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Expression of mir-25-3p, CARD9 and surviving in acute pancreatitis and predictive value for patient outcome

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ABSTRACT

The clinical data of 246 patients with acute pancreatitis who met the inclusion and exclusion criteria in our hospital from May 2018 to May 2020 were collected as the modeling group, and 96 patients were used as the model validation group. To observe the expression of mir-25-3p, CARD9 and Survivin in patients with acute pancreatitis. To analyze the prognostic factors of acute pancreatitis by univariate and multivariate analysis, and to establish and validate the prognostic model of acute pancreatitis. Results: There was no significant difference in general data between the two groups (P>0.05). Of 246 AP patients, 217 survived and 29 died. The APACHEI score, BISAP score, CRP, lipase, lactate, mir-25-3p, CARD9 and Survivin in the survival group were lower than those in the death group, and the differences were statistically significant (P<0.05). There was no significant difference in other indexes (P>0.05). APACHEI score, BISAP score, CRP, lipase, lactate, mir-25-3p, CARD9 and Survivin were included in multivariate logistic regression analysis. Survival =1 and death =0 were the dependent variables. BISAP score, CRP, lipase, lactate, mir-25-3p, CARD9 and Survivin were all protective factors for the survival of AP patients. Log(P)=-1.648×BISAP score -0.045×CRP-0.013× lipase-0.205 × lactate-1.339 × Mir-25-3P-2.701 ×CARD9-1.663×Survivin+43.925. The survival protective factors of AP patients were incorporated into R software to establish the nomogram prediction model.

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Introduction

Acute pancreatitis (AP) is a disorder of pancreatic secretory function caused by abnormal activation of pancreatic precursors. Acute pancreatitis is a very complex disease (1), which may lead to severe septic shock, accompanied by severe inflammation, organ failure and other complications (2). Therefore, it is of great significance to find indicators that can assess the prognosis of AP. There are many factors affecting the prognosis of AP. Studies have shown that Acute Physiology and Chronic Health (APACHEI) score, Bedside Index for Severity in Acute Pancreatitis (BISAP) score and C-reactive protein (CRP) are prognostic factors of AP (3). In addition, lipase and lactate are higher in patients with poor prognoses than in patients with good prognoses (4).

MiRNA is a single-stranded RNA containing 18-24 nucleotides, which has no ability to encode proteins and can participate in cell proliferation, apoptosis, autophagy and other activities (5). Studies have shown (6) that Mir-25-3p can promote the proliferation of kidney cells by regulating ATG (ATG) 14-Beclin 1, thereby inhibiting renal autophagy. Autophagy plays an important role in the occurrence and development of AP. In addition, decreased autophagy flux correlated with the severity of the inflammatory response. In addition, Mir-25-3p can participate in the occurrence and development of AP by regulating autophagy, so as to detect the level of Mir-25-3p in AP and judge the prognosis of AP (7). Caspase polymerase zone protein 9 (CARD9) is a highly expressed anti-inflammatory protein, which plays a key role in the activation of inflammatory reactions such as NF-XB and P38 MAPK, and participates in inflammatory reactions. Previous studies have reported that the expression of CARD9 protein and mRNA in the peripheral blood of patients with early AP is significantly increased, and there is a certain relationship with the severity of pancreatitis (8). Other studies have found (9) that the expression of Survivin in AP tissues is related to apoptosis and is closely related to its clinicopathology. In addition, the expression of Survivin was also significantly changed in AP rats (10). However, most studies on the expression of Survivin in pancreatitis have been carried out in animal experiments, and few clinical reports have been reported. Therefore, this study focused on analyzing the expression of mir-25-3p, CARD9 and Survivin in acute pancreatitis, and exploring the predictive value of the three in the prognosis of AP patients, and establishing a predictive model for the prognosis of AP patients, in order to improve the prognosis of AP patients.

