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Predictive value of peripheral blood TLR4/NF-κB/NLRP3 mRNA for in-hospital death in patients with severe acute pancreatitis

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Abstract: Objective To analyze the expression changes of peripheral blood Toll-like receptor 4 (TLR4) , nuclear factor- κB (NF-κB) , NOD-like receptor thermal protein domain associated protein 3 (NLRP3) mRNA in patients with severe acute pancreatitis (SAP) at different clinical disease stages, and to explore their predictive value for in-hospital death risk in SAP patients. **Methods** A prospective cohort study was conducted. A total of 100 SAP patients admitted to the Department of Critical Care Medicine, Affiliated Hospital of Inner Mongolia Medical University from January 2024 to January 2025 were enrolled. They were divided according to clinical stages into the acute response phase (n=50) , systemic infection phase (n=30) , and residual infection phase (n=20) .Based on in-hospital prognosis, patients were categorized into an in-hospital death group (n=24) and an in-hospital survival group (n=76) .The mRNA expression levels of TLR4, NF-κB, and NLRP3 were compared among groups. Multivariate logistic regression was used to assess the correlation of these indicators with in-hospital death, and receiver operating characteristic (ROC) curves were used to evaluate their predictive efficacy for in-hospital death. **Results** The expression levels of peripheral blood TLR4, NF-κB, and NLRP3 mRNA were significantly different among SAP patients at different disease stages (P<0.01). Levels in the systemic infection phase were significantly higher than those in the acute response and residual infection phases (P<0.05). Multivariate logistic regression showed that high Acute Physiology and Chronic Health Evaluation Ⅱ (APACHE Ⅱ) score within 24 h, high Sequential Organ Failure Assessment (SOFA) score within 24 h, systemic infection phase, low serum calcium level, high CRP level, and high expression of TLR4 mRNA (OR=1.893, 95%CI: 1.365-2.637), NF-κB mRNA (OR=1.278, 95%CI: 1.046-1.560), and NLRP3 mRNA (OR=1.962, 95%CI: 1.379-2.791) were independent risk factors for in-hospital death (P<0.05) . ROC curve analysis showed that the area under the curve (AUC) for predicting in-hospital death risk in SAP patients based on peripheral blood TLR4, NF-κB, and NLRP3 mRNA expression levels were 0.736, 0.708, and 0.782, respectively. The AUC for combined detection of the three indicators was 0.892, which was significantly higher than that of any single indicator (P<0.05). **Conclusion** The expression levels of TLR4, NF-κB, and NLRP3 mRNA in peripheral blood significantly differ across disease stages in SAP patients, and the expression levels are significantly elevated in patients in the systemic infection phase. This elevated expression is closely associated with an increased risk of in-hospital death. Combined detection of these three markers demonstrates higher predictive efficacy for death risk compared to any single indicator.

Keywords: Severe acute pancreatitis; Toll-like receptor 4; Nuclear factor-κB; NOD-like receptor thermal protein domain associated protein 3; In-hospital death risk

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Severe acute pancreatitis (SAP) is a common acute abdominal condition in clinical practice, characterized by rapid disease progression and an extremely poor prognosis. The all-cause in-hospital mortality rate is high, ranging from 15% to 30% [1-2]. In the early stage, SAP is dominated by systemic inflammatory response syndrome; while in the late stage, it is prone to complications such as infection, sepsis, and multiple organ failure. The pathophysiological mechanisms differ across disease stages, leading to distinctly different clinical intervention outcomes and prognoses [3]. Therefore, identifying specific biomarkers that reflect disease progression and assess mortality risk is of great clinical significance for optimizing stratified treatment strategies and improving patient outcomes in SAP. The toll-like receptor 4 (TLR4)/ nuclear factor (NF)-κB/ NOD-like receptor thermal protein domain associated protein 3 (NLRP3) pathway is a core signaling axis of the body's inflammatory response. Its aberrant activation can release pro-inflammatory cytokines such as interleukin (IL)-1β, IL-6, and tumor necrosis factor (TNF)-α, amplifying the systemic inflammatory cascade and participating in the pathological processes of pancreatic necrosis and multiple organ injury in SAP [4]. A study confirmed that TLR4, NF-κB, and NLRP3 are abnormally expressed in the peripheral blood of SAP patients [5]. However, their changing patterns across different disease stages (acute reaction phase, systemic infection phase, and residual infection phase) and their association with the risk of in-hospital all-cause mortality have not yet been clarified. Based on this, the present study aims to measure the mRNA expression levels of TLR4, NF-κB, and NLRP3 in the peripheral blood of SAP patients at different disease stages and to explore their value in assessing the risk of in-hospital mortality, thereby providing a theoretical basis for disease monitoring, risk stratification, and targeted therapy in SAP.

1 Subjects sand Methods

1.1 Study Subjects

This study was a prospective study. A total of 100 patients with SAP admitted to the Department of Critical Care Medicine of Affiliated Hospital of Inner Mongolia Medical University from January 2024 to January 2025 were collected.

Inclusion criteria:

(1) Met the diagnostic criteria for SAP according to the *Guidelines for Diagnosis and Treatment of Acute Pancreatitis in China (2021)* [6];

(2) Age ≥18 years;

(3) Time from onset to admission ≤72 h, with a clearly defined disease course stage;

(4) Voluntary participation in this study with signed informed consent;

(5) With complete clinical medical records and the ability to complete full follow-up until discharge or in-hospital death.

Exclusion criteria:

(1) Presence of other acute or chronic pancreatitis conditions (such as acute exacerbation of chronic pancreatitis or autoimmune pancreatitis);

(2) Previous history of pancreatic surgery or severe pancreatic insufficiency;

(3) Presence of malignant tumors, severe hepatic or renal failure, hematological diseases, immunodeficiency diseases, or long-term use of immunosuppressive agents;

(4) Pregnant or lactating women;

(5) Automatic discharge, transfer to another hospital, or abandonment of treatment within 24 h after admission;

(6) Systemic inflammatory response syndrome caused by other factors such as severe trauma, burns, or sepsis;

(7) Allergy to reagents related to the detection methods used in this study.

1.2 Detection of Peripheral Blood TLR4, NF-κB, and NLRP3 mRNA Expression Levels

Peripheral venous blood (5 mL) was collected from all patients within 24 h after admission and divided into two tubes: (1) 2 mL were placed into an anticoagulant tube containing EDTA. After gentle inversion and mixing, the sample was centrifuged at 4 °C and 3,000 r/min (centrifugal radius 10 cm) for 10 min. Peripheral blood mononuclear cells were isolated for total RNA extraction. (2) 3 mL were placed into a procoagulant tube, centrifuged to separate serum, and stored at −80 °C for later use.

Total RNA was extracted from peripheral blood mononuclear cells using the Trizol method. RNA concentration and purity were determined using an ultra-micro spectrophotometer, ensuring that the A260/A280 ratio ranged from 1.8 to 2.0. Subsequently, according to the instructions of the reverse transcription kit, 1 μg of total RNA was reverse-transcribed into complementary DNA (cDNA). The reaction conditions were 42 °C for 60 min and 70°C for 10 min.

Quantitative real-time polymerase chain reaction (qRT-PCR) was used to detect the mRNA expression levels of TLR4, NF-κB, and NLRP3, with GAPDH used as the internal reference gene. Primers were designed and synthesized by Sangon Biotech (Shanghai), and the sequences are shown in **Table 1**.

Tab.1 Primer sequence

Indicator	Upstream Sequence 5′→3′	Downstream Sequence 5′→3′
TLR4	TCG ATA GCT ACG TAG CTA T	CGA TTA TAG CTA GCT AAT
NF-κB	TCA TAT CGA TTA GCT AGC T	GCA TAT ATC GTA GCA TGC A
NLRP3	GCT ATA TGC TAG CTA GCT T	TAG CTA TAA GCT AGC TAT A
GAPDH	GAG TCC ACT GGC GTC TTC AC	TGG TTC ACA CCC ATG ACG AA

The qRT-PCR reaction system (20 μL) consisted of: 2 μL cDNA,0.8 μL each of forward and reverse primers, 10 μL SYBR Green Mix, and 6.4 μL RNase-free water. The reaction conditions were as follows: 95 °C pre-denaturation for 30 s; 95 °C denaturation for 5 s and60 °C annealing for 30 s for 40 cycles; melting curve analysis at 95 °C for 15 s, 60 °C for 1 min, and 95 °C for 15 s.

