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ORIGINAL RESEARCH

Clinical characteristics and molecular mechanisms of thrombocytopenia in patients with community-acquired *Klebsiella pneumoniae* and *Escherichia coli* bloodstream infections

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Abstract

Background: To explore clinical characteristics, risk factors, and potential molecular mechanisms of thrombocytopenia in patients with community-acquired *Enterobacterales* bloodstream infections (CA-EBSI). **Methods**: The dynamic changes of platelets were analyzed in CA-EBSI patients in a tertiary teaching hospital. The LASSO regression model was established to screen the risk factors of severe thrombocytopenia during hospitalization and a nomogram model constructed to predict thrombocytopenia. Mouse models of bloodstream infections with *Klebsiella pneumoniae* (*K. pneumoniae*) and *Escherichia coli* (*E. coli*) were established to dynamically monitor changes in platelet counts. Scanning electron microscopy was used to observe platelet morphological changes and bacterial adhesion characteristics. Transcriptome sequencing technology was employed to analyze the platelet gene expression profile and signal pathway enrichment patterns. **Results**: A total of 164 patients (*Klebsiella pneumoniae* n = 53 and *Escherichia coli* n = 111) were included, among whom 80.5% developed thrombocytopenia during hospitalization. The incidence of severe thrombocytopenia (PLT $<50 \times 10^9$/L) was numerically higher in the *K. pneumoniae* group than in the *E. coli* group (22.6% *vs.* 14.4%, $p = 0.24$). Age (OR = 1.15, $p = 0.049$) and mechanical ventilation (OR = 5.28, $p = 0.037$) were independent risk factors for severe thrombocytopenia, and the nomogram model presented excellent predictive performance (AUC = 0.975). In the animal infection model, the platelet counts of mice decreased to 44%–56% of the baseline 48 hours after infection. Scanning electron microscopy showed that platelets in mice with bloodstream infections exhibited aggregation, pseudopod extension, and bacterial adhesion. Transcriptome analysis revealed that processes such as adhesion disruption, enhanced pro-inflammatory signaling, and apoptosis induction collectively drive platelet consumption. **Conclusions**: Thrombocytopenia in CA-EBSI patients is characterized by early and rapid progression, and the baseline platelet level significantly affects the progression of the disease. Advanced age and mechanical ventilation are independent risk factors for severe reduction, and thrombocytopenia may be driven by multiple factors.

Keywords

Thrombocytopenia; Community-acquired infection; *Enterobacterales*; Bloodstream infection; Platelets

1. Introduction

Bloodstream infection (BSI) is a major global public health threat, with annual mortality of 2.91 million [1, 2]. Among them, community-acquired *Enterobacterales* bloodstream infection (CA-EBSI) accounts for more than 70% of community-acquired BSI [3]. CA-EBSI is not only closely related to urinary system, biliary tract, and abdominal infections but also prone to causing serious complications such as septic shock and multiple organ dysfunction syndrome (MODS) [4, 5]. *Escherichia coli* and *Klebsiella pneumoniae* are the main pathogens among *Enterobacterales*. Thrombocytopenia is a common complication of CA-EBSI and is closely related to the progression of sepsis, multiple organ failure, and the risk of death [6–9]. Moreover, the severity of thrombocytopenia is a critical determinant of patient outcomes. Notably, a recent large-scale retrospective study confirmed that severe thrombocytopenia (nadir $<50 \times 10^9$/L) in patients with sep-

tic shock was independently associated with both intensive care unit (ICU) mortality and a markedly increased risk of hemorrhagic complications [10]. The 28-day mortality rate of BSI patients with a platelet count (PLT) $<100 \times 10^9$/L is 2.3 times higher than that of the non-decreased group. The dynamic PLT level is significantly negatively correlated with the Sequential Organ Failure Assessment (SOFA) score [11]. However, the differences in the dynamic evolution of platelets caused by clinical *E. coli* and *K. pneumoniae* bloodstream infections are still unclear, and existing scoring systems such as SOFA and Acute Physiology and Chronic Health Evaluation II (APACHE II) have insufficient specificity in predicting thrombocytopenia.

Therefore, this study intends to reveal the epidemiological characteristics of thrombocytopenia in CA-EBSI patients by comparing the platelet decline trajectories of patients with *E. coli* and *K. pneumoniae* bloodstream infections, screen the influencing factors of severe thrombocytopenia during hospitalization using the LASSO regression model, and construct a nomogram early warning model. Furthermore, animal infection models and bacterial-cell interaction transcriptomics studies were used to clarify the possible mechanisms of platelet decline caused by EBSI, in order to improve the clinical management of such patients.

2. Materials and methods

2.1 Study population

This is a retrospective study involving all adult patients with community-acquired bloodstream infections admitted to Sir Run Run Shaw Hospital, Zhejiang University School of Medicine from 2021 to 2023. Community-acquired bloodstream infection is defined as a bloodstream infection that occurs before admission or within 48 hours after admission. The institutional review board of Sir Run Run Shaw Hospital approved the study protocol and waived from the need for a consent form (20240215-576).

The specific inclusion criteria for this study are: (1) meeting the diagnostic criteria for community-acquired bloodstream infection [8, 12], and the blood culture results are only positive for *E. coli* or *K. pneumoniae*; (2) aged $\geq$18 years; (3) having complete clinical data. The exclusion criteria are: (1) patients with a hospital stay of less than 24 hours; (2) patients with underlying diseases that may influence platelet count [13]: splenomegaly, bone marrow failure syndrome (aplastic anemia, Fanconi anemia, *etc.*), hematological malignancies, myelodysplastic syndrome, solid tumor bone marrow infiltration, hereditary thrombocytopenia (giant platelet syndrome, gray platelet syndrome, *etc.*), primary immune thrombocytopenia, autoimmune thrombocytopenia (systemic lupus erythematosus, antiphospholipid syndrome, thyroid diseases, *etc.*), thrombotic thrombocytopenic purpura; (3) pregnancy.

2.2 Treatments

Patients routinely underwent blood culture, urine culture, and routine hematological examinations in the emergency department. Antibiotic treatments were given as soon as possible based on the experience prior to the culture results.