Materials and Methods

General information

The clinical data of 246 patients with acute pancreatitis who met the inclusion and exclusion criteria in our hospital from May 2018 to May 2020 were collected as the modeling group, and 96 patients were used as the model validation group.

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Inclusion criteria

(a) All included subjects met the definition of acute pancreatitis in the Chinese Guidelines for the Diagnosis and Treatment of Acute pancreatitis (2021) (11) and were hospitalized within 24 hours after the onset of initial symptoms;

(b) None of the patients received any medical treatment before admission and were treated in our hospital after admission;

(c) Complete clinical data.

Exclusion criteria:

(a) Other acute or chronic infectious diseases within 1 month before the onset of AP;

(b) Major surgery, acute or chronic infection, or major traumatic stress in the 30 days prior to AP;

(c) A history of liver, kidney, lung, heart insufficiency or diabetes mellitus;

(d) Patients with malignant tumours;

(e) Those without Mir-25-3p, CARD9 and Survivin detection.

(f) Loss of follow-up or withdrawal.

This study was reviewed and approved by the Ethics Committee of our hospital. Informed consent was obtained from the patient and his family.

Material and methods

The clinical data of AP patients were collected by a self-made questionnaire. Cronbach's A =0.886. The contents of the questionnaire included age, gender, body mass index (BMI), etiology, Acute Physiology and Chronic Health (APACHEI) score (12), BISAP score (13), and experimental examination indexes: White blood cell (WBC), C-reactive protein (CRP), lipase, amylase, lactic acid, Mir-25-3p, CARD9, Survivin expression.

Questionnaire investigators were trained, passed the examination and went to work. Before the questionnaire survey, they explained the research purpose, research significance and questionnaire-filling methods to the patients. The questionnaire was filled in half an hour, and the questionnaires were collected on time to ensure that the patients completed all the questionnaires.

Patients were divided into a survival group and a death group according to their prognosis after 1 month.

Observation indicators

The expression of Mir-25-3p, CARD9 and Survivin in patients with acute pancreatitis was observed, and the expression differences of Mir-25-3p, CARD9 and Survivin in the two groups were compared. The predictive value of Mir-25-3p, CARD9 and Survivin in the prognosis of AP patients was established and verified.

Statistical methods

SPSS 25.0 software and R software were used to analyze the data, and the collected measurement data were tested for normality by the Shapiro-Wilk method. P>0.05 was the normal distribution data represented by (mean ± standard deviation), and t-test was used to show that P<0.05 was the non-normal distribution data described by the median (quartile). Mann-Whitney U test. The count data collected were expressed as (%). The 2 or Fisher exact test was used for disordered data, and the Mann-Whitney U test was used for ordered data. Univariate and multivariate logistic regression analyses were used to analyze the prognostic factors of AP, and the nomogram prediction model was established. The discriminant ability of the validation set and the calibration map were used to evaluate the accuracy of the nomogram. The area under the receiver operating characteristic (ROC) curve (AUC) was used to evaluate the discrimination ability of the nomogram. The calibration curve of the model was calculated, and the consistency of the model was verified by the Hosmer-Lemmeshaw test. The decision curve analysis (DCA) is also carried out to evaluate the discriminative ability of the model. P<0.05 was considered statistically significant.

Results

General information

There was no significant difference in general data

Table 1. Comparison of general data between the modeling group and validation group.