The relative expression levels of target genes were calculated using the $2^{-\Delta\Delta Ct}$. All experiments were performed in triplicate, and the average value was used as the final result.

1.3 Disease Course Staging

According to the *Guidelines for Diagnosis and Treatment of Acute Pancreatitis in China (2021)* [6] and the clinical characteristics of disease progression, patients were divided into three stages.

(1) Acute response stage:

Occurred within 1–2 weeks after SAP onset and was characterized primarily by systemic inflammatory response syndrome and organ dysfunction, without definite evidence of infection.

(2) Systemic infection phase:

Occurred within 3–6 weeks after SAP onset and manifested as systemic infection or sepsis, possibly accompanied by infected pancreatic necrotic tissue. Laboratory examinations showed significantly elevated C-reactive protein (CRP) and procalcitonin (PCT) levels, or positive blood culture/necrotic tissue culture results.

(3) Residual infection phase:

Occurred at 7 weeks or later after SAP onset and was characterized mainly by residual pancreatic necrotic tissue, formation of infectious abscesses, or sinus tract formation, requiring targeted anti-infective treatment or drainage.

Two senior intensive care physicians independently determined the disease stage according to the patients' clinical manifestations, imaging findings, and micro-biological examination results. In cases of disagreement, consensus was reached through departmental case discussions.

According to clinical disease staging, the patients were divided into the acute response phase group (50 cases), systemic infection phase group (30 cases), and residual infection phase group (20 cases).

1.4 In-Hospital Mortality Assessment and Outcome Grouping

In-hospital death was used as the primary outcome indicator. All patients entered the observation period from the time of admission. Their clinical status was continuously monitored through daily medical records, nursing records, and vital sign monitoring systems. The observation endpoint was patient discharge or in-hospital death. The criteria for death determination were in-hospital death caused by SAP itself or its complications (such as multiple organ failure, severe infection, or septic shock). Deaths caused by non-SAP-related accidental events (such as falls from bed or suicide) were excluded. According to the survival status at the end of the observation period, the study subjects were divided into the in-hospital death group (24 cases) and the in-hospital survival group (76 cases) for subsequent prognostic analysis.

1.5 Statistical Methods

SPSS 26.0 software was used for data processing and figure generation. Continuous variables conforming to normal distribution were expressed as $\bar{x} \pm s$. Comparisons between two groups were performed using the independent-samples *t*-test, while comparisons among multiple groups were performed using one-way analysis of variance (ANOVA). Pairwise comparisons were conducted using the LSD-*t* test. Continuous variables not conforming to normal distribution were expressed as M (Q_1, Q_3), and comparisons between two groups were performed using the Mann-Whitney *U* test. Categorical variables were expressed as cases (%), and comparisons between-groups were performed using the χ^2 test. Multivariate logistic regression analysis was used to investigate the correlations between the mRNA expression levels of TLR4, NF-κB, and NLRP3 and in-hospital death in patients with SAP. Receiver operating characteristic (ROC) curves were plotted to evaluate the predictive performance of each indicator for the risk of in-hospital death. A *P* value <0.05 was considered statistically significant.

2 Results

2.1 Comparison of peripheral blood TLR4, NF-κB, and NLRP3 mRNA expression in SAP patients at different disease stages

The mRNA expression levels of TLR4, NF-κB, and NLRP3 in peripheral blood showed statistically significant differences among SAP patients at different disease stages (P<0.01). The expression levels of TLR4, NF-κB, and NLRP3 mRNA in the systemic infection phase were significantly higher than those in the acute response phase and the residual infection phase (P<0.05). There was no statistically significant difference in the above indicators between the acute response phase and the residual infection phase (P>0.05). See **Table 2.**

2.2 Comparison of clinical data between the in-hospital death group and the in-hospital survival group

The in-hospital death group had significantly higher APACHE II score within 24 hours of admission, SOFA score, proportion of patients in the systemic infection phase, CRP and PCT levels, as well as mRNA expression levels of TLR4, NF-κB, and NLRP3 compared with the in-hospital survival group (P<0.05). The serum calcium level was lower in the in-hospital death group than in the in-hospital survival group (P<0.05). There was no statistically significant difference in the remaining indicators between the two groups (P>0.05). See **Table 3**.

2.3 Multivariate logistic regression analysis of in-hospital mortality in SAP patients

Taking the prognosis during hospitalization of SAP patients as the dependent variable (death = 1, survival = 0) and the indicators with statistically significant differences in the univariate analysis as independent variables, a multivariate logistic regression model was performed. The results showed that a high APACHE II score within 24 hours of admission, a high SOFA score within 24 hours of admission, the systemic infection phase, a low serum calcium level, a high CRP level, and high mRNA expression levels of TLR4, NF-κB, and NLRP3 were independent risk factors for in-hospital mortality in SAP patients (P<0.05). See **Table 4.**

2.4 Predictive value of peripheral blood TLR4, NF-κB, and NLRP3 mRNA expression for in-hospital mortality risk in SAP patients

ROC curve analysis showed that the areas under the curve (AUCs) of peripheral blood TLR4, NF-κB, and NLRP3 mRNA expression levels for predicting in-hospital mortality risk in SAP patients were 0.736, 0.708, and 0.782, respectively. The AUC of the combined detection of the three markers was 0.892, which was greater than that of each individual marker (Z = 3.254, 3.286, 2.997; P<0.05). See **Figure 1** and **Table 5**.

Tab.2 Comparison of the mRNA expression levels of TLR4, NF-κB and NLRP3 in the peripheral blood of SAP patients at different disease stages ($\bar{x}\pm s$)

Disease stage	Case	TLR4 mRNA	NF-κB mRNA	NLRP3 mRNA
Acute Response Phase	50	3.25±0.85	2.97±0.89	3.51±1.24
Systemic Infection Phase	30	6.88±1.52[ab]	5.96±1.31[ab]	7.23±1.35[ab]
Residual Infection Phase	20	2.82±0.79	2.53±0.76	3.00±0.89
***F* value**		126.662	100.663	107.511
***P* value**		<0.001	<0.001	<0.001

Note: Compared with acute response phase, [a]P<0.05; Compared with residual infection phase, [b]P<0.05.

Tab.3 Comparison of clinical data of SAP patients in the in-hospital death group and the in-hospital survival group