2.3 Data collection

The following data of patients were collected: (1) General information: age, gender, department admitted, body mass index (BMI), history of drinking, smoking, bleeding, blood transfusion, trauma, antiplatelet treatment, underlying diseases such as chronic obstructive pulmonary disease (COPD), cerebrovascular disease, hypertension, diabetes mellitus, coronary heart disease, heart failure, chronic kidney disease, recent hospitalization history, and the source of infection in this time. (2) Clinical laboratory indicators: including the first test results of patients at admission: white blood cell count (WBC), red blood cell count (RBC), hemoglobin (Hb), platelet count (PLT), absolute lymphocyte count (L), platelet to lymphocyte ratio (PLR), neutrophil percentage (N%), procalcitonin (PCT), C-reactive protein (CRP), serum creatinine (Scr), estimated glomerular filtration rate (eGFR), lactate dehydrogenase (LDH), lactate level (Lac), prothrombin time (PT), activated partial thromboplastin time (APTT), D-dimer, international normalized ratio (INR), albumin (ALB), *etc*. (3) Main treatment intervention measures: the first antibiotic use after admission, clinical treatment measures received within 48 hours after admission, such as central vein catheterization (CVC), mechanical ventilation (MV), continuous renal replacement therapy (CRRT), albumin infusion, immunoglobulin infusion, endoscopic retrograde cholangiopancreatography, anticoagulant therapy, corticosteroids therapy, *etc*.

2.4 Variable definition and grouping

All patients were divided into the *K. pneumoniae* bloodstream infection (KP-BSI) group and *E. coli* bloodstream infection (EC-BSI) group. Referring to the SOFA score, this study defines PLT $<150 \times 10^9$/L as thrombocytopenia, and according to the degree of platelet count reduction, thrombocytopenia is divided into: mild decrease (100×10^9/L $\leq$ PLT $< 150 \times 10^9$/L), moderate decrease (50×10^9/L $\leq$ PLT $< 100 \times 10^9$/L), severe decrease (20×10^9/L $\leq$ PLT $< 50 \times 10^9$/L), and extremely severe decrease (PLT $<20 \times 10^9$/L). According to the minimum platelet count detected in the laboratory after admission, the subjects were divided into two groups: the severe thrombocytopenia group (sTCP): PLT $<50 \times 10^9$/L, and the non-severe thrombocytopenia group (nTCP): PLT $\geq 50 \times 10^9$/L.

2.5 Animal models and cell experiments

2.5.1 Strain preparation and quantification

E. coli American Type Culture Collection (ATCC) 25922 and *K. pneumoniae* ATCC 700603 were inoculated on Mueller-Hinton (MH) solid medium and cultured at 37 °C for 16–18 hours. Single colonies were picked and transferred to Luria-Bertani (LB) liquid medium, cultured with shaking at 180 rpm until logarithmic growth phase. The bacterial cells were then resuspended with phosphate-buffered saline (PBS). The final bacterial concentration was determined by optical density. The concentration was adjusted to 10^7 colony forming unit (CFU)/200 μL PBS (intraperitoneal injection dose).

2.5.2 Mouse bloodstream infection model

Specific pathogen free (SPF)-grade C57BL/6 mice (male, 6–8 weeks old, weight 20 ± 2 g) were randomly divided into three groups (n = 5/group), namely the PBS control group, the *E. coli* infection group, and the *K. pneumoniae* infection group. 200 μL of bacterial suspension or an equal amount of PBS was injected intraperitoneally. 20 μL of blood was collected from the tail vein, anticoagulated with ethylenediaminetetraacetic acid (EDTA), and the platelet counts were measured with a cell analyzer, before infection (0 h) and 6 h, 24 h, and 48 h after infection. Six hours after infection, blood was collected from the heart of mice with bloodstream infection, anticoagulated with sodium citrate, and platelet plasma was obtained by gradient centrifugation; fixed with 2.5% glutaraldehyde at 4 °C overnight, post-fixed with 1% osmium tetroxide for 1.5 h, dehydrated with ethanol gradient (50%–100%); after critical point drying, observed with Nova Nano 450 scanning electron microscope (Thermo Fisher Scientific, FEI Company, Brno, Czech Republic). The institutional review board of Sir Run Run Shaw Hospital approved the animal study protocol (SRRSH202402688).

2.5.3 Transcriptome sequencing and analysis

Total RNA was extracted from platelets by TRIzol method, and purity was detected by NanoDrop. After strand-specific library construction, differential gene screening and functional enrichment were performed.

2.6 Statistical analysis

R 4.4.1 software was used for statistical analysis. Quantitative data with normal distribution were expressed as $\bar{x} \pm s$, and independent sample *t*-test was used for comparison between groups. Quantitative data with non-normal distribution were expressed as Median (interquartile range, IQR), and Wilcoxon rank-sum test was used for comparison between groups. Categorical data were expressed as cases and percentages, and chi-square test was used for comparison between groups of unordered categorical data. A $p < 0.05$ was considered statistically significant. The least absolute shrinkage and selection operator (LASSO) regression model was used to screen the influencing factors of severe thrombocytopenia during hospitalization, and the optimal λ value (lambda.1se) was determined by 10-fold cross-validation to screen the influencing factors. The screened influencing factors were included in the multivariate logistic regression model to calculate the odds ratio (OR) and 95% confidence interval (CI). The Hosmer-Lemeshow test was used to evaluate the goodness of fit of the model, and a $p > 0.05$ indicated a good fit of the model. According to the results of multivariate logistic regression analysis, the nomogram was drawn using the rms package of the R 4.4.1 software to visually display the prediction model. The receiver operating characteristic curve (ROC) was used to evaluate the discrimination ability of the model, the area under the curve (AUC) was calculated. The Bootstrap method was used for 1000 repeated samplings for internal validation to evaluate the robustness of the model. The model was further externally validated by using the publicly available MIMIC-IV (Medical Information Mart for Intensive Care) database, version 3.1. The calibration curve was used to evaluate the accuracy of the model, and the Brier score was calculated to test the consistency between the predicted probability of the model and the actual observed probability.

3. Results

3.1 Clinical characteristics

From January 2021 to December 2023, a total of 164 patients with CA-EBSI were included (Fig. 1). Among the included CA-EBSI patients, 29 patients (17.7%) developed severe or extremely severe thrombocytopenia during hospitalization (sTCP