Index		Validation group (n=96)	Modeling group (n=246)	t/χ^2/U	P
Age (years)		51.6±2.9	51.9±2.9	0.860	0.391
Gender (case)	Male	67	150	2.314	0.128
	Female	29	96		
BMI (kg/ m^2)		25.28±3.49	25.65±3.51	0.939	0.349
Etiology (case)	Cholelith disease	37	89	0.407	0.939
	Alcohol	26	75		
	Hyperlipidemia	30	75		
	Other	3	7		
APACHEIscore (points)		9.7±1.9	9.5±1.8	0.909	0.364
BISAP		3.1±0.8	3.0±0.8	1.039	0.300
WBC (×10^9/L)		18.61±3.28	18.84±3.29	0.507	0.612
CRP (mg/ L)		92.65±17.31	95.89±16.22	1.629	0.104
Amylase (U/ L)		511.84±29.58	506.92±30.16	1.363	0.174
Lipase (U/ L)		526.75±62.51	529.41±59.21	0.368	0.714
Lactic_acid (mg/ L)		26.95±4.51	27.84±4.56	1.919	0.056
miR-25-3p		3.89±0.89	3.91±0.88	1.627	0.105
CARD9		3.78±0.62	3.82±0.59	0.555	0.579
Survivin		3.75±0.49	3.79±0.50	0.669	0.504

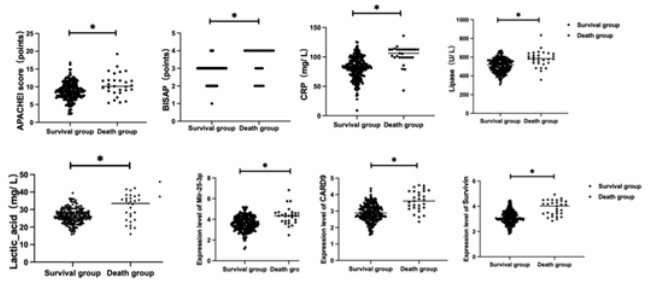

Figure 1. Univariate analysis of the modeling group.

between the two groups (P>0.05). Showing Table 1.

Univariate analysis of modeling group

Of 246 AP patients, 217 survived and 29 died. The APACHEI score, BISAP score, CRP, lipase, lactate, Mir-25-3p, CARD9 and Survivin in the survival group were lower than those in the death group, and the differences were statistically significant (P<0.05). There was no significant difference in other indexes (P>0.05) (Figure 1).

Multivariate analysis

APACHEI score, BISAP score, CRP, lipase, lactate, Mir-25-3p, CARD9 and Survivin were included in multivariate logistic regression analysis. Survival =1 and death =0 were the dependent variables. BISAP score, CRP, lipase, lactate, Mir-25-3p, CARD9 and Survivin were all protective factors for the survival of AP patients (Figure 2). Log(P)=-1.648×BISAP score -0.045×CRP-0.013× lipase-0.205 × lactate-1.339 × Mir-25-3P-2.701 ×CARD9-1.663×Survivin+43.925.

Nomogram model establishment and verification.

The protective factors of AP patients' survival were incorporated into R software to establish a nomogram prediction model (Figure 3), and the prediction probability corresponding to the sum of the integral of each protective factor was the probability of survival. The nomogram showed that the survival probability increased with the decrease of BISAP score, CRP, lipase, lactate, Mir-25-3p, CARD9 and Survivin. If the predicted probability of a patient is greater than the maximum Youden index (0.865), the patient's survival is predicted.

The area under the ROC curve of the nomogram prediction model of the modeling group was 0.964(95%CI :0. 922-1). The AUC areas of BISAP, CRP, LIPase, Lactic acid, Mir-25-3p, CARD9 and Survivin were 0.739, 0.843, 0.753, 0.793, 0.759, 0.808 and 0.825, respectively (Figure 4). In the validation group, 80 of 96 AP patients survived and 16 died. The nomogram was used to predict the survival probability of the validation group, and the ROC curve was drawn. The AUC area of the model was 1. The areas of BISAP, CRP, LIPase, Lactic acid, miR_253p, CARD9 and SurvivinAUC were 0.845, 0.998, 0.805, 0.975, 0.861,