Indicator	In-hospital death group (*n*=24)	In-hospital survival group (*n*=76)	$t/\chi^2/H$ value	*P* value
Age (years)[a]	61.25±11.36	58.89±12.34	0.832	0.408
BMI (kg/m^2)[a]	28.86±3.55	27.22±3.64	1.935	0.056
Gender [case (%)]				
Male	15(62.50)	45(59.21)	0.082	0.774
Female	9(37.50)	31(40.79)		
Etiology[b]				
Choledocholithiasis	10(41.67)	32(42.10)		
Alcoholic	7(29.17)	20(26.32)	0.153	0.985
Lipogenic	4(16.67)	15(19.74)		
Others	3(12.50)	9(11.84)		
Combined hypertension [case (%)]	8(33.33)	18(23.68)	0.883	0.347
Combined diabetes [case (%)]	6(25.00)	15(19.74)	0.305	0.581
Time from onset to hospitalization(h)[a]	38.21±13.12	36.52±12.35	0.576	0.566
APACHE II score within 24 hours of admission[a]	18.75±4.52	12.56±3.32	7.268	<0.001
SOFA score within 24 hours of admission[a]	8.52±2.13	4.23±1.56	10.709	<0.001
Disease Stage [case (%)]				
Acute response phase	5(20.83)	45(59.21)		
Systemic infection phase	18(75.00)	12(15.79)	30.647	<0.001
Residual infection phase	1(4.17)	19(25.00)		
Treatment measures [case (%)]				
Surgery	12(50.00)	22(28.94)	3.603	0.058
Mechanical ventilation	20(83.33)	50(65.79)	2.673	0.102
Renal replacement indicators	10(41.67)	17(22.37)	3.446	0.063
WBC(×10^9/L)[a]	18.45±4.52	16.87±3.56	1.772	0.079
Neutrophil percentage(%)[a]	80.15±8.32	77.23±8.67	1.452	0.150
ALT(U/L)[a]	69.65±25.31	62.32±18.54	1.561	0.122
AST(U/L)[a]	75.32±28.45	66.45±20.12	1.695	0.093
Total bilirubin(μmol/L)[b]	25.62(16.15, 36.87)	22.35(18.56, 28.42)	0.672	0.601
Serum creatinine(μmol/L)[b]	82.35(55.67, 98.23)	78.52(65.34, 92.45)	0.742	0.610
Blood urea nitrogen(mmol/L)[a]	7.32±3.56	6.54±2.12	1.315	0.191
Serum calcium(mmol/L)[a]	1.72±0.28	2.15±0.32	5.904	<0.001
Serum sodium(mmol/L)[a]	138.45±14.52	132.56±13.21	1.859	0.066
Serum amylase(U/L)[a]	1 178.56±325.45	1 028.45±342.19	1.895	0.061
CRP(mg/L)[a]	106.32±35.21	85.23±25.67	3.194	0.002
PCT(ng/mL)[a]	8.75±3.21	2.56±1.23	13.980	<0.001
TLR4 mRNA[a]	6.19±1.48	3.64±1.02	9.514	<0.001
NF-κB mRNA[a]	5.17±1.25	3.34±0.98	3.828	<0.001
NLRP3 mRNA[a]	6.15±1.32	4.01±1.15	7.667	<0.001

Note: [a], data was expressed as $\overline{x}\pm s$; [b], data was expressed as $M(Q_1, Q_3)$.

Tab.4 Multivariate logistic regression analysis of mortality during hospitalization in SAP patients

Variable	*β*	*SE*	Wald χ^2	*OR* value	95%*CI*	*P* value
APACHE II score within 24 hours of admission	0.345	0.148	5.434	1.412	1.053-1.841	0.021
SOFA score within 24 hours of admission	0.795	0.196	16.425	2.217	1.503-3.272	<0.001
Disease staging (systemic infection phase *vs* acute response phase)	1.986	0.618	10.321	7.295	2.156-24.460	<0.001
Disease staging (residual infection phase *vs* acute response phase)	0.523	0.841	0.388	1.687	0.320-8.780	0.498
Serum calcium	-0.382	0.151	6.400	0.682	0.208-0.916	0.017
CRP	0.108	0.045	5.760	1.114	1.022-1.216	0.020
PCT	0.156	0.098	2.528	1.169	0.967-1.412	0.112
TLR4 mRNA	0.638	0.168	14.426	1.893	1.365-2.637	<0.001
NF-κB mRNA	0.245	0.102	5.769	1.278	1.046-1.560	0.020
NLRP3 mRNA	0.674	0.181	13.852	1.962	1.379-2.791	<0.001

Note: The assignment was as follows: disease staging (acute response phase=1, systemic infection phase=2, residual infection phase=3); APACHE II score, SOFA score within 24 hours of admission, serum calcium, CRP, PCT, TLR4 mRNA, NF-κB mRNA, and NLRP 3mRNA were substituted with their original values.

Tab.5 The predictive value of the expression levels of TLR4, NF-κB and NLRP3 mRNA in peripheral blood for the risk of death during hospitalization in SAP patients

Indicator	AUC	95%*CI*	Cut-off value	Sensitivity	1-Specificity
TLR4 mRNA	0.736	0.617-0.855	3.98	0.875	0.408
NF-κB mRNA	0.708	0.577-0.839	4.42	0.792	0.553
NLRP3 mRNA	0.782	0.676-0.888	5.64	0.708	0.671
Combination	0.892	0.798-0.985	—	0.875	0.842

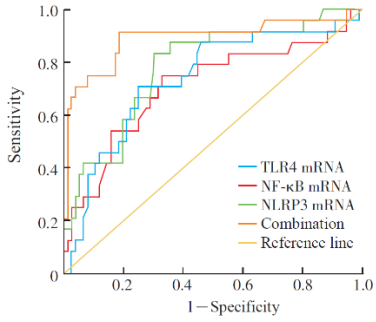

Fig.1 The ROC of predictive value of the expression levels of TLR4, NF-κB and NLRP3 mRNA in peripheral blood for the risk of in-hospital death of SAP patients

3 Discussion

SAP is a systemic critical illness driven by a cytokine storm, and its disease progression is often accompanied by the development of multiple organ dysfunction. Recently, activation of the innate immune key pathway——TLR4/NF-κB/NLRP3——has been considered to play a central role in the amplification of the early inflammatory response and systemic injury in SAP [4-5]. In this study, by observing the expression changes of key molecules in this pathway in the peripheral blood of SAP patients at different stages of disease progression and evaluating their association with the risk of in-hospital death, we aimed to identify new laboratory evidence for disease assessment and prognosis prediction in SAP.

In the same cohort of SAP patients, this study systematically analyzed the evolution pattern of TLR4, NF-κB, and NLRP3 mRNA expression levels throughout the clinical course. The results showed that the mRNA expression levels of all three reached their peak during the systemic infection phase, significantly higher than those in the acute response phase and residual infection phase. This finding is highly consistent with the disease progression of SAP. During the acute response phase, although pancreatic necrotic tissue can activate the TLR4/NF-κB signaling pathway and induce the release of a large number of pro-inflammatory factors, the inflammatory response at this stage is still in the initial outbreak phase[7]. After entering the systemic infection phase, pancreatic and peri pancreatic necrotic tissues become a “culture medium” for bacterial and fungal infections. The pathogen-associated molecular patterns released by these tissues (such as endotoxins) and damage-associated molecular patterns continuously and intensely stimulate TLR4 on the surface of immune cells, thereby maximally activating downstream NF-κB signaling and promoting the transcription of various inflammatory mediators, including the precursors of IL-1β and IL-18 [8-9] . At the same time, persistent inflammatory signals and intracellular danger signals (such as reactive oxygen species bursts) provide the necessary conditions for the assembly of the NLRP3 inflammasome. Activated NLRP3 inflammasomes cleave Gasdermin D to mediate pyroptosis and promote Caspase-1 to process IL-1β and IL-18 precursors into mature cytokines with strong pro-inflammatory activity, thereby driving further deterioration of the septic state and organ dysfunction [10-11]. Therefore, the significantly elevated expression of TLR4, NF-κB, and NLRP3 mRNA in the peripheral blood of patients during the systemic infection phase directly reflects the persistent and intense immune-inflammatory storm existing in vivo at this stage and represents a marker of the most critical disease condition. In the residual infection phase, infection foci are mostly localized and the systemic inflammatory response is alleviated; therefore, the activity of this pathway also declines to levels similar to those in the acute response phase.

This study demonstrated that high expression of TLR4, NF-κB, and NLRP3 mRNA are independent risk factors for in-hospital death in SAP patients. In the univariate analysis, the APACHE II score, SOFA score, CRP, PCT, and the three target mRNA levels in the death group were all significantly higher than those in the survival group, whereas blood calcium levels were significantly lower, which is consistent with previous studies and confirms the predictive value of traditional severity scores, inflammatory markers, and electrolyte disturbances for prognosis [12-13]. However, in the multivariate logistic regression analysis, after adjustment for important confounding factors such as APACHE II score, SOFA score, disease stage, and CRP, high expression of TLR4,NF-κB, and NLRP3 mRNA remained independently associated with the risk of in-hospital death. These results suggest that the pathological damage caused by excessive activation of the TLR4/NLRP3 pathway may exceed the scope that traditional scoring systems and inflammatory indicators can fully capture. APACHE II and SOFA scores mainly reflect the outcome of organ dysfunction, whereas CRP and PCT are broad inflammatory markers [14-15]. As initiating and key amplification components of the inflammatory response, TLR4 and NLRP3 expression levels may more specifically and earlier reflect the degree of immune microenvironment imbalance and the tendency toward loss of control in SAP patients. In particular, pyroptosis mediated by the NLRP3 inflammasome, as a novel form of programmed cell death, directly causes cellmembrane rupture and the release of a large number of damage-associated molecular patterns, thereby aggravating tissue injury and systemic inflammation [16-17], and is closely related to the severity of SAP. This mechanism has also been verified in animal experiments. Studies have shown that in SAP rat models, the expression levels of TLR4, NF-κB, and NLRP3 proteins and mRNA in pancreatic tissues were significantly increased, while serum levels of inflammatory cytokines such as IL-8,IL-12, IL-17, and IL-18 were markedly elevated, accompanied by obvious pathological changes in pancreatic tissues, including edema, necrosis, and hemorrhage [18]. Therefore, detecting the expression levels of key molecules in this pathway can provide incremental information for prognosis evaluation from an etiological perspective and also provide a theoretical basis for therapeutic strategies targeting this pathway, with the potential to improve the prognosis of SAP patients at the mechanistic level.

