.

According to the etiologies of CP that we have known, hereditary CP refers to two first-degree relatives or three or more second-degree relatives in two or more generations with recurrent acute pancreatitis and/or CP without any precipitating factors (Howes et al., 2004). The abnormal anatomy of the pancreatic duct refers to anomalous pancreaticobiliary junction and pancreas divisum (Lu, 1998). For patients who have a history of abdominal injury with imaging confirmation of pancreatic injury and subsequent ductal dilation, this was defined as post-traumatic CP. When the blood triglycerides are >1,000 mg/dl, hyperlipidemia is considered as an etiology (Yadav and Pitchumoni, 2003). Alcoholic CP was considered when the alcohol consumption exceeded 80 g/day for men or 60 g/day for women for at least 2 years without other causes (Lankisch et al., 1995). In the present study, patients were defined as smoking-related CP when they smoke for more than 30 pack-years. As the toxic effect has already impaired the pancreas, patients who met the criteria of alcoholic intake, whether they have quit drinking or not, were all regarded as alcoholic CP. Similarly, patients who met the criteria of tobacco intake, whether they have quit smoking or not, were regarded as smoking-related CP. When none of the above-mentioned causes was found, idiopathic CP was considered.

To explore the proper cutoff value of smoking consumption to define smoking-related CP, the distribution of cigarette consumption was calculated in the CP patients. In all the smokers, it peaked at 30–35 pack-year and gradually declined (Supplementary Figure S1A). After the exclusion of patients who ever drunk, it peaked at 20–25 pack-years (Supplementary Figure S1B). A comparison of complications at the diagnosis of CP between different cutoffs was also calculated (Table 1). For patients who smoke 10 to 30 pack-years, the development of pancreatic pseudocyst (PPC) is significantly different from that of the drinking patients and controls. For patients who smoke 20 to 30 pack-years, the development of steatorrhea is significantly different from that of the controls. Thus, the cutoff for smoking-related CP of 30 pack-years was selected according to the aforementioned findings and previous studies (Park et al., 2014; Tammemagi et al., 2014).

Patients with the following features were excluded (Figure 1): patients diagnosed with pancreatic cancer within 2 years after the onset of CP (Li et al., 2014), autoimmune pancreatitis, and groove pancreatitis (Malde et al., 2011). Patients with other etiologies (including hereditary, hyperlipidemic, abnormal anatomy of pancreatic duct, and post-traumatic) were also excluded. In order to exclude confounding factors, patients who both drink and smoke, patients who smoke <30 pack-years, and patients who drink <80 g/day (men) or 60 g/day (women) were further excluded.

Thus, patients with 30 or more pack-years of smoking history were defined as the smoking patients. Patients with alcohol consumption that exceeded 80 g/day for men or 60 g/day for women for at least 2 years were defined as the drinking patients. Idiopathic CP patients who never smoke or drink were assigned to the controls.

TABLE 1 Comparison between different cutoffs of smoking.

Cutoff (pack-year)	Smoker, *n*	Drinker, *n*	Control, *n*	Stone		DM		Steatorrhea		Biliary stricture		PPC	
				P^a	P^b	P^a	P^b	P^a	P^b	P^a	P^b	P^a	P^b
40	15	83	1,189	0.584	0.309	0.831	0.744	0.450	0.316	0.733	0.885	0.313	0.288
35	26	83	1,189	0.996	0.520	0.771	0.690	0.290	0.143	0.513	0.696	0.513	0.461
30	55	83	1,189	0.285	0.648	0.896	0.090	0.140	0.029	0.385	0.622	0.009	0.017
20	90	83	1,189	0.349	0.604	0.459	0.273	0.087	0.011	0.102	0.083	0.010	0.035
15	114	83	1,189	0.238	0.361	0.432	0.309	0.256	0.073	0.174	0.184	0.008	0.025
10	133	83	1,189	0.266	0.374	0.359	0.296	0.363	0.112	0.127	0.072	0.030	0.153
5	147	83	1,189	0.212	0.310	0.319	0.241	0.514	0.238	0.129	0.080	0.055	0.307

DM, diabetes mellitus; PPC, pancreatic pseudocyst.
[a]Comparison between the smoker and the drinker groups.
[b]Comparison between the smoker and the control groups.

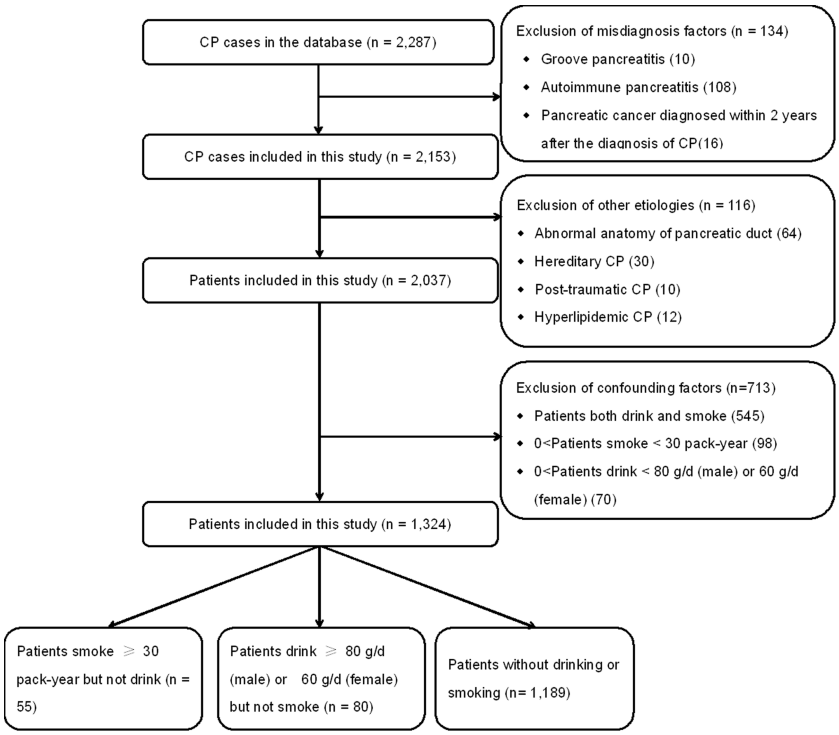

FIGURE 1
Flow diagram of the patients' enrollment and study design.

Statistical analysis

Continuous variables are expressed as mean ± standard deviation. Comparisons between smokers and drinkers and between smokers and controls were made using an unpaired two-tailed *t*-test or Mann–Whitney test. The categorical variables were compared using χ^2 test or Fisher's exact test. The Kaplan–Meier method was used to calculate the cumulative rate of DM, steatorrhea, pancreatic stones, PPC, and biliary stricture after CP onset. Log-rank test was used to analyze between groups for any significant differences.

Results

General characteristics of CP patients

After the exclusion of 250 patients, a cohort of 2,037 CP patients was included, which are listed in Figure 1. In the present study, 545 patients who both drink and smoke were excluded to reduce the confounding bias. Moreover, 98 patients who smoke <30 pack-years and 70 patients who drink <80 g/day (men) or 60 g/day (women) were also further excluded. Finally, a cohort of 1,324 patients was enrolled.

As shown in Table 2 show, the general features of these CP patients are listed. The median follow-up duration was 7.6 (range, 0.0–53.2) years. In the 55 smokers, the median follow-up duration was 5.3 (range, 0.2–39.0) years. Among the 80 drinkers, the median follow-up duration was 11.0 (range, 1.5–43.2) years, while in the 1,189 controls, the median follow-up time was 7.4 (range, 0.0–53.2) years. Age at onset of CP, initial manifestations, age at diagnosis of CP, age at pancreatic stone, DM, biliary stricture diagnosis, and the pain type were significantly different between smokers and drinkers (all P <0.05). Gender, age at the onset and diagnosis of CP, body mass index, initial manifestations, age at stone and steatorrhea, PPC, and type of pain were significantly different between smokers and controls (all P <0.05).

Cumulative rates in smokers, drinkers, and control

Cumulative rates of DM

DM developed in 26.5% (351/1,324) of the included patients in this study. The rate was 32.7% (18/55) in smokers, 45.0% (36/80) in drinkers, and 25.0% (297/1,189) in the control patients. DM was diagnosed in 10, 11, and 14 patients at 3, 5, and 10 years in the smokers after the onset of CP, the cumulative rates of

TABLE 2 General characteristics of 1,324 patients with CP.

Items	Smoker $N = 55$	Drinker $N = 80$	Control $N = 1,189$	P^a	P^b
Male sex	55 (100.0%)	80 (100.0%)	594 (50.0%)	–	<0.001
Age at the onset of CP, years[c]	49.669 ± 11.064	37.879 ± 12.131	37.106 ± 18.770	<0.001	<0.001
Age at the diagnosis of CP, years[c]	55.120 ± 7.972	45.198 ± 10.342	41.369 ± 17.763	<0.001	<0.001
Adolescent	0	2 (2.5%)	231 (19.3%)	0.237	<0.001
Body mass index[c]	23.096 ± 4.501	21.515 ± 4.149	20.474 ± 3.407	0.063	<0.001
Initial manifestations				0.001	<0.001
Abdominal pain	39 (70.9%)	70 (87.5%)	985 (82.8%)		
Endocrine/exocrine dysfunction	4 (7.3%)	8 (10.0%)	133 (11.2%)		
Others	12 (21.8%)	2 (2.5%)	71 (6.0%)		
Pancreatic stones[d]	42 (76.4%)	68 (85.0%)	828 (69.6%)	0.204	0.288
Age at pancreatic stone diagnosis[c]	55.038 ± 7.808	46.815 ± 10.842	38.074 ± 17.505	<0.001	<0.001
Time between onset and pancreatic stone[c]	6.182 ± 7.338	8.562 ± 8.350	6.097 ± 6.766	0.090	0.928
DM	18 (32.7%)	36 (45.0%)	297 (25.0%)	0.153	0.196
Age at diabetes[c]	50.276 ± 9.803	44.599 ± 9.687	46.378 ± 13.097	0.048	0.216
Time between onset and DM[c]	4.484 ± 11.063	8.449 ± 6.846	4.438 ± 7.438	0.111	0.980
Steatorrhea	16 (29.1%)	23 (28.8%)	232 (19.5%)	0.966	0.082
Age at steatorrhea[c]	50.252 ± 11.677	43.108 ± 9.753	41.389 ± 14.292	0.064	0.016
Time between onset and steatorrhea[c]	3.707 ± 6.843	5.792 ± 6.405	4.848 ± 8.334	0.373	0.593
Biliary stricture	9 (16.4%)	13 (16.3%)	177 (14.9%)	0.986	0.764
Age at CBD stenosis[c]	56.128 ± 10.122	46.259 ± 11.043	53.895 ± 14.440	0.046	0.648
Time between onset and CBD stenosis[c]	3.721 ± 4.749	6.889 ± 9.722	5.136 ± 8.949	0.378	0.639
Pancreatic pseudocyst	13 (23.6%)	10 (12.5%)	166 (14.0%)	0.091	0.046
Age at pseudocyst[c]	49.203 ± 8.650	48.628 ± 9.264	44.110 ± 17.263	0.881	0.081
Time between onset and pseudocyst formation[c]	4.543 ± 5.925	7.383 ± 8.817	3.004 ± 5.467	0.366	0.381
Pancreatic cancer	0	0	18 (1.5%)	–	0.358
Death	0	3 (3.8%)	57 (4.8%)	0.146	0.096
Morphology of MPD				0.619	0.376
Pancreatic stone alone	21 (38.2%)	31 (38.8%)	339 (28.5%)		
MPD stenosis alone	18 (32.7%)	19 (23.8%)	384 (32.3%)		
MPD stenosis and stone	12 (21.8%)	24 (30.0%)	342 (28.8%)		
Complex pathologic changes	4 (7.3%)	6 (7.2%)	124 (10.4%)		
Type of pain				0.041	0.018
Recurrent acute pancreatitis	17 (30.9%)	41 (51.3%)	346 (29.1%)		
Recurrent pain	8 (14.5%)	14 (17.5%)	400 (33.6%)		

(Continued)

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TABLE 2 Continued

Items	Smoker $N = 55$	Drinker $N = 80$	Control $N = 1,189$	P^a	P^b
Recurrent acute pancreatitis and pain	17 (30.9%)	16 (20.0%)	296 (24.9%)		
Chronic pain	5 (9.1%)	1 (1.3%)	54 (4.5%)		
Without pain	8 (14.5%)	8 (10.0%)	93 (7.8%)		
Severe acute pancreatitis	2 (3.6%)	5 (6.3%)	36 (3.0%)	0.501	0.798
Successful drainage[e]	34 (61.8%)	53 (66.3%)	798 (67.1%)	0.597	0.414
Overall treatment				0.689	0.621
Endotherapy alone	39 (70.9%)	57 (71.3%)	753 (63.3%)		
Surgery alone	6 (10.9%)	5 (6.3%)	170 (14.3%)		
Both endotherapy and surgery	5 (9.1%)	11 (13.8%)	102 (8.6%)		
Conservative treatment	5 (9.1%)	7 (8.8%)	164 (13.8%)		
DM in first-/second-/third-degree relatives	5 (9.1%)	5 (6.3%)	49 (4.1%)	0.536	0.077
Pancreatic diseases in first-/second-/third-degree relatives (excluding hereditary CP)	1 (1.8%)	0	14 (1.2%)	0.226	0.670

CP, chronic pancreatitis; DM, diabetes mellitus; ICP, idiopathic chronic pancreatitis; ACP, alcoholic chronic pancreatitis; HCP, hereditary chronic pancreatitis.
[a]Comparison between the smoker and the drinker groups.
[b]Comparison between the smoker and the control groups.
[c]Mean ± SD.
[d]Pancreatic calcifications were also regarded as stones that are located in a branch of the pancreatic duct or ductulus.
[e]Patients with successful main pancreatic duct (MPD) drainage are those whose CP was established after ERCP or pancreatic surgery or those who underwent successful MPD drainage during administration when CP diagnosis was established.

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which were 18.2% [95% confidence interval (CI): 12.0–24.4%], 20.0% (95% CI: 12.6–27.4%), and 25.5% (95% CI: 18.0–32.9%), and in 10, 13, and 24 patients in the drinking patients, the cumulative rates of which were 12.5% (95% CI: 8.9–16.1%), 16.3% (95% CI: 12.1–20.4%), and 30.0% (95% CI: 24.0–36.0%), while in 194, 210, and 250 patients in the controls, the cumulative rates were 16.3% (95% CI: 15.2–17.4%), 17.7% (95% CI: 16.5–18.8%), and 21.0% (95% CI: 19.6–22.5%), respectively. The rates of DM after the onset of CP showed a significant difference between the three groups (P = 0.011, Figure 2A).

Cumulative rates of steatorrhea

Steatorrhea was diagnosed in 20.5% (271/1,324) of the included patients in this study. The rate was 29.1% (16/55) in smokers, 28.8% (23/80) in drinkers, and 19.5% (232/1,189) in the control patients. Steatorrhea developed in 12, 13, and 13 patients at the third, fifth, and 10th year in the smoking patients after the onset of CP, the cumulative rates of which were 21.8% (95% CI: 16.0–27.6%), 23.6% (95% CI: 17.4–30.0%), and 23.6% (95% CI: 17.4–30.0%), and in 9, 10, and 12 patients in the drinking patients, the cumulative rates of which were 11.3% (95% CI: 7.8–14.7%), 12.5% (95% CI: 8.8–16.2%), and 15.0% (95% CI: 10.8–19.2%), while in 138, 149, and 189 patients in the controls, the cumulative rates were 11.6% (95% CI: 10.6–12.6%), 12.5% (95% CI: 11.6–13.5%), and 15.9% (95% CI: 14.5–17.3%), respectively. The rate of steatorrhea after the onset of CP showed no significant difference between the three groups (P = 0.084, Figure 2B).

Cumulative rates of pancreatic stone

Pancreatic stone was diagnosed in 70.8% (938/1,324) of the included patients in this study. The rate was 76.4% (42/55) in smokers, 85.0% (68/80) in drinkers, and 69.6% (828/1,189) in the control patients. Pancreatic stone was diagnosed in 19, 25, and 30 patients at the third, fifth, and 10th year in the smoking patients after the onset of CP, the cumulative rates of which were 34.5% (95% CI: 28.2–40.9%), 45.5% (95% CI: 38.4–52.5%), and 54.5% (95% CI: 47.3–61.8%), and in 25, 33, and 51 patients in the drinking patients, the cumulative rates of which were 31.3% (95% CI: 26.2–36.3%), 41.3% (95% CI: 35.9–46.6%), and 63.8% (95% CI: 58.4–69.1%), while in 438, 524, and 672 patients in the controls, the cumulative rates were 36.8% (95% CI: 35.5–38.2%), 44.1% (95% CI: 42.6–45.5%), and 56.5% (95% CI: 55.0–58.1%), respectively. The rates of pancreatic stone after the onset of CP showed no significant difference between the three groups (P = 0.713, Figure 2C).

Cumulative rates of biliary stricture

Biliary stricture was diagnosed in 15.0% (199/1,324) of the included patients in this study. The rates were 16.4% (9/55) in smokers, 16.3% (13/80) in drinkers, and 14.9% (177/1,189) in

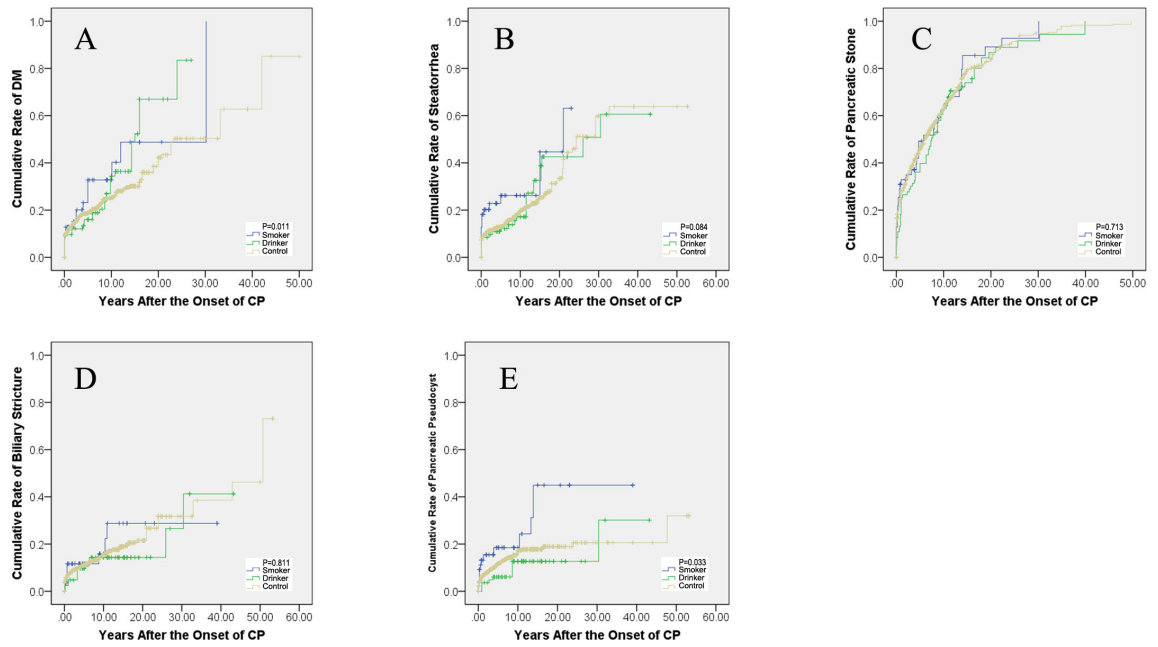

FIGURE 2
Cumulative rates after the onset of CP. **(A)** The cumulative rates of diabetes mellitus. **(B)** Cumulative rates of steatorrhea. **(C)** Cumulative rates of pancreatic stone. **(D)** Cumulative rates of biliary stricture. **(E)** Cumulative rates of pancreatic pseudocysts. CP, chronic pancreatitis; DM, diabetes mellitus.

the control patients. Biliary stricture developed in six, six, and seven patients at the third, fifth and 10th year in the smoking patients after the onset of CP, the cumulative rates of which were 10.9% (95% CI: 6.5–15.3%), 10.9% (95% CI: 6.5–15.3%), and 12.7% (95% CI: 6.9–18.5%), and in four, eight, and 11 patients in the drinking patients, the cumulative rates of which were 5.0% (95% CI: 2.6–7.4%), 10.0% (95% CI: 6.7–13.3%), and 13.8% (95% CI: 9.6–17.9%), while in 108, 124, and 149 patients in the controls, the cumulative rates were 9.1% (95% CI: 8.2–10.0%), 10.4% (95% CI: 9.4–11.4%), and 12.5% (95% CI: 11.3–13.7%), respectively. The rates of biliary stricture after the onset of CP showed no significant difference between the three groups (P = 0.811, Figure 2D).

Cumulative rates of PPC

PPC developed in 14.3% (189/1,324) of the included patients in this study. The rate was 23.6% (13/55) in smokers, 12.5% (10/80) in drinkers, and 14.0% (166/1,189) in the control patients. PPC developed in eight, nine, and nine patients at the third, fifth, and 10th year in the smoking patients after the onset of CP, the cumulative rates of which were 14.5% (95% CI: 9.5–19.5%), 16.4% (95% CI: 10.8–21.9%), and 16.4% (95% CI: 10.8–21.9%), and in four, five, and 11 patients in the drinking patients, the cumulative rates of which were 5.0% (95% CI: 2.6–7.4%), 6.3% (95% CI: 3.6–8.9%), and 11.3% (95% CI: 7.1–15.4%), while in 105, 126, and 160 patients in the controls, the cumulative rates were 8.8% (95% CI: 7.9–9.7%), 10.6% (95% CI: 9.6–11.6%), and 13.5% (95% CI: 12.2–14.7%), respectively. The rates of PPC after the onset of CP showed a significant difference between the three groups (P = 0.033, Figure 2E).

Discussion

This is a research about the natural course of CP smokers and drinkers based on a large sample of CP patients. A new type of CP, smoking-related CP, was put forward. In this study, the characteristics of CP, initial performance, natural course of the disease, and complications were accessed. The study included patients who only smoke or only drink, which ruled out the combined effects of tobacco and alcohol.

Age at the onset of CP and age at the diagnosis of CP were significantly different in these three groups. Smokers tended to have a later onset and diagnosis of CP than the drinkers and controls (both $P < 0.001$). However, smoking was reported to hasten the age of first diagnosis in a previous study (Maisonneuve et al., 2005), which is contradictory to the present finding. It may be due to the fact that only patients who smoke >30 pack-years were included in the present study, which needed several years to reach the criteria. A significant difference was observed in the initial manifestations in these three groups (P = 0.001 and $P < 0.001$ respectively). Smokers are less likely to have abdominal pain or endocrine/exocrine dysfunction at the onset of CP. It can also be proved by the type of pain (P = 0.041 and P = 0.018 respectively). Patients without pain occupied a larger proportion in the smokers. At the diagnosis of CP, steatorrhea developed more in the smoking patients than in the controls (P = 0.029), PPC developed more in the smoking groups than in the other two groups (P = 0.009 and P = 0.017, respectively). After the follow-up, DM developed more and faster in the smoking patients than in the drinkers and controls (P = 0.011); PPC developed more and faster as well in the smoking patients than in the drinking patients and controls (P = 0.033). Smokers tend to have a later formation of pancreatic stones than the other two groups (all P <0.001) and delayed occurrence of steatorrhea than controls (P=0.016).

Smoking was reported as a definite risk factor for CP, accelerating disease progression both from the onset of CP and within CP in numerous studies (Cote et al., 2011; Rebours et al., 2012; Sadr-Azodi et al., 2012; Ahmed Ali et al., 2016; Setiawan et al., 2016). Cigarette smoking is reported to accelerate pancreatic calcification and functional impairment (Talamini et al., 1996; Maisonneuve et al., 2005; Yadav et al., 2009; Law et al., 2010), which is in accordance with the present study. Cigarette smoking will also enhance ethanol-induced pancreatic injury (Hartwig et al., 2000). In the present study, patients who only smoke or only drink were included, which excluded the combined effect of tobacco and alcohol. According to the results of the comparison, the toxic impairment of pancreas caused by tobacco and alcohol is not exactly the same. Smoking may accelerate the damage of pancreatic endocrine and exocrine function as well as development of PPCs. Accordingly, less pain was observed in the smoking patients. Thus, cigarette smoking may be an independent etiology of CP. According to our present study, patients with a 30 or more pack-year smoking history in the absence of other CP etiologies should be identified as smoking-related CP. Smoking-related CP is a unique subgroup of CP which is different from other types of CP with other etiologies. Thus, smoking-related CP should be separated from idiopathic CP.

The identification of modifiable etiology provides evidence for guiding clinical practice and patient education—for example, lifestyle modifications such as tobacco abstinence, as recommended for CP patients, have been further confirmed by identifying smoking as an etiology of CP. Patients with smoking-related CP should be screened more frequently for DM, steatorrhea, and PPC. Once DM or steatorrhea occurred, insulin or pancreatic enzyme replacement therapy should be performed.

There are some limitations in our research. First, the data was collected retrospectively from 2000 to 2004, which may lead to recall bias. However, patients admitted to our hospital before and after January 2005 showed no significant difference in clinical characteristics. Based on the statistical analysis presented above, the recall bias has little effect on the results of

the study. Second, 149 patients diagnosed as CP have a follow-up time of less than 2 years after the diagnosis. Among the 149 patients, several of them with pancreatic cancer may have been misdiagnosed as CP. However, given the relatively large sample size of the study, these limitations have a little effect on the results. Third, as tobacco and alcohol are dose-dependent factors for CP development, toxins accumulation may cost years. The smoking and drinking patients are older than the controls. Adjustment of ages may be needed in a further study. Furthermore, the number of patients included in the smoking and drinking group is relatively small, which may increase the risk of selection and data collection bias and limit the possibility of making definite conclusions. A further study in a large sample prospective cohort is needed.

Conclusion

In conclusion, there is a really different clinical course of CP caused by smoking from that caused by other etiologies. Smoking may accelerate the damage of pancreatic endocrine and exocrine function as well as development of PPCs. Therefore, less pain was observed. A new type of CP, smoking-related CP, was put forward. Smoking-related CP may be separated from idiopathic CP and thus defined as a new independent subtype of CP different from alcoholic CP or idiopathic CP. Further studies focused on smoking-related CP are needed.

Data availability statement

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

Ethics statement

The studies involving human participants were reviewed and approved by the Ethics Committee of Changhai Hospital, The Second Military Medical University, Shanghai, China. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

Author contributions

LHao and YL participated in the acquisition, analysis, and interpretation of data as well as in manuscript drafting. Z-QD, J-HY, H-LG, DW, LHe, Y-WB, J-TJ, LX, TW, T-TD, J-HL, DZ, X-PZ, W-BZ, HC, JP, and ZL participated in data acquisition and manuscript drafting. G-QX, Z-SL, and L-HH contributed to the conception, design, and data interpretation as well as revised the manuscript for important intellectual content. All authors contributed to the article and approved the submitted version.

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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/fcimb.2022.939910/full#supplementary-material

SUPPLEMENTARY FIGURE 1
Distribution of cigarette consumption in CP patients. **(A)** Distribution of cigarette consumption in all CP patients. **(B)** Distribution of cigarette consumption in non-drinkers of CP. CP, chronic pancreatitis.

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Endoscopic treatment of pancreaticopleural fistulas

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Introduction: Pancreaticopleural fistula (PPF) is a serious complication of acute and chronic pancreatitis.

Objective: To evaluate the effectiveness of various endoscopic techniques for the treatment of patients with PPFs.

Methodology: Prospective analysis of the results of endoscopic treatment of 22 patients with PPF due to pancreatitis was conducted at the Department of General, Gastroenterological, and Oncological Surgery, Ludwik Rydygier Collegium Medicum in Bydgoszcz, Nicolaus Copernicus University in Torun, between 2018 and 2021.

Results: PPF was diagnosed in 22 patients (21 men and 1 woman; mean age 49.52 [30–67] years) with pancreatitis. In 19/22 (86.36%) patients, PPF communicated with the left pleural cavity and in 3/22 (13.64%) patients with the right pleural cavity. Chronic pancreatitis was diagnosed in 14/22 (63.64%) patients. Symptomatic pancreatic fluid collections were found in 15/22 (68.18%) patients with PPF (pancreatic pseudocyst in 11 and walled-off pancreatic necrosis in four patients). Endoscopic retrograde cholangiopancreatography was performed in 21/22 (95.45%) patients, confirming the diagnosis of PPF. All 21 patients underwent endoscopic sphincterotomy with prosthesis implantation in the main pancreatic duct (passive transpapillary drainage). In 1/22 (4.55%) patients, active transmural/transgastric drainage of the PPF was necessary due to inflammatory infiltration of the peripapillary region, precluding endoscopic pancreatography. Endoscopic transmural drainage was performed in all the 15 patients with pancreatic fluid collection. Clinical success was achieved in 21/22 (95.45%) patients. The mean total time of endotherapy was 191 (range 88–712) days. Long-term success of endoscopic treatment of PPFs during one year follow-up period was achieved in 19/22 (86.36%) patients.

Conclusions: Endoscopic treatment is effective for managing post-inflammatory PPFs. The preferred treatment method is passive transpapillary drainage (prosthesis of the main pancreatic duct). If transpapillary drainage is not feasible, transmural drainage of the PPF remains the preferred method. Endoscopic transmural drainage leads to closure of the fistula canal in patients with pancreatic fluid collection complicated by PPF.

KEYWORDS

pancreaticopleural fistula, pancreatic fistula, transpapillary drainage, transmural drainage, pancreatitis, endotherapy

Introduction

Fistulation, that is, the formation of pancreatic fistulas (PFs), may occur in the course of acute or chronic pancreatitis as the inflammatory process spreads (Jagielski et al., 2018a; Jagielski et al., 2018b; Larsen and Kozarek, 2014). A PF is an abnormal connection of the pancreatic ducts with another epithelium-covered surface, that is, with another organ, structure, or anatomical space (Larsen and Kozarek, 2014; Jagielski et al., 2018a; Jagielski et al., 2018a; Bassi et al., 2005; Morgan and Adams, 2007; Butturini et al., 2008). Regardless of the etiology, disruption of the main pancreatic duct (MPD) or smaller pancreatic ducts, defined as a break in the continuity of the duct leading to leakage of pancreatic juice, is at the root of the development of PF (Larsen and Kozarek, 2014; Jagielski et al., 2018a; Jagielski et al., 2018b). Disruption of MPD occurs in over 80% patients with post-inflammatory pancreatic and peripancreatic fluid collections (PPFCs) during the course of acute or chronic pancreatitis (Tay and Chang, 2013; Jagielski et al., 2017; Jagielski et al., 2020; Jagielski and Jackowski, 2021).