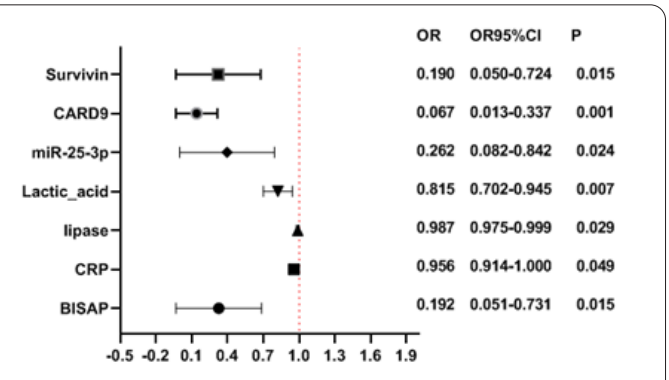

Figure 2. Multivariate analysis of APACHEI score, BISAP score, CRP, lipase, lactate, Mir-25-3p, CARD9 and Survivin.

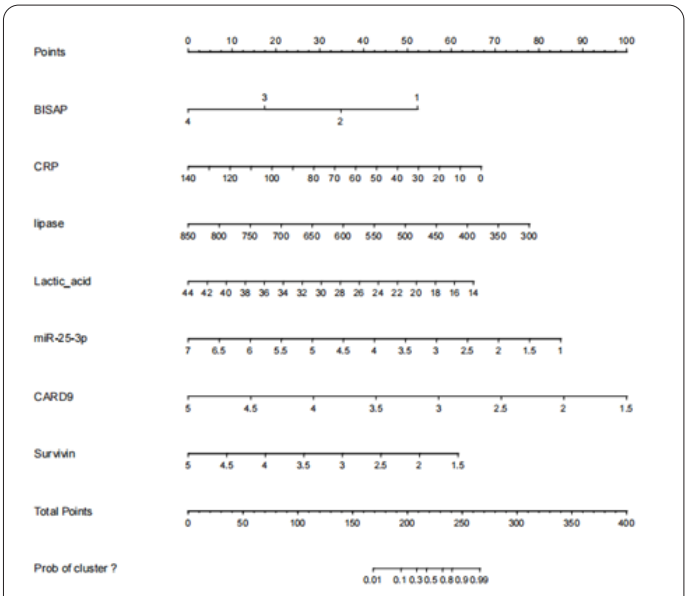

Figure 3. Nomogram of APACHEI score, BISAP score, CRP, lipase, lactate, Mir-25-3p, CARD9 and Survivin.

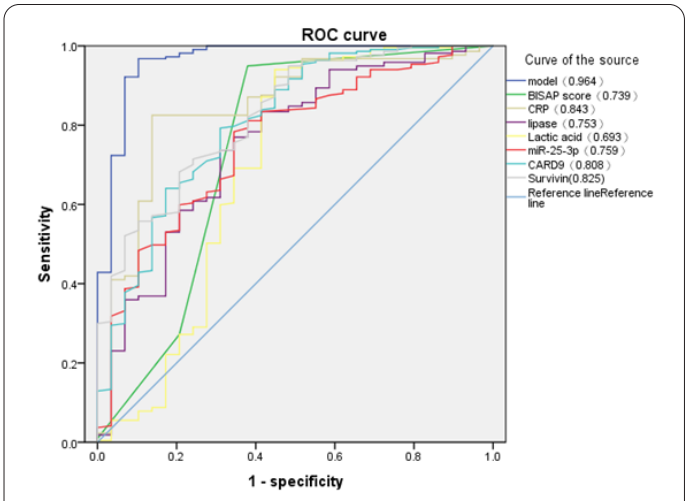

Figure 4. ROC curve of the modeling group.