ROC curve analysis showed that whenTLR4, NF-κB, and NLRP3 mRNA were detected individually, their AUC were all greater than 0.7, indicating moderate to high predictive efficacy [19]. Among them, NLRP3 mRNA had the highest AUC (0.782), suggesting that activation of the NLRP3 inflammasome may play a more critical role in mortality outcomes. The predictive efficacy of combined detection of the three markers (AUC = 0.892) was significantly superior to that of any single indicator and higher than the AUC (0.877) ofthe model constructed by Zhang *et al.*[20]. This fully demonstrates the advantage of the TLR4/NF-κB/NLRP3 pathway, as a continuously activated and synergistically functioning whole, in

prognosis evaluation. Combined detection can more comprehensively assess the activity status of the entire inflammatory cascade pathway, from inflammatory signal recognition (TLR4), signal transduction and transcriptional activation (NF-κB), to inflammasome assembly and effector molecule release (NLRP3), thereby more accurately identifying critically ill patients at extremely high risk of "cytokine storm." This provides important theoretical and experimental data support for the future development of SAP prognosis prediction models based on multigene expression profiles.

Inconclusion, the mRNA expression levels of TLR4, NF-κB, and NLRP3 in the peripheral blood of SAP patients peak during the systemic infection phase. Their high expression levels are independent predictors of in-hospital death, and combined detection of the three can significantly improve the predictive efficacy for mortality risk. These findings not only deepen the understanding of the immune pathogenesis of SAP but also suggest that monitoring the activity of the TLR4/NF-κB/NLRP3 pathway may become a powerful tool for evaluating disease severity and prognosis in SAP, providing potential targets for future precision medicine interventions.

Conflict of Interest None

References

[1] Mihoc T, Pirvu C, Dobrescu A, et al. Comparative analysis of laboratory markers, severity scores, and outcomes in 179 patients with severe acute pancreatitis[J]. Biomedicines, 2025, 13(4):797.

[2] Li XT, Zhang YY, Wang WW, et al. An inflammation-based model for identifying severe acute pancreatitis: a single-center retrospective study[J]. BMC Gastroenterol, 2024, 24(1):63.

[3] Saullo P, Caronna R, Angelici AM, et al. Value of continuous hemofiltration in patients with severe acute pancreatitis at onset: single centre experience on 48 patients[J]. J Clin Med, 2025, 14(18):6647.

[4] Mattke J, Darden CM, Lawrence MC, et al. Toll-like receptor 4 in pancreatic damage and immune infiltration in acute pancreatitis[J]. Front Immunol, 2024, 15:1362727.

[5] Lin XY, Zhang X, Fan L, et al. MARCH9 is down-regulated and negatively correlates with severity, NLRP3 inflammasome expression and NLRP3 inflammasome-derived inflammatory cytokines expression in acute pancreatitis patients[J]. Bratislava Med J, 2025, 126(8):1756-1769.

[6] Chinese Pancreatic Surgery Association. The guideline of diagnosis and treatment of severe acute pancreatitis [J]. Chin J Surg, 2007, 45(11):727-729. **[In Chinese]**

[7] Chen HW, Wang YH, Zippi M, et al. Oxidative stress, DAMPs, and immune cells in acute pancreatitis: molecular mechanisms and therapeutic prospects[J]. Front Immunol, 2025, 16:1608618.

[8] Shah J, Fernandez Y Viesca M, Jagodzinski R, et al. Infected pancreatic necrosis-Current trends in management[J]. Indian J Gastroenterol, 2024, 43(3):578-591.

[9] Cicchinelli S, Pignataro G, Gemma S, et al. PAMPs and DAMP sin sepsis: a review of their molecular features and potential clinical implications[J]. Int J Mol Sci, 2024, 25(2):962.

[10] Pinzón-Fernández MV, Saavedra-Torres JS, López Garzón NA, et al. NLRP3 and beyond: inflammasomes as central cellular hub and emerging therapeutic target in inflammation and disease[J]. Front Immunol, 2025, 16:1624770.

[11] Zhu LJ, Hu ML, Xu HM, et al. The key players of inflammasome sand pyroptosis in sepsis-induced pathogenesis and organ dysfunction[J]. Front Pharmacol, 2025, 16:1586364.

[12] Shi PN, Song ZZ, He XN, et al. Evaluation of scoring systems and hematological parameters in the severity stratification of early phase acute pancreatitis[J]. World J Gastroenterol, 2025, 31(15):105236.

[13] Selin D, Maret-Ouda J, Oskarsson V, et al. Long-term mortality in acute pancreatitis-a population-based cohort study[J]. United European Gastroenterol J, 2025, 13(4):640-649.

[14] Padula D, Mauro A, Maggioni P, et al. Practical approach to acute pancreatitis:from diagnosis to the management of complications[J]. Intern Emerg Med, 2024, 19(8):2091-2104.

[15] Bhat A, Alsadhan N, Alsadhan N, et al. Procalcitonin and C-reactive protein as early diagnostic markers of sepsis or septic shock in children who presented with fever to the pediatric emergency department at a tertiary hospital, in Riyadh, Saudi Arabia[J]. Int J Emerg Med, 2025, 18(1):87.

[16] Zhang LL, Wang KP, Huang L, et al. Ganoderic acid a alleviates severe acute pancreatitis by modulating gut homeostasis and inhibiting TLR4-NLRP3 signaling[J]. J Agric Food Chem, 2025, 73(2):1563-1579.

[17] Liu YF, Pan RJ, Ouyang YZ, et al. Pyroptosis in health and disease: mechanisms, regulation and clinical perspective[J]. Signal Transduct Target Ther, 2024, 9(1):245.

[18] Zhu XD, Liu KR, Zhang D, et al. Exploration on the mechanism of action of *Qingjie Huagong* Decoction on the inflammatory response of severe acute pancreatitis based on the P2X7R/TLR4/NF-κB/NLRP3 axis [J]. China Journal of Traditional Chinese Medicine and Pharmacy, 2024, 39(5):2498-2503. **[In Chinese]**

[19] Tang ZH, Zhou XW, Rao YJ, et al. Nucleotide-bound oligomeric domain-like receptor protein 3 as a serological biomarker in relation to disease severity and delirium after acute pancreatitis: a two center prospective cohort study[J]. Int J Gen Med, 2025, 18:3423-3440.