Pancreaticopleural fistula (PPF) is a rare complication of pancreatitis resulting from disruption of the MPD and leakage of pancreatic juice into the pleural cavity (Ali et al., 2009; Tay and Chang, 2013; Ramahi et al., 2019). Unlike pleural effusions seen in pancreatitis, which are usually clinically insignificant, PPFs often cause large, recurring pleural effusions (Ali et al., 2009).

Endoscopic treatment of disruption in the continuity of the MPD, and consequently, PFs caused by pancreatitis, involves performing endoscopic retrograde cholangiopancreatography (ERCP) with endoscopic sphincterotomy and implantation of the prosthesis into the MPD (passive transpapillary drainage) to ensure physiological outflow of pancreatic juice into the duodenum (Jagielski et al., 2017; Jagielski et al., 2018a; Jagielski et al., 2018b; Jagielski et al., 2020; Jagielski and Jackowski, 2021b)

The use of endoscopic techniques in the treatment of MPD disruption caused by pancreatitis remains controversial (Boxhoorn et al., 2021). Most of the evidence on the diagnosis and therapy of post-inflammatory PPFs is derived from single case reports (Wee et al., 2017; Ramahi et al., 2019). Moreover, the management of patients with pancreatitis and hindered access to the MPD through the major duodenal papilla during ERCP due to swelling of the duodenal wall or altered anatomy of the upper gastrointestinal tract remains challenging.

Therefore, this study presents the results of treatment of patients with PPF due to pancreatitis using various endoscopic techniques.

Materials and methods

This was a prospective analysis of treatment outcomes in patients with pancreatitis hospitalized at the Department of General, Gastroenterological, and Oncological Surgery, Ludwik Rydygier Collegium Medicum in Bydgoszcz, Nicolaus Copernicus University in Torun between 2018 and 2021. A significant number of these patients had previously been treated for pancreatitis in other clinical centers and were subsequently transferred to our referral center to treat the sequelae and complications of pancreatitis (Jagielski and Jackowski, 2021b).

The study was approved by the Ethics Committee at the Collegium Medicum of Nicolaus Copernicus University and was conducted in accordance with the Declaration of Helsinki. All patients provided oral and written informed consent to participate in the study. All patients received detailed information regarding the study.

The diagnosis of pancreatitis, the criteria of clinical and morphological categorization, and all definitions of local and systemic complications were based on the 2012 revised Atlanta classification (Sarr et al., 2013; Thoeni, 2012; Banks et al., 2013). The standards for conservative treatment of pancreatitis were based on international guidelines (Tenner et al., 2013; Working Group IAP/APA Acute Pancreatitis Guidelines, 2013). Conservative treatment relies primarily on dietary treatment with intensive intravenous fluid therapy and analgesia. Moreover, additional treatment methods were used depending on concomitant organ impairment and the patient's overall clinical condition. The decision to use interventional treatment for complications of pancreatitis was made after careful consideration of the clinical picture and imaging results, mostly contrast-enhanced abdominal computed tomography (CECT) images. In the case of qualification for interventional treatment, endoscopic techniques are the method of choice at our center (Jagielski and Jackowski, 2021a; Jagielski and Jackowski, 2021b).

Study inclusion criteria

All symptomatic patients with PPFs in the course of acute or chronic pancreatitis were included in the study. Qualification for endoscopic treatment was based on the clinical picture and imaging results, mainly based on CECT of the abdomen and magnetic resonance imaging (MRI).

Study exclusion criteria

Patients with PPFs without clinical signs associated with the presence of a fistula or with PPFs that were not a consequence of pancreatic inflammatory disease (acute or chronic pancreatitis) were excluded from the study. Patients who underwent surgery in the pancreatic region were also excluded.

Management strategy in patients with post-inflammatory pancreaticopleural fistula

Pleural fluid puncture with determination of amylase levels and passive drainage of the pleural cavity was performed in all patients with pancreatitis and suspected PPF based on the clinical picture and imaging. If pleural amylase activity exceeded 1000 U/l, the patient was diagnosed with PPF and referred for endoscopic treatment with transpapillary ERCP (through the major duodenal papilla). If transpapillary access was not possible, transmural access (through the wall of the upper gastrointestinal tract) was obtained under endoscopic ultrasound (EUS) guidance. Endoscopic drainage of the collection was performed in patients with PPFs and PPFCs. Somatostatin infusion was administered at a dose of 3.5 micrograms/kg body weight/hour from the time of diagnosis of the PPF until completion of pleural drainage.

Endoscopic procedures

Endoscopic procedures were performed under general anesthesia with tracheal intubation. All patients provided informed consent for the endoscopic procedures. All procedures were performed by a single endoscopist, and entailed carbon dioxide insufflation and the use of a linear echoendoscope (Pentax EG3870UTK, Pentax Medical, Tokyo, Japan), duodenoscope (Olympus TJF-Q180V, Olympus Corporation, Tokyo, Japan), and gastroscope (Olympus GIF-H185, Olympus Corporation) [16].

Transpapillary drainage

Attempts to perform ERCP to assess the morphology and integrity of the MPD and to employ possible endoscopic treatment were made in all patients with post-inflammatory PPF treated in our center (Figures 1A–F). In the case of disruption of the MPD, sphincterotomy (Fusion OMNI Sphincterotome FS-OMNI-35-480, Cook Endoscopy Inc., North Carolina, USA) was performed and a pancreatic 5 Fr, 7 Fr, 8.5 Fr, or 10 Fr endoprosthesis (Zimmon Pancreatic Stent, Cook, Endoscopy Inc., North Carolina, USA) was introduced into the MPD and subsequently replaced every 1, 3, 6, 12, or 24 months or until no contrast leakage outside the duct was identified.

Transmural drainage

If transpapillary access was not possible, transmural access (through the wall of the upper gastrointestinal tract) was obtained using the single transluminal gateway technique (SGT) (Figures 2A–D). Placement of the pancreaticogastric anastomosis in the form of a transmural cystostomy was performed under EUS guidance. Anastomosis between the lumen of the gastrointestinal tract and the fistula canal was created using a 10 Fr cystotome (Cystotome CST-10, Cook Endoscopy Inc., North Carolina, USA) and then dilated using a high-pressure balloon with a diameter of up to 15 mm (Cook Endoscopy Inc., North Carolina, USA). A transmural 7 Fr or 8 Fr double-pigtail stent (Cook Endoscopy Inc., North Carolina, USA) was inserted through pancreaticogastrostomy. For active transmural drainage, a 7 Fr or 8.5 Fr nasal drain (Cook Endoscopy Inc., North Carolina, USA) was inserted into the canal of the fistula through pancreaticogastric anastomosis. In cases of passive transmural drainage, only 7 Fr or 8 Fr double-

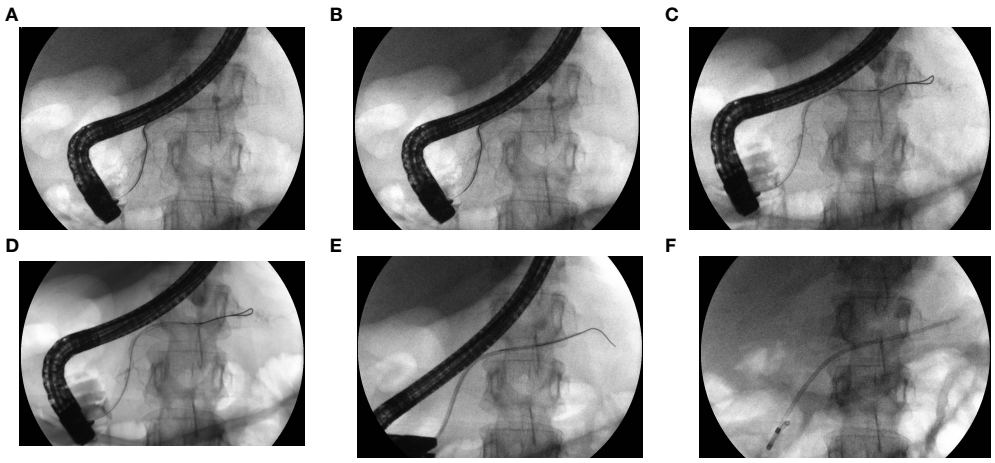

FIGURE 1
(A–F). ERCP with passive transpapillary drainage. The patient with MPD partial disruption (PPF) in pancreatic tail. Contrast medium and guidewire were introduced to MPD during ERCP **(A–C)**. MPD partial disruption in form of PPF in pancreatic tail became visible as leakage of contrast beyond the MPD **(A–C)**. During subsequent steps of ERCP the pancreatic stent was introduced along the guidewire **(D, E)**. Pancreatic stent created bridged the partial disruption of MPD in pancreatic tail **(F)**.

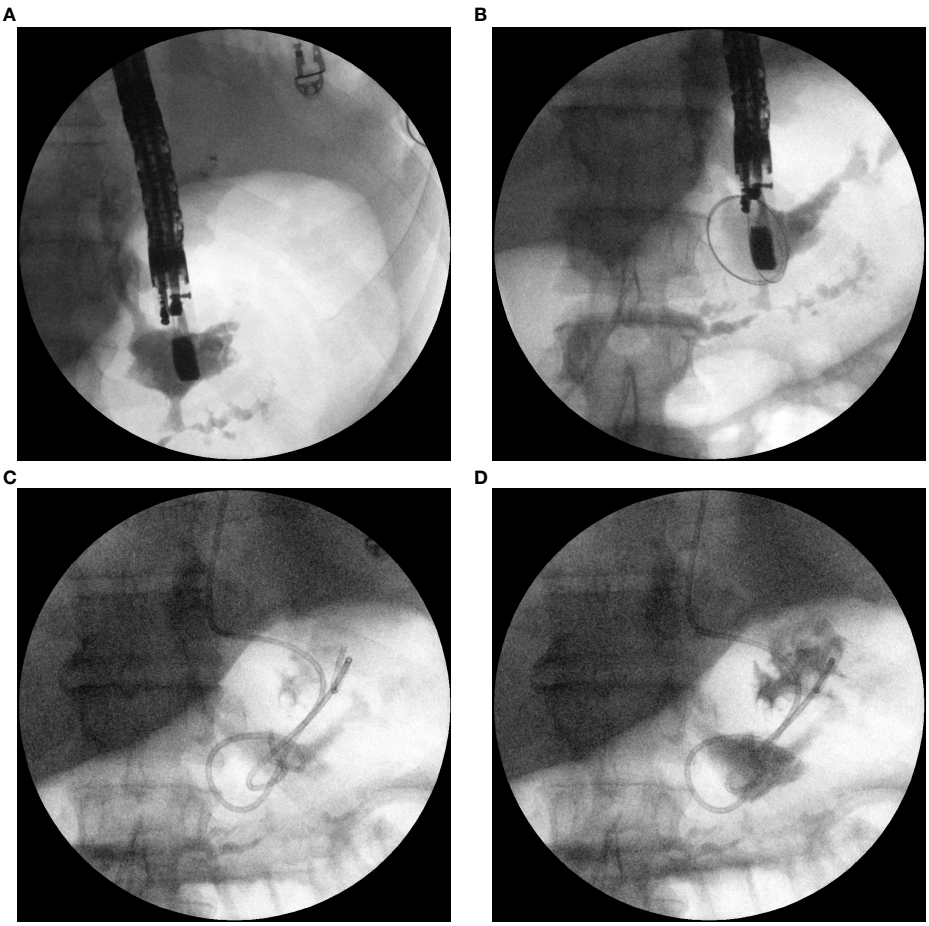

FIGURE 2
(A–D). Transmural drainage of PPF. Antegrade endoscopic pancreatography. Fluoroscopic images taken during the endoscopic procedure after the transmural puncture of the PPF canal **(A, B)**. The administered contrast filled the pleural fistula with a visible infiltration of the contrast into the pancreatic duct by partial disruption to the MPD **(A, B)**. Transmural drainage of PPF- plastic transmural stent and nasal drain introduced through the transmural fistula is visible **(C, D)**. Contrast medium administered through the nasal drain filled the PPF canal and was leaking through the stent into stomach **(D)**.

pigtail stents (Cook Endoscopy Inc., North Carolina, USA) were used through the transmural anastomosis.

Postoperative period

In the postoperative period, all patients with post-inflammatory PPF continued to receive somatostatin as a continuous intravenous infusion at a dose of 3.5 micrograms/kg body weight/hour until pleural drainage was complete, i.e., when the amount of drained pleural fluid did not exceed 50 ml per day without underwater seal drain.

In patients with active transmural drainage of the PPF, the nasal drain was rinsed with 50 ml of saline every 4 h. Active transmural drainage was considered as completed at the end of pleural drainage, the nasal drain was removed, and a double-pigtail plastic transmural endoprosthesis was left for passive transmural drainage of the PPF.

CECT of the chest and abdominal cavity was performed at the time of completion of the endoscopic treatment. During follow-up after the end of endotherapy, control imaging tests (mainly CECT) were performed every 3, 6, 12, and 24 months and subsequently every two years if no symptoms were reported. During follow-up no patient developed contrast induced nephropathy.

Definitions

Partial disruption of the MPD was defined as the flow of contrast, during ERCP, outside the MPD with contrast filling of the part of the duct distal to the disruption site.

Complete disruption of the MPD was defined as the flow of contrast outside the duct without contrast filling of the distal part of the MPD during ERCP.

Closure of the PPF was defined as a lack of visualization on imaging of a communication passage that existed earlier between the lumen of the pleural cavity and the MPD.

Complications of endoscopic treatment were divided into early complications (occurring up to 30 days after treatment), evaluated in line with the Clavien-Dindo classification (Clavien et al., 2009).

Clinical success of endoscopic treatment of post-inflammatory PPFs was defined as closure of the PPF, absence of pleural effusion, and lack of clinical signs associated with PPF.

The long-term success of endoscopic treatment of PPFs was defined as closure of the PPF, absence of pleural effusion, and lack of clinical signs associated with the PPF during the follow-up period.

Statistics

All statistical calculations were performed using STATISTICA data analysis software (StatSoft Inc., 2014). Quantitative variables are presented as arithmetic means and minimal and maximal values (ranges), whereas qualitative data are presented as means of numbers and percentages.

Results

A total of 882 (556 men, 326 women; mean age 54.44 [19–101] years) patients with pancreatitis were treated at the Department of General, Gastroenterological and Oncological Surg+ery, Ludwik Rydygier Collegium Medicum in Bydgoszcz, Nicolaus Copernicus University in Torun between 2018 and 2021.

Post-inflammatory PPF was diagnosed in 22/882 (2.49%) patients (21 men, 1 woman; average age 49.52 [30–67] years). None of the patients underwent any surgical intervention before a diagnosis of PPF was made. Chronic pancreatitis was diagnosed in 14/22 (63.64%) patients with PPF. The etiology of pancreatitis in this group of patients was alcohol-related in 16 patients and non-alcoholic in 6 (5 – biliary, 1 – iatrogenic). The average time from the beginning of the pancreatitis episode until the diagnosis of PPF was 52 (23–119) days. No further PFs were found in any of the patients. Detailed clinical characteristics of the patients are presented in Table 1.

Symptoms suggestive of PPFs were reported in 20/22 (90.91%) patients. Shortness of breath was reported by 17 patients, chest pain by 11 patients and abdominal pain by 13 patients. Furthermore, fever was observed in 4 patients in this group. Sepsis was diagnosed in 3 patients with PPF (*Escherichia coli* and *Enterococcus faecalis* were the most commonly grown pathogens). These symptoms are non-specific for PPFs.

Symptomatic PPFCs were found in 15/22 (68.18%) patients with PPF (pancreatic pseudocyst in 11 patients; walled-off pancreatic necrosis in 4 patients). The mean size of collection was 7.96 (5.44–16.3) cm. Infected PPFCs was revealed in 4/15 (26.67%) patients. The collection was initially sterile in 11/15 (73.34%) patients.

In all 22 patients, the diagnosis of PPF due to pancreatitis was made based on CECT of the chest, abdomen, and pelvis, as well as amylase high activity in the pleural fluid. The average amylase activity in the pleural fluid was 9883 (1221–230000) U/l. In 19/22 (86.36%) patients, PPF communicated with the left pleural cavity; in 3/22 (13.64%) patients with the right pleural cavity. Pleural cavity drainage was performed in all 22 patients. The mean drainage time was 5 (3–17) days.

All 22 patients with post-inflammatory PPFs were referred for endoscopic treatment. Transpapillary access to the PPF (anatomically through the major duodenal papilla) was achieved in 21 patients. In one patient, extraanatomical transmural/transgastric access was performed due to inflammatory infiltration of the peripapillary region of the descending duodenum, which prevented transpapillary access.

TABLE 1 Characteristics of the patients from study group.

	All patients (n=22)
Age, mean [range] years	49.52 [30–67]
Sex, n men (%)	21 (95.45%)
Chronic pancreatitis, n (%)	14 (63.64%)
Etiology of pancreatitis, n (%)	
Alcoholic	16 (72.73%)
Non-alcoholic	6 (27.27%)
Symptomatic PPFCs, n (%)	15 (68.18%)
Pancreatic pseudocyst	11
Walled-off pancreatic necrosis	4
CTSI (computed tomography severity index), mean [range] points	6 [4-10]
Time from the beginning of the pancreatitis episode until the diagnosis of PPF, mean [range] days	52 [23–119]
PPF localization, n	
Pancreatic head	3
Pancreatic body	15
Pancreatic tail	4
Symptoms related with PPF, n	
Shortness of breath	17
Chest pain	11
Abdominal pain	13
Fever	4
Method of minimally invasive treatment of PPF, n (%)	
Transpapillary drainage (ERCP)	21 (95.45%)
Transmural drainage	1 (4.55)
Complications of interventional treatment, n (%)	
Upper gastrointestinal bleeding	3 (13.63%)
Sepsis	1 (4.55%)

ERCP was performed in 21/22 (95.45%) patients, confirming the presence of PPF. Partial disruption of the MPD (pancreatic head, 3 patients; pancreatic body, 12 patients; pancreatic tail, 4 patients) communicating with the pleural cavity through a fistula was found in 19/21 (90.48%) patients. In the remaining 2/21 (9.52%) patients, complete disruption of the MPD was found in the pancreatic body. All 21 patients underwent endoscopic sphincterotomy with prosthesis implantation into the MPD (passive transpapillary drainage). The mean number of transpapillary replacements of the pancreatic endoprostheses was 2.55 (1–7). The mean total time endoscopic prostheses remained in the MPD was 191 (88–712) days.

In 1/22 (4.55%) patients, active transmural/transgastric drainage of the PPF was used because of inflammatory infiltration of the peripapillary region preventing ERCP. Active drainage of the fistula lasted for seven days, followed by passive drainage for the following 102 days.

Endoscopic transmural drainage, lasting 13 (4–36) days on average, was performed in all 15 patients with PPFC, followed by 72 (33–367) days of passive transmural drainage.

Procedure-related complications occurred in 4/22 (18.18%) patients. Three patients required transfusion of packed red blood cells because of gastrointestinal bleeding (Clavien–Dindo grade II). Sepsis requiring intravenous broad-spectrum antibiotic therapy (Clavien–Dindo grade II) was observed during endotherapy in one patient only. None of the patients required surgical treatment for complications of endotherapy.

Closure of PPF was confirmed by imaging studies and ERCP in 21/22 (95.45%) patients. The mean time from diagnosis to PPF closure was 66 (33–171) days.

Clinical success of endoscopic treatment of post-inflammatory PPFs was achieved in 21/22 (95.45%) patients. One patient out of the 22 (4.55%) patients was still undergoing endotherapy due to complete disruption of the MPD.

Recurrence of PPF was identified in two patients. One patient with recurrence of PPFC complicated by PPF required repeated endoscopic treatment. One patient required thoracosurgical treatment because of recurrence of a PPF complicated by pleural empyema. Long-term success of endoscopic treatment of PPFs during one year of follow-up was achieved in 19/22 (86.36%) patients.

Discussion

The current literature lacks clear guidelines defining an algorithm for performing diagnostic and therapeutic procedures in patients with PPFs. Most of the data are derived from individual case reports or case series, described in this publication (Ali et al., 2009; Wee et al., 2017; Ramahi et al., 2019). The present study is the largest case series demonstrating the effectiveness of various endoscopic techniques in patients with post-inflammatory PPF available in the literature to date.

Post-inflammatory PPF is an uncommon but serious complication of acute and more often chronic alcohol-induced pancreatitis (Dhebri and Ferran, 2005; Ali et al., 2009; Tay and Chang, 2013; Wee et al., 2017; Ramahi et al., 2019). The precise incidence rate of PPF is unknown and its estimation remains difficult. According to the literature, PPF is diagnosed in approximately 0.4% of patients with pancreatitis (Dhebri and Ferran, 2005; Tay and Chang, 2013). In our study, the incidence rate of PPF in patients with pancreatitis was 2.49% (Dhebri and Ferran, 2005; Tay and Chang, 2013). However, this is difficult to interpret in the context of the general incidence rate of PPF in the entire population of patients with pancreatitis because our facility is a reference center for the minimally invasive treatment of inflammatory diseases of the pancreas. A significant number of patients from the study group had previously been treated in other clinical centers for pancreatitis and were transferred to our referral center for the treatment of the sequelae and complications of pancreatitis.

There are no typical clinical features of post-inflammatory PPF, making diagnosis difficult. Patients with post-inflammatory PPF often report severe abdominal pain as the predominant symptom, accompanied by shortness of breath and chest pain, which does not unequivocally point to the suspicion of post-inflammatory PPF based on the clinical picture alone. As shown in this study, abdominal pain typical of pancreatitis often masks chest symptoms, which are more characteristic of PPF. According to the available literature, shortness of breath is the most common clinical symptom associated with PPF, followed by abdominal pain, chest pain, cough, hemoptysis, weight loss, fever, and other non-specific symptoms (Ondrejka et al., 2000; Dhebri and Ferran, 2005; Oh et al., 2006; Ali et al., 2009; Tay and Chang, 2013; Wee et al., 2017; Ramahi et al., 2019).

Diagnostic difficulties in this group of patients represent one of the main problems in determining the exact incidence of PPF often delaying the correct diagnosis. Non-invasive magnetic resonance cholangiopancreatography (MRCP) is the most sensitive and specific diagnostic test for PFs resulting from disruption of the MPD. On the other hand, suspicion of PF due to MPD disruption is an indication for ERCP (Devière et al., 1995; Varadarajulu et al., 2005). In cases where MPD disruption and the presence of PPF are confirmed during ERCP, an endoprosthesis may be inserted into the MPD to secure physiological outflow of pancreatic juice into the lumen of the duodenum and subsequent PPF closure (Devière et al., 1995; Varadarajulu et al., 2005; Jagielski et al., 2018a; Jagielski et al., 2018b). It is also recommended that secretin-stimulated MRCP (secretin MRCP) should be performed to evaluate MPD when there is no suspicion of PPF as a result of MPD disruption and there is no need to apply endoscopic treatment (Matos et al., 1997; Soto et al., 2001; Punwani et al., 2003). Secretin MRCP is considered a safe and non-invasive imaging technique that enables visualization of the entire anatomy of the pancreas, including the pancreatic ducts (Matos et al., 1997; Soto et al.,

2001; Punwani et al., 2003). Despite the availability of MRCP in our center, if PPF was suspected based on high amylase activity in the pleural fluid, we performed ERCP without confirming the diagnosis using MRCP. If the presence of PPF was confirmed during ERCP, endoscopic sphincterotomy was performed with implantation of a prosthesis into the MPD. This study demonstrates that such a diagnostic and therapeutic algorithm in patients with post-inflammatory PPF is associated with effective treatment and low likelihood of complications.

Disruption to the MPD or smaller pancreatic ducts, leading to leakage of pancreatic juice outside the pancreatic ducts, is the most important factor in the pathophysiology of PFs (Larsen and Kozarek, 2014; Jagielski et al., 2018a; Jagielski et al., 2018b). Disruption of the pancreatic ducts on the anterior surface of the pancreas usually leads to the development of a PPF and manifests as pancreatic ascites. Disruption of the pancreatic ducts on the posterior surface of the pancreas leads to the formation of a PPF with pancreatic juice leaking into the pleural cavity. In the case of PFs, pancreatic juice usually spreads retroperitoneally to pleural cavities through the paths of least resistance, that is, through the aortic or esophageal hiatus (Dhebri and Ferran, 2005; Ali et al., 2009; Tay and Chang, 2013). Transdiaphragmatic communication is very rare (Dhebri and Ferran, 2005; Ali et al., 2009; Tay and Chang, 2013).In most patients with PPFs, fluid is found in the left pleural cavity, and less often on the right side or bilaterally (Ondrejka et al., 2000; Dhebri and Ferran, 2005; Oh et al., 2006; Ali et al., 2009; Tay and Chang, 2013).As presented above, with post-inflammatory PPFs, pancreatic juice can leak directly into the pleural cavities from the site of the pancreatic ducts disruption. The pathophysiology is different in patients with post-inflammatory PPFC complicated by PPF. In such cases, pancreatic juice leaks into the lumen of the collection, which communicates with the pleural cavity through a PF. In this study, symptomatic PPFCs were found in most patients with PPFs caused by pancreatitis, the most common finding being a pancreatic pseudocyst in the course of chronic pancreatitis.

In this study, it was shown that in patients with PPFCs complicated by PPF, endoscopic transmural drainage of the collection resulted in closure of the fistula canal. Similar observations were found in a group of patients with PPFCs complicated by pancreaticocolonic fistula, in which effective drainage of the collection resulted in regression of the intestinal fistula (Jagielski et al., 2018).

In the largest study on the endotherapy of post-inflammatory PPFs available in the literature, Wroński et al. presented the results of treatment of eight patients (Wronski et al., 2011). Endoscopic treatment was applied during ERCP in seven patients; in one patient, the major duodenal papilla could not be found and cannulation failed, and the patient underwent surgical treatment (Wronski et al., 2011). In our study, 22 patients with post-inflammatory PPFs were treated endoscopically. Transpapillary drainage was performed during ERCP in 21 patients; in one patient, the duodenal papilla major could not be accessed, and effective extraanatomical (transmural) drainage of the PPF was performed. Thus, it was demonstrated that surgical treatment can be prevented through the application of advanced endoscopic techniques such as extraanatomical transgastric drainage.

In the aforementioned work, Wroński et al. (Wronski et al., 2011) noted the technical success of the ERCP procedure in seven (87.5%) patients, although three patients required subsequent surgery due to failed endoscopic treatment (ineffective transpapillary drainage and superinfection of the pleura or pancreatic collections). Ultimately, clinical success of endotherapy was achieved in 4 (50%) patients (Wronski et al., 2011). In our study, technical success of endoscopic surgery was achieved in all 22 patients. However, clinical success of endotherapy was achieved in 21 (95.45%) patients, and long-term success of endoscopic treatment of PPFs was noted in 19 (86.36%) patients.

In our opinion, the poor results of endoscopic treatment reported by Wroński et al. (Wronski et al., 2011) are associated with difficult anatomical conditions due to chronic pancreatitis found during ERCP in the study population. In only one (12.5%) patient, it was possible to properly introduce pancreatic endoprosthesis during ERCP so that it covered the site of disruption (Wronski et al., 2011). In the remaining six patients, placement of the pancreatic stent failed because intraductal stones and ductal strictures precluded its passage or the stent was too short to reach the fistula located in the distal part of the pancreas (Wronski et al., 2011). In our study, no such abnormalities were encountered during ERCP, which would prevent the proper introduction of a pancreatic endoprosthesis. Moreover, Wroński et al. (Wronski et al., 2011) showed that in patients with post-inflammatory PPF, complete disruption of the MPD in the pancreatic body and leakage into the left pleural cavity were the most common findings. In our study, both the body and tail were the most common locations of PPF with leakage into the left pleural cavity. However the majority (90.48%) of patients suffered from partial disruption of the MPD, which enabled stenting of the disruption using pancreatic endoprosthesis and, consequently, led to better results of endoscopic treatment. Partial disruption of the MPD is associated with better outcomes of endotherapy compared to complete disruption of the MPD (Devière et al., 1995; Varadarajulu et al., 2005; Jagielski et al., 2017; Jagielski et al., 2018a; Jagielski and Jackowski, 2021a). Although endotherapy is a more effective treatment method in cases of partial disruption of the pancreatic duct compared with total disruption in patients with pancreatitis, we believe that stenting of the MPD should also be applied in patients with complete duct disruption (Jagielski et al., 2018b; Jagielski et al., 2021a; Jagielski et al., 2017). However, it should be noted that most patients with complete MPD disruption require permanent passive transmural drainage in addition to passive

transpapillary drainage (stenting of the MPD), especially in cases of pancreatic fragmentation (disconnected duct syndrome) (Jagielski et al., 2017; Jagielski et al., 2018a; Jagielski et al., 2018b; Jagielski and Jackowski, 2021a).

The results of our study make an important contribution to the current state of knowledge, as there is currently no consensus regarding the optimal treatment for patients with post-inflammatory PPFs. According to literature, conservative treatment should be the first-line treatment (Dhebri and Ferran, 2005; Ali et al., 2009; Tay and Chang, 2013). Interventional treatment using minimally invasive techniques may only be initiated if conservative management is ineffective (Dhebri and Ferran, 2005; Ali et al., 2009; Tay and Chang, 2013). In the case of ineffective minimally invasive methods, surgery remains the treatment of choice (Dhebri and Ferran, 2005; Ali et al., 2009; Tay and Chang, 2013). The diagnostic and therapeutic algorithms presented in this study appear optimal for patients with post-inflammatory PPFs. In patients with confirmed pancreatitis, the detection of a pleural effusion on imaging requiring thoracocentesis with high amylase activity in the drained fluid is sufficient for the diagnosis of post-inflammatory PPF and for the implementation of treatment, particularly when this coexists with post-inflammatory PPFCs. In our study, the first-line treatment in patients with post-inflammatory PPF was endotherapy in combination with pharmacotherapy. In the literature, treatment often begins with conservative management, and a decision to perform ERCP is made only where conservative management is ineffective (Tay and Chang, 2013; Dhebri and Ferran, 2005; Ali et al, 2009). In contrast to previous reports, in this study, ERCP was performed in all patients with post-inflammatory PPFs, which confirms the diagnosis and also allows the implementation of treatment to decompress the pancreatic duct system and restore the outflow of pancreatic juice to the duodenum.

The main limitations of our study include the lack of randomization and the fact that the study was conducted on a selected group of patients from a single center. Although our study presents the experience of a single center, the fact that all endoscopic procedures were performed by a single endoscopist may be considered a strength of the study as this enables a reliable comparison of the results of endoscopic treatment.