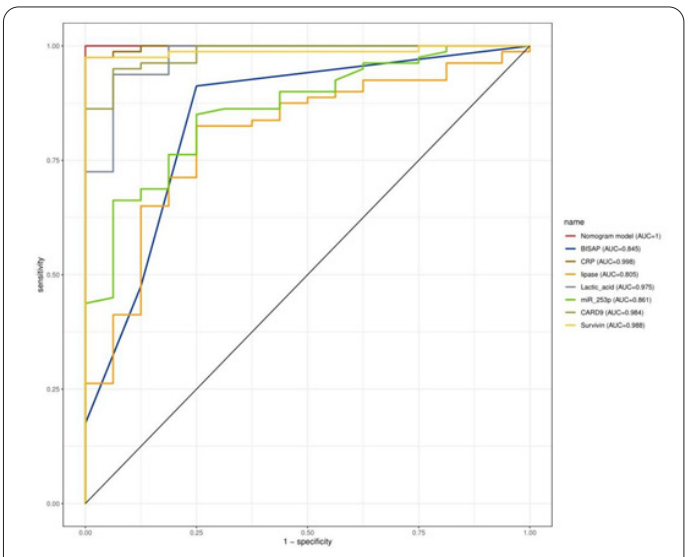

Figure 5. ROC curve of validation group.

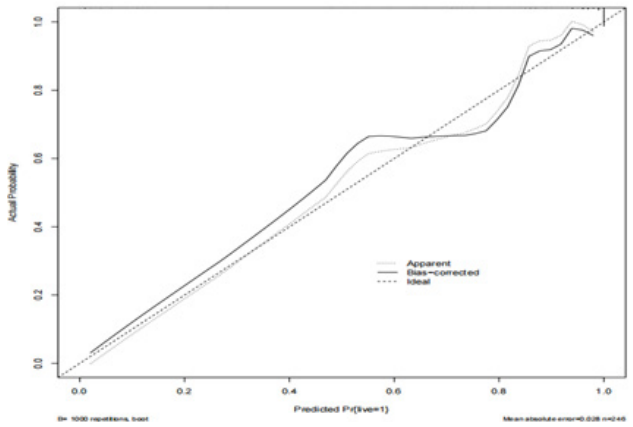

Figure 6. Calibration curve of the nomogram of the verification group.

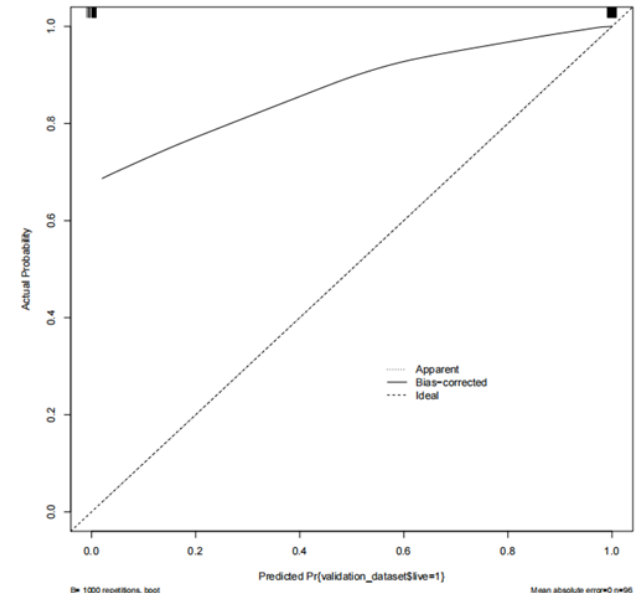

Figure 7. Calibration curve of the nomogram of the verification group.

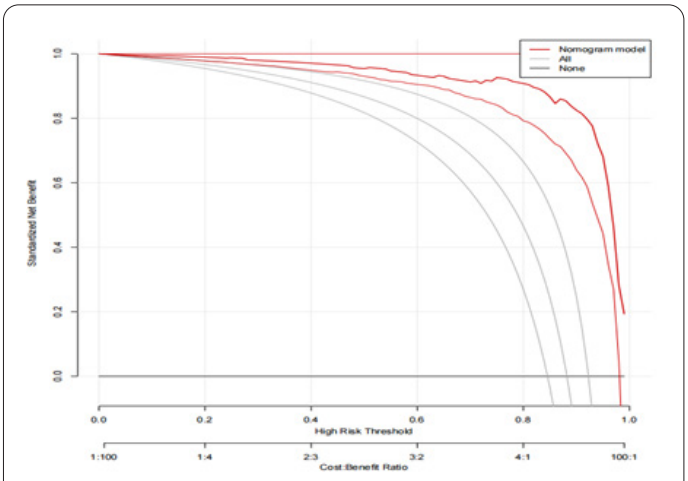

Figure 8. Modeling group prediction model DCA.