[20] Zhang JX, Yang T, Li R. Construction and validation of a predictive model for in-hospital mortality in elderly patients with severe acute pancreatitis [J]. Journal of Clinical Medicine in Practice, 2024, 28(17):51-55. **[In Chinese]**

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·急性胰腺炎专题·论著·

外周血TLR4、NF-κB、NLRP3 mRNA对重症急性胰腺炎患者院内死亡的预测价值

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摘要：目的 分析不同病程分期重症急性胰腺炎(SAP)患者外周血Toll样受体4(TLR4)、核因子κB(NF-κB)、NOD样受体热蛋白结构域相关蛋白3(NLRP3)mRNA的表达变化，并探讨其对SAP患者住院期间死亡风险的预测价值。**方法** 采用前瞻性队列研究方法，选取内蒙古医科大学附属医院重症医学科2024年1月至2025年1月收治的100例SAP患者，按临床病程分期分为急性反应期(*n*=50)、全身感染期(*n*=30)和残余感染期(*n*=20)。根据患者住院期间预后分为院内死亡组(*n*=24)和院内存活组(*n*=76)，比较各组外周血TLR4、NF-κB、NLRP3的mRNA表达水平。采用多因素logistic回归分析各指标与SAP患者住院期间死亡的关系，采用受试者工作特征(ROC)曲线评价各指标对SAP患者住院期间死亡的预测效能。**结果** 外周血TLR4、NF-κB、NLRP3的mRNA表达水平在不同病程分期的SAP患者中差异有统计学意义(*P*<0.01)，其中全身感染期患者外周血TLR4、NF-κB、NLRP3的mRNA表达水平均显著高于急性反应期和残余感染期(*P*<0.05)。多因素logistic回归分析显示，高入院24 h内急性生理学与慢性健康状况评分系统Ⅱ(APACHEⅡ)评分、高入院24 h内序贯器官衰竭评估(SOFA)评分、全身感染期、低血钙水平、高CRP水平及TLR4 mRNA高表达(*OR*=1.893，95%*CI*：1.365~2.637)、NF-κB mRNA高表达(*OR*=1.278，95%*CI*：1.046~1.560)、NLRP3 mRNA高表达(*OR*=1.962，95%*CI*：1.379~2.791)是SAP患者住院期间死亡的独立危险因素(*P*<0.05)。ROC曲线显示，外周血TLR4、NF-κB、NLRP3 mRNA表达水平预测SAP患者住院期间死亡风险的曲线下面积(AUC)分别为0.736、0.708、0.782，三者联合检测的AUC为0.892，均大于各指标单独检测(*P*<0.05)。**结论** 不同病程分期SAP患者外周血TLR4、NF-κB、NLRP3的mRNA表达水平存在显著差异，全身感染期患者表达水平显著升高，其升高与患者住院期间死亡风险密切相关，三者联合检测较单一指标具有更高的死亡风险预测效能。

关键词： 重症急性胰腺炎；Toll样受体4；核因子-κB；NOD样受体热蛋白结构域相关蛋白3；住院死亡风险

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Predictive value of peripheral blood TLR4/NF-κB/NLRP3 mRNA for in-hospital death in patients with severe acute pancreatitis

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Abstract: Objective To analyze the expression changes of peripheral blood Toll-like receptor 4 (TLR4), nuclear factor-κB (NF-κB), NOD-like receptor thermal protein domain associated protein 3 (NLRP3) mRNA in patients with severe acute pancreatitis (SAP) at different clinical disease stages, and to explore their predictive value for in-hospital death risk in SAP patients. **Methods** A prospective cohort study was conducted. A total of 100 SAP patients admitted to the Department of Critical Care Medicine, Affiliated Hospital of Inner Mongolia Medical University from

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January 2024 to January 2025 were enrolled. They were divided according to clinical stages into the acute response phase (n=50), systemic infection phase (n=30), and residual infection phase (n=20). Based on in-hospital prognosis, patients were categorized into an in-hospital death group (n=24) and an in-hospital survival group (n=76). The mRNA expression levels of TLR4, NF-κB, and NLRP3 were compared among different groups. Multivariate logistic regression was used to assess the correlation of these indicators with in-hospital death, and receiver operating characteristic (ROC) curves were used to evaluate their predictive efficacy for in-hospital death. **Results** The expression levels of peripheral blood TLR4, NF-κB, and NLRP3 mRNA were significantly different among SAP patients at different disease stages (P<0.01). Levels in the systemic infection phase were significantly higher than those in the acute response and residual infection phases (P<0.05). Multivariate logistic regression showed that high Acute Physiology and Chronic Health Evaluation Ⅱ (APACHE Ⅱ) score within 24 h, high Sequential Organ Failure Assessment (SOFA) score within 24 h, systemic infection phase, low serum calcium level, high CRP level, and high expression of TLR4 mRNA (OR=1.893, 95%CI: 1.365-2.637), NF-κB mRNA (OR=1.278, 95%CI: 1.046-1.560), and NLRP3 mRNA (OR=1.962, 95%CI:1.379-2.791) were independent risk factors for in-hospital death (P<0.05). ROC curve analysis showed that the areas under the curve (AUCs) for predicting in-hospital death risk in SAP patients based on peripheral blood TLR4, NF-κB, and NLRP3 mRNA expression levels were 0.736, 0.708, and 0.782, respectively. The AUC for combined detection of the three indicators was 0.892, which was significantly higher than that of any single indicator (P<0.05). **Conclusion** The expression levels of TLR4, NF-κB, and NLRP3 mRNA in peripheral blood significantly differ across disease stages in SAP patients, and the expression levels are significantly elevated in patients in the systemic infection phase. This elevated expression is closely associated with an increased risk of in-hospital death. Combined detection of these three markers demonstrates higher predictive efficacy for death risk compared to any single indicator.

Keywords: Severe acute pancreatitis; Toll-like receptor 4; Nuclear factor-κB; NOD-like receptor thermal protein domain associated protein 3; In-hospital death risk

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重症急性胰腺炎(severe acute pancreatitis,SAP)是临床常见的急腹症,病情进展迅猛且预后极差,住院期间死亡率高,为15%~30%[1-2]。SAP早期以全身炎症反应综合征为主,后期易并发感染、脓毒症及多器官衰竭,不同病程阶段的病理生理机制存在差异,导致临床干预效果和预后截然不同[3]。因此,探寻能反映病程进展、评估死亡风险的特异性生物标志物,对优化SAP分层治疗策略、改善患者预后具有重要临床意义。Toll样受体4(toll-like receptor 4,TLR4)/核因子(nuclear factor,NF)-κB/NOD样受体热蛋白结构域相关蛋白3(NOD-like receptor thermal protein domain associated protein 3,NLRP3)通路是机体炎症反应的核心信号轴,其异常激活可增加白细胞介素(interleukin,IL)-1β、IL-6、肿瘤坏死因子(tumor necrosis factor,TNF)-α等促炎因子水平,放大全身炎症级联反应,参与SAP胰腺坏死及多器官损伤的病理过程[4]。有研究证实,TLR4、NF-κB及NLRP3在SAP患者外周血中存在异常表达[5],但关于其在不同病程分期(急性反应期、全身感染期和残余感染期)中的变化规律,以及与住院全因死亡风险的关联性尚未明确。基于此,本研究拟通过检测不同病程分期SAP患者外周血TLR4、NF-κB、NLRP3的mRNA表达水平,探讨其对SAP患者住院期间死亡风险的评估价值,为SAP的病情监测、风险分层及靶向治疗提供理论依据。

1　资料与方法

1.1　*研究对象*　本研究为前瞻性研究。收集内蒙古医科大学附属医院重症医学科2024年1月至2025年1月收治的SAP患者100例。纳入标准:(1)符合《中国急性胰腺炎诊治指南(2021)》[6]中SAP的诊断标准;(2)年龄≥18岁;(3)发病至入院时间≤72 h,可明确病程分期;(4)自愿参与本研究并签署知情同意书;(5)临床病历资料完整,可完成全程随访至出院或住院期间死亡。排除标准:(1)合并其他急慢性胰腺炎(如慢性胰腺炎急性发作、自身免疫性胰腺炎);(2)既往有胰腺手术史或严重胰腺功能不全病史;(3)合并恶性肿瘤、严重肝肾功能衰竭、血液系统疾病、免疫功能缺陷病或长期使用免疫抑制剂;(4)妊娠期或哺乳期女性;(5)入院后24 h内自动出院、转院或放弃治疗;(6)严重创伤、烧伤、脓毒症等其他原因导致的全身炎症反应综合征者;(7)对本研究检测方法相关试剂过敏者。