In summary, regardless of the clinical situation, the management of patients with post-inflammatory PPFs should begin with the use of minimally invasive techniques, often combined with intensive conservative treatment. The study showed that endoscopic techniques, such as minimally invasive treatment, in patients with post-inflammatory PPF are effective. Transpapillary drainage (stenting of the MPD) was the preferred method. If transpapillary drainage is not possible, transmural drainage of the PPF remains the management of choice. In patients with PPFS complicated by PPF, drainage of the collection through endoscopic transmural drainage leads to closure of the fistula canal.

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 study was approved by the Ethics Committee at the Collegium Medicum of Nicolaus Copernicus University and was conducted in accordance with the Declaration of Helsinki. All patients provided oral and written informed consent to participate in the study. All patients received detailed information regarding the study.

Author contributions

Conceptualization was performed by MatJ, JP and MarJ. Formal analysis was performed by MatJ, JP, and MarJac. The methodology was performed by MatJ, JP and MarJ. Project administration was performed by MatJ. Endoscopic procedures was performed by MatJ. The writing of the original draft was performed by MatJ, and JP. Editing was performed by MatJ, and MarJ. All authors (MatJ, JP, and MarJ) revised and approved the submitted version of the manuscript.

Conflict of interest

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

Publisher's note

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Interleukin-6 is better than C-reactive protein for the prediction of infected pancreatic necrosis and mortality in patients with acute pancreatitis

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Introduction: This study aimed to identify whether interleukin-6 (IL-6) is better than C-reactive protein (CRP) for the prediction of severe acute pancreatitis (SAP), infected pancreatic necrosis (IPN), and mortality.

Methods: Sixty-seven patients with acute pancreatitis (AP) who were hospitalized within 48 h of onset and received serum CRP and IL-6 tests from September 2018 to September 2019 were included. Spearman's correlation was performed to assess their associations with severity. The areas under the curve (AUCs) for the prediction of SAP, organ failure, pancreatic necrosis, IPN, and mortality were estimated using receiver operating characteristic curves.

Result: Serum CRP and IL-6 levels were significantly positively correlated with the severity of AP ($p < 0.05$). The AUC for the prediction of SAP based on the CRP level was 0.78 (95% CI, 0.66–0.89) and that based on the IL-6 level was 0.69 (95% CI, 0.56–0.82). For the prediction of organ failure and pancreatic necrosis, CRP was more accurate than IL-6 (AUC 0.80 vs. 0.72 and 0.75 vs. 0.68, respectively). However, CRP was less accurate than IL-6 for predicting mortality and IPN (AUC 0.70 vs. 0.75 and 0.65 vs. 0.81, respectively). Systemic inflammatory response syndrome plus CRP was more accurate than systemic inflammatory response syndrome plus IL-6 (AUC 0.79 vs. 0.72) for the prediction of SAP.

Conclusions: IL-6 was more accurate than CRP for predicting mortality and IPN in patients with AP.

KEYWORDS

C-reactive protein, interleukin 6, infected pancreatic necrosis, mortality, organ failure, severe acute pancreatitis

Introduction

Acute pancreatitis (AP) is a common gastrointestinal disease with an increasing morbidity rate (Tenner et al., 2013). According to the 2012 revised Atlanta classification, the severity of AP is defined as mild acute pancreatitis (MAP), moderately severe acute pancreatitis (MSAP), and severe acute pancreatitis (SAP). Approximately 20% of patients with AP have severe disease, and 36%–50% of those with SAP die (Banks et al., 2013). It is critical to identify patients at risk of severe disease to facilitate the implementation of early active interventions to improve the prognosis of patients (Whitcomb, 2006; Talukdar and Swaroop Vege, 2011; He et al., 2017). Several multifactor scoring systems predict the severity of AP, such as the Acute Physiology and Chronic Health Evaluation II, the Ranson, the Bedside Index of Severity in Acute Pancreatitis, and the Glasgow scoring systems (He et al., 2017); however, the limitations of these scoring systems are the inability to obtain a complete score until at least 48 h into the illness, the complexity of the scoring system, or the poor accuracy (Matull et al., 2006; He et al., 2017). Moreover, clinicians also use individual laboratory parameters to assist in the prediction of which patients with AP will develop severe disease. C-reactive protein (CRP) and interleukin-6 (IL-6) are laboratory markers most commonly used to predict disease severity and prognosis (Meher et al., 2015).

CRP is an acute-phase reactant. A CRP level of >150 mg/L within the first 72 h strongly correlates with the presence of pancreatic necrosis (PN), and it was recommended in some guidelines for the prediction of SAP (Banks and Freeman, 2006; Greenberg et al., 2016; Leppaniemi et al., 2019). Clinicians widely consider CRP to be the gold standard for disease severity assessment at 48 h after disease onset (Staubli et al., 2015). IL-6 is released by a wide range of cells in response to tissue injury, and it stimulates the synthesis of acute-phase proteins, including CRP, by hepatocytes *in vitro* and *in vivo* (Bhatia and Moochhala, 2004). Nieminen et al. (2014) found that IL-6 levels on admission have prognostic value for SAP, and when measured within 48 h of AP onset, an IL-6 level of ≥28.90 pg/ml was reported to be the best biomarker among those tested (IL-8, IL-10, and CRP) in a prospective cohort study on the prediction of SAP (Jain et al., 2018). Moreover, Jain et al. found that the additional consideration of IL-6 significantly improved the predictive value of systemic inflammatory response syndrome (SIRS) for the prediction of SAP (Jain et al., 2018). In view of the finding that IL-6 is highly accurate for the prediction of SAP, our pancreatic disease center routinely detected IL-6 in patients with AP admitted in the early stage and recorded it in the AP database since September 2018. We designed this cohort study based on this prospective database to compare the accuracy of IL-6 detected within 48 h of onset with that of CRP for the prediction of SAP, organ failure (OF), PN, infected pancreatic necrosis (IPN), and mortality.

Abbreviations: AP, acute pancreatitis; CRP, C-reactive protein; IL-6, interleukin-6; MAP, mild acute pancreatitis; MSAP, moderately severe acute pancreatitis; SAP, severe acute pancreatitis; IPN, infected pancreatic necrosis; OF, organ failure; PN, pancreatic necrosis; SIRS, systemic inflammatory response syndrome.

Methods

Ethical approval

The construction of the AP database and the performance of this study was conducted according to the Declaration of Helsinki and was approved by the Clinical Ethics Committee of the First Affiliated Hospital of Nanchang University (Approval No. (2011)001). Informed consent was waived.

Patients

This study retrospectively screened 1,280 AP cases admitted to the First Affiliated Hospital of Nanchang University from September 2018 to September 2019. We selected patients who were admitted to the hospital within 48 h of disease onset and had values for IL-6 and CRP. All of the patient data in this retrospective cohort study were collected from the AP database. Serum IL-6 and CRP were tested using enzyme-linked immunosorbent assays at the Inspection Center of the First Affiliated Hospital of Nanchang University. Briefly, coated microtiter plates with anti-human IL-6 antibody and CRP antibody, and detected by double-antibody sandwich ELISA.

Classification of acute pancreatitis severity

In this study, we classified the severity of AP at the time of discharge based on the occurrence of OF (respiratory, cardiovascular, and renal), systemic complications, and local pancreatic complications during the period from onset to hospital discharge. SIRS scores were calculated daily in the 7 days after admission. Patients with AP were divided into groups with MAP, MSAP, and SAP according to the revised Atlanta classification (Banks et al., 2013). The above definitions are explained in Table 1.

Statistics

Demographic and baseline characteristics were analyzed using descriptive statistics. Qualitative variables are described as numbers and percentages. Quantitative variables are

TABLE 1 Definitions of endpoints.

Endpoint	Definition
Systemic inflammatory response syndrome (SIRS)	Presence of two or more criteria: * Heart rate, >90 beats/min * Core temperature, <36°C or >38°C * White blood count, <4,000 or >12,000/mm * Respirations, >20/min or pCO_2 <32 mm Hg
Persistent SIRS	SIRS lasted more than 48 h
Respiratory failure	$PaO_2/FiO_2 \leq 300$, or requirement for mechanical ventilation
Cardiovascular failure	Circulatory systolic blood pressure <90 mm Hg, despite adequate fluid resuscitation, or requirement for inotropic catecholamine support
Renal failure	Creatinine level > 177 μmol/L after rehydration or new need for hemofiltration or hemodialysis
Persistent organ failure	Organ failure persists for >48 h
Systemic complications	Exacerbation of preexisting comorbidity
Necrotising pancreatitis.	Inflammation associated with pancreatic parenchymal necrosis and/or peripancreatic necrosis
Local complications	Include acute peripancreatic fluid collection, pancreatic pseudocyst, acute necrotic collection, walled-off necrosis, gastric outlet dysfunction, splenic and portal vein thrombosis, and colonic necrosis
Mild acute pancreatitis	Absence of organ failure and absence of local or systemic complications
Moderately severe acute pancreatitis	Presence of transient organ failure or local or systemic complications
Severe acute pancreatitis	Persistent organ failure.
Mortality	Patients who died during hospitalization or within 30 days of discharge

described as the means ± standard deviations. Medians and interquartile ranges (IQRs) were reported if the distribution of the variable was not normal. Kruskal–Wallis tests were performed for nonnormally distributed variables. The predictive accuracy was evaluated using the area under the receiver operating characteristic (ROC) curve (AUC). The best cutoff value was selected according to the largest value of the Youden index. The diagnostic characteristics were assessed with the AUC, sensitivity, specificity, positive likelihood ratio (+LR), and negative likelihood ratio (−LR). When analyzing the combination of SIRS and serum IL-6 or CRP levels for the prediction of SAP, univariate analyses were performed with SAP as the dependent variable and predefined prognostic factors as independent variables, including SIRS and the serum IL-6 and CRP levels. Logistic regression analysis was used to assess the combinations, and then ROC curve analysis was performed to evaluate the diagnostic value of the combinations of SIRS with IL-6 and SIRS with CRP for the severity of AP. Epi Info 7 (Centers for Disease Control and Prevention, Atlanta, GA, USA) and Microsoft Excel® 2013 (Microsoft, Inc., Redmond, WA, USA) were used to collect and process the data, which were then analyzed with IBM SPSS statistics version 25.0 (IBM Corp., Armonk, NY, USA).

Results

Patient characteristics

A total of 1,280 AP patients were admitted to the First Affiliated Hospital of Nanchang University from September 2018 to September 2019. Of these, 67 were eventually enrolled in this study after the application of the exclusion criteria (the details are listed in Figure 1). Their characteristics and the clinical outcomes of AP are shown in Table 2. The mean age of the included AP patients was nearly 48 years, and most patients were female. The causes of AP included hyperlipidemia (44.8%), biliary causes (37.3%), alcohol (14.9%), and idiopathic pancreatitis (3.0%). According to the 2012 Atlanta classification criteria, there were 35 (52.2%) patients with SAP, 22 (32.8%) patients with MSAP, and 10 (14.9%) patients with MAP. A total of 80.6% of the patients received treatment in the pancreatic intensive care unit, 46 (68.7%) patients developed OF, 34 patients developed persistent OF, and 9% died (Table 2).

Serum interleukin-6 and C-reactive protein levels in patients with differing severities of acute pancreatitis

The levels of IL-6 were significantly elevated in AP patients within 48 h of onset. Compared with MAP (median 52.34 [IQR 29.4–121.5]) and MSAP (median 108.0 [IQR 42.6–206.1]) patients, SAP patients had the highest serum IL-6 level (median 173.3 [IQR 65.7–321.3], $p < 0.05$). Similarly, SAP patients had the highest serum CRP level (median 296.0 [IQR 153.0–377.0] whereas MSAP patients had a moderate serum CRP level (median 110 [IQR 42.3–260.0]), and MAP patients had the lowest serum CRP level (median 81.3 [IQR 43.7–102.5], $p < 0.05$) (Table 3). Spearman correlation analysis showed that the IL-6 level was significantly positively correlated with the severity of AP ($p < 0.05$) and the same with that of CRP ($p < 0.05$). CRP had a stronger correlation with severity than IL-6 (0.513 vs. 0.327) (Table 3).

Predictive value of interleukin-6 and C-reactive protein for severe acute pancreatitis, organ failure, pancreatic necrosis, infected pancreatic necrosis, and mortality

Figure 2 and Table 4 show the predictive value of IL-6 and CRP for SAP, OF, PN, IPN, and mortality. The AUC for the use

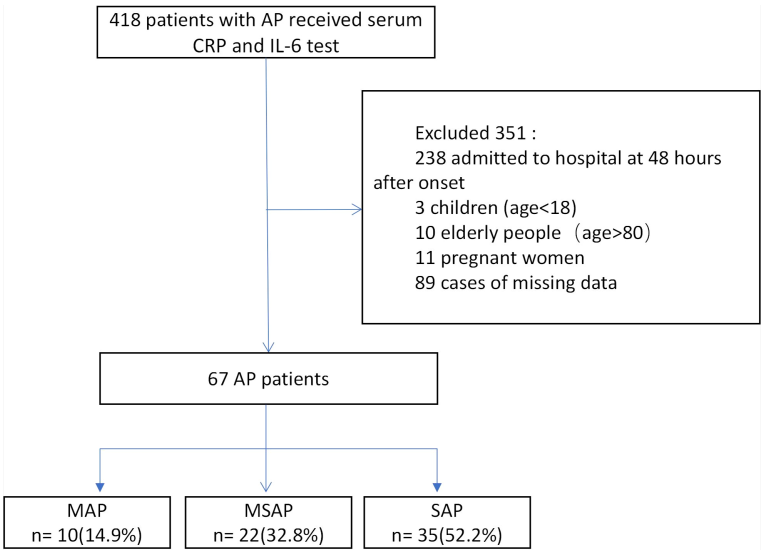

FIGURE 1
Study flowchart.

of IL-6 (cutoff = 121.1 pg/ml) measured within 48 h of onset for the prediction of SAP was 0.69 (95% CI, 0.56–0.82), with a sensitivity of 67.65%, a specificity of 67.74%, a +LR of 2.10, and a −LR of 0.48. The AUCs for the use of IL-6 for the prediction of OF, PN, IPN, and mortality were 0.72 (95% CI, 0.58–0.85), 0.68 (95% CI, 0.55–0.82), 0.81 (95% CI, 0.69–0.94), and 0.75 (95% CI, 0.52–0.99), respectively. The ROC curve analysis showed that CRP was more accurate (AUC= 0.78; 95% CI, 0.66–0.89) for the prediction of SAP than IL-6 (AUC= 0.69; 95% CI, 0.56–0.82) (Figure 2A). The AUCs for the use of CRP for the prediction of OF (AUC 0.80; 95% CI, 0.69–0.91) and PN (AUC 0.75; 95% CI, 0.63–0.87) were also higher than those for the use of IL-6 (Figures 2B, C). With regard to the prediction of IPN and mortality, IL-6 was superior (AUC 0.81 and 0.75, respectively) to CRP (Figures 2D, E).

Systemic inflammatory response syndrome plus interleukin-6 and systemic inflammatory response syndrome plus C-reactive protein for the prediction of severe acute pancreatitis, infected pancreatic necrosis, and mortality

The AUC for the use of SIRS on the first day of admission for the prediction of SAP was 0.69 (0.56–0.82), with a sensitivity of 94.29% and a specificity of 43.33% (Figure 2F and Table 5). The AUC for the use of persistent SIRS for the prediction of SAP was 0.77 (0.65–0.89), and specificity increased to 60.00%. The combination of SIRS at admission and serum IL-6 (>121.1 pg/ml) within 48 h improved the accuracy of the prediction of SAP (AUC = 0.72), but the sensitivity (82.86%) was lower than that for persistent SIRS. The accuracy (AUC = 0.79) and specificity (73.33%) of the use of SIRS at admission combined with CRP (>142.5 mg/L) within 48 h for the prediction of SAP were higher than those of the use of SIRS plus IL-6 (Figure 2F). The AUC of SIRS plus IL-6 in predicting IPN and mortality was lower than that of IL-6 alone (Supplementary Tables S1 and S2)

Discussion

This study, which was based on data from a prospectively collected AP database, found that the levels of serum IL-6 and CRP increased in proportion to the severity of AP, and both had a strong correlation with severity. Moreover, we found that compared with CRP, serum IL-6 has higher predictive accuracy for IPN and mortality but lower predictive accuracy for SAP, OF, and PN. We confirmed that the additional consideration of IL-6 improved the accuracy of the use of SIRS for the prediction of SAP at admission, although accuracy and sensitivity were lower than those obtained with the use of persistent SIRS and specificity was lower than that obtained with the use of SIRS combined with CRP.

AP is an inflammatory reaction in pancreatic tissue related to the inappropriate activation of trypsinogen to trypsin and a lack of the prompt elimination of active trypsin inside the pancreas. The activation of digestive enzymes causes pancreatic injury and results in an inflammatory response. The

TABLE 2 Patient demographics and baseline clinical.

Characteristic		*n* = 67
Age in years, mean (*SD*)		48 (17)
Sex, *n* (%)		
	Female	47 (70.1%)
	Male	20 (29.9%)
BMI		25 (23–28)
Cause of pancreatitis		
	Biliary	25 (37.3%)
	Hyperlipidemic	30 (44.8%)
	Alcoholic	10 (14.9%)
	Idiopathic	2 (3.0%)
Coexisting condition		
	Hypertension	14 (20.9%)
	Coronary heart disease	1 (1.5%)
	Diabetes	10 (14.9%)
Smoking history		24 (35.8%)
Drinking history		25 (37.3%)
Disease severity		
	Admission to ICU	54 (80.6%)
	SIRS	2 (2–3)
	APACHE II score	11 (8–13)
	CTSI within 1 week of onset	4 (3–6)
	IL-6	122.0 (45.5–261.0)
	C-reactive protein (mg/L)	162.5 (82.3–317.3)
	White cell count ($\times 10^{-9}$/L)	13.8 (5.9)
	AMY	921 (691)
	Organ failure	46 (68.7%)
	Persistent organ failure	33 (49.3%)
	Respiratory	34 (50.7%)
	Cardiovascular	0
	Renal	8 (11.9%)
	Pancreatic necrosis	26 (38.8%)
	Infected pancreatic necrosis	12 (17.9%)
	MAP	10 (14.9%)
	MSAP	22 (32.8%)
	SAP	35 (52.2%)
	Death	6 (9%)

ICU, intensive care unit; SIRS, systemic inflammatory response syndrome; APACHE II, Acute Physiology and Chronic Health Evaluation II; IL-6, interleukin-6; MAP, mild acute pancreatitis; MSAP, moderately severe acute pancreatitis; SAP, severe acute pancreatitis; CTSI: computed tomography severity index; AMY, amylase.

acute inflammatory response itself causes substantial tissue damage and may progress outside the pancreas to SIRS, multiorgan failure, or death (Whitcomb, 2006). Serum cytokine levels reflect the magnitude of the inflammatory response. IL-6 is a prototypical cytokine that has redundant and pleiotropic activity, the synthesis of which is promptly induced to aid in host defense when tissue damage or inflammation because of infections or injuries occurs (Tanaka and Kishimoto, 2014). Several studies have demonstrated an association between IL-6 and AP and found that IL-6 is a useful marker for the assessment of the severity of AP in its early stages (Inagaki et al., 1997; Jiang et al., 2004; Stimac et al., 2006; Karpavicius et al., 2016). In agreement with these previous studies, our results show that the IL-6 level is correlated with the severity of AP; the higher the IL-6 level in a patient is, the more likely the development of SAP in that patient. The present study identified a cutoff value of ≥121.10 pg/ml for IL-6, with a sensitivity of 67.65% and a specificity of 67.74%, for the prediction of SAP. This result is consistent with the results of previous studies (Schütte and Malfertheiner, 2008). Sathyanarayan et al. found that, at a cutoff value of 122 pg/ml on day 3, IL-6 has a sensitivity of 81.8% and a specificity of

TABLE 3 Serum interleukin-6 (IL-6) and C-reactive protein (CRP) levels in patients with acute pancreatitis and their correlation with severity.

	IL-6 (pg/ml)	CRP (mg/L)
MAP	52.34 (29.4–121.5)	81.3 (43.7–102.5)
MSAP	108.0 (42.6–206.1)	110 (42.3–260.0)
SAP	173.3 (65.7–321.3)	296.0 (153.0–377.0)
p-value[§]	<0.05	<0.05
Spearman's rho	0.327	0.513
p-value*	<0.05	<0.05

MAP, mild acute pancreatitis; MSAP, moderately severe acute pancreatitis; SAP, severe acute pancreatitis.
[§]Kruskal–Wallis H.
*Spearman's rho.

77.7%, for the prediction of SAP (Sathyanarayan et al., 2007). Because the serum IL-6 concentration decreases very rapidly over time, a prospective study showed that serum IL-6 detected within 48 h of onset was the most accurate for the prediction of SAP (Jain et al., 2018). Considering the urgent need to predict the severity as soon as possible, we chose IL-6 detected within 48 h of onset for the prediction of SAP, which has more clinical relevance.

CRP has been widely adopted as a nonspecific indicator of inflammation; a number of clinical studies have reported that CRP plays an important role in the prediction of SAP (Leser et al., 1991; Jiang et al., 2004; Papachristou and Whitcomb, 2004; Sternby et al., 2017; Zheng et al., 2018). Serum CRP levels increase during the first 24 h and peak between 36 and 48 h after the onset of AP (Heath et al., 1993). Viedma et al. found that the serum CRP level was relatively high and remained high for a long time in patients with SAP. A serum CRP level of >300 mg/l can be used to predict SAP (Viedma et al., 1994). Another study indicated that a CRP level of >150 mg/l can be used to predict severe attacks of AP with a sensitivity of 90% and a specificity of 79% (Heath et al., 1993). Many studies have shown that the use of CRP to predict SAP has a sensitivity and a specificity of approximately 80% (Dervenis et al., 1999). The present study found that a CRP level greater than 142.50 mg/l could be used to distinguish between severe and mild attacks, with a sensitivity of 76.47% and a specificity of 74.19%, which was consistent with previous studies.

Over the years, several studies have been conducted to compare the use of different serum markers for the early identification of patients with AP who are at risk for severe disease. Heath et al. found that IL-6 had a sensitivity of 100% and a specificity of 71% for the prediction of SAP; CRP had a sensitivity of 90% and a specificity of 79%, indicating similar predictive value (Heath et al., 1993). Pezzilli et al. reported that CRP had a lower prognostic efficiency than IL-6 (sensitivity of 100% and specificity of 83% vs. sensitivity of 87% and specificity of 46%) (Pezzilli et al., 1999), and recently, a systematic review and meta-analysis also reported the superiority of IL-6 for the early prediction of MSAP/SAP (van den Berg et al., 2020). However, in 2012, the revised Atlanta classification recommended SIRS as one index indicating the potential for SAP and did not mention any laboratory markers that were available in clinical practice and consistently accurate for the prediction of SAP; the accuracy of IL-6 and CRP for the prediction of SAP is unclear (Banks et al., 2013). A recent study showed that IL-6 was closely related to the severity of AP, whereas CRP had low predictive accuracy for SAP (Nieminen et al., 2014). Duarte-Rojo et al. found that during the first 48 h after admission, IL-6 was more accurate than CRP (Duarte-Rojo et al., 2009). Our study revealed that IL-6 and CRP both have a strong correlation with severity, but CRP has a higher predictive value than IL-6 for the prediction of SAP (AUC 0.78 vs. AUC 0.69). Because of their low cost, ease of performance, and widespread availability, tests for CRP are generally considered to be the "gold-standard" biochemical marker for the severity of AP (Wilson et al., 1989; Papachristou and Whitcomb, 2004; Staubli et al., 2015). Our results are in accordance with this statement; a CRP level of >142.50 mg/l had a sensitivity of 76% and a specificity of 74% for the prediction of SAP. Several studies have demonstrated the predictive value of Il-6 and CRP for OF, PN, IPN, and mortality (Teerenhovi and Nordback, 1988; Ueda et al., 1997; Mándi et al., 2000; Kaya et al., 2007; Cardoso et al., 2013; Khanna et al., 2013; Karpavicius et al., 2016; Kolber et al., 2018; Vasudevan et al., 2018). A study found that IL-6 is a good marker of peripancreatic necrosis (Karpavicius et al., 2016), and previously published results showed that IPN can aggravate prognosis (Kolber et al., 2018). Our study also compared the predictive values of Il-6 and CRP for OF, PN, IPN, and mortality. The results indicated that serum IL-6 was more accurate than CRP for the prediction of IPN and mortality, but not for the prediction of OF and PN.

To mitigate the limitations inherent in the use of individual prognostic markers, some studies used combinations of multiple laboratory markers to predict the severity of AP. In 1999, Pezzilli et al. found that combining IL-6 and lipase obtained a good result with regard to the prediction of SAP (Pezzilli et al., 1999). Recently, Tian et al. reported that the combination of CRP, Procalcitonin (PCT), IL-6, and lactate dehydrogenase (LDH) is a good predictor of the severity of AP (Cardoso et al., 2013; Tian et al., 2020). SIRS is superior for the early identification of SAP; because of the low accuracy of SIRS on admission and the need to wait for 48 h for persistent SIRS, Jain et al. combined early SIRS (on admission) and IL-6 for the prediction of SAP and found that IL-6 significantly improved the predictive ability (Jain et al., 2018). Similarly, we combined early SIRS with IL-6 and CRP and compared the accuracy of those combinations with regard to the prediction of SAP; we found that both IL-6 and CRP improved the accuracy of the prediction of SAP, but SIRS at admission combined with CRP within 48 h was more accurate than SIRS plus IL-6.

This study has some limitations. First, it was a single-center study, and patients admitted to our center had relatively more

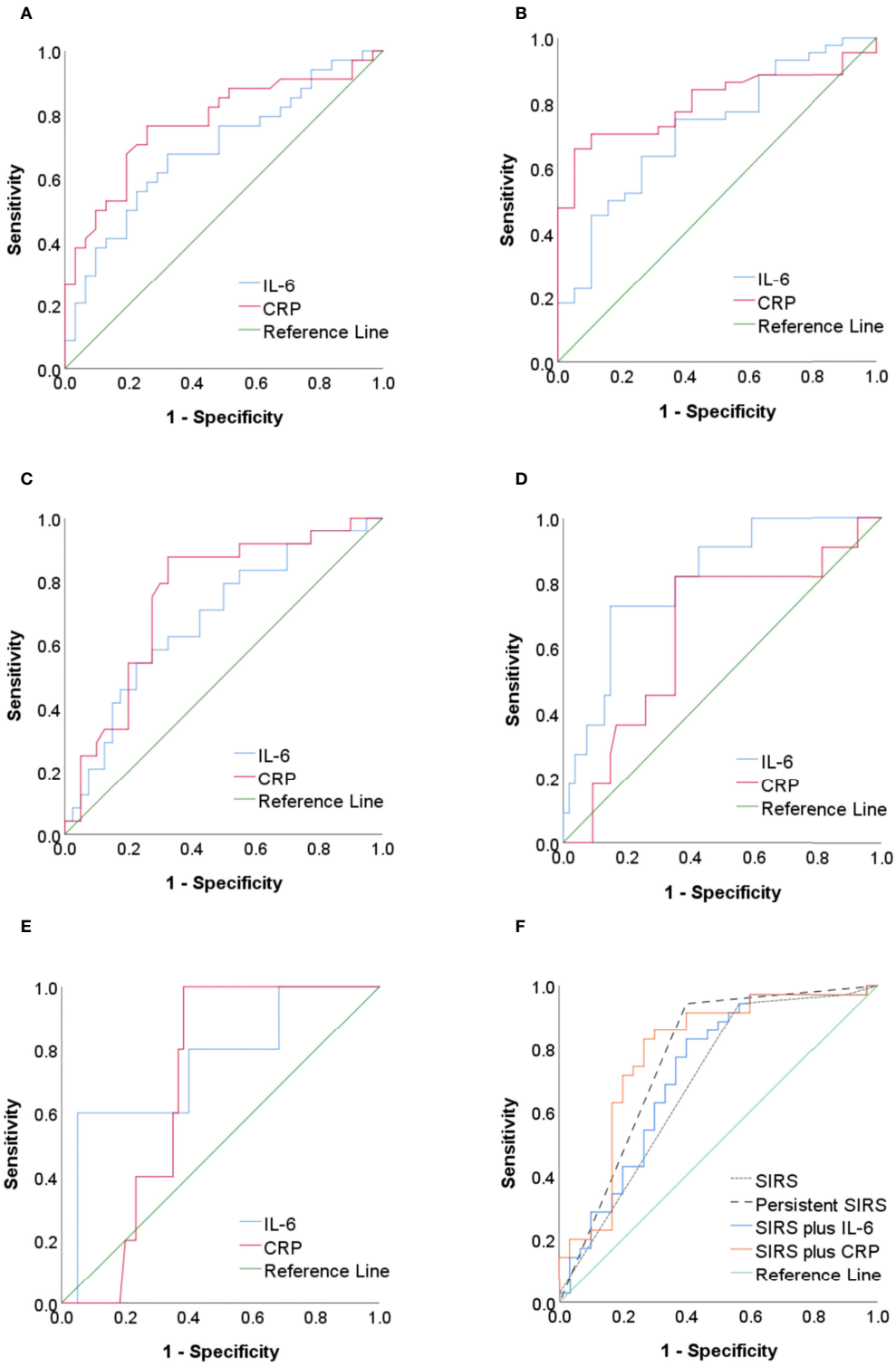

FIGURE 2
(A–E) The ROC curve of IL-6 and CRP as predictors of SAP, OF, PN, IPN, and mortality in AP. **(F)** The ROC curve of SIRS, persistent SIRS, IL-6 combined with SIRS, and CRP combined with SIRS as predictors of SAP. ROC, receiver operating characteristic; IL-6, interleukin-6; CRP, C-reactive protein; SAP, severe acute pancreatitis; OF, organ failure; PN, pancreatic necrosis; IPN, infected pancreatic necrosis; AP, acute pancreatitis; SIRS, systemic inflammatory response syndrome.

TABLE 4 Comparison of IL-6 and CRP for the prediction of SAP, OF, PN, IPN, and death.

	Predictive method	AUC (95% CI)	p-value	Cutoff value	Sensitivity	Specificity	+LR	−LR
SAP	IL-6	0.69 (0.56–0.82)	0.008	121.10	67.65%	67.74%	2.10	0.48
	CRP	0.78 (0.66–0.89)	0.000	142.50	76.47%	74.19%	2.96	0.32
OF	IL-6	0.72 (0.58–0.85)	0.006	54.16	75.00%	63.16%	2.04	0.40
	CRP	0.80 (0.69–0.91)	0.000	117.00	70.45%	89.47%	6.69	0.33
PN	IL-6	0.68 (0.55–0.82)	0.016	122.95	62.50%	67.50%	1.92	0.56
	CRP	0.75 (0.63–0.87)	0.001	117.00	87.50%	67.50%	2.69	0.19
IPN	IL-6	0.81 (0.69–0.94)	0.001	219.30	72.73%	85.19%	4.91	0.32
	CRP	0.65 (0.47–0.82)	0.13	170.00	81.82%	64.81%	2.33	0.28
Death	IL-6	0.75 (0.52–0.9)	0.06	122.95	80.00%	60.00%	2.00	0.33
	CRP	0.70 (0.57–0.82)	0.15	170.00	100.00%	61.67%	2.61	0.00

AUC, area under the ROC curve; CI, confidence interval; +LR, positive likelihood ratio; −LR, negative likelihood ratio; IPN, infected pancreatic necrosis; OF, organ failure; PN, pancreatic necrosis; SAP, severe acute pancreatitis; IL-6, interleukin-6; CRP, C-reactive protein.