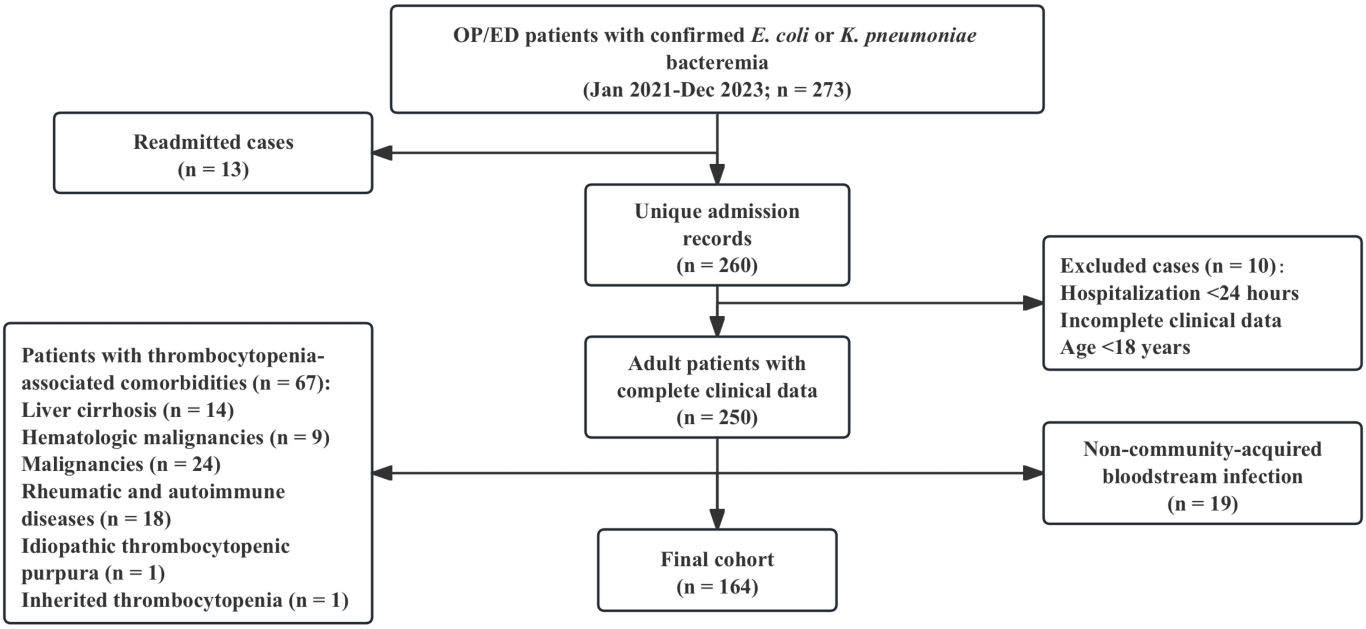

FIGURE 1. Flow diagram of participant selection for CA-EBSI cohort study. *K. pneumonia*: *Klebsiella pneumoniae*; *E. coli*: *Escherichia coli*; OP/ED: Outpatient/Emergency Department.

group, Table 1). The average age of patients in the sTCP group was 71.3 ± 13.8 years, slightly higher than that in the nTCP group (67.7 ± 14.0 years, $p = 0.215$). Among the 29 patients in the sTCP group, 18 (62.1%) were admitted to the ICU, and the median ICU stay time was 2 days (IQR: 0, 4). Compared with the nTCP group without severe or extremely severe thrombocytopenia, the ICU admission rate was significantly increased ($p = 0.003$). The median hospital stay of patients in the sTCP group was 12 days, which was significantly longer than that in the nTCP group (9 days, $p = 0.041$). In terms of the distribution of pathogenic bacteria, the proportion of *K. pneumoniae* infection in the sTCP group (41.4%) was higher than that in the nTCP group (30.4%), but the difference was not statistically significant ($p = 0.352$). 48.3% of the sTCP group were emergency surgery patients, higher than that in the nTCP group (32.6%), while the proportion of non-surgical patients in the nTCP group was higher (59.3% *vs.* 44.8%). The median time to positive blood culture in the sTCP group was 11.0 hours (IQR: 8.4–13.7), slightly shorter than that in the nTCP group (14.2 hours, IQR: 9.6–16.6), with the difference not statistically significant ($p = 0.078$). In terms of infection sources, patients in the sTCP group were mainly biliary tract infection (37.9%) and liver abscess (27.6%), while patients in the nTCP group were mainly urinary tract infection (43.7%) ($p = 0.011$).

3.2 Characteristics of thrombocytopenia in CA-EBSI patients

Among the 164 patients, about 4/5 (132/164) had thrombocytopenia during hospitalization, and most patients (61.0%, 100/164) had thrombocytopenia at admission, of which 17.7% (29/164) progressed to severe reduction (PLT $<50 \times 10^9$/L) (Fig. 2A). Both at admission and during hospitalization, there was no statistically significant difference in the rate of severe to extremely severe thrombocytopenia between patients with KP-BSI and EC-BSI (7.55% *vs.* 4.50%, and 22.64% *vs.* 14.41%, $p > 0.05$ for both). Overall, the incidence of thrombocytopenia in CA-EBSI patients during hospitalization increased significantly, and the proportion of KP-BSI patients with severe or extremely severe thrombocytopenia was higher than that of EC-BSI patients. It is worth noting that 84.7% (111/131) of patients developed the lowest platelet count within the first 3 days of admission (D0–D3), with D2 being the most (33.6%, 44/131) (Fig. 2B,C). The average platelet count began to rise from D4. The platelet change patterns of patients with EC-BSI and KP-BSI were similar. Overall, thrombocytopenia in CA-EBSI patients mainly occurred in the early stage of hospitalization and gradually recovered after a few days, and this recovery trend was consistent in different pathogen infection groups (Fig. 2C). The platelet level in the first test at admission was a key predictor of platelet changes after admission (Fig. 3). Only 12.5% (8/64) of patients with normal platelet count at admission ($\geq 150 \times 10^9$/L, n = 64) progressed to moderate to severe reduction, and none deteriorated to severe. While the risk of deterioration in patients with initial moderate reduction ($50–99 \times 10^9$/L, n = 43) increased significantly, and 37.2% (16/43) progressed to severe or more reduction. Overall, patients with higher initial platelet count at admission (normal or mild reduction) had a risk of deterioration mainly from mild to moderate during hospitalization, and the progress range was limited.

3.3 Construction and validation of nomogram prediction model

To screen important variables, we used LASSO regression analysis. The screening process was shown by regression coefficient path diagram and cross-validation curve (Fig. 4). We finally screened 10 variables with significant contributions, including age, platelet count on admission day, high-sensitivity C-reactive protein, international normalized ratio, D-dimer, lactate, use of antiplatelet drugs within 3 months, urinary tract infection source, initial antibiotics: β-lactam/β-lactamase inhibitor compound preparation, and mechanical ventilation within 48 hours of admission. The 10 screened variables were included in the multivariate logistic regression model for analysis. The analysis results showed that age (OR = 1.15, $p = 0.049$) and mechanical ventilation (OR = 5.28, $p = 0.037$) were independent risk factors for severe thrombocytopenia (Table 2).