1.2　*外周血TLR4、NF-κB、NLRP3 mRNA表达水平检测*　所有患者于入院后24 h内采集外周静脉血5 mL,

分为两管：(1) 2 mL置于含乙二胺四乙酸(EDTA)的抗凝管中，颠倒混匀后，4 ℃、3 000 r/min(离心半径10 cm)离心10 min，分离外周血单个核细胞，用于提取总RNA；(2) 3 mL置于促凝管中，离心分离血清，-80 ℃冰箱冻存备用。采用Trizol法提取外周血单个核细胞总RNA，使用超微量分光光度计测定RNA浓度与纯度，保证A260/A280比值为1.8~2.0。随后，按照逆转录试剂盒操作说明书，将1 μg总RNA反转录为cDNA。反应条件：42 ℃ 60 min，70 ℃ 10 min。采用实时荧光定量聚合酶链反应(quantitative real-time polymerase chain reaction，qRT-PCR)检测TLR4、NF-κB、NLRP3 mRNA表达水平，以GAPDH为内参。引物由生工生物工程(上海)设计与合成，序列见表1。qRT-PCR反应体系(20 μL)：cDNA 2 μL，上下游引物各0.8 μL，SYBR Green Mix 10 μL，无酶水6.4 μL。反应条件：95 ℃预变性30 s；95 ℃变性5 s，60 ℃退火30 s，40个循环；熔解曲线分析95 ℃ 15 s，60 ℃ 1 min，95 ℃ 15 s。用$2^{-\Delta\Delta Ct}$法计算目标基因相对表达量。所有实验均设置3个复孔，取平均值作为最终结果。

1.3 病程分期 参照《重症急性胰腺炎诊治指南(2021)》[6]及临床病程特点，将患者分为3期。(1) 急性反应期：发病1~2周，以全身炎症反应综合征、器官功能障碍为核心表现，无明确感染证据；(2) 全身感染期：发病3~6周，出现全身感染或脓毒症表现，可合并胰腺坏死组织感染，实验室检查提示C反应蛋白(C-reactive protein，CRP)、降钙素原(procalcitonin，PCT)显著升高，或血培养/坏死组织培养阳性；(3) 残余感染期：发病7周及以上，以胰腺坏死组织残留、感染性脓肿形成或窦道形成为主要表现，需针对性抗感染或引流治疗。由2名高年资重症医学科医师根据患者的临床表现、影像学及微生物学检查结果独立进行病程分期判定，意见不一致时通过科室病例讨论达成共识。按临床病程分期分为急性反应期(50例)、全身感染期(30例)和残余感染期(20例)。

1.4 住院期间死亡评估与结局分组 以患者住院期间死亡为主要结局指标。所有患者自入院起进入观察期，通过每日病历记录、护理记录及生命体征监测系统实时追踪其临床状态，观察终点为患者出院或住院期间死亡。死亡判定标准为因SAP本身或其并发症(如多器官功能衰竭、严重感染、脓毒症休克等)导致的住院期间死亡，排除因非SAP相关意外事件(如坠床、自杀等)导致的死亡。根据观察期结束时患者生存状态，将研究对象分为院内死亡组(24例)和院内存活组(76例)，用于后续预后分析。

表1 引物序列

Tab.1 Primer sequences

引物	上游序列5′→3′	下游序列5′→3′
TLR4	TCG ATA GCT ACG TAG CTA T	CGA TTA TAG CTA GCT AAT
NF-κB	TCA TAT CGA TTA GCT AGC T	GCA TAT ATC GTA GCA TGC A
NLRP3	GCT ATA TGC TAG CTA GCT T	TAG CTA TAA GCT AGC TAT A
GAPDH	GAG TCC ACT GGC GTC TTC AC	TGG TTC ACA CCC ATG ACG AA

1.5 统计学方法 采用SPSS 26.0软件进行数据处理与图表绘制。符合正态分布的连续变量以$\bar{x}\pm s$表示，两组间比较采用独立样本t检验，多组间比较采用单因素方差分析，两两比较采用LSD-t检验；不符合正态分布的连续变量以$M(Q_1, Q_3)$表示，两组间比较采用Mann-Whitney U检验。分类变量以例(%)表示，组间比较采用χ^2检验。采用多因素logistic回归分析探讨TLR4、NF-κB、NLRP3 mRNA表达水平与SAP患者住院期间死亡的相关性。绘制受试者工作特征(receiver operating characteristic，ROC)曲线评价各指标对住院期间死亡风险的预测效能。P<0.05为差异有统计学意义。

2 结 果

2.1 不同病程分期SAP患者外周血TLR4、NF-κB、NLRP3 mRNA表达水平比较 外周血TLR4、NF-κB、NLRP3 mRNA表达水平在不同病程分期的SAP患者差异有统计学意义(P<0.01)，其中全身感染期患者外周血TLR4、NF-κB、NLRP3 mRNA表达水平均显著高于急性反应期和残余感染期(P<0.05)；急性反应期与残余感染期上述指标表达水平比较，差异无统计学意义(P>0.05)。见表2。

2.2 院内死亡组与院内存活组患者临床资料比较 院内死亡组入院24 h内APACHE Ⅱ评分、SOFA评分、全身感染期比例及CRP、PCT水平和TLR4、NF-κB、NLRP3的mRNA表达水平均高于院内存活组(P<

表2 不同病程分期SAP患者外周血TLR4、NF-κB、NLRP3 mRNA表达水平比较 ($\bar{x}\pm s$)

Tab.2 Comparison of the mRNA expression levels of TLR4, NF-κB and NLRP3 in the peripheral blood of SAP patients at different disease stages ($\bar{x}\pm s$)

病程分期	例数	TLR4 mRNA	NF-κB mRNA	NLRP3 mRNA
急性反应期	50	3.25±0.85	2.97±0.89	3.51±1.24
全身感染期	30	6.88±1.52[ab]	5.96±1.31[ab]	7.23±1.35[ab]
残余感染期	20	2.82±0.79	2.53±0.76	3.00±0.89
F值		126.662	100.663	107.511
P值		<0.001	<0.001	<0.001

注：与急性反应期比较，[a]P<0.05；与残余感染期比较，[b]P<0.05。

0.05)，血钙水平低于院内存活组(P<0.05)，其余指标两组比较差异无统计学意义(P>0.05)。见表3。

2.3　SAP患者住院期间死亡的多因素logistic回归分析　以SAP患者住院期间预后情况为因变量(死亡=1，存活=0)，将单因素分析中差异有统计学意义的指标作为自变量，纳入多因素logistic回归模型，结果显示，高入院24 h内APACHE Ⅱ评分、高入院24 h内SOFA评分、全身感染期、低血钙水平、高CRP水平及TLR4、NF-κB、NLRP3的mRNA高表达是SAP患者住院期间死亡的独立危险因素(P<0.05)。见表4。

表3　院内死亡组与院内存活组SAP患者临床资料比较

Tab.3　Comparison of clinical data of SAP patients between the in-hospital death group and the in-hospital survival group