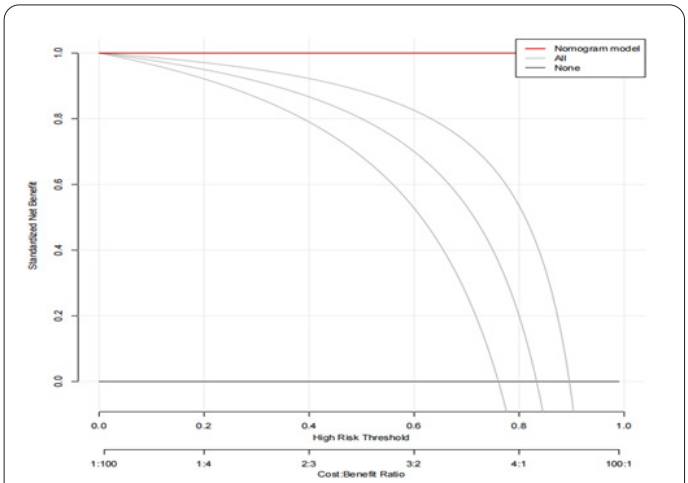

Figure 9. DCA of the prediction model in the validation group.

0.984 and 0.988, respectively (Figure 5). The slope of the nomogram calibration curve in the modeling group was close to 1 (Figure 6), and P>0.05 in the degree of fit test, indicating a high consistency between the predicted event and the actual event. The slope of the nomogram calibration curve in the validation group was close to 1 (Figure 7), and P>0.05 in the degree of the fitting test.

The decision analysis curves of the modeling group and the validation prediction model (Figure 8 and Figure 9). The X-axis represents the threshold probability, the Y-axis represents the net revenue, and the solid black line represents the net revenue of the nomogram prediction model, which also confirms the effectiveness of the nomogram prediction model.

Discussion

AP patients present with necrosis, edema, and bleeding of the pancreas as a whole or locally, and the clinical manifestations of this disease vary greatly (14). After the onset of AP and pancreatic injury, a large amount of pancreatic enzymes are released, thus causing damage to its own tissue, and then causing related inflammatory and immune reactions (15). Mild AP is more common in clinical practice. If the condition deteriorates to severe AP, the prognosis is poor, and severe AP may lead to death (16). In this study, the predictive value of Mir-25-3p, CARD9 and Surviving for AP was clarified to establish a prediction model.

In this study, the modeling group and the validation group were selected to establish and verify the prediction model for the prognosis of AP patients. The general data of the two groups were not statistically significant, and subsequent experiments could be carried out. The univariate analysis of survival and death patients in the modeling group showed that The PACHEI score, BISAP score, CRP, lipase, lactate, Mir-25-3p, CARD9 and Surviving were lower than those in the death group, and the differences were statistically significant (P<0.05). APACHEI score, BISAP score, CRP, lipase, lactate, Mir-25-3p, CARD9 and Surviving were included in multivariate logistic regression analysis. Survival =1 and death =0 were the dependent variables. BISAP score, CRP, lipase, lactate, Mir-25-3p, CARD9 and Surviving were all protective factors for the survival of AP patients. APACHEI score and BISAP score play an important role in disease progression. The higher the score, the more severe the disease may be (17). Pynnonen L et al. also showed in their study (18) that CRP, lipase and lactate were related to the prognosis of AP patients, and the levels of CRP, lipase and lactate were lower in surviving patients.