A nomogram prediction model for severe thrombocytopenia in patients with CA-EBSI was established by including 10 influencing factors as mentioned above (Fig. 5). The individual score for each influencing factor can be obtained through the scale above the model. The result risk corresponding to the total score obtained by summing all individual scores is the probability of severe thrombocytopenia in patients with CA-EBSI.

According to the calculation results of the regression equation, ROC curves were drawn using the data of the modeling population and the validation population respectively (Fig. 6A). The AUC value of the modeling population was 0.975, and the AUC value of the validation population was 0.813, indicating that the nomogram prediction model had good discrimination ability. Calibration curves were drawn using the data of the training set population and the validation set population respectively (Fig. 6B). The horizontal axis represents the predicted value of severe thrombocytopenia in patients, and the vertical axis represents the actual proportion of severe thrombocytopenia in patients. This result showed that in both the training set population and the validation set population, the "Bias-corrected" curve of the calibration curve is close to the "Ideal" curve, indicating that the result of the model predicting severe thrombocytopenia in patients with CA-EBSI is highly consistent with the actual situation. For the training set, the mean absolute error of the calibration curve was 0.041, and the mean square error was 0.00507. The mean absolute error of the calibration curve of the validation set was 0.049, and the mean square error was 0.00275, which further supported the good performance of the model. A total of 372 patients with CA-EBSI from MIMIC-IV database were included for external validation. Among the included CA-EBSI patients, 254 patients were infected with *E. coli*, while 118 were infected with *K. pneumoniae*. Of note, 42 patients (11.3%) developed severe or extremely severe thrombocytopenia during hospitalization. The AUC value of the external validation model was 0.783, indicating that the nomogram

TABLE 1. Baseline characteristics of CA-EBSI patients.

	nTCP (n = 135)	sTCP (n = 29)	*p*-value
Age, yr	67.7 (14.0)	71.3 (13.8)	0.215
BMI (kg/m^2)	23.1 (3.1)	23.3 (3.8)	0.749
Escherichia coli, n (%)	94 (69.6)	17 (58.6)	0.352
Klebsiella pneumoniae, n (%)	41 (30.4)	12 (41.4)	0.352
ESBL+, n (%)	43 (31.9)	7 (24.1)	0.551
Admitting department, n (%)			
Hepatology & Infectious Diseases	53 (39.3)	11 (37.9)	0.264
General Surgery	30 (22.2)	13 (44.9)	
Urology	16 (11.9)	2 (6.9)	
Gastroenterology	17 (12.6)	2 (6.9)	
Emergency Medicine	14 (10.4)	0.0 (0.0)	
Other departments	5 (3.7)	1 (3.4)	
Surgical intervention, n (%)			
No surgery	80 (59.3)	13 (44.8)	0.275
Emergency surgery	44 (32.6)	14 (48.3)	
Elective surgery	11 (8.1)	2 (6.9)	
Time to blood culture positivity (h), median (IQR)	14.2 (9.6, 16.6)	11 .0 (8.4, 13.7)	0.078
Length of hospital stay (d), median (IQR)	9 (7, 12)	12 (7, 18)	**0.041**
ICU admission, n (%)	41 (30.4)	18 (62.1)	**0.003**
ICU length of stay (d), median (IQR)	0 (0, 1.5)	2 (0, 4)	**0.001**
Comorbidities, n (%)			
Hypertension	73 (54.1)	15 (51.7)	0.980
Diabetes mellitus	42 (31.1)	10 (34.5)	0.893
Cardiovascular disease	26 (19.3)	6 (20.7)	1.000
Renal insufficiency	21 (15.6)	3 (10.3)	0.667
Cerebrovascular disease	16 (11.9)	5 (17.2)	0.630
Infection source, n (%)			
Primary bloodstream infection	4 (3.0)	4 (13.8)	**0.011**
Pulmonary infection	7 (5.2)	2 (6.9)	
Liver abscess	20 (14.8)	8 (27.6)	
Biliary tract infection	38 (28.1)	11 (37.9)	
Gastrointestinal infection	4 (3.0)	1 (3.4)	
Urinary tract infection	59 (43.7)	3 (10.3)	
Vertebral infection	3 (2.2)	0 (0.0)	
Evidence level of infection source, n (%)			
Definite	121 (89.6)	25 (86.2)	0.477
Probable	13 (9.6)	3 (10.3)	
Possible	1 (0.7)	1 (3.4)	

Continuous variables with normal distribution (e.g., Age, BMI) are presented as mean ± standard deviation and compared using t-tests or ANOVA. Non-normally distributed variables (e.g., Blood culture positive time, Length of hospital stay, ICU stay time) are expressed as median (interquartile range) and analyzed with the Mann-Whitney U test. Categorical variables (e.g., Clinical departments, Patient categories) are shown as n (%) and compared by Chi-square test or Fisher's exact test where appropriate. Values in bold font denote statistical significance ($p < 0.05$). nTCP: non-severe thrombocytopenia group; sTCP: severe thrombocytopenia group; BMI: body mass index; ESBL: Extended-Spectrum β-Lactamase; IQR: interquartile range; ICU: intensive care unit.