TABLE 5 Comparison of SIRS, persistent SIRS, SIRS plus IL-6, and SIRS plus CRP for the prediction of SAP.

Predictive method	AUC (95% CI)	Sensitivity	Specificity	+LR	−LR	DOR
SIRS	0.69 (0.56–0.82)	94.29%	43.33%	1.66	0.13	12.77
Persistent SIRS	0.77 (0.65–0.89)	94.29%	60.00%	2.36	0.10	23.60
SIRS plus IL-6	0.72 (0.59–0.85)	82.86%	60.00%	2.07	0.29	7.14
SIRS plus CRP	0.79 (0.67–0.91)	82.86%	73.33%	3.11	0.23	13.52

AUC, area under the ROC curve; CI, confidence interval; +LR, positive likelihood ratio; −LR, negative likelihood ratio; DOR, diagnostic odds ratio; SIRS, systemic inflammatory response syndrome; IL-6, interleukin-6; CRP, C-reactive protein.

severe disease (Tian et al., 2020; Yu et al., 2020). Most of the patients in our study needed intensive care, and the percentage of mild cases was lower than that observed in general hospitals. Second, hypertriglyceridemia (44.8%) was the major etiology of AP in our study, whereas previous studies showed that biliary etiology was the most common (He et al., 2017; Zhu et al., 2017; Fan et al., 2018). Severe Hypertriglyceridemic (HTG) significantly increases the severity of AP (Zhang et al., 2019), which is consistent with the observation of relatively more severe cases in our study. Finally, the small sample size is also a limitation, and a large-sample prospective study is needed for validation. Whatever, our study provides some guidance for clinicians seeking to identify patients early who are at risk for SAP, enabling them to promptly initiate therapy.

Conclusions

In conclusion, our study found that CRP and IL-6 had diagnostic value for the severity of AP, and for predicting IPN and mortality, IL-6 had some advantages over CRP.

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 construction of the AP database and the performance of this study was conducted according to the Declaration of Helsinki and was approved by the Clinical Ethics Committee of the First Affiliated Hospital of Nanchang University (approval number (2011)001). Written informed consent from the patients/participants was not required to participate in this study in accordance with the national legislation and the institutional requirements.

Author contributions

WH designed the research. JL, ZC, LL, TL, HP and LG collected data. JL, ZC and WH analyzed the data. JL drafted the manuscript. YZ reviewed and revised the manuscript. All authors contributed to the article and approved the submitted version.

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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/fcimb.2022.933221/full#supplementary-material

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Non-linear correlation between amylase day 2 to day 1 ratio and incidence of severe acute pancreatitis

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Background: This study aimed to assess whether the amylase day 2/amylase day 1 ratio was associated with severe acute pancreatitis (SAP).

Methods: We retrospectively enrolled 464 patients with acute pancreatitis. Serum amylase was measured on admission (day 1) and 24 h later (day 2). Univariable logistic regression with restricted cubic spline analysis, multivariable logistic analysis, and receiver operating characteristic curve analysis was used to evaluate the relationship between the amylase day 2/amylase day 1 ratio and SAP.

Results: A non-linear association between the amylase day 2/amylase day 1 ratio and SAP was observed. The multivariable logistic analysis confirmed that a high amylase day 2/amylase day 1 ratio (≥0.3) was independently associated with the development of SAP (OR: 6.62). The area under the receiver operating characteristic curve (AUC) of the amylase day 2/amylase day 1 ratio, as a predictive factor for SAP, was 0.65. When amylase ratio ≥0.3 was counted as 1 point and added to the BISAP score to build a new model named the BISAPA (BISAP plus Amylase ratio) score (AUC = 0.86), it improved the diagnostic power of the original BISAP score (AUC = 0.83) for SAP. With a cut-off value of 3, the BISAPA score achieved a sensitivity of 66.0%, a specificity of 86.7%, and diagnostic accuracy of 84.48%.

Conclusions: There is a non-linear correlation between the amylase day 2/amylase day 1 ratio and the incidence of SAP. BISAPA score might also be a useful tool for the same purpose.

KEYWORDS

amylases, early diagnosis, risk factors, acute pancreatitis, severe acute pancreatitis

Highlights

① There is a nonlinear relationship between the amylase day 2/amylase day 1 ratio and the incidence of SAP.

② With a cut-off value of 0.3, amylase ratio achieved a sensitivity of 92.0% and a specificity of 33.8% for the prediction of SAP.

③ The advantages of amylase estimation are its technical simplicity, easy availability, and high sensitivity.

④ When amylase ratio ≥0.3 is counted as 1 point and added to the BISAP score, it significantly improves diagnostic power compared to the original BISAP score (AUC, 0.86 versus 0.83).

Introduction

Acute pancreatitis (AP) is a common gastrointestinal disorder with marked variation in severity. In most patients, AP has a self-limiting and mild course. However, a subset of 10%–20% of patients might progress to SAP with high mortality (Hong et al., 2019).

Early identification of high-risk patients on admission may help physicians to stratify the patients who would benefit the most from close surveillance or aggressive intervention (2). Early risk assessment of patients with AP through reliable methods is necessary to potentially improve the clinical outcome, while reduce the treatment cost and length of hospitalization. A great deal of effort has focused on the development of approaches for early risk stratification of AP. However, existing scoring systems, such as the Bedside index of severity in acute pancreatitis (BISAP), have only moderate diagnostic accuracy in the prediction of SAP (Mounzer et al., 2012). More recently, attention has also focused on assessing the association between SAP and individual laboratory parameters, such as admission albumin, blood urea nitrogen (BUN), high-density lipoprotein cholesterol level, and total cholesterol level on admission (Koutroumpakis et al., 2015; Hong et al., 2017b; Hong et al., 2017c; Hong et al., 2020).

Repeating serum amylase tests is thought to have no value in assessing the clinical progress of the patients or the ultimate prognosis once the diagnosis of AP has been made (Yadav et al., 2002). However, recently, Kumaravel et al. suggest a 10% decrease in the percentage of amylase during the first 2 days after admission was associated with a significantly decreased odds of SAP (Odds ratio, 0.93, 95% CI 0.87–0.98) (Kumaravel et al., 2015). However, it is not clear whether an increase in amylase from day 1 to day 2 would be associated with an increase in the incidence of SAP. On the other hand, the amylase increase is usually first detected 2–12 h after the onset of symptoms in AP. The level then peaks at 12–72 h and usually normalizes within 5 days (Frank and Gottlieb, 1999; Hong et al., 2017a). In clinical practice, serum amylase may increase or decrease during the first 2 days after admission. In our opinion, evaluation of the amylase day 2/amylase day 1 ratio may be more appropriate and comprehensive rather than that of an increase or decrease of amylase from day 1 to day 2 after admission as a potential predictor of severe acute pancreatitis. In addition, Fallah et al. suggested that nonlinear modeling procedures can prevent model misspecification and can provide information between prognostic factors and disease risk that is not revealed by the use of standard modeling techniques (Fallah et al., 2009). To the best of our knowledge, the non-linear correlation between the amylase day 2/amylase day 1 ratio and SAP has not been evaluated in the literature. Therefore, the current study aimed to assess the relationship between the amylase day 2/amylase day 1 ratio and SAP.

Methods

Inclusion and exclusion criteria

Patients with AP admitted to our hospital within 72 h of the onset of symptoms from 1 January 2012 to 31 December 2015 were retrospectively enrolled in the study. The AP diagnosis was based on the presence of two of the three features (pancreatic pain, amylase, and/or lipase ≥three times the upper limit of

Abbreviations: AUC, area under the receiver operating characteristic curve; BISAP, Bedside index of severity in acute pancreatitis; BMI, body mass index; BUN, blood urea nitrogen; CI, confidence interval; ERCP, endoscopic retrograde cholangiopancreatography; HRS, hours; IQR, interquartile range; OR, odds ratio; ROC, receiver operating characteristic curve; SAP, severe acute pancreatitis; SD, standard deviation.

normal and characteristic findings on abdominal imaging) (Hong et al., 2011). The disease severity was stratified into mild, moderately severe, and severe according to the revised Atlanta classification (Banks et al., 2013). SAP consists of persistent organ failure (at least one of the three organs involved: cardiovascular failure, respiratory failure, and renal failure) for more than 48 h (Hong et al., 2017c). Exclusion criteria were: previous pancreatic surgery, pancreatitis due to endoscopic retrograde cholangiopancreatography (ERCP) or trauma, chronic pancreatitis, pancreatic cancer, patients receiving surgery or therapeutic ERCP during hospitalization, chronic renal disease, previous albuminuria, hepatitis, liver cirrhosis, and incomplete data records.

Data collection

Age, gender, body mass index (BMI), and time from symptom onset to patient admission were recorded within 12 h of hospitalization. Serum amylase was measured on admission (day 1) and 24 h later (day 2) (Banks et al., 2013; Hong et al., 2017a). The amylase ratio was calculated as amylase day 2/amylase day 1. In addition, the BISAP score was calculated according to the laboratory and clinical data (Wu et al., 2008).

This study protocol was approved by the Ethics Committee of our hospital (date: 18-10-2016; number: 2016-211). This study was performed according to the principles expressed in the Declaration of Helsinki and written informed consent was obtained from all the subjects.

Sample size

The sample size was calculated based on the identification of an independent dichotomous predictor in multivariable logistic regression analysis for SAP (Hong et al., 2020). With an α risk of 0.05 and β risk of 0.1, the prevalence of SAP was estimated to be 10% with a bilateral test, assuming a low correlation between the predictor and other covariates (R2 = 0.20). A sample of 309 patients was predicted to provide 80% power of detecting of an adjusted odds ratio (OR) of 3.0 for a dichotomous predictor with an overall prevalence of 70%.

Statistical analysis

Categorical values were described by count and proportions and compared by Pearson's χ^2 test or Fisher's exact test if there were few observations. A Shapiro–Wilk test was used to evaluate whether the continuous data had a normal distribution (Hong et al., 2017b). According to the results of the Shapiro–Wilk test, continuous values were expressed using mean ± standard deviation (SD), or median and interquartile range (IQR). Continuous data were compared using Student's t-test or one-way analysis of variance if normality and homogeneity of variance. Conversely, the nonparametric Mann–Whitney test or Kruskal–Wallis non-parametric test was used if there was no normality and homogeneity of variance for continuous data (Hong et al., 2020). Nonlinearity in the relationship between the amylase ratio and SAP was assessed by univariable logistic regression with restricted cubic spline analysis (Hong et al., 2020). We used the default (5 knots) number of knots when performing restricted cubic spline analysis (knot points for amylase ratio levels: 0.096, 0.261, 0.432, 0.681, and 1.445). The cutoff of amylase ratio levels used to differentiate SAP from non-SAP was determined according to expected incidences of SAP predicted by restricted cubic spline analysis (Hong et al., 2020). Multivariable logistic analysis was also used to evaluate the relationship between the amylase ratio and SAP adjusted for potential confounders. We used our clinical experience, knowledge and previous study to select possible confounders for their potential association with amylase levels as follows: age, gender, body mass index, biliary etiology, and time interval before admission (Hong et al., 2017a). Odds ratios (OR) were calculated with 95% confidence intervals (CI). In order to evaluate the clinical usefulness of amylase ratio as an early predictor of SAP, the area under the receiver operating characteristic (ROC) curve (AUC) was used to evaluate the performance of predictions. Differences were assessed as being relevant when the two-tailed P-value <0.05 was reached.

Results

Clinical characteristics

A total of 464 patients, of whom 281 (60.6%) were male with a median age of 48 (37–63) years old, were included in the study (Table 1). The interval between the onset and admission was 1.8 ± 0.8 days. There was no significant difference with respect to the interval between the onset and admission among patients with different severity of disease (P = 0.73). It was 1.8 ± 0.8 days, 1.9 ± 0.8 days, and 1.9 ± 0.8 days for patients with mild, moderate severe, and severe AP, respectively. The median amylase levels on day 1 and day 2 were 743 (IQR 273–1,660) IU/L and 268 (IQR 119.5–587) IU/L, respectively. The most common cause of AP was the involvement of the biliary system (42.5%). Of all 464 patients, 348 (75.0%), 66 (14.2%), and 50 (10.8%) patients developed mild, moderately severe, and severe AP, respectively. Eight (1.72%) died during hospitalization. Patients with SAP had higher median serum amylase levels (941 IU/L, IQR 536–2,098 IU/L) on admission (day 1) compared to patients without SAP (666 IU/L, IQR 262–1,619 IU/L) (P = 0.0237).

TABLE 1 Demographic and Clinical Characteristics of 464 patients.

Characteristic	Value
Median age, years	48 (37–63)
Male sex, N (%)	281 (60.6%)
Time from pain onset to admission, days	1.8 ± 0.8
Mean BMI	23.5 (21.2–26.0)
Etiology	
Biliary, N (%)	197 (42.5)
Alcohol, N (%)	68 (14.7)
Hypertriglyceridemia, N (%)	27 (5.8)
Idiopathic, N (%)	157 (33.8)
Other, N (%)	15 (3.2)
Amylase at day 1 (IU/L)	743 (273–1,660)
Amylase at day 2 (IU/L)	268 (119.5–587)
Hematocrit	0.43 (0.38–0.46)
BUN, mmol/L	4.8 (3.7–6.4)
Outcomes	
Severity of acute pancreatitis	
Mild, N (%)	348 (75)
Moderately severe, N (%)	66 (14.2)
Severe, N (%)	50 (10.8)
Mean hospital days	10 (7–14)
Persistent organ failure N (%)	50 (10.8)
Death, N (%)	8 (1.72)

Data are shown either as number of observations, mean ± standard deviation (SD), percentage, median or interquartile range (IQR).

Non-linear correlation between amylase day 2 to day 1 ratio and incidence of SAP

Based on univariable logistic regression with restricted cubic spline analysis, a non-linear association between the amylase ratio and SAP was observed (Figure 1). In patients with an amylase ratio of <0.3, the expected incidence of SAP was low and did not significantly change with amylase ratio. While in patients with an amylase ratio of ≥0.3, the expected incidence of SAP increased rapidly with amylase ratio and reached a peak in patients with an amylase ratio of 0.6. Therefore, 0.3 was used as the cutoff of the amylase ratio to divide patients into different study groups.

Amylase ratio was independently associated with SAP

When 0.3 was used as the cut-off of amylase ratio, as shown in Figure 2, 14.4% (46/320) patients developed SAP with a high amylase ratio (≥0.3) as compared to 2.8% (4/144) with a low amylase ratio (<0.3) (P <0.001). Similarly, patients with a high amylase ratio (≥0.3) had a trend towards higher mortality (7/320, 2.2%) than patients with a low amylase ratio (<0.3) (1/144, 0.7%) during hospitalization, although it did not reach statistical significance (P = 0.445) (Figure 2).

Multivariable logistic regression indicated that a high amylase ratio (≥0.3) (OR: 6.62; 95% CI: 2.27–19.36; P = 0.001) was independently associated with the development of SAP after adjustment for age, gender, BMI, biliary etiology, and time from pain onset to admission.

Amylase ratio as predictor of SAP

Based on ROC analysis, the AUC for the amylase ratio for the prediction of SAP was 0.65 ± 0.04 (Figure 3). With a cut-off value of 0.3, the amylase ratio achieved a sensitivity of 92.0% and a specificity of 33.8%. Overall, the diagnostic performance of the amylase ratio was inferior to the BISAP score (AUC = 0.83 ± 0.03) (Figure 3). When amylase ratio ≥0.3 was counted as 1 point and added to the BISAP score to build a new model named the BISAPA (BISAP plus Amylase ratio) score (AUC = 0.86 ± 0.02), it significantly improved diagnostic power compared to the original BISAP score (Figure 3). With a cut-off value of 3, the BISAPA score achieved a sensitivity of 66.0%, a specificity of 86.7%, and diagnostic accuracy of 84.48%.

Discussion

Although amylase is useful in the diagnosis of pancreatitis, it correlates poorly with the severity of the illness (Yadav et al., 2002). As a result, the level of elevation of amylase is not included in the major tools used to assess the severity of illness, such as Ranson's criteria and BISAP score. The American College of Gastroenterology guidelines states that daily measurement of amylase after the initial diagnosis has limited value in assessing the clinical progress of the illness or ultimate prognosis (Tenner et al., 2013). However, recently, Kumaravel et al. (2015) suggested that the percentage change in amylase from admission to day 2b (which was calculated as ([amylase day 1 − amylase day2]/amylase day 1)) was associated with the severity of AP (Kumaravel et al., 2015). However, serum amylase may increase or decrease during the first 2 days after admission. Therefore, the amylase ratio, in clinical practice, which was calculated as (amylase day 2/amylase day 1), might be more suitable than the percentage change in amylase as a potential predictor of SAP.

Our study suggested that patients with a high amylase day 2/day 1 ratio (≥0.3) had a higher incidence of SAP than patients with a low amylase day 2/day 1 ratio (<0.3) (14.4% vs. 2.8%, P <0.001) (Figure 2). These results indicate that 97.2% of patients would not develop SAP if their amylase on day 2 decreased by 70% compared to amylase on day 1. However, 14.4% of patients would develop SAP if their amylase on day 2 either decreased by less than 70% compared to day 1 or increased. Multivariable

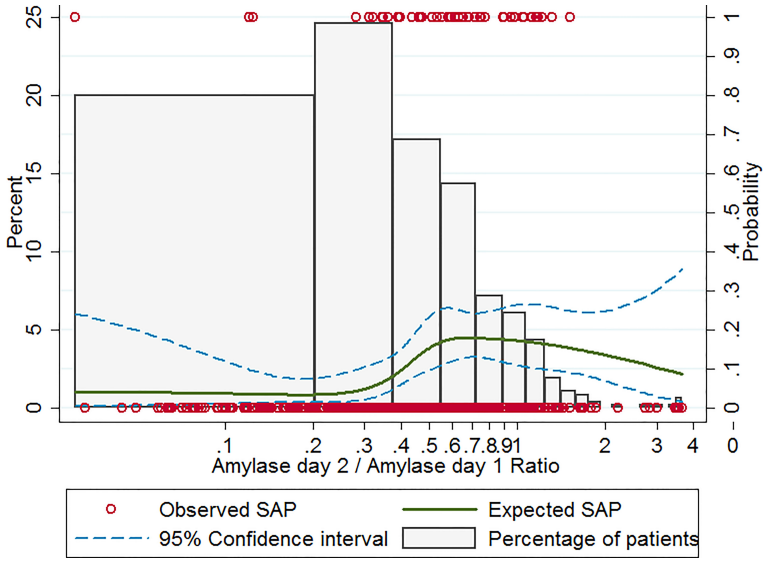

FIGURE 1
Observed and expected incidence of SAP in patients with different amylase ratio levels. Histograms show the percent distribution of study patients at different amylase ratio levels (the left-hand y-axis). The red circles give the observed incidence of SAP for each patient (the right-hand y-axis). The solid curve gives the expected incidence of SAP based on restricted cubic spline analysis (the right-hand y-axis). The dashed curves represent the 95% confidence intervals for the expected incidence of SAP (the right-hand y-axis). SAP, severe acute pancreatitis.

logistic regression indicated that a high amylase ratio (≥0.3) (OR: 6.62; 95% CI: 2.27–19.36; P = 0.001) was independently associated with the development of SAP after adjusting age, gender, BMI, biliary etiology, and time from pain onset to admission.

Based on ROC analysis, the AUC for the amylase ratio for the prediction of SAP was 0.65 (95% CI: 0.58–0.73) (Figure 3), which means that a serum amylase day 2/amylase day 1 ratio had moderate diagnostic accuracy for the prediction of SAP. With a cut-off of 0.3, the serum amylase day 2/amylase day 1 ratio

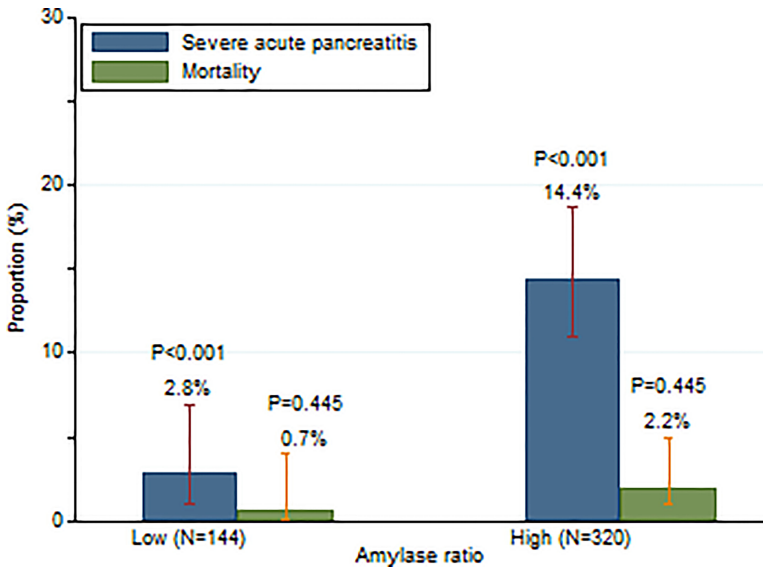

FIGURE 2
Incidence of SAP and mortality in patients with high and low serum amylase day 2/amylase day 1 ratios.

achieved a sensitivity of 92.0% and specificity of 33.8%. Therefore, patients with a serum amylase day 2/amylase day 1 ratio of >0.3 should be transferred to an intensive care unit setting. The advantages of amylase ratio estimation are its technical simplicity, easy availability, and high sensitivity. However, its disadvantage is its low specificity with this cut-off value. The diagnostic performance of the amylase ratio for the prediction of SAP was significantly inferior to the BISAP score (AUC: 0.65 vs.0.83; P <0.001) (Figure 3). Therefore, the serum amylase day 2/amylase day 1 ratio may be used as an additional supplement tool but not a substitute when compared to BISAP score. When amylase ratio ≥0.3 was counted as 1 point and added to the BISAP score to build a new model named the BISAPA score (AUC = 0.86), it significantly improved diagnostic power compared to the original BISAP score (AUC = 0.83) (Figure 3). With a cut-off value of 3, the BISAPA score achieved a sensitivity of 66.0%, specificity of 86.7%, and diagnostic accuracy of 84.48%.

One of the novelties of our study is that we fully investigated the relationship between amylase day 2/amylase day 1 ratio and SAP and determined the best cut-off value of serum amylase day 2/amylase day 1 ratio for the prediction of SAP by using a nonlinear model (restricted cubic spline analysis) (Figure 1). The restricted cubic spline model can transform an independent continuous variable and analyze the nonlinear effects of an independent variable on disease severity (Desquilbet and Mariotti, 2010). It generally provides a better fit to the data and also has the effect of reducing the degrees of freedom (Desquilbet and Mariotti, 2010). The other novelty is that we developed a BISAPA score, which incorporated both the serum amylase day 2/amylase day 1 ratio and the BISAP score. It could help us better stratify the severity of AP and predict outcomes in AP, as well as achieve high diagnostic accuracy. Early staging of SAP is critical to enable adequate triage of these patients to the intensive care unit and a timely treatment plan (Dar et al., 2021). Recently, Choi et al. proposed that serum phosphate level after ERCP can be used as a reliable prognostic marker in predicting the severity of post-ERCP pancreatitis (Choi et al., 2018). Agarwala et al. identified that gastrointestinal failure is an independent predictor of mortality in patients with AP (Agarwala et al., 2020). Trikudanathan et al. suggested that decreased skeletal muscle density was independently associated with in-hospital mortality in necrotizing pancreatitis patients and can be usefully incorporated into computed tomography-based predictive scoring models as a prognostic marker (Trikudanathan et al., 2021). Liu et al. suggested that the volume and mean computed tomography density of necrosis based on contrast-enhanced computed tomography can provide early prediction of organ failure and the need for intervention in patients with acute necrotizing pancreatitis (Liu et al., 2021). It will be necessary and interesting to compare the performance of our BISAPA score with the aforesaid indexes in predicting the disease severity of AP in the future.

Nevertheless, our study has some limitations. First, the important limitation is that serum lipase on day 1 and day 2 was not tested in most patients in our study. Therefore, we did not investigate the relationship between SAP and lipase. As a result, our results may not be generalized to some countries, such

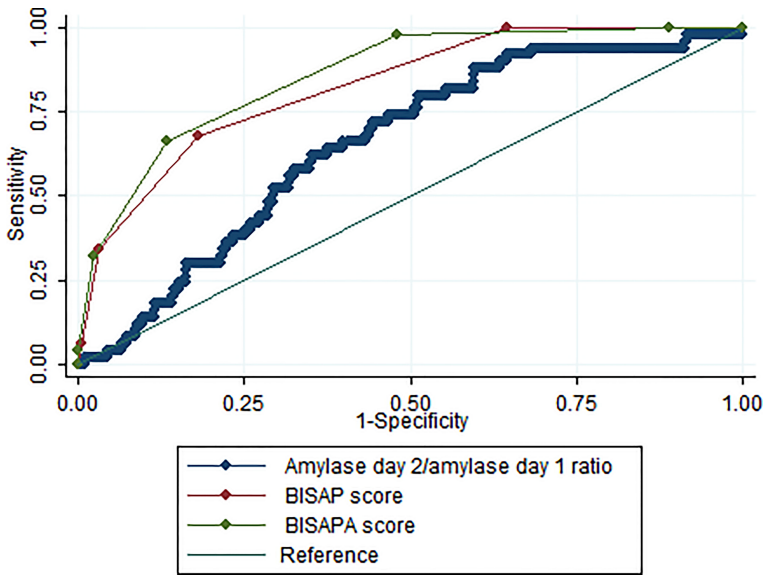

FIGURE 3
The ROC curves for serum amylase day 2/amylase day 1 ratio, BISAP score, and BISAPA score for SAP prediction. BISAP, bedside index of severity in AP; SAP, severe acute pancreatitis; BISAPA, bedside index of severity in acute pancreatitis and amylase day 2/amylase day 1 ratio.

as Germany, where amylase measurements have largely been substituted for lipase measurements. However, according to the current international consensus (Banks et al., 2013), an increase in amylase activity is still one of the primary criteria for the diagnosis of AP. Amylase testing other than lipase is still routinely performed at many healthcare institutions in clinical practice in most countries (such as mainland China in the world), although lipase is believed to offer superior sensitivity and specificity compared to amylase for the diagnosis of AP (Hong et al., 2017a). In addition, Chase et al. suggested that checking for lipase and amylase simultaneously does not result in improved diagnostic accuracy because amylase and lipase are closely correlated (Chase et al., 1996). Second, not all patients had records of C-reactive protein, serum lipase, and disease severity scores such as Acute Physiology and Chronic Health Evaluation (APACHE) II due to the retrospective study design. This made it difficult for us to further analyze the relationship between the amylase day 2 to day 1 ratio and these indexes. It will be necessary and interesting to evaluate these correlations in the future. Third, in AP, the levels of amylase usually peak at 48 h. Over the period of the next 5–7 days after onset, amylase levels typically tend to normalize (Hong et al., 2017a). Only patients who were admitted to the hospital within 72 h of the onset of symptoms were enrolled in our study. Therefore, our results may not be generalized to patients with a delay in time from pain onset to admission. Additionally, previous studies suggest that hospital volume influences the clinical outcome in both patients with mild and those with SAP (Singla et al., 2009; Murata et al., 2011). In our study, there were very few patients referred from other hospitals. Therefore, the results from our hospital may not be generalized to other hospitals with a high proportion of interhospital transfers. Last, similar to the change in amylase and body mass index (CAB) score (Kumaravel et al., 2015; Zheng et al., 2019), the new Japanese severity score (Ueda et al., 2009) and the modified Glasgow score (Mounzer et al., 2012), our amylase day 2 to day 1 ratio also requires 48 h to complete, which means it cannot be used at bedside as a predictor of SAP within 24 h of admission in clinical practice. However, AP is a dynamic and evolving process that involves multiple systems and the risk of organ complications (Papachristou et al., 2010). Therefore, the amylase day 2 to day 1 ratio could still be useful on day 2 after admission.

Conclusion

In conclusion, there is a non-linear correlation between the amylase day 2/amylase day 1 ratio and SAP incidence. The serum amylase day 2/amylase day 1 ratio represents an additional tool that is easy to perform and could serve as a cheap marker to stratify patients at risk of SAP without any need for complex calculations. When the amylase ratio of ≥0.3 was counted as 1 point and added to the BISAP score to build a new model named the BISAPA score, it significantly improved diagnostic power compared to the original BISAP score.

Data availability statement

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

Ethics statement

This study protocol was approved by the Ethics Committee of our hospital (date: 2016-10-18; number: 2016-211). The patients/participants provided their written informed consent to participate in this study.

Author contributions

WH conceived the study and carried out majority of the work. WH and LZ participated in data collection and conducted data analysis. WH, YL, and MQ drafted and revised the manuscript. YY, ZB, MZ, VZ, and WG helped to finalize the manuscript. All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

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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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Impact of multiple drug-resistant Gram-negative bacterial bacteraemia on infected pancreatic necrosis patients

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Introduction: Multiple drug-resistant Gram-negative bacterial (MDR-GNB) bacteraemia poses a serious threat to patients in hospital. Infected pancreatic necrosis (IPN) patients are a vulnerable population to infectious complications during hospitalization. This study aims to evaluate the impact of MDR Gram-negative bacteraemia on IPN patients.

Methods: A case–control study was performed with data collected from 1 January 2016 to 1 July 2022 in a Chinese tertiary teaching hospital. Clinical data of the IPN patients with MDR-GNB bacteraemia were analyzed and compared to those of a matched control group without MDR-GNB bacteraemia (case–control ratio of 1:2). Comparisons were performed between with/without MDR-GNB bacteraemia and different severities of acute pancreatitis (AP). Independent predictors of overall mortality were identified *via* univariate and multivariate binary logistic regression analyses.

Results: MDR-GNB bacteraemia was related to a higher mortality rate (62.5% vs. 8.3%, $p < 0.001$). Severe AP combined with MDR-GNB bacteraemia further increased mortality up to 81.3% ($p = 0.025$). MDR-GNB bacteraemia (odds ratio (OR) = 8.976, 95% confidence interval (CI) = 1.805 –44.620, $p = 0.007$) and severe AP (OR = 9.414, 95% CI = 1.742 –50.873, $p = 0.009$) were independent predictors of overall mortality. MDR- *Klebsiella pneumoniae* was the most common causative pathogen.