The expressions of Mir-25-3p, CARD9 and Survivin in AP death patients were significantly higher than those in survival patients. Studies have shown (19) that the levels of Mir-25-3p in the survival group and the death group increased with the extension of treatment time, and the

levels of Mir-25-3p in the TO, T1 and T2 phases of the survival group were all higher than those in the death group, suggesting that Mir-25-3p is related TO the progression of AP, and its level may be used TO evaluate the prognosis of AP. The ROC curve of Mir-25-3p was constructed TO evaluate the prognosis of AP. The results showed that the AUC and sensitivity of Mir-25-3p in TO phase were high, but the specificity was low, suggesting that Mir-25-3p in TO phase had certain value in the prognosis assessment of AP, and it could assist in the assessment of the prognosis of AP. This is consistent with the conclusion of this study. In addition, CARD9 is a novel adaptor protein with high expression in macrophages, which can resist bacterial and fungal infections. It can also form a complex with B-cell lymphoma factor-10 (Bcl-10), activate downstream NF-XB and P38 MAPK pathways, and its expression is increased in early AP patients (20). Survivin is one of the most potent inhibitors of apoptosis, which can inhibit angiogenesis, apoptosis and promote cell transformation. Under normal circumstances,Survivin is expressed only in embryonic tissues, thymus, and gonads and is not expressed or very low in adult pancreatic tissues (21). Studies have reported (22) that Survivin can play an inhibitory role in apoptosis through various regulatory pathways, and such an inhibitory effect is premised on the negative regulation of Survivin by the P53 gene at MR-NA and protein levels. The BIR unit is the key structure of Survivin to play the role of apoptosis. The BIR unit contains a variety of amino acid residues, which can bind to caspase-3 to inhibit its activity, prevent apoptotic signal and inhibit cell apoptosis. Moreover, Sur-Vivin contains a double helix structure, which has a certain relationship with its anti-apoptosis effect (23). The expression intensity of Survivin in pancreatitis mice gradually increased with the extension of modeling time. The expression intensity of Survivin in tissue samples obtained at 24h after modeling was significantly stronger than that at 6 and 12 h after modeling, which confirmed that Survivin expression was associated with the degree of inflammation in pancreatitis (24). Some studies have suggested (25) that Survivin can affect the severity of patients' diseases by inhibiting the apoptosis of inflammatory cells. This process may be attributed to the effect of Survivin on the apoptosis of acinar cells in patients with acute pancreatitis through its anti-apoptotic effect, which aggravates the condition of pancreatic cells affected by inflammation from apoptosis to necrosis.

In this study, a prognostic model for AP patients was established based on Mir-25-3p, CARD9, Survivin, BISAP score, CRP, lipase, and lactate. The nomogram results showed that The survival probability increased with the decrease of BISAP score, CRP, lipase, lactate, Mir-25-3p, CARD9 and Survivin. In the validation group, the nomogram was used to predict the survival probability of the validation group, and the ROC curve was drawn. The AUC area of the model was 1, and the slope of the nomogram calibration curve of the modeling group and the validation group was close to 1, and the fitting degree test $P>0.05$ showed that the consistency between the predicted event and the actual event was high. The DCA of the two groups also further confirmed the validity of the nomogram prediction model. It shows that the model has good predictive value.

In conclusion, the expressions of Mir-25-3p, CARD9 and Survivin are increased in patients with acute pancreatitis death, and the predictive value of Mir-25-3p, CARD9 and Survivin for the prognosis of AP patients is high. The prediction model based on Mir-25-3p, CARD9 and Survivin had the largest AUC area and the highest predictive value for the prognosis of AP patients, and the predicted value was consistent with the actual value. For patients with low predicted survival probability of AP patients, timely intervention should be conducted to improve the prognosis of patients. However, the sample size of this study is small, and it is necessary to further expand the large sample size and prospective studies to verify and improve the model.

Data Availability

The experimental data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declared that they have no conflicts of interest regarding this work.

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