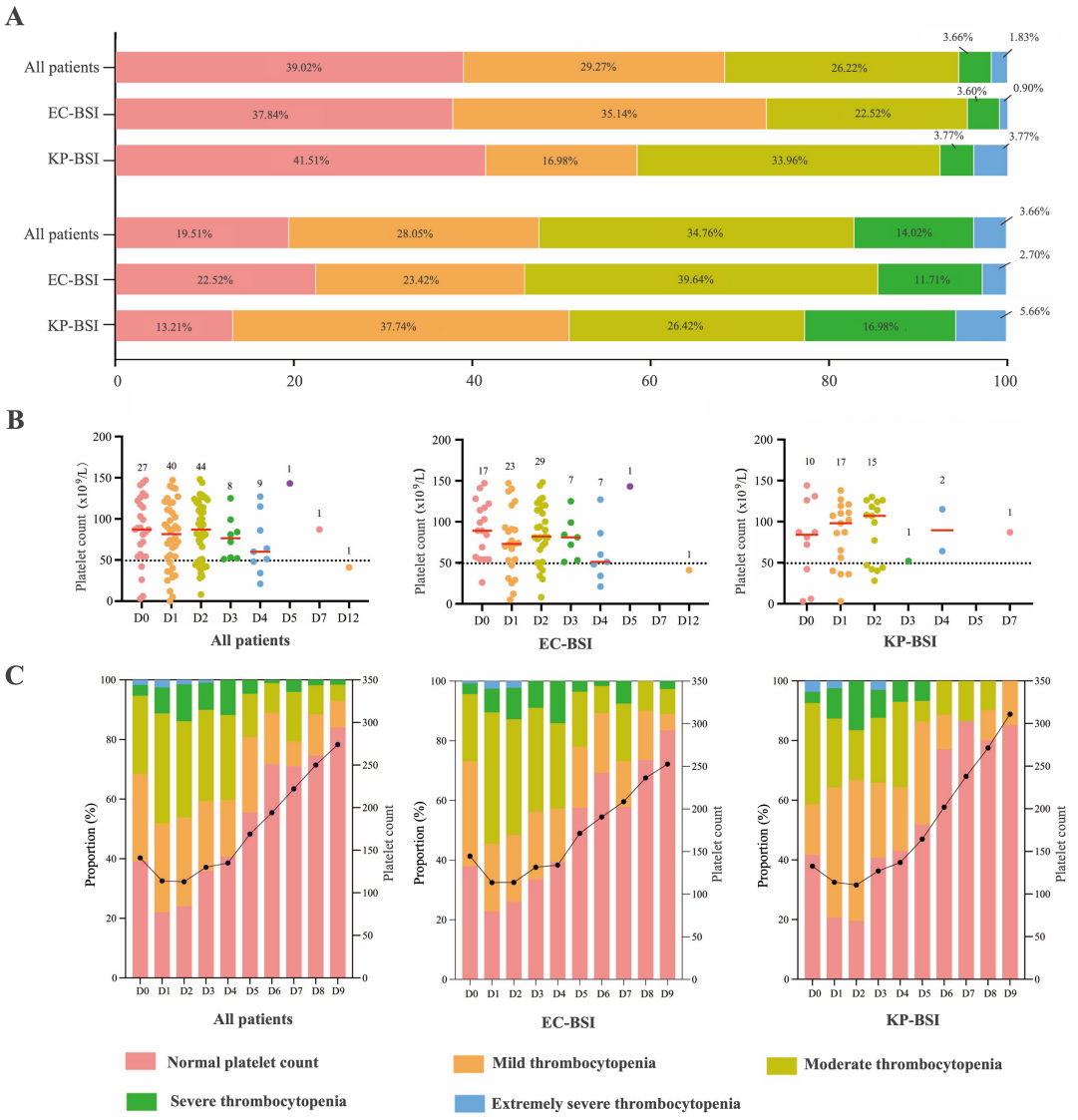

FIGURE 2. Incidence and temporal dynamics of thrombocytopenia in hospitalized patients with *Escherichia coli* or *Klebsiella* pneumoniae bloodstream infection. (A) Stacked bar chart comparing the maximum severity of thrombocytopenia (based on nadir platelet count during hospitalization) against the baseline severity at admission. (B) Scatter plot showing the relationship between the timing (days since admission) and the value of the platelet count nadir. The horizontal dashed line indicates the threshold for severe thrombocytopenia (50×10^9/L). (C) Line graph (right y-axis) illustrating the mean platelet count trajectory over time. The stacked area chart (left y-axis) depicts the daily changing proportion of patients by thrombocytopenia severity category. Data are stratified by the causative pathogen throughout all panels. KP-BSI: *K. pneumoniae* bloodstream infection; EC-BSI: *E. coli* bloodstream infection.

prediction model is stable and adaptable (Fig. 6C). The AUC value of the external validation was limited, which could be attributed to the substantial missing data for critical predictors (D-dimer and CRP) in the MIMIC-IV dataset.

3.4 Possible molecular mechanism of thrombocytopenia in *Enterobacterales* infections

A mouse model of bloodstream infection with *E. coli* ATCC 25922 and *K. pneumoniae* ATCC 700603 was constructed. Intraperitoneal injection of 10^7 CFU was the optimal infection dose, which could stably induce mouse bloodstream infection (100% infection rate at 6 hours, ≥80% survival rate at 48 hours), and the blood bacterial load increased significantly (*E. coli* ATCC 25922: 750 CFU/mL; *K. pneumoniae* ATCC 700603: 900 CFU/mL). It was also confirmed that bloodstream infection could lead to a significant decrease in platelet count. The platelet counts of mice with bloodstream infection began to decrease 6 hours after infection (a decrease of 24%–43%) and dropped to 44%–56% of the baseline value at 48 hours (Fig. 7A). Scanning electron microscopy showed that platelets in the infected group had pathological changes such as aggregation, pseudopod extension, and membrane rupture, and some platelets had bacterial adhesion on their surfaces (Fig. 7B).

Transcriptomic data analysis showed that *E. coli* ATCC 25922 infection caused 1449 differential genes (681 up-regulated/768 down-regulated), *K. pneumoniae* ATCC

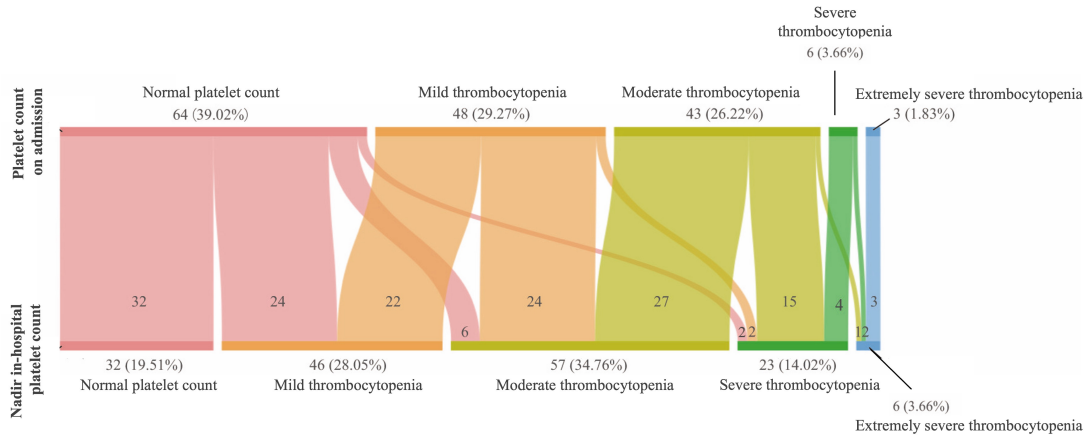

FIGURE 3. Temporal evolution and worsening of thrombocytopenia during hospitalization. Stacked bar charts depict the shift in severity categories from admission (Top) to the platelet count nadir (Bottom). At admission, most patients (39.0%) had normal platelet counts. By the time of the nadir, this proportion was halved (19.5%), with a corresponding marked increase in the prevalence of moderate (26.2% to 34.8%), severe (3.7% to 14.0%), and extremely severe (1.8% to 3.7%) thrombocytopenia.