Conclusion: A higher mortality rate in IPN patients was related to MDR-GNB bacteraemia and further increased in severe AP patients combined with MDR-GNB bacteraemia.

KEYWORDS

multiple drug-resistant, Gram-negative bacterial bacteraemia, infected pancreatic necrosis, acute pancreatitis, mortality

Introduction

Acute pancreatitis (AP) is an unpredictable and potentially lethal disease of the gastrointestinal system with increasing occurrence within the past decade (Boxhoorn et al., 2020; Szatmary et al., 2022). Approximately 30% of AP patients will develop severe acute pancreatitis (SAP) and infected pancreatic necrosis (IPN), which are respectively the major causes for the first and second peaks of mortality in AP (Brown et al., 2014; Werge et al., 2016; Baron et al., 2020).

Pancreatic necrosis is sterile in the early disease stages, but as many as 30% of cases will develop into IPN as the disease progresses (Garret et al., 2020). Gram-negative bacteria account for more than half of all bacterial infections in hospitalized patients, including AP patients. Specifically, moderately SAP and SAP patients are the most susceptible populations to multiple drug-resistant Gram-negative bacterial (MDR-GNB) infections due to prolonged hospitalization and frequent use of invasive interventions and antibiotics (Diekema et al., 2019; Ning et al., 2021b). The overuse of antibiotics for AP patients in China, which ranks first in the world over the most recent decade, may be the main reason for MDR-GNB infections (Parniczky et al., 2019).

The emergence of MDR-GNB infections with increasing prevalence merits more attention because limited antibiotic treatment choices for MDR-GNB strains result in a greater potential of developing severe sepsis (Wolbrink et al., 2020). The impact of MDR-GNB infectious complications on the outcomes of AP patients is not consistent between different centers in other countries (Jain et al., 2018; Shi et al., 2020). Some studies have highlighted an increase in the risk of MDR bacterial infections among AP patients, which aimed to differentiate between MDR and non-MDR bacterial infections but failed to investigate the role of MDR-GNB bacteraemia (Moka et al., 2018; Ning et al., 2019; Garret et al., 2020; Li et al., 2020). Other studies have revealed that organ failure (OF) and IPN may be two independent risk factors for a poor AP prognosis, but IPN has been regarded as less important than organ failure (Shi et al., 2020; Windsor and de-Madaria, 2021). An infectious complication of AP cannot be arbitrarily defined as IPN, and it also needs to include bacteraemia or pulmonary infection (Wu et al., 2022a). Moreover, whilst it is often overlooked, bacteraemia, especially MDR-GNB bacteraemia, may pose a serious threat to IPN patients (Ning et al., 2021a). MDR-GNB bacteraemia mortalities have been reported in several studies of hospital- onset infections, but they still have not been fully addressed in IPN patients (Tsai et al., 2014). In addition, given clear MDR-GNB differences and the many variables associated with adverse outcomes, it may be more useful to investigate the mortality rates in IPN patients with MDR-GNB bacteraemia.

Currently, no report exists that focuses on the MDR-GNB bacteraemia in IPN patients, which suggests that large gaps remain in this area. The purpose of the present study was as follows: 1) to investigate the role of MDR-GNB bacteraemia in IPN patients, 2) to determine the relationship between AP severity and MDR-GNB bacteraemia, 3) to identify the independent predictors of mortality among IPN patients, and 4) to describe the distribution of MDR-GNB strains in IPN patients. Our findings may shed light on the improvement of treatment and prognosis among this population.

Methods

Study design, setting, and ethics

This retrospective study was performed at Xiangya Hospital, a 3,500-bed tertiary-care teaching hospital, affiliated with Central South University, Changsha, Hunan, China. Data from the electronic patient record system of IPN patients with and without MDR-GNB bacteraemia, from 1 February 2016 to 1 July 2022, were collected. A case–control study with a case–control ratio of 1:2 was performed. The control group was age (± 2) and gender- matched MDR-GNB IPN patients without MDR-GNB bacteraemia. IPN patients with missing data or without positive MDR-GNB culture at the pancreas (Figure 1) were excluded. The clinical characteristics included severity, aetiology, age, sex, referral timing from the onset of AP, length of hospitalization including also intensive care unit (ICU) stay, bedside index of severity in acute pancreatitis (BISAP) at admission, major complications, mortality, and laboratory variables at the onset of MDR-GNB bacteraemia. The role of both severity and MDR-

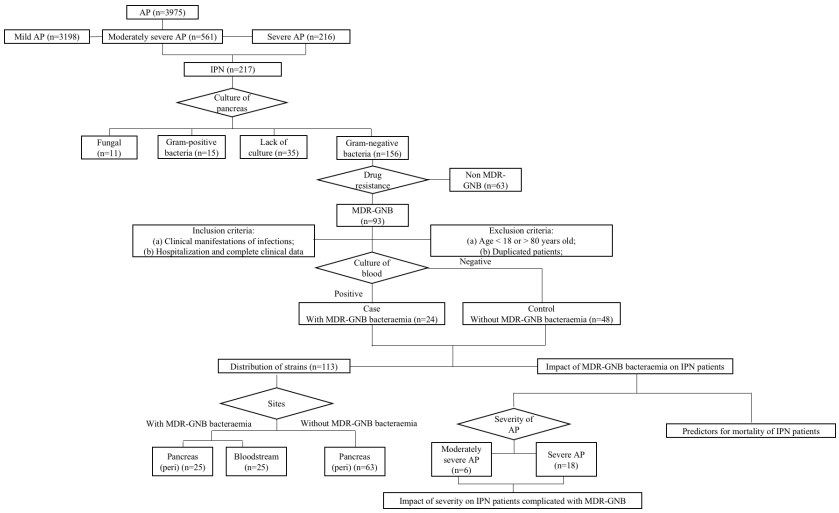

FIGURE 1
Flow chart of the study.

GNB bacteraemia among IPN patients, predictors of overall mortality, and distribution of causative pathogens were investigated. This study was approved by the Institutional Review Board of Xiangya Hospital (No. 202103047). Informed consent was waived due to the study's retrospective nature, and confidentiality was assured throughout the study.

Definitions

The presence of bacteraemia was diagnosed based on the clinical manifestations and positive results of specimens according to the criteria of the Centers for Disease Control (CDC) (Horan et al., 2008; Wu et al., 2021a). The onset of MDR-GNB bacteraemia was defined as the collection date of the first positive culture for blood, which was divided into three time periods including pre-intervention and post-intervention (≤5 and >5 days, respectively) (Carey et al., 2011). The diagnosis, severity, and classification of AP followed the criteria of the Revised Atlanta Classification: 1) mild AP: the absence of either OF or local/systemic complications; 2) moderately SAP: the presence of transient OF (less than 48 h) and/or local or systemic complications; 3) SAP: the presence of persistent OF (more than 48 h) (Banks et al., 2013). OF was defined for the three organ systems (respiratory, cardiovascular, and renal) according to the modified Marshall Score (Marshall et al., 1995). IPN was defined as peri-pancreatic specimens obtained with positive MDR-GNB culture during the first intervention of pancreatic necrosis. The criteria of AP aetiology were as follows: 1) biliary: radiological evidence of abdominal ultrasonography with increased serum alanine aminotransferase; 2) alcoholism: regular drink over 50 g/day; 3) hypertriglyceridemia: triglycerides of more than 5.6 mmol/L without any other clear aetiology. Gram-positive bacterial or fungal infections were defined as the presence of concomitant infections (Wu et al., 2022b). MDR- GNB is defined as strains not sensitive to three or more categories of antibiotics (Wu et al., 2021b). Combination antibiotic therapy was defined as a regimen with at least two categories of antibiotics based on the condition of patients and approved by infectious disease specialists.

Patients, management, and microbiology

All patients were assessed and managed at admission according to the latest international guidelines *via* the multi-disciplinary team (Crockett et al., 2018). The antibiotic regimen we recorded was the initial therapy (3–5 days) after obtaining the culture results, which could potentially change or be combined with new antibiotics, dependent on whether the patients' condition worsened. If there was an AP patient with suspected bacteraemia, we would perform a blood culture in accordance with the standard protocols from the CDC (Horan et al., 2008). A conventional blood culture set consists of aerobic and anaerobic bottles. A collection of 20–30 ml of blood requires more than two bottles and is obtained within a few hours of each other *via* peripheral venipuncture when obtaining blood cultures for a total volume of 40–60 ml of blood to optimize the detection of pathogens (Doern et al., 2019). Identification and drug-resistant test of MDR-GNB were performed *via* the Vitek-2 system and broth micro-dilution method, respectively (Wu et al., 2021a).

Statistical analysis

The continuous variables expressed using medians with standard deviations were compared with Student's t- test, Welch's t- test, Mann–Whitney U test, or Fisher's exact test, as appropriate. Categorical variables described in absolute numbers and percentages were compared with the χ^2 test or Fisher's exact tests. The binary logistic regression analysis was used to determine independent predictors of mortality *via* univariate and multivariate analyses. The odds ratio (OR) and 95% confidence interval (CI) were calculated to evaluate the associations. p-Value < 0.05 (two-tailed) was considered statistically significant. Statistical analyses were performed using SPSS 26.0.

Results

Clinical characteristics and outcome of infected pancreatic necrosis patients

As seen in Table 1, 56 patients (77.8%) were male with a mean age of 46.8 ± 11.4 years. Hypertriglyceridemia (n = 32, 44.4%) was the leading aetiology of total patients; however, biliary source is significantly higher in the MDR-GNB bacteraemia group when compared to the contrast group (45.8% vs. 18.8%, p = 0.027). The MDR-GNB bacteraemia group showed significantly longer ICU stays (21.6 days vs. 4.1 days, p < 0.001) and a higher BISAP score at admission (3.0 vs. 2.1, p = 0.001) than the contrast group. SAP (66.7% vs. 25.0%, p < 0.001) more frequently occurred in the MDR-GNB bacteraemia group. The mortality rate was significantly higher among patients with MDR-GNB bacteraemia than those without MDR-GNB bacteraemia infections (62.5% vs. 8.3%, p < 0.001).

Comparison between moderately severe acute pancreatitis and severe acute pancreatitis among multiple drug-resistant Gram-negative bacterial bacteraemia group

In Table 2, MDR-GNB bacteraemia was observed in eight moderately SAP and 16 SAP patients. SAP patients showed a significantly higher procalcitonin level when compared to the contrast group (35.5 vs. 5.1 ng/L, p = 0.020). Inappropriate empirical therapy was not significantly different between the moderately SAP and SAP groups (37.5% vs. 31.3%, p = 0.761). The mortality rate was significantly higher in the SAP group when compared with the moderately SAP group (81.3% vs. 25.0%, p = 0.025). There were three and nine patients diagnosed with bacteraemia during the post-intervention (≤5 days) for IPN with no significant difference in the moderately SAP and SAP groups, respectively (p = 0.096).

Predictors of mortality

The association of the variables with mortality *via* the univariate and multivariate analyses is shown in Table 3. The univariate analysis identified the variables, including BISAP ≥ 3, SAP, MDR-GNB bacteraemia, and major complications, as predictors of mortality. MDR-GNB bacteraemia (OR = 8.976, 95% CI = 1.805 –44.620, p = 0.007) and SAP (OR = 9.414, 95% CI = 1.742 –50.873, p = 0.009) were both statistically significant in the multivariate logistic regression analysis.

Distribution of multiple drug-resistant Gram-negative bacterial strains

In Figure 2, all 113 respective bacterial strains in 72 IPN patients were divided into with and without bacteraemia groups (a total of 50 and 63, respectively). In the pancreas, *Klebsiella pneumoniae* is the most common species in both with (11 of 25) and without MDR-GNB bacteraemia (18 of 63) followed by *Acinetobacter baumannii* (5 of 25 and 15 of 63, respectively) and *Escherichia coli* (3 of 25 and 14 of 63, respectively). In the blood, *K. pneumoniae* is also the primary bacterium (15 of 25).

Discussion

The emergence of MDR-GNB is increasingly becoming a major and global public health issue with rising antibiotic resistance and increased mortality (Mills and Marchaim, 2021). The incidence of bacteraemia due to MDR-GNB is higher than that of other infections (Diekema et al., 2019). Uncontrollable bacteraemia, secondary to AP, may play a key role in this prognosis. This is the first case–control study that has focused on the impact of MDR-GNB bacteraemia on IPN patients, and the mortality of IPN in this study was up to 26.4% in accordance with previous studies, which highlighted that MDR-GNB has become a serious pathogen of infections among AP patients (Jain et al., 2018; Firsova et al., 2020).

We found that MDR-GNB bacteraemia resulted in higher mortality and longer ICU stays and was related to higher BISAP scores at admission, SAP, and biliary AP. In line with Li et al., hyperlipidaemia was the primary aetiology in the IPN patients, but it was interesting that the biliary aetiology was more frequent in patients within the MDR-GNB bacteraemia group than the contrast group, thus suggesting that MDR-GNB bacteraemia could be secondary to bacterial translocation through the gut (Flint and Windsor, 2003; Li et al., 2020; Yang and McNabb-Baltar, 2020). In addition, these invasive treatments may result in

TABLE 1 Clinical characteristics and comparison between IPN patients with and without MDR-GNB bacteraemia.

Characteristics	Total	Control, without MDR-GNB bacteraemia (n = 48)	Case, with MDR-GNB bacteraemia (n = 24)	*p*-Value
Age, years (mean ± SD)	46.8 ± 11.4	45.8 ± 10.9	49.0 ± 12.4	0.253
Sex, n (%)				0.841
Male	56 (77.8)	37 (77.1)	19 (79.2)	
Female	16 (22.2)	11 (22.9)	5 (20.8)	
Classification of AP, n (%)				<0.001*
Moderately SAP	44 (61.1)	36 (75.0)	8 (33.3)	
SAP	28 (38.9)	12 (25.0)	16 (66.7)	
Aetiology, n (%)				0.027*
Hypertriglyceridemia	32 (44.4)	21 (43.8)	11 (45.8)	
Biliary	20 (27.8)	9 (18.8)	11 (45.8)	
Alcoholism	4 (5.6)	4 (8.3)	0	
Others	16 (22.2)	14 (29.2)	2 (8.2)	
Recurrent AP, n (%)	11 (15.3)	6 (12.5)	5 (20.8)	0.563
Concomitant infections, n (%)	18 (25.0)	11 (22.9)	7 (29.2)	0.564
Referred patient, n (%)	68 (94.4)	45 (93.8)	23 (95.8)	0.998
Intensive care units stay, days (mean ± SD)	9.9 ± 13.8	4.1 ± 4.8	21.6 ± 18.0	<0.001*
Hospitalization, days (mean ± SD)	40.8 ± 22.1	37.6 ± 19.3	47.1 ± 26.0	0.085
Intervention, n (%)				0.067
PCD	8 (11.1)	5 (10.4)	3 (12.5)	
PCD to minimal access retroperitoneal necrosectomy	56 (77.8)	41 (85.4)	15 (62.5)	
OPN	2 (2.8)	0	2 (8.3)	
Step-up to OPN	6 (8.3)	2 (4.2)	4 (16.7)	
BISAP score at admission (mean ± SD)	2.4 ± 1.0	2.1 ± 0.8	3.0 ± 1.1	0.001*
Details of BISAP score, n (%)				
Blood urea nitrogen >25 mg/dl	27 (37.5)	11 (22.9)	16 (66.7)	<0.001*
Impaired mental status	8 (11.1)	0	8 (33.3)	<0.001*
Age > 60	12 (16.7)	7 (14.6)	5 (20.8)	0.737
Pleural effusion present	65 (90.3)	44 (91.7)	21 (87.5)	0.769
SIRS criteria (>2)	60 (83.3)	38 (79.2)	22 (91.7)	0.314
Major complications, n (%)				
Hemorrhage	17 (23.6)	8 (16.7)	9 (37.5)	0.051
Intestinal leakage	10 (13.9)	3 (6.3)	6 (25.0)	0.059
Pancreatic fistula	11 (15.3)	5 (10.4)	6 (25.0)	0.203
Mortality	19 (26.4)	4 (8.3)	15 (62.5)	<0.001*

Note. SD, standard deviation; PCD, percutaneous catheter drainage; OPN, open necrosectomy; SIRS, systemic inflammatory response syndrome; MDR-GNB, multiple drug-resistant Gram-negative bacterial; IPN, infected pancreatic necrosis; AP, acute pancreatitis; SAP, severe acute pancreatitis; BISAP, bedside index of severity in acute pancreatitis.
*p-Values are statistically significant between with and without MDR-GNB bacteraemia groups.

bacteraemia, especially MDR-GNB bacteraemia (Wu et al., 2021b). In our study, there were also 12 of 24 patients diagnosed with bacteraemia during the post-intervention (≤5 days), which may be possibly related to translocation secondary to intervention and needs to be validated in the larger sample size study. According to Hagjer et al., a high BISAP score, similar to SAP, indicated more severe OF, which needed invasive treatments, such as ventilators, central venous catheter support, and hemodialysis (Hagjer and Kumar, 2018). However, bacteraemia, especially sepsis shock, could worsen OF and mimic SAP. In the subgroup comparison (moderately SAP vs. SAP) in the MDR-GNB bacteraemia, the mortality was significantly increased by the severity of AP, which was also accompanied by high procalcitonin in accordance with previous studies investigating markers of severe infections (Lin et al., 2017; Hong et al., 2019; Wu et al., 2021c; Wu et al., 2022b). It is worth

TABLE 2 Clinical characteristics and comparison between different categories among 24 IPN patients with MDR-GNB bacteraemia.

Characteristics	Moderately SAP (n = 8)	SAP (n = 16)	*p*-Value
Age, years (mean ± SD)	53.3 ± 13.9	46.9 ± 11.4	0.247
Sex, n (%)			1.000
Male	6 (75.0)	13 (81.2)	
Female	2 (25.0)	3 (18.8)	
Aetiology, n (%)			0.113
Hypertriglyceridemia	6 (75.0)	5 (31.3)	
Biliary	2 (25.0)	9 (56.2)	
Others	0	2 (12.5)	
Concomitant infections, n (%)	3 (37.5)	6 (37.5)	1.000
Onset of bacteraemia, n (%)			0.096
Pre-intervention	0	3 (18.8)	
Post-intervention (≤5 days)	3 (37.5)	9 (56.2)	
Post-intervention (> 5 days)	5 (62.5)	4 (25.0)	
Intervention, n (%)			0.789
PCD	1 (12.5)	2 (12.5)	
PCD—minimal access retroperitoneal necrosectomy	4 (50.0)	11 (68.8)	
OPN	1 (12.5)	1 (6.2)	
Step-up to OPN	2 (25.0)	2 (12.5)	
Laboratory and clinical variables (mean ± SD)			
Albumin, g/L	29.8 ± 3.2	30.6 ± 4.0	0.631
Neutrophil count, $10^3/mm^3$	7.2 ± 4.6	12.3 ± 9.1	0.152
Lymphocyte count, $10^3/mm^3$	0.8 ± 0.7	1.0 ± 1.6	0.807
Procalcitonin, ng/L	5.1 ± 5.2	35.5 ± 46.2	0.020*
Highest temperature, °C	39.2 ± 0.7	39.0 ± 1.0	0.690
Inappropriate empirical therapy	3 (37.5)	5 (31.3)	0.761
Antibiotic therapy, n (%)			0.885
Monotherapy	5 (62.5)	8 (50.0)	
Polytherapy	3 (37.5)	8 (50.0)	
Mortality, n (%)	2 (25.0)	13 (81.3)	0.025*

IPN, infected pancreatic necrosis; MDR-GNB, multiple drug-resistant Gram-negative bacterial; SAP, severe acute pancreatitis; PCD, percutaneous catheter drainage; OPN, open necrosectomy.
*p-Values are statistically significant between moderately SAP and SAP groups among MDR-GNB bacteraemia patients.

noting that MDR-GNB bacteraemia had a mortality rate of 62.5% resultant from untimely detection and inappropriate antibiotic treatment, which has also been revealed to be associated with a greater risk of hospital mortality among critically ill patients with bacteraemia (Diekema et al., 2019). We revealed that both MDR-GNB bacteraemia and SAP were independent predictors of overall mortality in multivariate analysis, which suggested that bacteraemia plays a key role in the severity of AP.

TABLE 3 Univariate and multivariate analyses of predictors associated with mortality in 72 IPN patients.

Variable, n (%)	Survival (n = 53)	Mortality (n = 19)	Univariate analysis		Multivariate analysis	
			OR (95% CI)	*p*	OR (95% CI)	*p*
BISAP ≥ 3	15 (28.3)	14 (73.7)	7.093 (2.173–23.157)	0.001*	1.779 (0.337–9.389)	0.497
Male	43 (81.1)	13 (68.4)	0.504 (0.154–1.651)	0.504		
SAP	12 (22.6)	16 (84.2)	18.222 (4.535–73.220)	<0.001*	9.414 (1.742–50.873)	0.009*
MDR-GNB bacteraemia	9 (17.0)	15 (78.9)	18.333 (4.919–68.223)	<0.001*	8.976 (1.805–44.620)	0.007*
Major complications	18 (34.0)	13 (68.4)	4.213 (1.372–12.938)	0.012*	1.736 (0.360–8.375)	0.492
Concomitant infections	11 (20.8)	7 (36.8)	2.227 (0.709–6.995)	0.170		

Note. IPN, infected pancreatic necrosis; MDR-GNB, multiple drug-resistant Gram-negative bacterial; BISAP, bedside index of severity in acute pancreatitis; SAP, severe acute pancreatitis.
*P values are statistically significant.

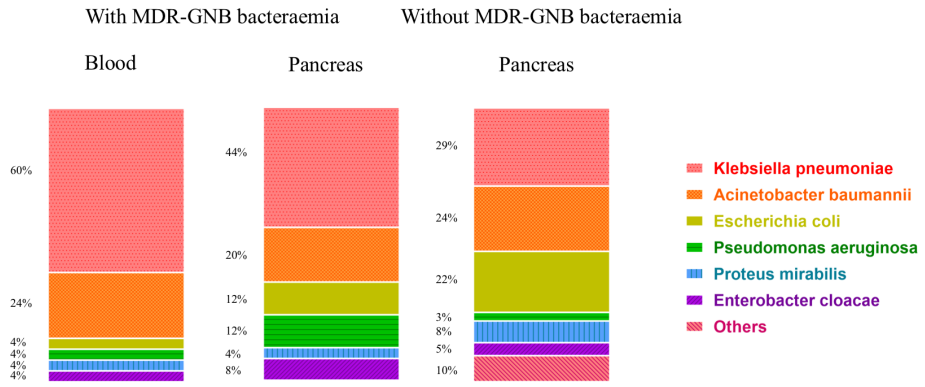

FIGURE 2
Distribution of 113 MDR-GNB strains among 72 IPN patients with and without bacteraemia. MDR-GNB, multiple drug-resistant Gram-negative bacterial; IPN, infected pancreatic necrosis.

For the distribution of pathogens, in accordance with previous studies, MDR-*K. pneumoniae* was revealed to be the most common pathogen of IPN, which enhances the theory of colonizing bacterial translocation (Tugal et al., 2015; Wu et al., 2021c). Interestingly, MDR-*K. pneumoniae* was also the primary species in the blood. However, this must be further validated in order to support the hypothesis that bacteria, released from invasive intervention and gut, may enter the bloodstream. The timing of MDR-GNB bacteraemia was not as in other studies, which suggests that extra-pancreatic infections develop before IPN (Lu et al., 2019; Garret et al., 2020). This may possibly be due to the delayed intervention in addition to a step-up strategy for IPN and untimely bacteraemia diagnosis (Boxhoorn et al., 2021). Routine bacterial culture, which relies on the number of bacteria in the specimen, was not sensitive enough in establishing the microbiological bacteraemia diagnosis without a specific clinical presentation different to the severity of AP itself (Carey et al., 2011). Novel molecular and phenotypic rapid tests for identification and antimicrobial susceptibility testing are recommended as one of the best choices for MDR-GNB bacteraemia, which reduce both the time of treatment and the misuse of antibiotics (Giacobbe et al., 2020). The problem of overuse of broad-spectrum antibiotics, especially carbapenem or third- generation cephalosporin β-lactams, has not been solved in line with the latest guideline (Baron et al., 2020). MDR-GNB screening, especially MDR or carbapenem-resistant *K. pneumoniae*, at ICU admission is considered an effective approach for the surveillance of MDR-GNB colonization in the high-incidence area, guiding clinicians to prescribe appropriate antibiotics in AP patients complicated with "suspected" bacteraemia when blood culture results are pending. In this era with the MDR bacteria, we must pay attention to protecting organ function and reducing invasive treatment or ICU stays instead of creating or overusing "new" antibiotics. Prevention is more important than treatments for MDR-GNB bacteraemia among AP patients.

Although many advances in understanding the role of MDR-GNB bacteraemia in AP patients have taken place in this study, there are also some limitations. Firstly, our study is limited to deficient variables and potential selection biases by its retrospective case–control nature. We performed the latest precise definition and criteria to decrease the suboptimal control selection biases, which may result in only a very small reduction in power. Secondly, the limited sample size of the case group reduced the power of risk analyses, as indicated by the wide range of confidence intervals, which is a common problem in studies identifying risk factors for mortality due to MDR organisms. Thirdly, the surgical aspects of IPN, including locations and subsequent choices of interventions, were not discussed due to the specific focus on the impact of bacteraemia. Fourthly, the percentage of SAP was significantly higher in the MDR-GNB bacteraemia group. The OF caused by both bacteraemia and the severity of AP could not be ignored, and SAP patients could suffer from more exposure to antibiotics and ICU stays, which would possibly result in MDR-GNB bacteraemia. There may be a mutual cause-and- effect relationship between the severity of AP and MDR-GNB bacteraemia, which may be hard to correct in multivariable analysis. Thus, we could only analyze the overall mortality, instead of infection- related mortality, which needs to be further investigated in the future. Finally, we chose the IPN patients without MDR-GNB bacteraemia as the control group and used concomitant infections as a variable, so our study could only assess the hazard of MDR-GNB bacteraemia on the IPN patients instead of patients who suffered from other infections. This effect needs to be further investigated.

Conclusion

Higher mortality and the possibility of SAP were observed in patients with MDR-GNB bacteraemia, and further efforts to prevent MDR-GNB bacteraemia are urgently needed. SAP combined with MDR-GNB bacteraemia could significantly increase mortality. SAP and MDR-GNB bacteraemia were two independent predictors of mortality in IPN patients. MDR- *K. pneumoniae* was the most frequent pathogen in both blood and pancreas, and this merits further attention in future studies.

Data availability statement

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

Ethics statement

The studies involving human participants were reviewed and approved by Institutional Review Board of Xiangya Hospital. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements. Written informed consent was not obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

Author contributions

DW and YJ contributed equally to this article. DW and YJ participated in the design of the study and drafted the manuscript. WC, YH, AK, DL, and RS participated in data collection and analysis. JP conceived of the study, and participated in design and coordination and helped to draft the manuscript. All authors contributed to the article and approved the submitted version.

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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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Elevated hypertriglyceridemia and decreased gallstones in the etiological composition ratio of acute pancreatitis as affected by seasons and festivals: A two-center real-world study from China

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Objective: The purpose of this study was to observe the annual variation in the etiology of acute pancreatitis (AP) and its relationship with seasons and festivals.

Methods: From 2011 to 2017, 5146 adult patients with AP were studied, including 4110 patients from the First Affiliated Hospital of Nanchang University (South center) and 1036 patients from the First Affiliated Hospital of Harbin Medical University (North center). We analyzed the overall annual variation in the etiology of AP and then compared the differences in etiology between the two regions, as well as the effects of seasons and festivals on the etiology of AP.

Results: Gallstones, hypertriglyceridemia (HTG) and alcohol were the top three etiologies of AP. Gallstone AP showed a downward trend (P<0.001), and HTG-AP and alcohol AP showed an upward trend (both P<0.01). Among the etiologies of AP, gallstones and HTG were affected by seasons and festivals. The composition ratio of HTG-AP increased, while gallstone AP decreased in winter and in months with long holidays (all P<0.01). The composition ratio of gallstone AP in the south center was higher than that in the north center (59.5% vs. 49%), especially in summer (62.9% vs. 44.0%) and autumn (61.5% vs. 45.7%, all P<0.001).

Conclusions: The composition ratio of HTG-AP increased while gallstone AP decreased in the past 7 years, and they were affected by seasons and festivals.

KEYWORDS

acute pancreatitis, epidemiology, season, etiology, hypertriglyceridemia

Introduction

Acute pancreatitis (AP) is a common gastrointestinal disease that is associated with substantial suffering and morbidity (Forsmark et al., 2016). The incidence of AP has been increasing in recent years worldwide (Lindkvist et al., 2004; Spanier et al., 2013; Peery et al., 2015; Peery et al., 2019). In the past 10 years, the number of hospitalized patients with AP in the United States has increased by at least 20% (Peery et al., 2015; Peery et al., 2019). Migrating gallstones cause transient obstruction of the pancreatic duct, and exposure of the pancreas to biliary constituents represents the most common cause of AP (Forsmark et al., 2016). Alcohol is the second most common cause of AP and accounts for approximately 30% of cases in Western countries (Gullo et al., 2002; Yadav and Lowenfels, 2013; Forsmark et al., 2016); however, it only accounts for approximately 5% of all cases in China (Zhu et al., 2017; Wu et al., 2017). Hypertriglyceridemia (HTG) is a well-established cause of AP (Rawla et al., 2018), and it has become the second leading cause of AP in China (Zhu et al., 2017; Mukherjee et al., 2019). It is worth evaluating the common regional characteristics and variation in the main causes of AP in China.

We speculate that the seasonal climates and food cultures of different countries are the main reasons for the differences in etiology. Seasonality is a well-known factor in the epidemiology of many diseases, such as peptic ulcers (Bendahan et al., 1992) and inflammatory bowel disease (Koido et al., 2013). A study from Germany explored the connection between AP and seasonality and found that there was no seasonal pattern in AP occurrence (Lankisch et al., 1998). However, subsequent studies have shown that there are periodic changes in the onset of AP, that gallstone and alcohol AP significantly increase in spring in Italy (Gallerani et al., 2004) and that alcohol AP is highest in summer and autumn in Finland (Räty et al., 2003). The occurrence of alcoholic AP also increases significantly during holidays. (Räty et al., 2003; Roberts et al., 2013) A recent study from eastern China found that the prevalence of AP is significantly higher in spring and autumn, especially for gallstone and HTG-induced AP (HTG-AP) (Wu et al., 2017). However, China is a vast territory, and different regions (such as the north and south) have different climates and diets. Both the composition ratio and trend of the etiologies of AP in these places are still unclear. This real-world, dual-center study collected data from AP patients from two pancreatic disease centers to observe changes in the main causes of AP in China over the past 7 years, along with the relationship of AP with seasons and festivals.