项目	院内死亡组(n=24)	院内存活组(n=76)	$t/\chi^2/Z$值	P值
年龄(岁)[a]	61.25±11.36	58.89±12.34	0.832	0.408
BMI(kg/m²)[a]	28.86±3.55	27.22±3.64	1.935	0.056
性别[b]				
男	15(62.50)	45(59.21)	0.082	0.774
女	9(37.50)	31(40.79)		
病因[b]				
胆源性	10(41.66)	32(42.10)		
酒精性	7(29.17)	20(26.32)	0.153	0.985
脂源性	4(16.67)	15(19.74)		
其他	3(12.50)	9(11.84)		
基础疾病[b]				
高血压	8(33.33)	18(23.68)	0.883	0.347
糖尿病	6(25.00)	15(19.74)	0.305	0.581
发病至入院时间(h)[a]	38.21±13.12	36.52±12.35	0.576	0.566
入院24h内APACHE Ⅱ评分(分)[a]	18.75±4.52	12.56±3.32	7.268	<0.001
入院24 h内SOFA评分(分)[a]	8.52±2.13	4.23±1.56	10.709	<0.001
病程分期[b]				
急性反应期	5(20.83)	45(59.21)		
全身感染期	18(75.00)	12(15.79)	30.647	<0.001
残余感染期	1(4.17)	19(25.00)		
治疗措施[b]				
手术	12(50.00)	22(28.94)	3.603	0.058
机械通气	20(83.33)	50(65.79)	2.673	0.102
肾脏替代疗法	10(41.67)	17(22.37)	3.446	0.063
白细胞计数(×10⁹/L)[a]	18.45±4.52	16.87±3.56	1.772	0.079
中性粒细胞比例(%)[a]	80.15±8.32	77.23±8.67	1.452	0.150
ALT(u/L)[a]	69.65±25.31	62.32±18.54	1.561	0.122
AST(u/L)[a]	75.32±28.45	66.45±20.12	1.695	0.093
总胆红素(μmol/L)[c]	25.62 (16.15, 36.87)	22.35 (18.56, 28.42)	0.672	0.601
血肌酐(μmol/L)[c]	82.35 (55.67, 98.23)	78.52 (65.34, 92.45)	0.742	0.610
血尿素氮(mmol/L)[a]	7.32±3.56	6.54±2.12	1.315	0.191
血钙(mmol/L)[a]	1.72±0.28	2.15±0.32	5.904	<0.001
血钠(mmol/L)[a]	138.45±14.52	132.56±13.21	1.859	0.066
血淀粉酶(u/L)[a]	1 178.56±325.45	1 028.45±342.19	1.895	0.061
CRP(mg/L)[a]	106.32±35.21	85.23±25.67	3.194	0.002
PCT(ng/mL)[a]	8.75±3.21	2.56±1.23	13.980	<0.001
TLR4 mRNA[a]	6.19±1.48	3.64±1.02	9.514	<0.001
NF-κB mRNA[a]	5.17±1.25	3.34±0.98	3.828	<0.001
NLRP3 mRNA[a]	6.15±1.32	4.01±1.15	7.667	<0.001

注：BMI，身体质量指数；APACHE Ⅱ，急性生理学与慢性健康状况评分Ⅱ；SOFA，序贯器官衰竭评估；ALT，丙氨酸氨基转移酶；AST，天门冬氨酸氨基转移酶；[a]为以$\bar{x}\pm s$表示；[b]为以例(%)表示；[c]为以$M(Q_1, Q_3)$表示。

2.4　外周血TLR4、NF-κB、NLRP3 mRNA表达水平对SAP患者住院期间死亡风险的预测价值　ROC曲线显示，外周血TLR4、NF-κB、NLRP3 mRNA表达水平预测SAP患者住院期间死亡风险的曲线下面积(area under the curve，AUC)分别为0.736、0.708、0.782，三者联合检测的AUC为0.892，均大于各指标单独检测(Z=3.254、3.286、2.997，P<0.05)。见图1、表5。

表4　SAP患者住院期间死亡的多因素logistic回归分析

Tab.4　Multivariate logistic regression analysis of in-hospital death in SAP patients

项目	β	SE	Waldχ^2	OR值	95%CI	P值
入院24 h内APACHE Ⅱ评分	0.345	0.148	5.434	1.412	1.053~1.841	0.021
入院24 h内SOFA评分	0.795	0.196	16.425	2.217	1.503~3.272	<0.001
病程分期(全身感染期*vs*急性反应期)	1.986	0.618	10.321	7.295	2.156~24.460	<0.001
病程分期(残余感染期*vs*急性反应期)	0.523	0.841	0.388	1.687	0.320~8.780	0.498
血钙	−0.382	0.151	6.400	0.682	0.208~0.916	0.017
CRP	0.108	0.045	5.760	1.114	1.022~1.216	0.020
PCT	0.156	0.098	2.528	1.169	0.967~1.412	0.112
TLR4 mRNA	0.638	0.168	14.426	1.893	1.365~2.637	<0.001
NF-κB mRNA	0.245	0.102	5.769	1.278	1.046~1.560	0.020
NLRP3 mRNA	0.674	0.181	13.852	1.962	1.379~2.791	<0.001

注：赋值，入院24 h内APACHE Ⅱ评分(原值代入)、SOFA评分(原值代入)、病程分期(急性反应期=1，全身感染期=2，残余感染期=3)、血钙(原值代入)、CRP(原值代入)、PCT(原值代入)、TLR4 mRNA(原值代入)、NF-κB mRNA(原值代入)、NLRP3 mRNA(原值代入)。

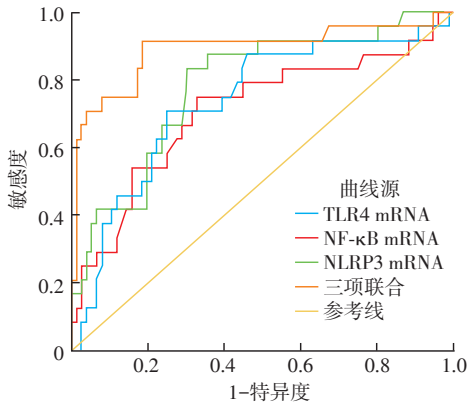

图1　外周血TLR4、NF-κB、NLRP3 mRNA表达水平对SAP患者住院期间死亡风险的预测价值ROC曲线

Fig.1　The ROC of predictive value of the expression levels of TLR4, NF-κB and NLRP3 mRNA in peripheral blood for the risk of in-hospital death of SAP patients

表5 外周血TLR4、NF-κB、NLRP3 mRNA表达水平对SAP患者住院期间死亡风险的预测价值

Tab.5 The predictive value of the expression levels of TLR4, NF-κB and NLRP3 mRNA in peripheral blood for the risk of in-hospital death of SAP patients

项目	AUC	95%*CI*	截断值	敏感度	特异度
TLR4 mRNA	0.736	0.617~0.855	3.98	0.875	0.408
NF-κB mRNA	0.708	0.577~0.839	4.42	0.792	0.553
NLRP3 mRNA	0.782	0.676~0.888	5.64	0.708	0.671
三项联合	0.892	0.798~0.985	—	0.875	0.842

3 讨 论

SAP是一种由细胞因子风暴驱动的全身性危重疾病，其病程进展往往伴随多器官功能障碍的发生。近年来，先天免疫关键通路TLR4/NF-κB/NLRP3的激活被认为在SAP早期炎症反应放大和系统性损伤中发挥核心作用[4-5]。本研究通过观察不同病程分期SAP患者外周血中该通路关键分子的表达变化，并评估其与住院死亡风险的关联，旨在为SAP的病情评估和预后预测寻找新的实验室依据。

本研究在同一SAP患者队列中系统分析了TLR4、NF-κB及NLRP3 mRNA表达水平随临床病程的演变规律。研究结果显示，三者mRNA表达水平在全身感染期达到峰值，显著高于急性反应期和残余感染期。这一发现与SAP的疾病进程高度吻合。在急性反应期，胰腺坏死组织虽可激活TLR4/NF-κB信号，诱发大量促炎因子释放，但此时炎症反应尚处于初始爆发阶段[7]。进入全身感染期后，胰腺及胰周坏死组织成为细菌和真菌感染的“培养基”，其释放的病原相关分子模式（如内毒素）和损伤相关分子模式，持续、强烈地刺激免疫细胞表面的TLR4，进而最大化地激活下游NF-κB信号，促进包括IL-1β、IL-18前体在内的多种炎性介质转录[8-9]。与此同时，持续的炎症信号和细胞内危险信号（如活性氧簇爆发）为NLRP3炎症小体的组装提供了必要条件，活化的NLRP3炎症小体切割Gasdermin D介导细胞焦亡，并促使Caspase-1将IL-1β和IL-18前体加工为成熟的、具有强致炎活性的细胞因子，从而驱动脓毒症状态和器官功能损伤的进一步恶化[10-11]。因此，全身感染期患者外周血中TLR4、NF-κB及NLRP3 mRNA的显著高表达，直观地反映了该阶段体内存在的持续、剧烈的免疫炎症风暴，是病情最为危重的标志。至残余感染期，感染灶多被局限，全身性炎症反应减轻，故该通路活性也随之回落至与急性反应期相近的水平。