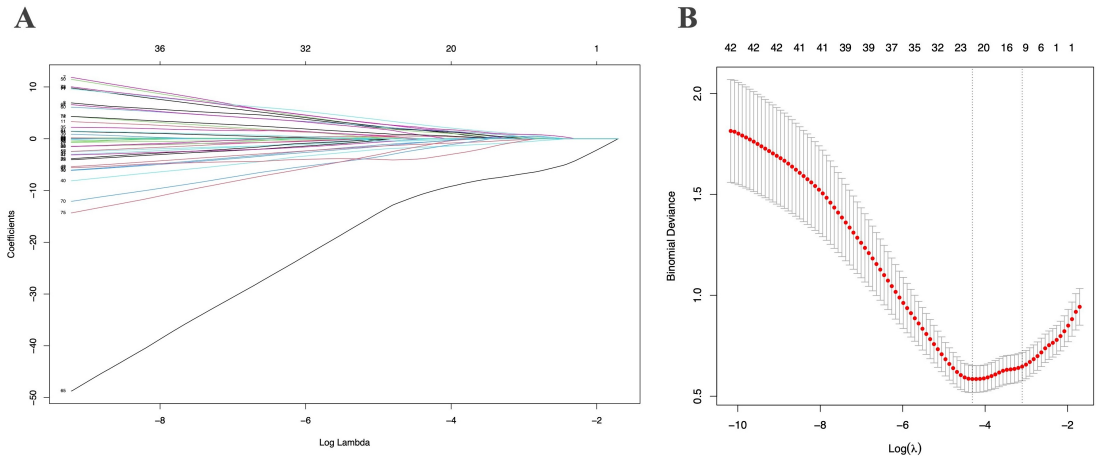

FIGURE 4. LASSO regression: coefficient paths and cross-validation curve. (A) LASSO coefficient paths. (B) LASSO cross-validation curve.

TABLE 2. Multivariable logistic regression analysis of severe thrombocytopenia in CA-EBSI patients.

Variable	*β*	SE	Wald *Z*	*p*-value
Intercept	−20.235	11.184	−1.81	0.070
Age	0.141	0.071	1.97	0.049
Antiplatelet drugs	−5.321	5.009	−1.06	0.288
Urinary tract infection	−9.786	28.450	−0.34	0.731
β-Lactam/BLI combination	−1.192	1.361	−0.88	0.381
Mechanical ventilation	5.284	2.526	2.09	0.037
Platelet count on admission	−0.033	0.012	−2.71	0.007
CRP	−0.007	0.005	−1.29	0.198
INR	4.500	2.941	1.53	0.126
D-dimer	0.334	0.199	1.68	0.093
Lac	0.938	0.567	1.65	0.098

Full variable names: Age; Platelet count on admission; High-sensitivity C-reactive protein (hs-CRP) on admission; International normalized ratio (INR) on admission; D-dimer on admission; Lactate on admission; Antiplatelet drug use within 3 months; Urinary tract infection source; Initial antibiotics: β-lactam/β-lactamase inhibitor combination; Mechanical ventilation within 48 h of admission. CRP: C-reactive protein; INR: international normalized ratio; Lac: lactate level; SE: standard error; BLI: β-Lactamase inhibitor.

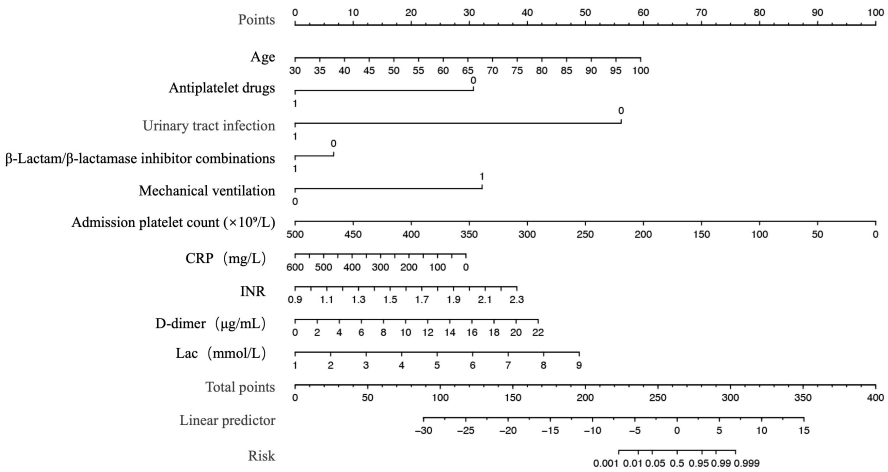

FIGURE 5. Nomogram for predicting severe thrombocytopenia in CA-EBSI patients. Each significant predictor is assigned points on the variable axis. Summing these points yields a total score on the "Total Points" scale. A vertical line drawn downward from the total score intersects the "Risk" axis, indicating the estimated risk. CRP: C-reactive protein; INR: international normalized ratio; Lac: lactate level.