Methods

Patients and locations

This dual-center, real-world retrospective cohort study was conducted at two pancreatic disease centers. The study was approved by the Medical Ethics Research Committee of the First Affiliated Hospital of Nanchang University (South center) and the Medical Ethics Research Committee of the First Affiliated Hospital of Harbin Medical University (North center). We selected AP patients with onset within 7 days from January 2011 to December 2017 from the AP databases of the two pancreatic disease centers. We excluded the following patients: 1) patients aged < 18 years, 2) patients transferred from another tertiary hospital, 3) patients with recurrent pancreatitis, 4) patients with an acute attack of chronic pancreatitis, and 5) patients with pancreatic cancer diagnosed after AP onset. The diagnosis and classification were based on the revised Atlanta classification. The diagnostic criteria for the various etiologies of AP are shown in S-Table 1.

Classification of seasons and festivals

In China, the four seasons are determined based on the combination of astronomical seasons and climate seasons: spring (March-May), summer (June–August), autumn (September–November), and winter (December–February). There are three statutory long holidays to celebrate festivals in China: the Spring Festival in February, the labor day in May and the National Day in October. People hold various banquets (such as wedding banquets, etc.), family reunions and dinners among friends in these three months.

Data collection and statistical analysis

Data on demographics, AP etiology, laboratory indicators, scoring systems, local and systemic complications, and clinical outcomes of patients admitted to the hospital from January 2011 to December 2017 were collected and analyzed. Demographic and baseline characteristics were analyzed using descriptive analysis. Qualitative variables were described using numbers and percentages. Quantitative variables are presented as the mean ± standard deviation (SD). The median and interquartile range (IQR) were reported if the distribution of the variable was not normal. A t test was performed for continuous variables when the data were normally distributed, and the Kruskal–Wallis test was performed when the data were not normally distributed. The X (Lindkvist et al., 2004) test was performed for categorical variables, and relative risk was calculated for dichotomous variables. A two-tailed $P < 0.05$ was considered to indicate statistical significance.

Results

Participant characteristics

During the 7-year study period, 5146 patients with AP were examined, with a median age of 49 years, and 57.2% were male.

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TABLE 1 Baseline Characteristics of the Patients.

Characteristic	Total 5146	South center n=4110	North center n=1036	P value
Age, median (IQR), y	49 (39-63)	50 (40-63)	46 (37-58)	<0.001
Male sex, no. (%)	2952 (57.2)	2293 (55.8)	659 (63.68)	<0.001
Smoking history, no. (%)	1119 (21.7)	764 (18.6)	355 (34.3)	<0.001
Drinking history, no. (%)	1124 (21.8)	723 (17.6)	401 (38.7)	<0.001
Coexisting condition, no. (%)				
Coronary heart disease, no. (%)	146 (2.8)	73 (1.8)	73 (7.0)	<0.001
Hypertension	891 (17.3)	700 (17.0)	191 (18.4)	0.29
COPD, no. (%)	49 (1.0)	47 (1.1)	2 (0.2)	0.17
Diabetes, no. (%)	498 (9.6)	360 (8.8)	138 (13.3)	0
Cirrhosis, no. (%)	21 (0.4)	17 (0.4)	4 (0.4)	0.87
Chronic renal failure, no. (%)	9 (0.2)	9 (0.2)	0 (0)	0.28
Cerebral infarction, no. (%)	92 (1.8)	46 (1.1)	46 (4.4)	<0.001
WBC (IQR),	12.0 (8.7-15.7)	12.0 (8.5-15.4)	12.3 (9.0,16.3)	<0.001
HCT (IQR),	40.0 (36.0,44.0)	40.0 (35.9,44.0)	41.0 (37.4,46.1)	<0.001
AMY (IQR),	251 (87,703)	279 (90,742)	184 (82,531)	<0.001
APACHEII (IQR),	6 (3,8)	6 (3,9)	5 (3,7)	<0.001
Disease severity				
MAP, no. (%)	1914 (37.1)	1485 (36.1)	429 (41.4)	0.01
MSAP, no. (%)	2382 (46.1)	1892 (46.0)	490 (47.3)	0.68
SAP, no. (%)	849 (16.4)	732 (17.8)	117 (11.3)	<0.001
Rate of ICU admission	1160 (22.5)	1045 (25.4)	115 (11.1)	<0.001
Duration of hospitalization (IQR),	8 (5,12)	8 (5,12)	9 (6,13)	0.001
Death, no. (%)	72 (1.4)	58 (1.4)	14 (1.4)	0.5

AMY, amylase; APACHE II, Acute Physiology and Chronic Health Evaluation II; COPD, Chronic obstructive pulmonary disease; HCT, Hematocrit; IQR, interquartile range; MAP, mild acute pancreatitis; MSAP, moderately severe acute pancreatitis; SAP, severe acute pancreatitis; WBC, White Blood Cell. The data collection time points for WBC, HCT, AMY, and APACHEII were within 24 hours of admission.

According to the revised Atlanta severity classification, the numbers of mild, moderate, and severe cases were 1914 (37.1%), 2382 (46.1%), and 849 (16.4%), respectively. The median length of hospital stay was 8 days, and the mortality rate was 1.4% (Table 1). Of the 5,146 patients, 4,110 were hospitalized in the South center, and 1,036 were hospitalized in the North center. Compared with patients in the South center, patients with AP in the North center were younger [median 46 years (IQR 37-58) vs. 50 years (IQR 40-63), P = 0.00] and tended to be male (63.68% vs. 55.8%, P = 0.00). More AP patients from the North center had a history of smoking (34.3% vs. 18.6%, P = 0.00) and drinking (38.7% vs. 17.6%, P = 0.00) than patients from the South center. In terms of coexisting conditions, more patients had coronary heart disease (7.0% vs. 1.8%, P = 0.00), diabetes (13.3% vs. 8.8%, P = 0.00) and cerebral infarction (4.4% vs. 1.1%, P = 0.00) in the North center than in the South center.

Etiology of AP

Table 2 shows that gallstones, HTG and alcohol were the top three etiologies of AP in both the north and south centers, and the total composition ratios of these causes were 57.4%, 23.9%, and 8.8%, respectively. Gallstone AP was higher, and idiopathic pancreatitis was lower in the south center than in the north center (59.5% vs. 49%, 6.1% vs. 14.2%, respectively, both P <0.001). In the south center, gallstone AP was more common in female and elderly patients, and HTG and alcohol AP were more common in male and young patients (all P<0.001, S-Table 2). In the North center, there was no sex difference in HTG-AP (P = 0.70, S-Table 2) and no age difference in alcohol AP (P = 0.09, S-Table 2).

Annual variation in major etiologies of AP

Figure 1 shows the trends of the top three etiologies of AP from 2011 to 2017. Gallstone AP showed a downward trend (P<0.001), but HTG-AP and alcohol AP showed an upward trend (P<0.001 and P = 0.006, respectively). We further compared the changes in etiology before and after 2015 and found that the composition ratio of gallstone AP in the South center decreased (60.2% vs. 55.1%, P<0.001, S-Table 3); HTG-AP increased (21.3% vs. 26.0%, P<0.001), but alcohol AP did not change significantly. The

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TABLE 2 Etiological composition of acute pancreatitis in South and North center.

Characteristic	Total n=5146	South center n=4110	North center n=1036	P value
Biliary, no. (%)	2952 (57.4)	2444 (59.5)	508 (49.0)	<0.001
Alcohol abuse, no. (%)	452 (8.8)	349 (8.5)	103 (9.9)	0.14
HTG, no. (%)	1231 (23.9)	989 (24.1)	242 (23.4)	0.65
Operative[#], no. (%)	17 (0.3)	8 (0.2)	9 (0.9)	0.03
PEP, no. (%)	22 (0.4)	20 (0.5)	2 (0.2)	0.15
Traumatic, no. (%)	12 (0.2)	10 (0.2)	2 (0.2)	0.55
Autoimmunity, no. (%)	7 (0.1)	1 (0.02)	6 (0.6)	<0.001
Parapapillary diverticulum of duodenum, no. (%)	17 (0.4)	17 (0.4)	0 (0)	0.02
Hypercalcaemia , no. (%)	16 (0.3)	2 (0.05)	14 (1.4)	<0.001
Drug-induced , no. (%)	15 (0.3)	15 (0.4)	0 (0)	0.03
Other etiologies*, no. (%)	7 (0.1)	4 (0.1)	3 (0.3)	0.13
Idiopathic , no. (%)	398 (7.7)	251 (6.1)	147 (14.2)	<0.001

HTG, Hypertriglyceridaemia; PEP, post-ERCP Pancreatitis. [#]It refers to the acute pancreatitis after surgery. *Other etiologies: pancreatic cancer, ampullary cancer, papillary sphincter dysfunction, pancreatic Division.

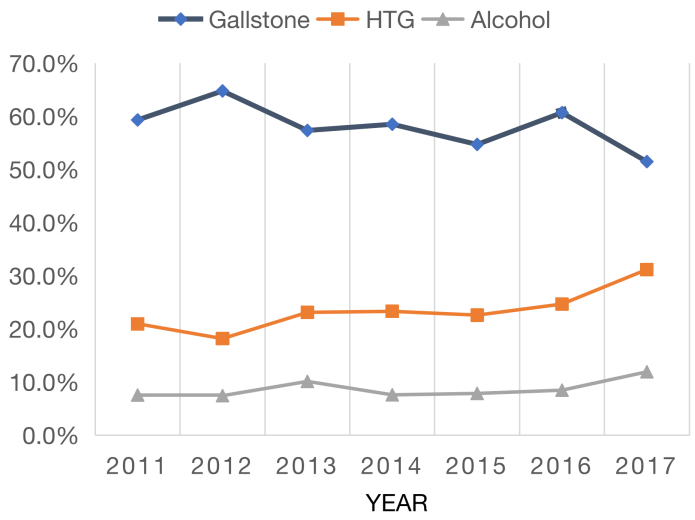

FIGURE 1
Annual variation in major etiologies of acute pancreatitis.

changes in the abovementioned etiological composition in the North center were consistent with those in the South center.

Seasonal variation in major etiologies of AP

Figure 2 shows that the composition ratio of the three major causes changes seasonally. In winter, the composition ratio of HTG-AP increased, while gallstone AP decreased (both $P < 0.05$), but alcohol AP did not show seasonal changes (Figure 2). There were seasonal differences in the etiological composition ratio between the two centers: the gallstone AP in the North center in summer and autumn was significantly lower than that in the South center (44.0% vs. 62.9%, 45.7% vs. 61.5%, respectively, both $P < 0.001$, Table 3). In summer, the HTG-AP was lower in the North center than in the South center (17.2% vs. 22.6%, $P = 0.04$).

Monthly variation in major etiologies of AP and the impact of festivals

Figure 3 shows the monthly variation trend of the composition ratio of the three major causes of AP, in which HTG-AP increased significantly, but gallstone AP decreased significantly in February, May and December (both $P < 0.001$).

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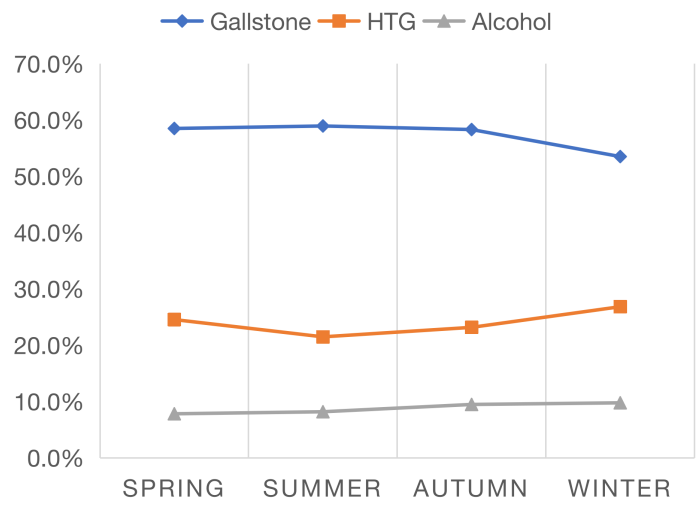

FIGURE 2
Seasonal variation in major etiologies of acute pancreatitis.

TABLE 3 The composition of AP etiology in different seasons in in South and North center.

Etiology	season	South center		North center		P-value
		(n)	constituent ratio	(n)	constituent ratio	
Gallstone	Spring	603	59.4%	131	54.6%	0.37
	Summer	687	62.9%	128	44.0%	0.00
	Autumn	627	61.5%	118	45.7%	0.00
	Winter	527	53.6%	131	53.0%	0.87
HTG	spring	247	24.3%	61	25.4%	0.67
	summer	247	22.6%	50	17.2%	0.04
	autumn	234	22.9%	62	24.0%	0.71
	winter	261	26.6%	69	27.9%	0.66
Alcohol	spring	82	8.1%	16	6.7%	0.48
	summer	84	7.7%	29	10.0%	0.21
	autumn	90	8.8%	31	12.0%	0.12
	winter	93	9.5%	27	10.9%	0.49

HTG, Hypertriglyceridaemia.

Alcohol AP showed no significant monthly change. The composition ratio of gallstone AP was lower in months with long holidays than in months without long holidays (52.0% vs. 59.3%, P<0.001, S-Table 4). In contrast, HTG-AP was higher in months with long holidays than in months without long holidays (29.5% vs. 21.9%, P<0.001), but alcohol AP was not significantly elevated in long holiday months (P=0.564).

Discussion

This study explored the variation in the etiology of AP at two pancreatic disease centers in China and found that gallstones, HTG and alcohol were the top three etiologies of AP. HTG-AP and alcohol AP showed an upward trend, while gallstone AP showed a downward trend. In addition, the composition ratios of gallstone AP and HTG-AP were affected by seasons and festivals. Compared with patients with AP in the South center, patients with AP in the North center were younger and tended to be male, and more patients had a history of smoking and drinking. In terms of comorbidities, AP patients with coronary heart disease, diabetes and cerebral infarction were more likely to be found in the North center than in the South center. The geographical environment, food culture, and dietary habits are substantially different between the southern and northern regions in China,

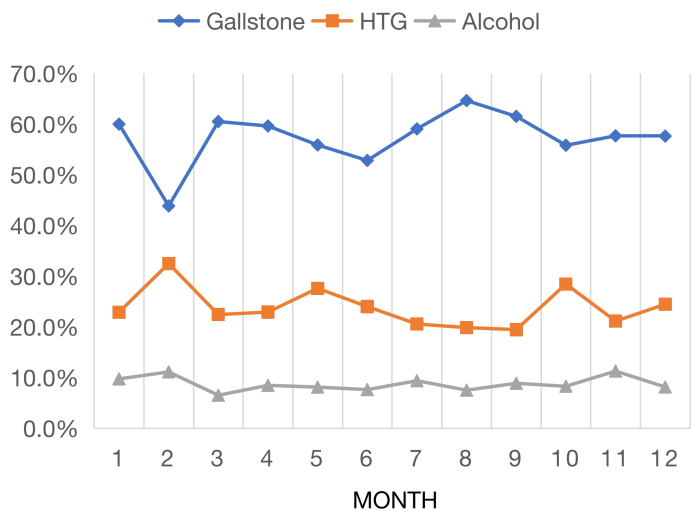

FIGURE 3
Monthly variation in major etiologies of acute pancreatitis.

and alcohol and Western eating patterns are more prevalent in the northern region (Song and Cho, 2017).

Although gallstones were the dominant etiology in the two regions, the composition of gallstones AP was higher in the south center than in the north center. Regional differences in gallstone AP have also been reported in Europe, with the highest proportion of gallstone AP in southern Europe and the lowest in eastern Europe (Roberts et al., 2017). We believe that the higher proportion of male and younger patients in the North center resulted in a lower proportion of gallstone APs in the North center than in the South center. Both sex and age have an important influence on the occurrence of gallstone AP; the risk of gallstones is twice as high in women than in men, and age further increases the risk for gallstone AP in both sexes (Shaffer, 2005). Previous studies have also shown that gallstone AP is more common in females and that its occurrence increases sharply with age (Roberts et al., 2013; Zhu et al., 2017; Wu et al., 2017). The composition ratio of alcohol AP in the South and North centers was 8.5% and 9.9%, respectively, which was lower than that in Europe and the United States (Yadav and Lowenfels, 2013; Roberts et al., 2017). Additionally, alcohol AP was more common in male and young patients, which is consistent with the results of studies from the United Kingdom and the United States (Yadav and Lowenfels, 2013; Roberts et al., 2013).

This study shows that HTG accounts for 23.9% of the etiological composition of AP, which is different from Western reports (Forsmark et al., 2016). HTG is a rare cause in European and American countries, accounting for approximately 2–5% of AP cases (Forsmark et al., 2016). However, two systematic reviews reported that HTG is the third most common cause of AP, accounting for 10% of cases worldwide (Carr et al., 2016; Adiamah et al., 2018). In China, with the development of the economy, 33.97% of adult residents developed dyslipidemia in 2010, almost twice the number in 2002 (Pan et al., 2016). Correspondingly, studies from China have shown that HTG was the second leading cause of AP (10.4%-14.3%) in 2010 (Zheng et al., 2015; Zhu et al., 2017). The increase in the proportion of HTG AP may lead to a relative decrease in gallstone AP. Gallstone AP decreased by 5%, consistent with a 5% increase in HTG in the South center after 2015. HTG is the main type of dyslipidemia in China, but hypercholesterolemia is the main type in Western countries (Pan et al., 2016). This may be the reason why the incidence rate of HTG-AP in China is higher than that in Western countries.

Seasonal variation in the etiology of AP remains uncertain. This study showed that the composition ratio of gallstone AP was significantly lower than in other seasons, which suggests that rising temperatures may increase the incidence of gallstone AP. A cross-sectional study found that the frequency of gallstone-induced acute cholecystitis is higher in summer than in other seasons (Hosseini et al., 2006). Cholecystectomy is also related to periodic changes in temperature, with the highest number of cases occurring in summer and the lowest in winter (Liu et al., 2014). In addition, the lower composition ratio of gallstones AP in the north center in summer and autumn may be related to the lower

temperature in northern China than in southern China in summer and autumn. The composition ratio of HTG-AP was the lowest in summer and the highest in winter, which is related to Chinese residents eating more high-fat food in winter. A high-fat diet is often considered a common secondary cause of hypertriglyceridemia and a risk factor for AP (Scherer et al., 2014). There was no significant seasonal change in alcoholic AP, which is consistent with data from eastern China (Wu et al., 2017).

Two studies from Europe found that increased alcohol consumption in months with holidays leads to the highest prevalence of acute alcoholic AP but not gallstone AP (Räty et al., 2003; Roberts et al., 2013). Our research shows that the composition ratio of HTG-AP was significantly higher and gallstone AP was relatively lower in months with long holidays. The long holidays are traditional Chinese family reunion festivals where relatives and friends frequently gather for meals and are more likely to overeat, consume a high-fat diet and abuse alcohol, leading to increased serum triglyceride levels and an increase in the prevalence of HTG-AP, which is consistent with a study from Shanghai, China (Wu et al., 2017). We speculate that the increase in the proportion of HTG led to a relative decrease in gallstone AP. Moreover, long holidays mainly occur in spring and autumn, and the incidence of gallstone disease is lower in cold climates. Given the increasing incidence of HTG-AP, it has become a major issue in China. Health education should emphasize a low-fat diet and alcohol abstinence, especially during festivals. The formulation of health policies should take into account the influence of regions, seasons and festivals.

The advantage of this study is that a large sample of cases was collected, including more than 5000 patients with AP from two pancreatic centers in southern and northern China. A limitation, however, is that these data do not fully represent all AP patients in these two regions. This selection bias may have been relatively small because most AP patients in lower-level hospitals are transferred to tertiary hospitals for better treatment.

In conclusion, our study revealed that the composition ratio of HTG-AP increased both in the south and north centers, while gallstone AP showed a downward trend. The etiology of AP was also affected by seasons and festivals. In winter and months with long holidays, the composition ratio of HTG-AP increased, and gallstone AP decreased significantly. Climate, a high-fat diet and alcohol consumption may be the main causes of the variation in the etiology of AP during seasons and holidays.

Data availability statement

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

Author contributions

WH: study design, statistical analysis, and manuscript preparation; GW: data collection, reviewed and revised the manuscript; BY: data abstraction and collection and manuscript preparation; LX: data collection; YoZ: data collection; PL: data collection; YiZ: data collection and data analysis; BS: study design, reviewed and revised the manuscript; NL: study design, reviewed and revised the manuscript. All authors contributed to the article and approved the submitted version.

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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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Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fcimb.2022.976816/full#supplementary-material

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Identification of novel immune-related targets mediating disease progression in acute pancreatitis

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Introduction: Acute pancreatitis (AP) is an inflammatory disease with very poor outcomes. However, the order of induction and coordinated interactions of systemic inflammatory response syndrome (SIRS) and compensatory anti-inflammatory response syndrome (CARS) and the potential mechanisms in AP are still unclear.

Methods: An integrative analysis was performed based on transcripts of blood from patients with different severity levels of AP (GSE194331), as well as impaired lung (GSE151572), liver (GSE151927) and pancreas (GSE65146) samples from an AP experimental model to identify inflammatory signals and immune response-associated susceptibility genes. An AP animal model was established in wild-type (WT) mice and Tlr2-deficient mice by repeated intraperitoneal injection of cerulein. Serum lipase and amylase, pancreas impairment and neutrophil infiltration were evaluated to assess the effects of *Tlr2 in vivo*.

Results: The numbers of anti-inflammatory response-related cells, such as M2 macrophages ($P = 3.2 \times 10^{-3}$), were increased with worsening AP progression, while the numbers of pro-inflammatory response-related cells, such as neutrophils ($P = 3.0 \times 10^{-8}$), also increased. Then, 10 immune-related AP susceptibility genes (SOSC3, ITGAM, CAMP, FPR1, IL1R1, TLR2, S100A8/9, HK3 and MMP9) were identified. Finally, compared with WT mice, Tlr2-deficient mice exhibited not only significantly reduced serum lipase and amylase levels after cerulein induction but also alleviated pancreatic inflammation and neutrophil accumulation.

Discussion: In summary, we discovered SIRS and CARS were stimulated in parallel, not activated consecutively. In addition, among the novel susceptibility genes, *TLR2*might be a novel therapeutic target that mediates dysregulation of inflammatory responses during AP progression.

KEYWORDS
acute pancreatitis, CARS, SIRS, neutrophil, Tlr2-deficient mice

Introduction

AP is the most common digestive disease and is characterized by inflammation and autodigestion of the pancreas (Vege et al., 2018). The incidence rate of AP is estimated to be 100 to 140 per 100,000 per person annually in developed countries (Mederos et al., 2021). Mild AP (MAP) is a self-limiting disorder that can be resolved within one week with finite treatment (Lee and Papachristou, 2019). However, approximately 10-20% of patients experience moderately severe AP (M-SAP) or severe AP (SAP), which is associated with pancreatic or peripancreatic tissue necrosis, local or systemic complications and persistent single or multiple organ failure and has a high mortality rate (Schepers et al., 2019; Boxhoorn et al., 2020; Mederos et al., 2021). Convincing evidence has demonstrated that infected necrosis is the greatest risk factor for severe AP and mortality, and the intensity of the immune response during this process exerts an enormous influence on systemic complications and disease severity (van Dijk et al., 2017; Sendler et al., 2020). AP is a complex inflammatory disease with diverse characteristics in terms of severity and course. While the therapeutic strategy and stratification systems currently used for AP are generally useful, the effectiveness and accuracy of these methods still need to be improved to reduce the mortality rate and improve early diagnosis of AP (Gukovskaya et al., 2017; Lee and Papachristou, 2019; Leppaniemi et al., 2019; Sun et al., 2021).

AP is triggered by premature activation of digestive enzymes in acinar cells, and the infiltration of inflammatory cells is induced simultaneously (Kono and Rock, 2008; Sendler et al., 2018). In particular, during these processes, immune-related cells undergo interactions under physiological and pathophysiological conditions. Macrophages and neutrophils initially reach the organ and cause pancreatic damage, monocyte induction is a key factor in systemic inflammation and worsening tissue injury, and T-cell activation plays an important role in inducing the adaptive immune response in AP (Gukovskaya et al., 2002; Zheng et al., 2013; Sendler et al., 2018; Xu et al., 2020). As a result of the initial immune response, pro-inflammatory cytokines are released, causing systemic inflammatory response syndrome (SIRS). Meanwhile, hyperinflammation is accompanied by compensatory anti-inflammatory response syndrome (CARS) during disease development (Zheng et al., 2013; Sendler et al., 2020). Abundant hyperinflammation during SIRS is associated with organ dysfunction syndrome or shock in patients, and inordinate immunosuppression during CARS is associated with bacterial translocation, resulting in pancreatic necrosis or severe sepsis; all of these factors dramatically increase the mortality rate of SAP patients (Bhatia and Moochhala, 2004; Lee and Papachristou, 2019). Therefore, elucidating the magnitude and order of induction of SIRS and CARS during different phases of the disease might provide an effective therapeutic strategy for AP.

As pattern-recognition receptors, Toll-like receptors (TLRs) seem to play an essential role in the development and severity of inflammatory diseases by mediating SIRS, regulating inflammatory cell recruitment, altering microvascular leakage and inducing cellular apoptosis (Iwasaki and Medzhitov, 2004; Vaz et al., 2013). Damage-associated molecular patterns (DAMPs) released in cellular contents exert their effects by specifically binding TLRs to activate the NF-kappa B signaling pathway. The induction of NF-kappa B signaling regulates the expression profiles of pro-inflammatory cytokines, chemokines and adhesion factors. Among the 13 subunits of the TLR family, most endogenous ligands and bacteria-derived compounds interact with *TLR2* and *TLR4* to induce the innate immune response (Gorskii et al., 2014; Gorsky et al., 2015). In previous studies, the main candidate gene investigated for targeting in AP was *TLR4*, while the potential functions of *TLR2* in the pathophysiology of AP are still elusive (Vaz et al., 2013). *TLR2* was overexpressed in the peripheral blood, glands and pancreas in a cerulein-induced experimental AP model and in human peripheral blood (Ding et al., 2013; Gorskii et al., 2014). In contrast, Awla and colleagues induced AP in wild-type, *Tlr2*-deficient and *Tlr4*-deficient mice and showed that *Tlr4* but not *Tlr2* regulates chemokine formation, neutrophil recruitment and tissue damage in the SAP mouse model (Awla et al., 2011). Hence, additional *in vivo* and *in vitro* studies are essential for elucidating the effects and biological mechanisms of *TLR2* in the pathogenesis and deterioration of AP.

In this study, we investigated the development and progression of AP on the basis of transcriptional profiles in human peripheral blood, as well as the corresponding signatures in tissue samples from an AP animal model. Consistent with a recent study based on immune cell infiltration analysis and cytokine and chemokine expression patterns in patients with AP of different severities, we found that pro-inflammatory and

anti-inflammatory responses were activated in parallel (Sendler et al., 2020), not consecutively, as previously reported for SIRS and CARS (Andersson et al., 2007). Furthermore, to make further progress in the diagnosis and treatment of AP, it is crucial to identify the etiological mechanisms and susceptibility genes of AP *via* comprehensive bioinformatics analysis. Ten immune-related hub genes and pathways for AP were identified based on an integrative analysis. In addition, to investigate the potential functions and biological mechanisms of these immune-related hub genes, AP mouse models were established in WT mice and *Tlr2*-deficient mice. *Tlr2*-deficient mice showed substantial amelioration of the pancreatic inflammatory response and pancreatic injury, with reduced activation of neutrophils. Therefore, these findings highlight that SIRS and CARS are activated simultaneously and that *Tlr2* and several interacting genes that regulate immune cell infiltration during AP progression are potential therapeutic targets for AP.

Materials and methods

Data and resources

The count-based gene expression profiles of peripheral blood and associated clinical information for the three AP severity levels were acquired from the Gene Expression Omnibus (GEO) database (accession number: GSE194331), including 32 healthy donors, 57 mild acute pancreatitis (MAP) patients, 20 moderately severe acute pancreatitis (M-SAP) patients and 10 severe acute pancreatitis (SAP) patients (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE194331). This dataset was deposited by Maryam N et al. from the Nepean Hospital (Nesvaderani et al., 2022). The severity levels of AP were evaluated according to the Revised Atlanta classification.

To validate the potential targets and etiology of AP in human blood samples, the transcriptome profiles of the AP experimental models GSE151572 (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE151572) and GSE151927 (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE151927) were downloaded from the GEO database. GSE151572 was designed to gain insight into the effect of emodin in Sprague–Dawley (SD) rats (8 weeks old) with SAP-induced lung injury (Xu et al., 2021a). Emodin- and dexamethasone-treated groups were filtered out, and we acquired the mRNA high-sequencing profiles of only the control group (n = 6), SAP-6 h group (n = 3) and SAP-24 h group (n = 3) to perform further analyses. The SAP model was induced by standard retrograde infusion of fresh 5.0% sodium taurocholate (0.1 mL/100 g body weight) into the biliopancreatic duct. An equal volume of sterile saline was injected into the rats in the control group. Necrosis, inflammation, hemorrhage and edema were more severe in the SAP-24 h group than in the SAP-6 h group according to previous research (Xu et al., 2021a). GSE151927 was designed to extend the understanding of metabolic gene changes during AP based on hepatic transcriptome profiles (Zhang et al., 2022a). The mice were divided into control (n = 8), AP (n = 8) and SAP (n = 8) groups.

Furthermore, to dynamically detect the fluctuating expression levels of causal genes with disease progression, the mRNA profile of pancreas tissue from the AP mouse model in GSE65146 was downloaded (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=gse65146). KrasG12D-mutated mice were not included in the subsequent analyses. Hence, data from 44 wild-type mice with cerulein injection-induced AP at 13 consecutive time points remained for further analyses. The expression profiles were evaluated by Affymetrix GeneChip Mouse Gene 1.0 ST arrays.

Principal component analysis

The count-based gene expression matrix of human and rat models was used to generate the PCA plot with the R packages gmodels (v. 2.18.1) and ggplot2 (v. 3.3.5). Because the 6-hour and 24-hour control groups for the rat model did not receive other treatments, we combined the rats from the two subtypes into one group.

Differential gene expression analysis

Differentially expressed genes (DEGs) between each severity level of AP (MAP, M-SAP, SAP) and one common healthy group were identified using the DESeq2 package (v. 3.6.3) (Anders and Huber, 2010) in R to detect gene expression changes related to AP aggravation. The genes with a false discovery rate (FDR) ≤0.05 and an absolute value of log2fold change ≥1 were considered to be deferentially expressed (Xu et al., 2021b; Nesvaderani et al., 2022). To limit potential false-positive results, nonexpressed or low-count (average counts ≤ 1) transcripts were filtered out for further analyses. Subsequently, the aberrant expression profiles from different comparison groups in the SAP rat model were analyzed with the same methods described above.