本研究显示，TLR4、NF-κB及NLRP3 mRNA高表达是SAP患者住院期间死亡的独立危险因素。在单因素分析中，院内死亡组患者的APACHE Ⅱ评分、SOFA评分、CRP、PCT以及三个目标mRNA水平均显著高于院内存活组，而血钙水平显著偏低，这与既往研究一致，证实了传统病情评分、炎症标志物和电解质紊乱对预后的预测价值[12-13]。然而，在多因素logistic回归分析中，调整APACHE Ⅱ评分、SOFA评分、病程分期、CRP等重要混杂因素后，TLR4、NF-κB及NLRP3的mRNA高表达依然与住院死亡风险独立相关。这一结果表明，TLR4/NLRP3通路的过度激活所带来的病理损害，可能超出了传统评分和炎症指标所能完全涵盖的范围。APACHE Ⅱ和SOFA评分主要反映的是器官功能失调的结果，而CRP、PCT是广义的炎症标志物[14-15]。TLR4和NLRP3作为炎症反应的始动和关键放大环节，其表达水平可能更早、更特异地反映了SAP患者免疫内环境的紊乱程度和失控倾向。特别是NLRP3炎症小体介导的细胞焦亡，作为一种新型的程序性细胞死亡方式，直接导致细胞膜破裂和大量损伤相关分子模式释放，从而加剧组织损伤和全身炎症[16-17]，与SAP的严重程度密切相关。这一机制也在动物实验中得到了验证，研究显示在SAP大鼠模型中，胰腺组织TLR4、NF-κB、NLRP3蛋白和mRNA的表达量显著升高，同时血清中IL-8、IL-12、IL-17、IL-18等炎症因子含量明显上升，且胰腺组织出现明显水肿、坏死出血等病理改变[18]。因此，检测该通路关键分子的表达水平，能够从病因学层面为预后评估提供增量信息，也为靶向该通路的治疗策略提供了理论依据，有望从机制层面改善SAP患者的预后。

ROC曲线分析结果显示，TLR4、NF-κB和NLRP3 mRNA单独检测时，其AUC均大于0.7，显示出中等以上的预测效能[19]，其中NLRP3 mRNA的AUC最高（0.782），提示NLRP3炎症小体的激活可能在死亡结局中起着更为关键的作用。三者联合检测的预测效能（AUC=0.892）显著优于任一单一指标，且高于张佳旭等[20]研究所构建模型的AUC（0.877）。这充分体现了TLR4/NF-κB/NLRP3通路作为一个连续激活、相互协同的功能整体在预后评估中的优势。联合检测能够更全面地评估从炎症信号识别（TLR4）到信号转导与转录激活（NF-κB），再到炎症小体组装与效应分子释放（NLRP3）的整个炎症级联通路的活性状态，从而更精准地识别出那些存在“炎症因子风暴”极高风险的危重患者。这为未来开发基于多基因表达谱的SAP预后预测模型提供了重要的

理论和实验数据支持。

综上所述，SAP 患者外周血中 TLR4、NF-κB 及 NLRP3 的 mRNA 表达水平在全身感染期达到高峰，其高表达是患者住院死亡的独立预测因子，三者联合检测可显著提高对死亡风险的预测效能。这些发现不仅深化了对 SAP 免疫发病机制的理解，也提示监测 TLR4/NF-κB/NLRP3 通路活性有望成为评估 SAP 病情和预后的有力工具，为未来的精准医疗干预提供了潜在的靶点。

利益冲突　无

参考文献

[1] Mihoc T, Pirvu C, Dobrescu A, et al. Comparative analysis of laboratory markers, severity scores, and outcomes in 179 patients with severe acute pancreatitis[J]. Biomedicines, 2025, 13(4): 797.

[2] Li XT, Zhang YY, Wang WW, et al. An inflammation-based model for identifying severe acute pancreatitis: a single-center retrospective study[J]. BMC Gastroenterol, 2024, 24(1): 63.

[3] Saullo P, Caronna R, Angelici AM, et al. Value of continuous hemofiltration in patients with severe acute pancreatitis at onset: single centre experience on 48 patients[J]. J Clin Med, 2025, 14(18): 6647.

[4] Mattke J, Darden CM, Lawrence MC, et al. Toll-like receptor 4 in pancreatic damage and immune infiltration in acute pancreatitis[J]. Front Immunol, 2024, 15: 1362727.

[5] Lin XY, Zhang X, Fan L, et al. MARCH9 is down-regulated and negatively correlates with severity, NLRP3 inflammasome expression and NLRP3 inflammasome-derived inflammatory cytokines expression in acute pancreatitis patients[J]. Bratislava Med J, 2025, 126(8): 1756-1769.

[6] 中华医学会外科学分会胰腺外科学组. 急性胰腺炎诊治指南[J]. 中华外科杂志, 2021, 59(7): 578-587.

[7] Chen HW, Wang YH, Zippi M, et al. Oxidative stress, DAMPs, and immune cells in acute pancreatitis: molecular mechanisms and therapeutic prospects[J]. Front Immunol, 2025, 16: 1608618.

[8] Shah J, Fernandez Y Viesca M, Jagodzinski R, et al. Infected pancreatic necrosis-Current trends in management[J]. Indian J Gastroenterol, 2024, 43(3): 578-591.

[9] Cicchinelli S, Pignataro G, Gemma S, et al. PAMPs and DAMPs in sepsis: a review of their molecular features and potential clinical implications[J]. Int J Mol Sci, 2024, 25(2): 962.

[10] Pinzón-Fernández MV, Saavedra-Torres JS, López Garzón NA, et al. NLRP3 and beyond: inflammasomes as central cellular hub and emerging therapeutic target in inflammation and disease[J]. Front Immunol, 2025, 16: 1624770.

[11] Zhu LJ, Hu ML, Xu HM, et al. The key players of inflammasomes and pyroptosis in sepsis-induced pathogenesis and organ dysfunction[J]. Front Pharmacol, 2025, 16: 1586364.

[12] Shi PN, Song ZZ, He XN, et al. Evaluation of scoring systems and hematological parameters in the severity stratification of early-phase acute pancreatitis[J]. World J Gastroenterol, 2025, 31(15): 105236.

[13] Selin D, Maret-Ouda J, Oskarsson V, et al. Long-term mortality in acute pancreatitis-a population-based cohort study[J]. United European Gastroenterol J, 2025, 13(4): 640-649.

[14] Padula D, Mauro A, Maggioni P, et al. Practical approach to acute pancreatitis: from diagnosis to the management of complications[J]. Intern Emerg Med, 2024, 19(8): 2091-2104.

[15] Bhat A, Alsadhan N, Alsadhan N, et al. Procalcitonin and C-reactive protein as early diagnostic markers of sepsis or septic shock in children who presented with fever to the pediatric emergency department at a tertiary hospital, in Riyadh, Saudi Arabia[J]. Int J Emerg Med, 2025, 18(1): 87.

[16] Zhang LL, Wang KP, Huang L, et al. Ganoderic acid a alleviates severe acute pancreatitis by modulating gut homeostasis and inhibiting TLR4-NLRP3 signaling[J]. J Agric Food Chem, 2025, 73(2): 1563-1579.

[17] Liu YF, Pan RJ, Ouyang YZ, et al. Pyroptosis in health and disease: mechanisms, regulation and clinical perspective[J]. Signal Transduct Target Ther, 2024, 9(1): 245.

[18] 朱晓东, 刘锟荣, 张鼎, 等. 基于 P2X7R/TLR4/NF-κB/NLRP3 轴探讨清解化攻方对重症急性胰腺炎炎症反应的作用机制[J]. 中华中医药杂志, 2024, 39(5): 2498-2503.

[19] Tang ZH, Zhou XW, Rao YJ, et al. Nucleotide-bound oligomeric domain-like receptor protein 3 as a serological biomarker in relation to disease severity and delirium after acute pancreatitis: a two-center prospective cohort study[J]. Int J Gen Med, 2025, 18: 3423-3440.

[20] 张佳旭, 杨婷, 李燃. 中老年重症急性胰腺炎患者住院期间死亡预测模型的构建与验证[J]. 实用临床医药杂志, 2024, 28(17): 51-55.

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