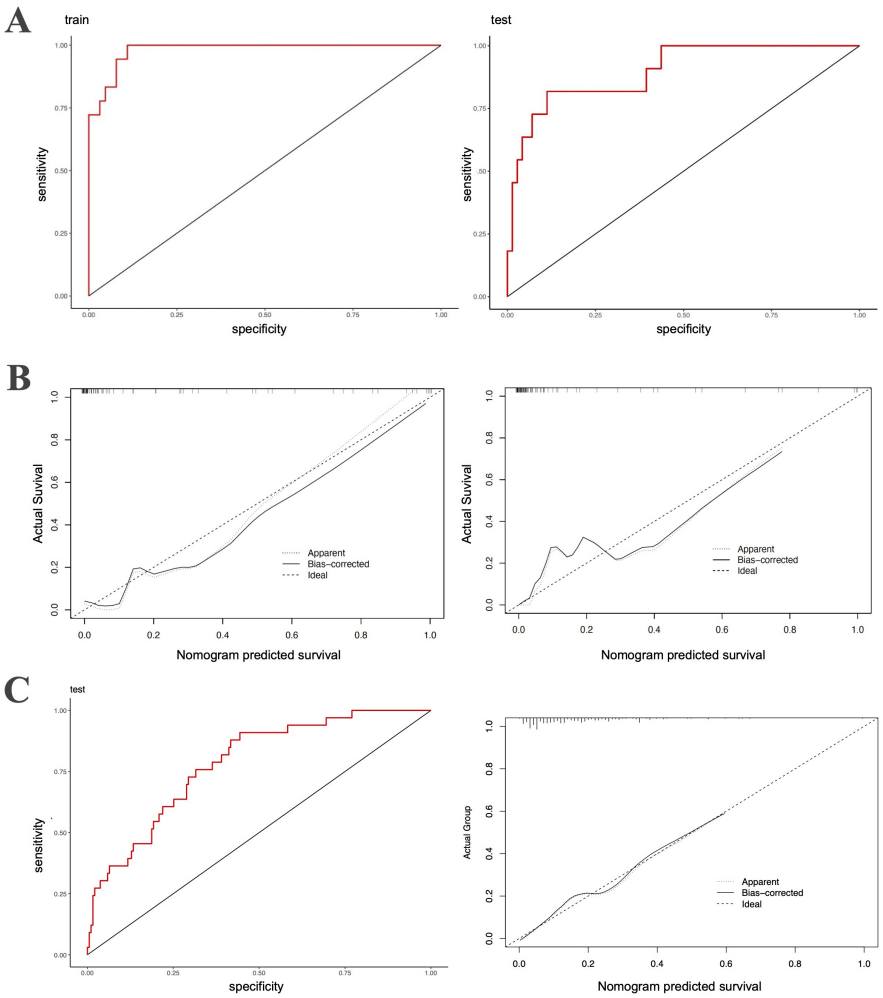

FIGURE 6. Validation of the nomogram for predicting severe thrombocytopenia in CA-EBSI patients. (A) ROC curves of the nomogram for severe thrombocytopenia prediction in CA-EBSI patients: Left—derivation cohort; Right—validation cohort. (B) Calibration curves of the nomogram for severe thrombocytopenia prediction in CA-EBSI patients: Left—derivation cohort; Right—validation cohort. (C) External validation of the nomogram in an independent test cohort: Left—ROC curve; Right—Calibration curve.

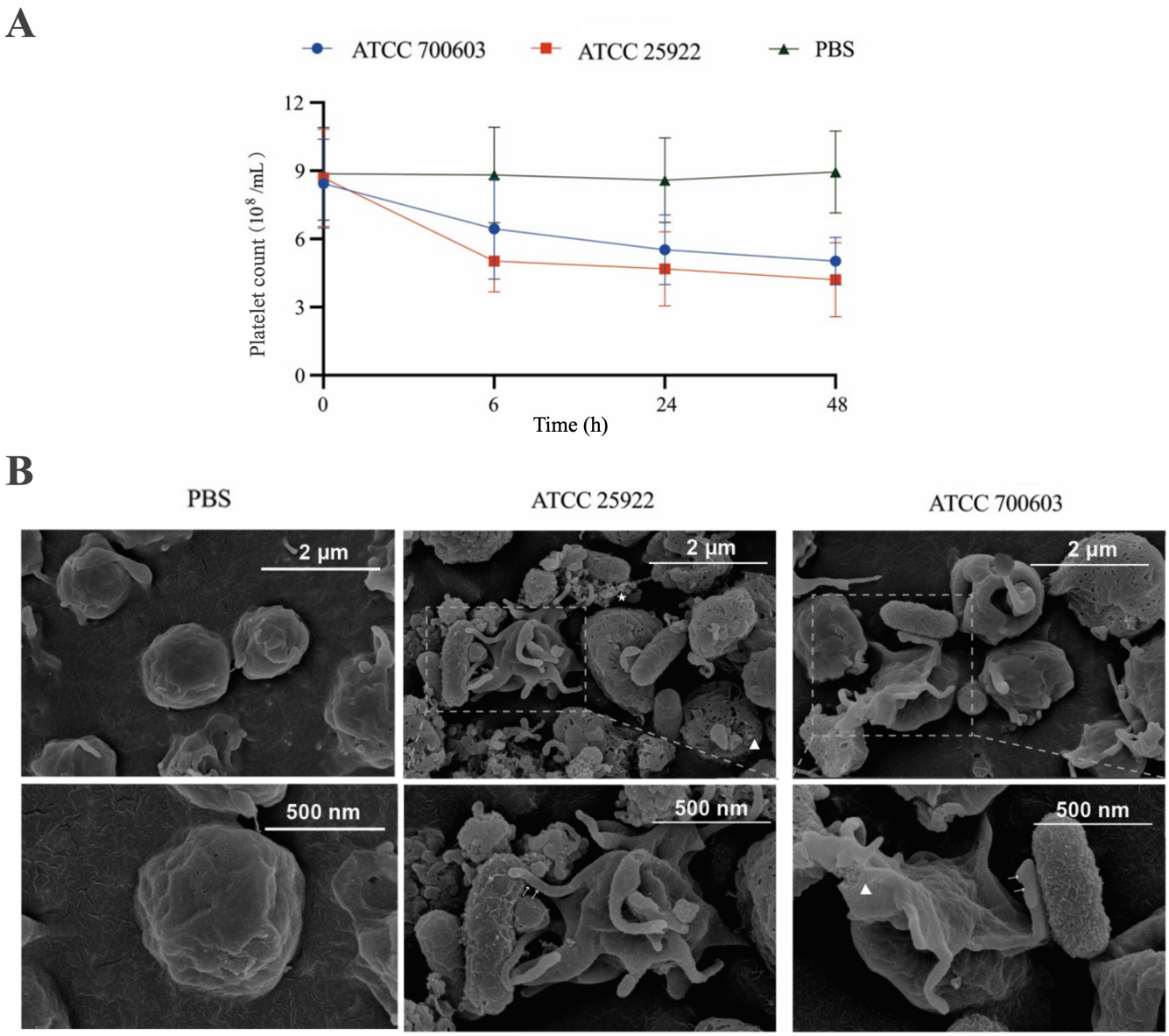

FIGURE 7. Platelet dynamics and morphological changes in murine bacteremia models. (A) Platelet count dynamics in bacteremic mice over time. (B) Platelet morphological changes in *K. pneumoniae* ATCC 25922 and *E. coli* ATCC 700603 bacteremic mice by SEM. Scale bars: low mag 2 μm, high mag 500 nm; arrows indicate platelet pseudopods. ATCC: American Type Culture Collection; PBS: phosphate-buffered saline.

700603 infection caused 468 differential genes (388 up-regulated/80 down-regulated), and there were 191 common differential genes between the two bacteria. Differential gene screening and functional enrichment analysis suggested that thrombocytopenia caused by bloodstream infection is a complex process driven by multiple factors, involving adhesion disruption, enhanced pro-inflammatory signals, and apoptosis signal induction (Fig. 8).

4. Discussion

This study revealed that 84.7% of CA-EBSI patients had the lowest platelet count within 3 days of admission, and the time of the lowest value was independent of the infecting pathogen. The first platelet level at admission is a key predictor of subsequent platelet changes. This finding suggests that clinical stratified monitoring of such patients should be based on the baseline level. The nomogram model (AUC = 0.975) constructed based on the above characteristics integrates independent risk factors such as age and mechanical ventilation, providing a quantitative tool for early identification of high-risk patients, and making up for the deficiency of traditional scoring systems (such as SOFA) in the prediction specificity of thrombocytopenia.