Immune cell infiltration analysis of the mRNA sequencing data

The CIBERSORTx algorithm was used to evaluate the relative abundance of 22 types of infiltrating immune cells according to the mRNA expression profile (Newman et al., 2015; Newman et al., 2019), which was assessed based on the expression levels of specific markers of immune cells. Before calculating the immune cell proportions, the expression count

data were normalized to reads per kilobase per million mapped reads (RPKM) format. The 22 immune cell types involved were naive B cells, memory B cells, $CD8^+$ T cells, T cells, follicular helper T regulatory cells, gamma delta T cells, $CD4^+$ memory resting T cells, $CD4^+$ memory activated T cells, $CD4^+$ naive memory T cells, plasma cells, resting natural killer cells, activated natural killer cells, monocytes, M0 macrophages, M1 macrophages, M2 macrophages, resting mast cells, activated mast cells, resting dendritic cells, activated dendritic cells, eosinophils, and neutrophils. Heatmaps were generated and DEG expression profile clustering was performed with gplot (v 3.1.1) and pheatmap (v. 1.0.12) in R.

Pathway and network analysis

To illuminate the potential mechanisms of AP, the R package ClusterProfiler (v. 3.14.3) was used to perform Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis (v. 3.14.3) (Yu et al., 2012). Terms with FDR values less than 0.05 were considered to represent significantly enriched pathways. To construct biomolecular networks and identify the causal genes for AP of different severity levels, protein–protein interaction network analysis with overlapping targets was performed using STRING (v. 10.5) with the default parameters (https://string-db.org) (Szklarczyk et al., 2021). Cytoscape (v. 3.9.1) was applied to visualize the biomolecular interactions of SAP-related candidate genes (Shannon et al., 2003). According to the interaction scores, which were calculated with the MCC algorithm scores from the Cytohubba plugin in Cytoscape, the top 10 genes were named hub targets for AP occurrence and severity risk.

The R package WGCNA was implemented to construct the network modules of highly correlated mRNAs (Langfelder and Horvath, 2008). This tool helps to find gene pairs with similar expression patterns and high topological overlap, and it is essential to identify causal genes and understand the etiology of complex diseases. First, using a threshold power of 30, we constructed a weighted network according to the gene pair correlations among all the transcriptomes. Second, 6 specific modules with module sizes from 41 to 10138 were hierarchically clustered using the default parameters to evaluate the network interconnection. In addition, we calculated the correlations among the hub genes' transcriptome expression profiles using the Spearman method with human and experiential models.

Mice

C57BL/6J mice were purchased from Charles River Laboratories (Beijing, China). C57BL/6-Tlr2^{em1Smoc} (*Tlr2*$^{-/-}$) mice were purchased from Shanghai Model Organisms Center, Inc. (Shanghai, China). Only male mice (6-8 weeks) were included in the studies. All mice were housed under conditions of controlled temperature (22-25°C) and humidity (40-60%) with a 12:12 hour light-dark cycle and were allowed free access to food and water. In addition, mice were kept in specific pathogen-free facilities, and all of the experiments were performed in accordance with the guidelines of the Zhejiang University Animal Care and Use Committee.

Experiential models and treatments

Age-matched wild-type and *Tlr2*$^{-/-}$ male mice were used in the following experiments. GraphPad software was used to randomize mice with a single sequence of random assignments before treatment. AP was induced by intraperitoneal injection of caerulein (50 μg/kg; Glpbio, Montclair, USA) 8 times at hourly intervals (Sendler et al., 2018; Xu et al., 2020). SAP was induced by hourly injection of caerulein (50 μg/kg) 8 times plus LPS (10mg/kg; Servicebio, Wuhan, China), and LPS was injected right after the last injection of caerulein. Control groups were administered an equivalent volume of phosphate-buffered saline (PBS). Then, the mice were sacrificed 4 hours, 12 hours, 24 hours and 36 hours after the last injection of caerulein. Mice that exhibited signs of suffering during the treatment process were excluded from the study.

Amylase and lipase measurement

Serum samples were collected from different groups of mice. The amylase and lipase levels were detected with a Beckman Coulter AU680 automatic biochemical analyzer. The remaining blood samples were stored at -80°C until use.

Histological examination

Hematoxylin and eosin (H&E) staining was applied to detect pancreatic and pulmonary tissue injury in mice with AP or SAP. Paraffin-embedded sections (5 μm) were stained with H&E. For histological examination, the pancreas and lungs were removed and fixed in 4% paraformaldehyde. To identify the pancreas and lungs injury level, multiple randomly selected microscopic fields from at least three mice per group were assessed by two pathologists in a blinded manner. The pancreatic injury score was utilized on the basis of pancreatic edema (0-2), inflammatory cell infiltrate (0-3), hemorrhage and fat necrosis (0-3) and acinar necrosis (0-3), as previously described (Miao et al., 2019). The pulmonary injury score was assessed using a scale for the interstitial and intra-alveolar edema (0-5), interstitial and intra-alveolar leukocyte infiltration (0-5), and fibrosis (0-5), as previously described (Hu et al., 2022).

Immunohistochemical analysis

Paraffin-embedded sections (5 μm) of pancreatic tissue were incubated with a rabbit polyclonal anti-Ly6G antibody (1:500, Servicebio, Wuhan, China) at 4°C overnight. Further experimental steps were performed according to the manufacturer's instructions for the Streptavidin/Peroxidase Histostain™ Plus Kit (ZSGQ-BIO, Beijing, China). The number of neutrophils was determined by counting $Ly6G^+$ cells per microscopic field (200×).

Statistical analysis

Statistical analyses and the creation of graphs were performed using the R package Tableone (v. 0.13.0) and GraphPad Prism 8 software. Statistically significant differences were calculated by an unpaired two-tailed Student's *t test* (2 groups) and one-way ANOVA (multiple groups). A *P* value less than 0.05 was considered to be statistically significant. The correlation matrix was constructed with R according to the Pearson correlation coefficient.

Results

DEG analysis and sample clustering for human AP

The mRNA high-throughput sequencing data were collected using blood samples from 32 healthy individuals, 57 MAP patients, 20 M-SAP patients and 10 SAP patients. After quality control, approximately 20,000 annotated transcriptomes were identified in each individual. A PCA plot generated using the gmodels (v. 2.18.1) and ggplot2 (v. 3.3.5) revealed that the characteristic expression profiles of SAP and M-SAP were largely discriminated from those of healthy donors and MAP patients (Figure 1A). In contrast, nearly half of the MAP patients

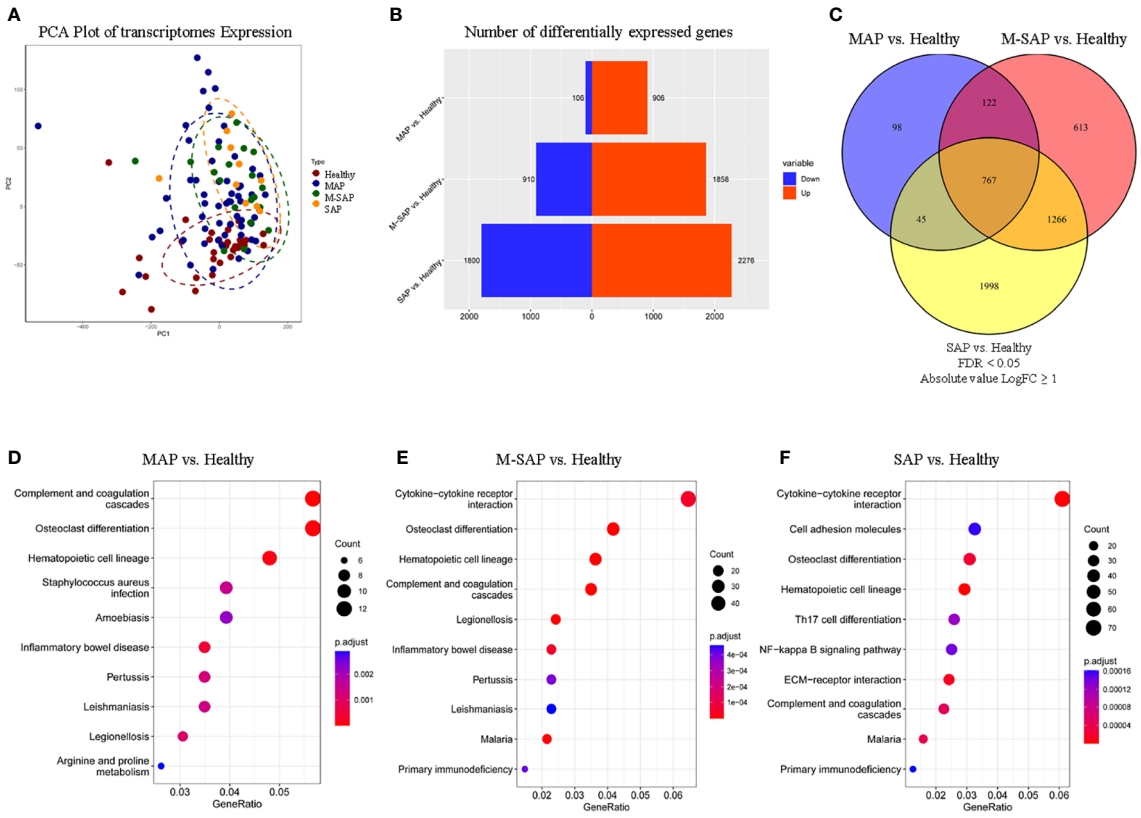

FIGURE 1
Shared DEGs and pathways of AP of different severity levels. **(A)** Principal component analysis of sub-subjects with MAP, M-SAP or SAP and healthy controls. **(B)** Distribution of the number of DEGs between each severity level of AP and healthy controls. The red rectangle indicates upregulated genes, and the blue rectangle indicates downregulated genes. **(C)** Venn diagram depicting the overlapping DEGs in each severity group compared with a common set of healthy groups. **(D–F)** Pathway enrichment analysis for three severity levels of AP and healthy controls using KEGG analysis. Only the top 10 significantly enriched pathways after sorting by the Bonferonni adjusted *P* value were selected. Dots represent significant pathways, and all of them were sorted and colored by adjusted *P* value.

were mixed with the healthy donors.

To better understand the molecular mechanisms underlying disease occurrence and risk severity, we conducted DEG analysis with transcriptome sequencing data. When compared directly with that of healthy controls, the number of DEGs dramatically increased with the disease severity, with MAP, M-SAP and SAP exhibiting 906, 1,858 and 2,276 upregulated DEGs and 106, 910, and 1,800 downregulated DEGs, respectively ($P_{adj} \leq 0.05$, $|\log2FC| \geq 1$; Figure 1B; Tables S1-3). In addition, there were 934 significant DEGs (92.9%) for MAP that were shared between M-SAP or SAP. Of these genes, 767 DEGs, which accounted for more than three-fourth of the MAP DEGs (75.8%), were abnormally expressed in a consistent direction in both M-SAP and SAP (27.7%, 18.8%; Figure 1C). These results suggested that the majority of DEGs for MAP also play essential roles in progression to M-SAP and SAP.

DEGs-based enrichment analysis for three severity levels of AP

To illuminate the potential pathogenesis and shared mechanisms of different severity levels of AP, the DEGs were subjected to KEGG enrichment analysis. Our pathway analysis of DEGs for different severity levels of AP (MAP, M-SAP, and SAP) compared against one common control group revealed 8, 13 and 26 significantly enriched pathways (FDR ≤ 0.05), respectively (Table S4; Figures 1D-F). Of these pathways, six of the eight pathways enriched in MAP (complement and coagulation cascades, osteoclast differentiation, hematopoietic cell lineage, inflammatory bowel disease, legionellosis and pertussis) were also significantly enriched in M-SAP and SAP. For MAP, the most significantly enriched pathway was the complement and coagulation cascades ($P = 6.02 \times 10^{-7}$); this pathway was significantly enriched in the M-SAP and AP groups ($P = 1.65 \times 10^{-7}$, $P = 2.74 \times 10^{-3}$). Interestingly, a previous study demonstrated that this pathway was significantly activated in acute necrotizing pancreatitis patients compared with interstitial edema pancreatitis patients, and crucial genes in this pathway were positively related to pancreatic necrosis and aggravated AP (Zhang et al., 2022b). The inflammatory bowel disease pathway was a common pathway in the three groups ($P = 4.39 \times 10^{-4}$, $P = 4.94 \times 10^{-5}$, $P = 6.54 \times 10^{-3}$), confirming an important role of this pathway in AP. According to the clinical presentation, pancreatic abnormalities are common in inflammatory bowel disease (IBD) patients, and nearly 20% of IBD patients have asymptomatic exocrine insufficiency and/or hyperamylasemia (Ramos et al., 2016). The wide spectrum of pancreatitis-related symptoms observed in IBD may reveal the shared etiology and shared common genes of the two diseases.

According to the pathway analysis, gradually aggravated immune dysfunction was identified. We identified 13 significantly dysregulated pathways in M-SAP, with P values ranging from 1.65×10^{-8} to 1.96×10^{-3}. Notably, 12 dysregulated pathways in MAP were significantly enriched in SAP, such as hematopoietic cell lineage and cytokine–cytokine receptor interaction (M-SAP: $P = 1.27 \times 10^{-7}$, $P = 4.72 \times 10^{-5}$; SAP: $P = 7.51 \times 10^{-5}$, $P = 4.25 \times 10^{-4}$). For SAP, 26 dysregulated pathways were detected, with P values ranging from 7.52×10^{-5} to 4.51×10^{-2}. In addition, the majority of these pathways were highly related to immune responses, such as Th17-cell differentiation and the NF-kappa B signaling pathway ($P = 5.00 \times 10^{-3}$, $P = 5.00 \times 10^{-3}$), which indicated a stronger perturbation of inflammation in SAP.

Distinct characteristics of immune infiltration in AP

Using CIBERSORT, we presented the landscape of 22 infiltrating immune-related cell populations in AP by analyzing the mRNA expression profile of the GSE194331 dataset (Figure 2A). Among the 22 types of immune cells, follicular helper T cells, gamma delta T cells, activated NK cells, M1 macrophages, activated mast cells, resting dendritic cells, and eosinophils were not detected based on the peripheral blood RNA-seq dataset (Table S5). We also illustrated the relationships between different types of immune-related cells in AP using a correlation matrix. Interestingly, neutrophils exhibited negative correlations with naive B cells, memory B cells, $CD8^+$ T cells, resting memory $CD4^+$ T cells, activated memory $CD4^+$ T cells, and resting natural killer cells and positive relationships with M0 and M2 macrophages (Figure 2B). The proportion of monocytes demonstrated a positive correlation with M0 macrophages and a negative correlation with naive B cells and resting memory $CD4^+$ T cells. Furthermore, we investigated the differences in these immune-related cell proportions among AP of three severity levels and healthy controls. Using ANOVA, the fractions of $CD8^+$ T cells and resting memory $CD4^+$ T cells were clearly decreased with increasing AP severity ($P = 2.9 \times 10^{-3}$, $P = 2.7 \times 10^{-5}$). The proportions of activated memory $CD4^+$ T cells, monocytes, M0 macrophages, M2 macrophages and neutrophils were significantly increased among the four groups ($P = 1.5 \times 10^{-3}$, $P = 4.0 \times 10^{-4}$, $P = 1.3 \times 10^{-2}$, $P = 3.2 \times 10^{-3}$ and $P = 3.0 \times 10^{-8}$, respectively; Figure 2C).

To investigate the immune response in more detail, we evaluated the whole-blood cytokine and chemokine mRNA expression profiles of different severity levels of AP and the healthy group (Table S6). During disease progression, cytokines and chemokines are released due to acinar cell injury and necrosis; these factors recruit and mediate the infiltration of immune cells into the injury area (Lee and Papachristou, 2019). Blood cytokine profiles revealed that the pro-inflammatory response indicators *TNFSF13β*, *IL1β* and *IL18* were significantly elevated with the

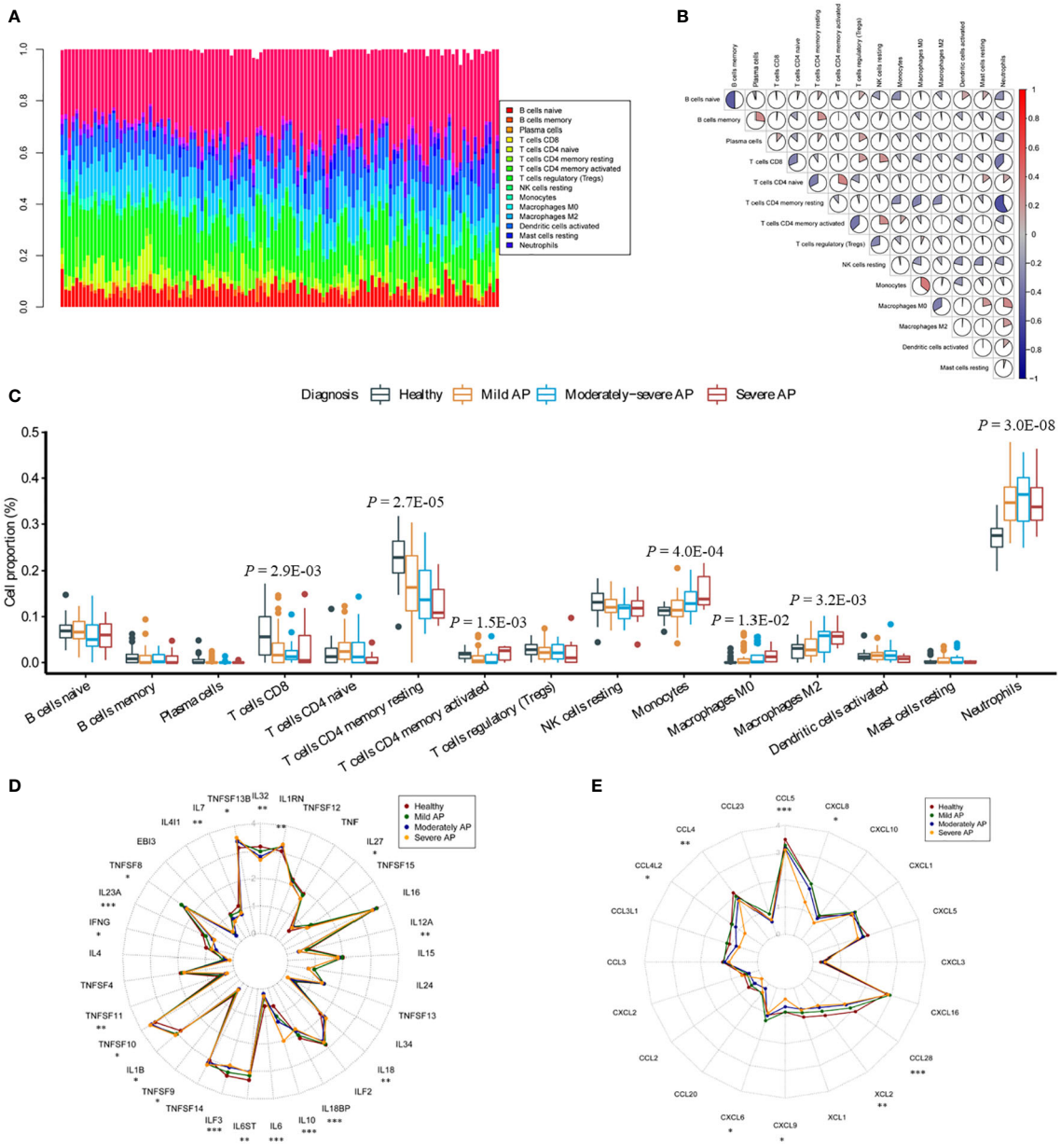

FIGURE 2
Analysis of immune cell infiltration and the transcription profile of cytokines and chemokines in AP. **(A)** The proportions of 22 immune-related cells were assessed through the CIBERSORTx algorithm in AP. The stacking plot indicates the distribution of 22 types of immune cells in AP. **(B)** The correlation matrix of 22 types of immune cells in AP. **(C)** Significant differences among the groups were evaluated utilizing ANOVA. The Y-axis indicates the percentage of immune cells in AP. **(D, E)** Dot plots represent the expression levels of cytokines and chemokines in the peripheral blood of humans. Asterisks indicate significant differences, red represents healthy donors, dark green represents MAP patients, dark blue represents M-SAP patients, and dark yellow represents SAP patients (*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$).

aggravation of AP ($P = 0.010$, $P = 0.031$, $P = 0.004$, respectively; Figure 2D). In contrast, compared with those in the healthy groups, the transcript levels of several pro-inflammatory cytokines, such as the *IL12* cytokine family *IL12α* and *IL23α*, *IL6*, *IL32* and *IFN-γ*($P = 0.007$, $P < 0.001$, $P < 0.001$, $P < 0.001$, $P = 0.001$, respectively) were markedly decreased. Furthermore, expression of the anti-inflammatory cytokine *IL10* ($P < 0.001$) was significantly elevated. In addition, compared with that in the healthy group, expression of *CCL4*, *CCL4L2*, *CCL5*, *CCL28*, *XCL2*, *CXCL6*, *CXCL8* and *CXCL9* was significantly decreased ($P = 0.003$, $P = 0.025$, $P < 0.001$, $P < 0.001$, $P = 0.003$, $P = 0.021$, $P = 0.029$, $P = 0.040$, respectively; Figure 2E).

Immune-related genes identified in humans and experimental AP models

To further understand the pathogenesis of AP, we searched for DEGs for the common control groups *vs.* the SAP-6 h groups and the common control groups *vs.* the SAP-24 h groups. According to the PCA plot, the characteristics of the control and SAP-6 h groups were dramatically different from those of the SAP-24 h group (Figure S1). A total of 20,342 high-quality rat transcripts remained after quality control. Compared with the control group and SAP-6 h group, the SAP-6 h group exhibited 123 significantly downregulated transcripts and 167 upregulated transcripts ($P_{adj} \leq 0.05$, $|\log2FC| \geq 1$; Figure S2a; Table S7). Next, compared with a common set of control groups and the SAP-24 h groups, 471 transcripts were significantly downregulated, and 652 transcripts were upregulated (Figure S2b; Table S8).

Furthermore, to comprehensively understand the etiology and mechanism of different severity levels of AP, the abovementioned DEGs were subjected to pathway enrichment analysis. In the KEGG analysis results for the control group *vs.* SAP-6 h group, we observed 9 significantly enriched KEGG terms with *P* values ranging from 1.71×10^{-5} to 2.27×10^{-3} (Figure S2c and Table S9). In addition, for the control group *vs.* SAP-24 h group, the number of enriched pathways was dramatically increased, and the majority of these pathways were strongly related to dysfunctional immune responses (Figure S2d). We identified 28 significant pathways with *P* values ranging from 6.19×10^{-15} to 1.72×10^{-3}. Four pathways, namely, hematopoietic cell lineage, complement and coagulation cascades, ECM-receptor interaction and proteoglycans in cancer, were identified as common pathways in the rat experimental AP model. Notably, the hematopoietic cell lineage and complement and coagulation cascade pathways were identified as common pathways in both the human and experimental AP models.

Based on the transcriptional profiles of humans and experimental rat models, there were 69 upregulated DEGs and 3 downregulated DEGs for AP that were shared among at least four different groups (Figures 3A-C). Next, protein–protein interaction network (PPI) analysis was conducted to identify the interactions among the overlapping genes using the STRING database (https://string-db.org/cgi/input.pl) with the default parameters (Figure 3D). The results showed that 43 genes strongly interacted with each other. The R package of WGCNA was applied to identify the gene pairs with highly topological overlap and strongly related expression patterns, and it is important to detecting promising targets genes and understanding the pathology of diseases. Performing with the default parameter, 6 specific modules were hierarchically clustered (Figure 3E). According to the weighted network module, among of the 43 highly related genes, 35 genes with similar expression profiles and located in blue module (n = 3,037; Figure S2e). Furthermore, the top ten highly interacting hub genes were screened based on the interaction score, which was calculated through the 12 different algorithms of Cytohubba software (Figures S3a-i). Interestingly, similar to the MCC algorithm analysis result, *TLR2* was annotated as one of top 10 hub genes in Betweenness, BottleNeck, Closeness, Degree, EPC, MNC, Radiality, Stress algorithms analyzed results, except under ClusteringCoefficient, DMNC and EcCentricity model. Therefore, these results indicated that *TLR2* might play important role in the etiology and mechanism of AP.

To infer the potential functions of the hub genes for AP, we first performed correlation analysis between the hub genes and immune-related infiltrating cells in AP. The heatmap revealed that *SOSC3, ITGAM, CAMP, FPR1, IL1R1, TLR2, S100A8/9, HK3* and *MMP9* were negatively correlated with $CD8^+$ T cells and resting memory $CD4^+$ T cells, and the majority of these genes were positively correlated with activated memory $CD4^+$ T cells, monocytes, M0 macrophages, M2 macrophages and neutrophils (Figure 4A). Second, since we were unable to annotate the GO terms for some of the 10 hub genes for AP, we performed GO analysis using ClusterProfiler with the 43 highly interacting hub genes of AP to identify overrepresented biological processes, and only terms with FDR values < 0.05 were considered significant. The GO analysis revealed enrichment of biological processes largely related to immune response (e.g., regulation of inflammatory response and macrophage activation; Figure 4B and Table S10). Interestingly, nine of ten hub genes were included in the gene set of the blue module; only *CAMP* was excluded. In addition, we utilized Spearman correlation analysis to assess the relationships among the 10 hub genes for AP. The hub gene expression levels were positively correlated with each other, with Spearman correlation coefficients ranging from 0.26 to 0.90 in humans and from 0.51 to 0.99 in the experimental AP model (Figure 4C; Figure S2f). Therefore, it can be inferred that the ten hub genes for AP closely interact and probably exert their effects by regulating the immune response in AP.

Deletion of TLR2 attenuates the severity of AP

Bioinformatic analyses showed that the hub genes for AP were overexpressed during the induction and progression of AP (Figures S4a-i). Compared with those in the healthy group, *TLR2* expression levels in blood samples from MAP, M-SAP and SAP patients were dramatically upregulated ($P = 4.19 \times 10^{-10}$, $P = 1.24 \times 10^{-14}$, $P = 5.19 \times 10^{-11}$; Figure 5A). In addition, in accordance with the results obtained for human blood samples, *Tlr2* was gradually increased in injured lung tissue from the caerulein-treated group compared with the control group at 6

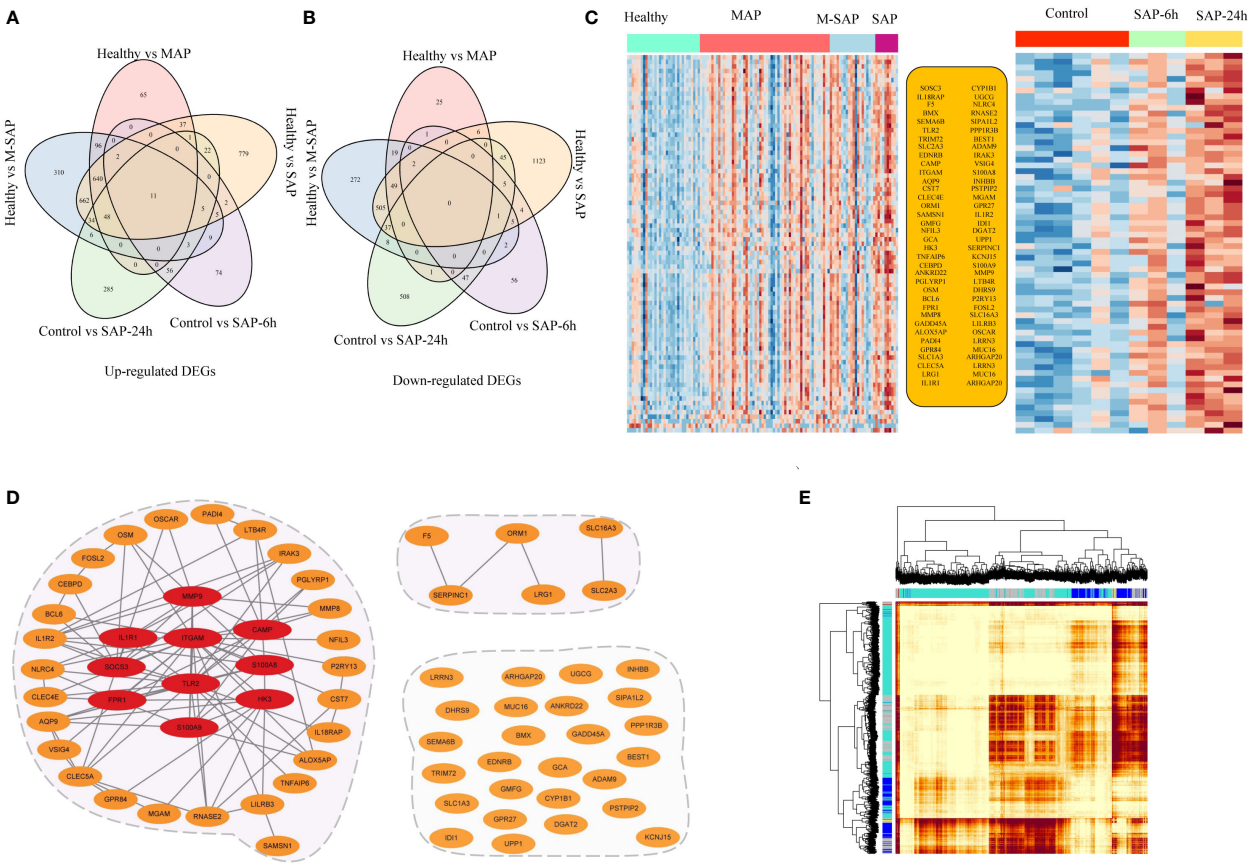

FIGURE 3
Specific transcriptional characteristics and potential interaction mechanisms of AP-associated genes. **(A, B)** The Venn diagram represents the overlapping genes from human blood samples and the animal AP model. **(C)** Expression of 71 shared transcriptomes according to DEG analysis of human blood samples and samples from the animal AP model. **(D)** Interaction network analysis was applied using the STRING database and WGCNA. Orange dots represent the 71 AP-related genes. The red ellipse denotes the 10 hub genes that were scored with the cytoHubba tool. The gray edge represents the interaction relationships annotated by the STRING database. **(E)** Topological overlap matrix plots for AP modules.

hours and 24 hours ($P = 0.02$, $P = 9.76 \times 10^{-05}$; Figure 5B). In addition, the dysregulated expression profile was identified in injured liver tissue from caerulein-treated mice at 7 hours and 12 hours ($P = 0.80$, $P = 3.3 \times 10^{-03}$; Figure 5C). Furthermore, during the recovery phase of AP, compared with 0 hour time point for cerulein-injected mice, the results from AP model mice at 13 consecutive time points indicated that the *Tlr2* expression level in pancreas tissue dramatically increased and peaked at 24 hours and then gradually recovered (0 h *vs.* 3 h: $P = 1.21 \times 10^{-3}$, 0 h *vs.* 12 h: $P = 1.20 \times 10^{-3}$, 0 h *vs.* 24 h: $P = 6.97 \times 10^{-5}$, 0 h *vs.* 36 h: $P = 1.67 \times 10^{-3}$; 0 h *vs.* 48 h: $P = 4.04 \times 10^{-3}$, 0 h *vs.* 60 h: $P = 2.81 \times 10^{-3}$, 0 h *vs.* 72 h: $P = 4.54 \times 10^{-3}$, 0 h *vs.* 84 h: $P = 0.093$, 0 h *vs.* 96 h: $P = 0.043$, 0 h *vs.* 120 h: $P = 0.081$, 0 h *vs.* 168 h: $P = 0.952$, 0 h *vs.* 336 h: $P = 6.85 \times 10^{-3}$, Figure 5D). Regarding other hub genes, *Itgam, Socs3, Mmp9, Il1r1, Hk3* and *Fpr1* expression levels were significantly increased after caerulein injection and peaked before 12 hours in the mouse model, but *S100a9* and *Camp* did not show the same pattern (Figures S5a-i).

To prove that *Tlr2* is a crucial gene for AP, an animal model of AP was established through repeated injection of cerulein 8 times at hourly intervals in C57BL/6J mice and *Tlr2*$^{-/-}$ mice (Figure 6A). As shown in Figure 6B, H, E staining of pancreatic tissues showed that inflammatory infiltration and edema continually increased and peaked at 12 hours as AP developed and then gradually recovered at 36 hours. The serum lipase and serum amylase activity levels were dramatically elevated in AP mice and peaked at 4 hours (Figures 6C, D). These data indicated that we successfully established a MAP mouse model with this method, resulting in intrapancreatic necrosis on histology and increased serum lipase and serum amylase compared with control mice. Next, to confirm *Tlr2* as a risk gene that plays an important role in the pathogenesis of AP, we induced AP in *Tlr2*-deficient mice and C57BL/6J mice. H&E staining indicated that *Tlr2*$^{-/-}$ mice exhibited a significant alleviation of pancreatic tissue damage compared to that in wild-type mice (Figures 6E, F). Furthermore, cerulein

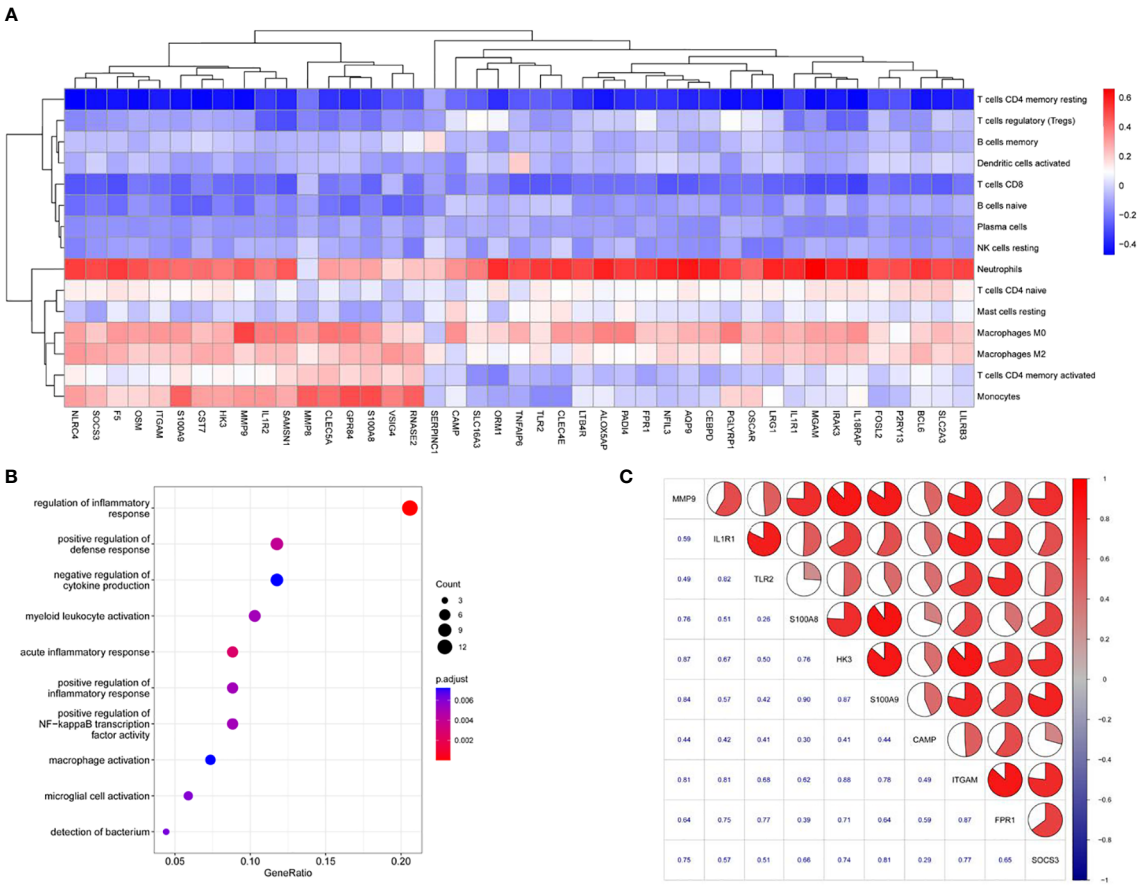

FIGURE 4
The potential functions of AP-related genes. **(A)** Heatmap denoting the relationships among the identified 43 highly connected candidate genes and immune cell proportions. Colored rectangles represent the Spearman correlation coefficient of transcript abundance for the highly connected candidate genes and fluctuating immune cell proportions. **(B)** GO term enrichment analysis of 43 highly connected candidate genes for AP. **(C)** Correlation matrix of the expression profiles of hub genes of AP in human blood samples.

treatment resulted in significantly increased serum lipase and amylase activity levels in both WT and *Tlr2*$^{-/-}$ mice. However, after cerulein induction, the serum lipase and amylase activity levels of *Tlr2*$^{-/-}$ mice were clearly lower at 4 hours and 12 hours than those of WT mice (Figures 6G, H). Next, immunohistochemical staining was performed to examine neutrophil infiltration in the pancreas. Significantly increased neutrophil numbers were observed in the pancreas in wild-type mice at 12 hours and 24 hours. In contrast, neutrophil infiltration in the pancreas was significantly decreased in the *Tlr2*$^{-/-}$ groups compared to the wild-type groups (Figures 6I, J).

To confirm the effect of *Tlr2* on SAP, an animal model of SAP that mimicking septic conditions with multiple organ failure was established through LPS (10mg/kg) superimposed on a caerulein*8 regimen right after the last injection of caerulein (Figure 7A). H&E staining indicated that *Tlr2*$^{-/-}$ mice exhibited a significant alleviation of pancreatic tissue damage compared to that in wild-type mice (Figures 7B, C). Lung injury is the most common extra pancreatic organ dysfunction induced by SAP. We also investigated the lung injury in the different groups. As shown in (Figures 7B, D), Tlr2−/− mice also exhibited a significant alleviation of lung tissue damage compared to that in wild-type mice. What is more, the serum lipase and amylase activity levels of *Tlr2*$^{-/-}$ mice were lower than those of WT mice (Figures 7E, F). Taken together, these results show that overexpression of *TLR2* increased disease severity and pancreatic damage in AP patients and animal models.

Discussion

AP is an inflammatory disease of the pancreas that can lead to SIRS and CARS, which are associated with a high mortality rate (Gentile et al., 2012; Zerem, 2014; Hawiger, 2018). This study advances two concepts: the order of induction of SIRS and CARS and immune-related susceptibility genes during AP

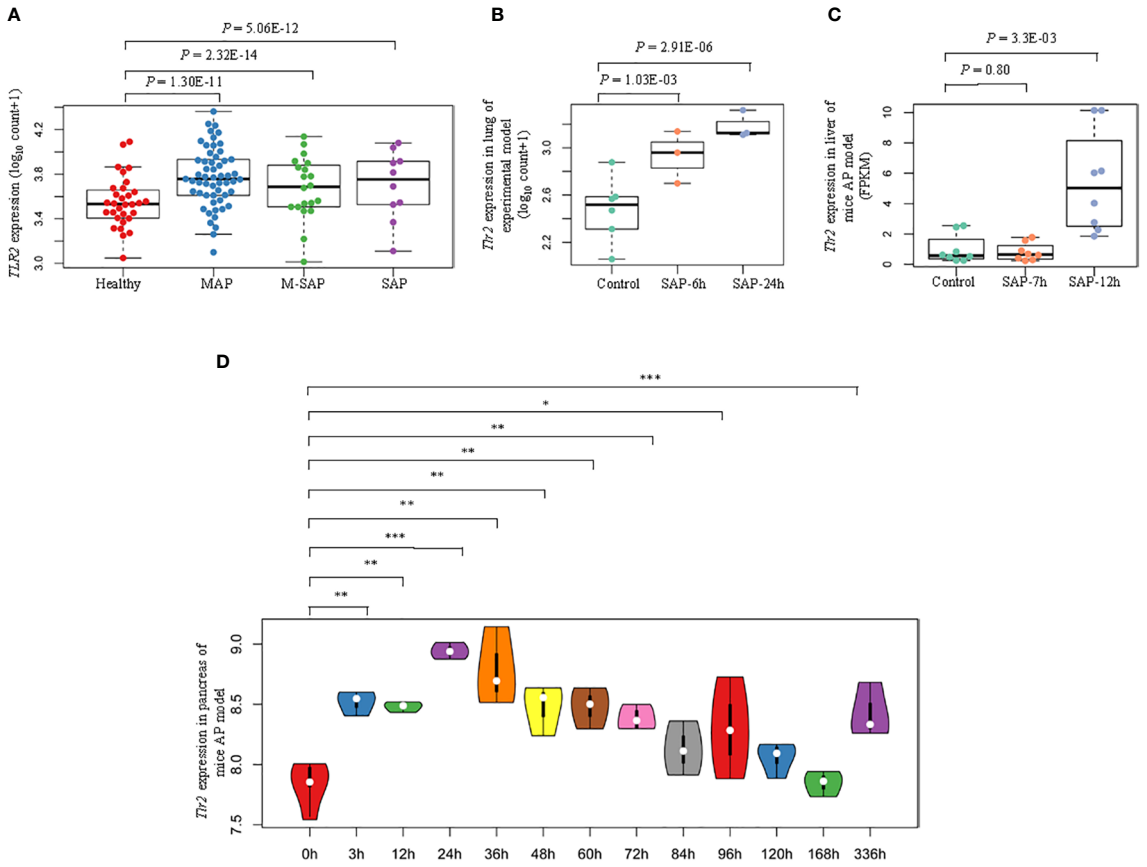

FIGURE 5
Schematic diagram of identified epigenetic aberrations of *TLR2* in AP. **(A)** Box plots indicate the differential expression patterns of *TLR2* between each severity level of AP (MAP, M-SAP, SAP) and normal controls in human peripheral blood. **(B)** Box plots represent the differential expression profiles of *Tlr2* at 6 hours and 24 hours after lung injury and **(C)** at 7 hours and 12 hours after liver injury in the cerulein-induced AP mouse model. **(D)** Violin plot indicating dynamic fluctuations in *Tlr2* expression at thirteen consecutive time points in pancreatic tissue from the AP mouse model: *P < 0.05; **P < 0.01; ***P < 0.001.

initiation and progression. On the one hand, our results strongly suggested that SIRS and CARS were induced concurrently, not consecutively, during the course and aggravation of AP. On the other hand, 10 immune-related hub genes for AP, namely, *SOSC3, ITGAM, CAMP, FPR1, IL1R1, TLR2, S100A8/9, HK3* and *MMP9*, were identified in patients with different severity levels of AP compared to a common set of healthy donors, and these dysregulated expression levels were validated in various tissues from an AP experimental model. Among the central genes, *S100A8/9, MMP9, SOCS3* and *IL1R1* have been widely reported to be associated with the progression or tissue injuries in AP (Norman et al., 1996; Farkas et al., 2014; Shi et al., 2018; Qin et al., 2019). Therefore, *CAMP, FPR1, TLR2, HK3* and *ITGAM* are novel targets for AP that need to be given more attention in future studies. The *TLR* family plays an essential role in AP by supporting recognition of conserved structures of microorganisms by immune cells and promoting SIRS to induce tissue damage; however, the effects of *TLR2* in AP remain controversial (Awla et al., 2011; Gorskii et al., 2014; Lee and Papachristou, 2019). Hence, we performed several experiments *in vivo* to highlight *TLR2* as a risk gene for AP that amplifies local inflammation and pancreatic injuries by inducing pro-inflammatory neutrophil activation.

Abnormal immune responses are an essential contributor to the pathogenesis of AP (Mayerle et al., 2012; Lee and Papachristou, 2019). Notably, previous studies indicated that AP patients' disease progression and treatment outcomes are strongly related to innate and adaptive immune cell infiltration (Bhatnagar et al., 2001; Hatano et al., 2001; Van Gassen et al., 2015; Caluianu et al., 2017; Schmidt et al., 2017; Zhao et al., 2018; Yang et al., 2021). However, because of the inability to obtain resected pancreatic tissue from AP patients and the limited number of immune cells obtained from the pancreas in the AP mouse model, a systemic analysis of infiltrating immune cells during the different phases of AP is currently lacking. Hence, we conducted microenvironment analysis with a large number of

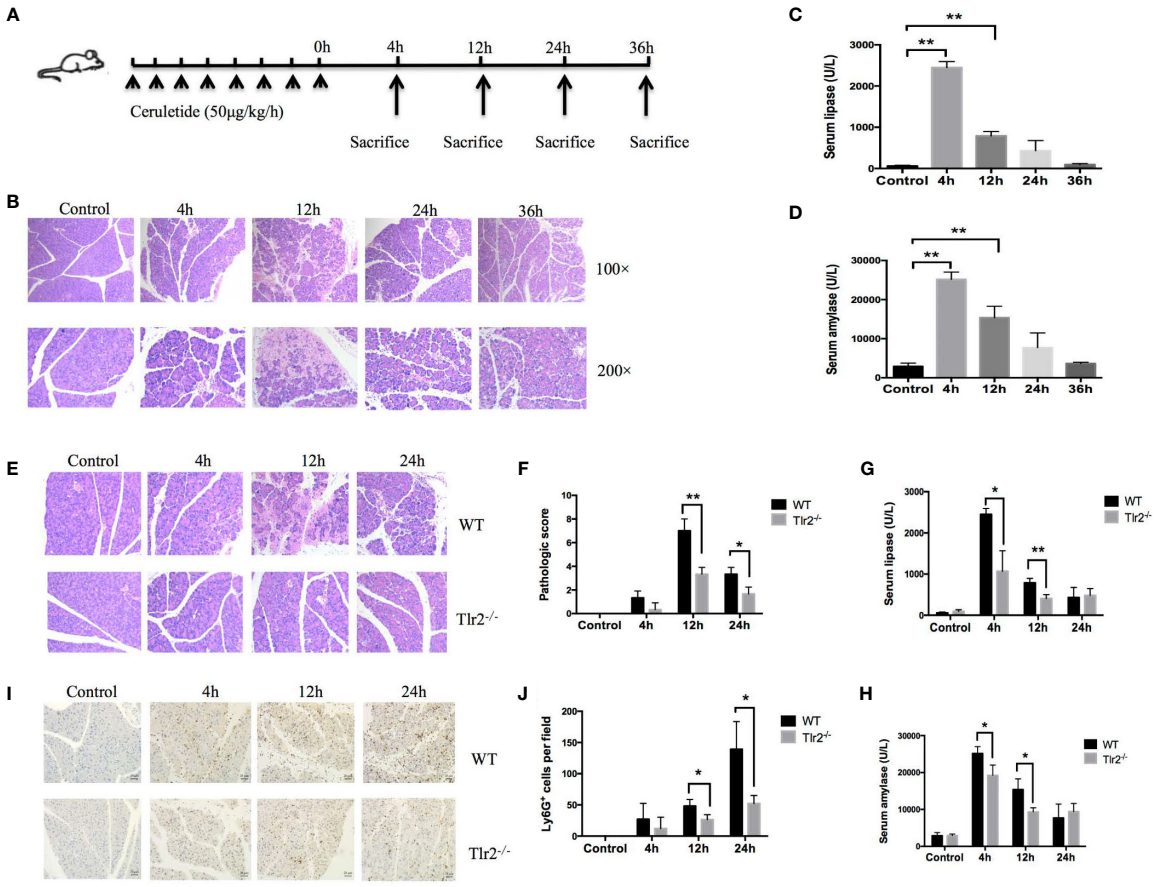

FIGURE 6
Tlr2 deficiency alleviated pancreatic inflammation in a cerulein-induced AP mouse model. **(A)** Illustration of the experimental protocol. **(B)** Histological analysis of mouse pancreases in the cerulein-induced acute pancreatitis mouse model (original magnification, 100× or 200×). **(C, D)** Serum lipase and amylase were detected in a cerulein-induced acute pancreatitis mouse model. **(E)** H&E staining of pancreas samples from wild-type (WT) and *Tlr2*$^{-/-}$ mice (original magnification, 200×). **(F)** Pancreas injury scores were evaluated based on H&E staining. **(G, H)** Serum lipase and amylase levels in the WT and *Tlr2* $^{-/-}$ groups are displayed. **(I)** Immunostaining for neutrophils was performed. The sections were stained for Ly6G to confirm the infiltration of neutrophils in the pancreas (original magnification, 200×). **(J)** The numbers of Ly6G + cells were counted based on immunostaining. Data are shown as the mean ± SD (n = 3/group). These data are representative of three independent experiments. *P < 0.05; **P < 0.01.

transcriptome profiles from AP patients' blood samples by using a transposed convolution algorithm in CIBERSORTx. This analysis provides integrative and dynamic insights into the regulatory network between AP progression and immune cell infiltration. CD4$^+$ T cells and CD8$^+$ T cells play an essential role in inducing pro-inflammatory cytokine production and provoking the immune response during the course of AP (Mayerle et al., 2012; Xu et al., 2020). In addition, activating CD4$^+$ T cells amplified the adaptive immune response and enlarged pathological changes in AP of different severity levels (Sendler et al., 2020; Xu et al., 2020). However, Yang et al. showed that CD4$^+$ T cells were significantly downregulated in pancreatic tissue in cerulein-induced or alcohol- and palmitoleic acid-induced MAP mouse models (Yang et al., 2021). In our study, we found that the numbers of CD8$^+$ T cells and resting memory CD4$^+$ T cells were significantly decreased and that those of activated memory CD4$^+$ T cells were significantly increased following disease aggravation. Previous studies indicated that the pro-inflammatory response is activated in the early stage and that the anti-inflammatory response is activated in the later stage of AP, which is characterized by high expression of anti-inflammatory cytokines and anti-inflammatory immune cell infiltration (Lee et al., 2017; Lee and Papachristou, 2019). We found not only anti-inflammatory cells, including M0 macrophages and M2 macrophages, were significantly activated, followed by the worsening of AP progression but also that production of anti-inflammatory related cytokines was significantly stimulated. For example, expression of the anti-inflammatory cytokine *IL10*, which plays an essential role in increasing the Treg response, was

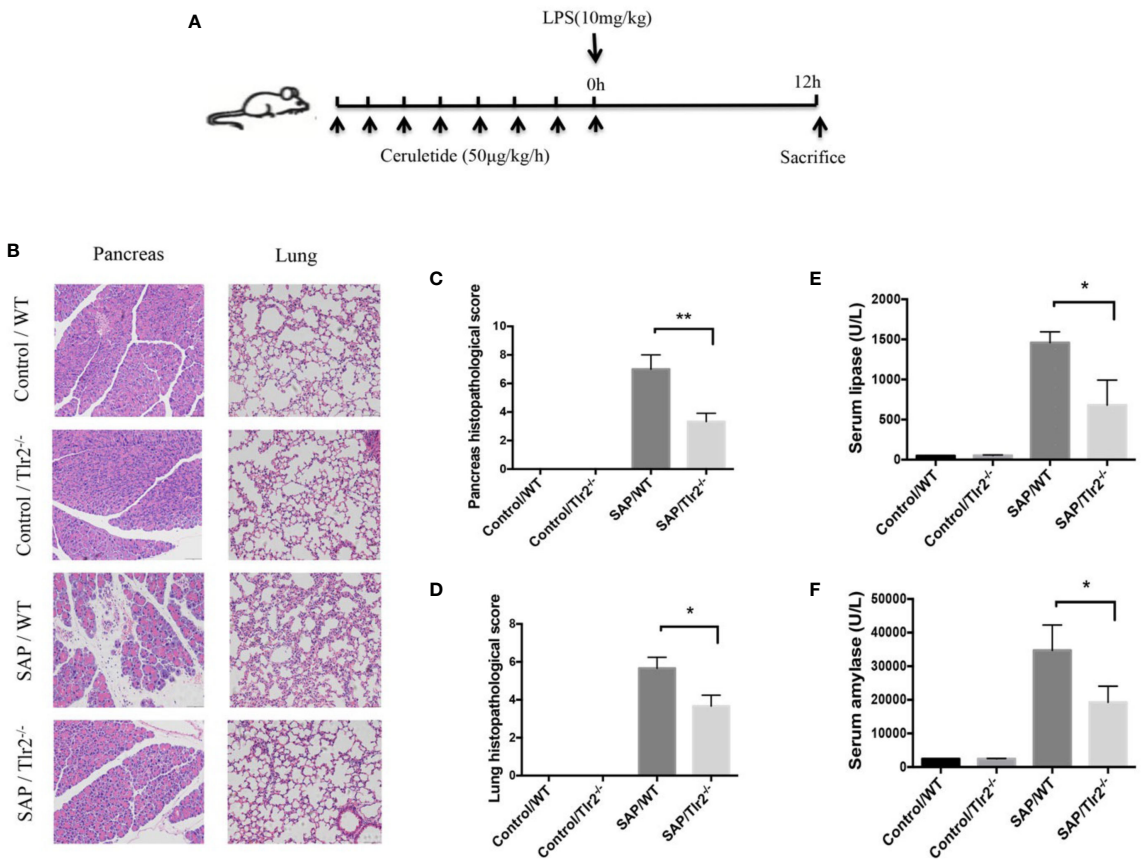

FIGURE 7
Tlr2 deficiency alleviated pancreatic and pulmonary injury in a SAP mouse model. **(A)** Illustration of the experimental protocol. **(B)** H&E staining of pancreas and lungs samples from wild-type (WT) and *Tlr2*$^{-/-}$ mice (original magnification, 200×). **(C)** Pancreatic injury scores were evaluated based on H&E staining. **(D)** Pulmonary injury scores were evaluated based on H&E staining. **(E, F)** Serum lipase and amylase levels in the WT and *Tlr2* $^{-/-}$ groups are displayed. Data are shown as the mean ± SD (n = 3/group). These data are representative of two independent experiments. *P < 0.05; **P < 0.01.

significantly elevated during AP aggravation (Sendler et al., 2020). In addition, transcriptional analysis of chemokines and chemokine receptors supported the idea that anti-inflammatory responses and inflammatory responses emerge simultaneously in AP. The significantly dysregulated chemokines and chemokine receptors were all downregulated. However, previous studies reported that inhibition of chemokines and chemokines in an AP mouse model exerted protective effects against pancreas and distant organ injury (Frossard et al., 2011; Malla et al., 2016). These inconsistent results suggest that SIRS and CARS are activated simultaneously during the progression of AP, and balancing the intensity and order of induction of the immune response might be an effective therapeutic approach for AP.

Many studies have shown that the course and severity of AP are largely determined by the crosstalk between innate and adaptive immune responses (Sendler et al., 2020; Xu et al., 2020; Nesvaderani et al., 2022), yet the essential genes remain largely unclear. In the current study, by conducting an integrative analysis of samples from AP patients and injured tissues from an AP animal model, we intended to explore the susceptibility genes and biological pathways involved in the pathogenesis of AP. As a result, we highlighted 43 highly interacting DEGs that were common to at least four different groups. Spearman correlation analysis indicated that the majority of 43 candidate genes were positively correlated with neutrophil, M0 and M2 macrophage cell proportions and negatively correlated with the proportions of adaptive immune cells, such as resting memory $CD4^+$ T cells and $CD8^+$ T cells. More interestingly, enrichment analysis showed that the 43 susceptibility genes in AP were significantly enriched in biological processes related to the immune response, which is consistent with the notion that function-related genes contribute to the risk of complex disease (Chen et al., 2011). In addition, the MCC algorithm scores from cytoHubba were used to select *SOSC3, ITGAM, CAMP, FPR1, IL1R1, TLR2, S100A8/9, HK3*

and *MMP9* as the top 10 enriched genes for AP. Notably, 5 of 10 hub genes have been reported to be associated with physiological and pathophysiological phenotypes of AP. For example, as a calcium sensor, *S100A8/9* forms a heterodimer that is constitutively expressed in neutrophils and monocytes, contributing to the induction of leukocyte recruitment and the stimulation of cytokine secretion during the inflammatory response (Wang et al., 2018). Several studies have shown that *MMP9* is stimulated by *S100A8/9* under inflammatory conditions to strengthen the immune response during the progression of AP (Shi et al., 2018; Wang et al., 2018). In the SAP experimental model, LPS-induced overexpression of *Sosc3* exacerbated MAP, leading to SAP (Zhou et al., 2015). In addition, we detected that a large proportion of the 10 genes were involved in the blue network module based on WGCNA, and the fluctuating expression patterns of these genes showed positive correlation in human and animal models. These results indicate that these targets may perform similar functions to mediate immune cell infiltration and the immune response and influence the initiation and progression of AP.

TLRs perform multiple functions in different types of immune cells, and TLR signaling is related to the activation of adaptive immunity. There are still large gaps in our understanding of TLRs (Fitzgerald and Kagan, 2020). Although *TLR4* has been widely reported to be associated with increased SIRS and tissue injury, the biological functions of *TLR2* in AP remain controversial (Awla et al., 2011; Gorskii et al., 2014; Lee and Papachristou, 2019). Interestingly, based on an integrative analysis, we found that *TLR2* was dramatically overexpressed in blood samples from MAP, M-SAP and SAP patients compared with healthy donors, with similar expression profiles in impaired pancreas and lung tissues from animal models of MAP and SAP. These findings are consistent with a previous study showing that *TLR2* was overexpressed in peripheral blood mononuclear cells from AP patients compared with healthy controls (Gorskii et al., 2014). However, a paradoxical result indicated that a *Tlr2*-deficient mouse model did not exhibit an obvious difference from WT mice after treatment with taurocholate (Awla et al., 2011). These contradictory results might result from the use of different methods to induce MAP. Our study showed that *Tlr2* deficiency in mice with cerulein-induced AP resulted in alleviated pancreas injury and reduced neutrophil accumulation within the pancreas during AP compared with those in WT mice. Herein, our findings imply that *TLR2* was a key factor and was significantly activated in MAP, M-SAP and SAP patients, contributing to the severity of AP, leading to the accumulation of neutrophil infiltration, and increasing pancreatic necrosis and distant organ impairment.

Several limitations of the current study are worth noting. First, although we presented a variety of evidence from human peripheral blood and animal models to support the conclusion that the 10 susceptibility genes of AP are linked to immune cell infiltration, we could not determine to what extent each gene contributes to regulating the immune response in AP, nor could we further assess the regulation of these targets. Second, we speculate that sustaining dysregulated *TLR2* expression would be even worse in clinical patients with SAP or in an aggravated AP animal model with more severe SIRS. Thus, more studies are needed to evaluate the contributions and functions of *TLR2* in SAP patients and SAP mouse models.

In summary, by conducting an integrative bioinformatics analysis of transcriptional sequencing data from human peripheral blood and AP animal models, we showed that pro-inflammatory and anti-inflammatory responses induce the progression of AP in parallel and synergistically. In addition, we discovered 10 highly connected susceptibility genes that regulate the immune response to influence the course and severity of AP. Subsequently, we found that knockout of *Tlr2* alleviated organ impairment and neutrophil accumulation in the pancreas. Therefore, by pinpointing which immune responses and susceptibility genes affect the initiation and aggravation of AP and investigating the underlying mechanisms, this study provides a solid foundation for understanding the progression of AP and identifies several novel therapeutic targets that mediate the dysregulation of pro-inflammatory and anti-inflammatory responses during AP.

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 animal study was reviewed and approved by The Zhejiang University Animal Care and Use Committee.

Author contributions

QL, LL, and DX collected the data, performed the bioinformatic analysis, AP animal model experiments, and wrote the manuscript. JZ, ZH, SC, LZ, and YG were involved in data collection and reviewed the manuscript. XZ and HS conceived the current study and reviewed the manuscript. All authors contributed to the article and approved the submitted version.

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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/fcimb.2022.1052466/full#supplementary-material

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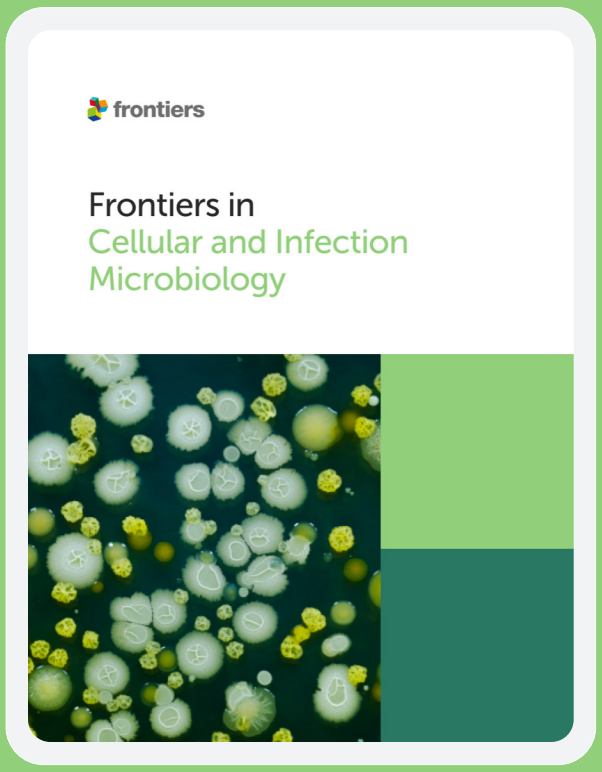

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