It is worth noting that although the incidence of severe thrombocytopenia in KP-BSI was higher than that in EC-BSI, there was no statistical difference. This trend may highly suggest that *K. pneumoniae* infection, especially hypervirulent strain infection, may be accompanied by more intense inflammatory response and more severe clinical outcomes. The severity of thrombocytopenia is an important biomarker for evaluating the disease burden

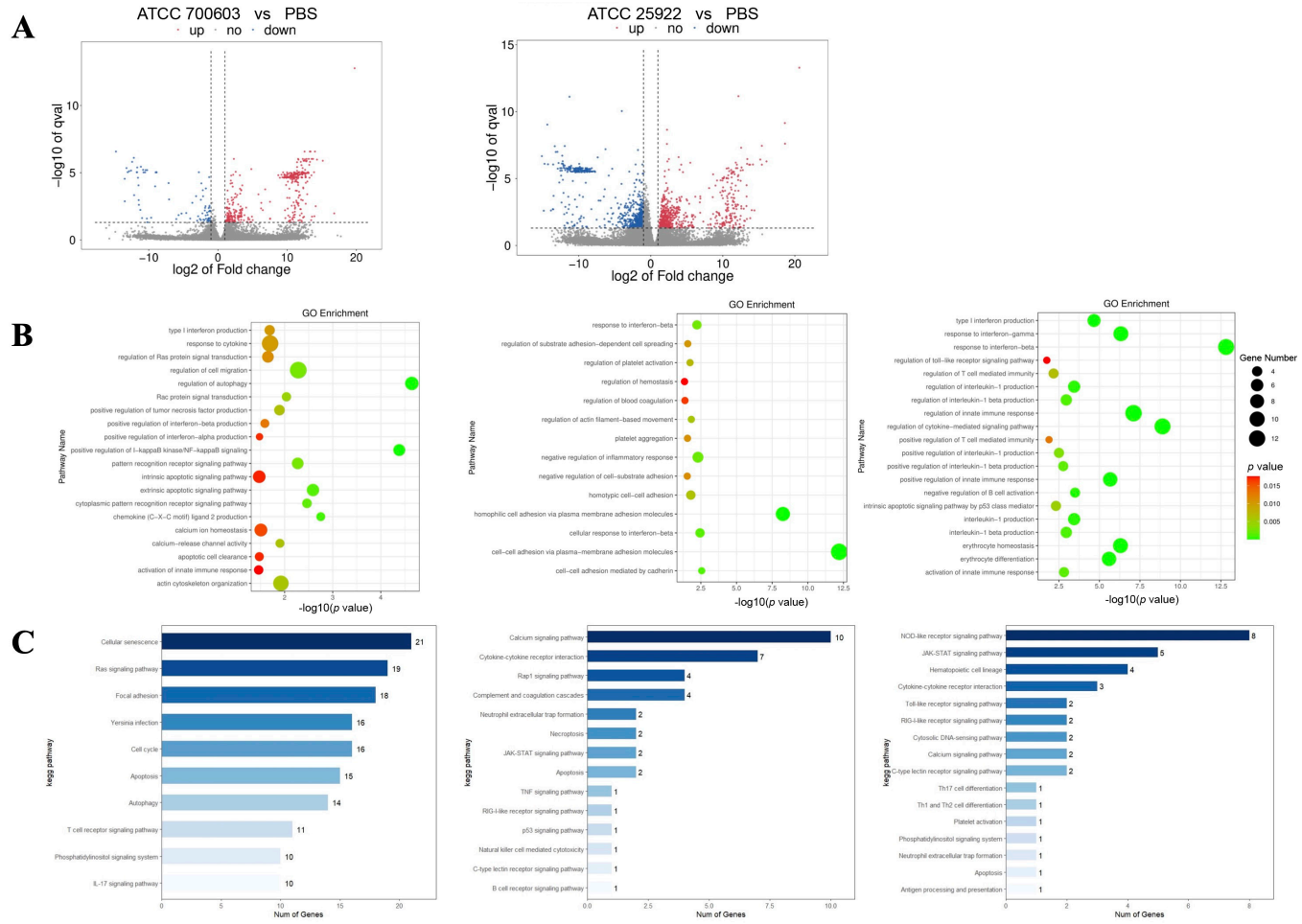

FIGURE 8. Transcriptomic profiling of platelets in murine bacteremia models. (A) Volcano plot of differentially expressed genes (DEGs). Red: significantly upregulated DEGs. Blue: significantly downregulated DEGs. Gray: non-significant genes. (B) GO enrichment analysis. Left: Unique DEGs in *E. coli* ATCC 25922 group. Center: Unique DEGs in *K. pneumoniae* ATCC 700603 group. Right: Shared DEGs in both pathogens. (C) KEGG pathway enrichment. Left: *E. coli*-specific pathways. Center: *K. pneumoniae*-specific pathways. Right: Shared pathways between pathogens. ATCC: American Type Culture Collection; PBS: phosphate-buffered saline; GO: gene ontology; IL: interleukin; JAK-STAT: Janus kinase-signal transducer and activator of transcription; TNF: tumor necrosis factor; NOD: nucleotide-binding oligomerization domain.

and predicting poor prognosis of such infections [14, 15]. This difference may be partially explained by the unique pathogenic mechanism of hypervirulent *K. pneumoniae* strains (such as hypermucoviscous phenotype K1/K2 types), Existing experiments have confirmed that they significantly increase lipopolysaccharide (LPS) release through capsular polysaccharides, strongly activate platelet toll-like receptor 4/myeloid differentiation primary response 88 (TLR4/MyD88) and c-Jun N-terminal kinase/mitogen-activated protein kinase (JNK/MAPK) pathways, induce platelet aggregation (maximum aggregation rate increased by 2.1 times) and mitochondrial membrane potential-dependent apoptosis, and further inhibit megakaryocyte cluster of differentiation (CD) 41 expression and polyploidization [16–19]. This multiple and potent mechanisms of platelet consumption and production inhibition leads to a more significant decrease in quantity and extensive intravascular platelet aggregation (immune thrombosis) and inflammatory mediator storm, which were key links driving organ microcirculation disorders, tissue hypoxia, and even MODS [20–22].

At the same time, the high consistency of platelet evolution patterns observed in the two groups of patients in this study (both rapidly dropped to the lowest value in D0–D3, and gradually recovered in D7–D9) may reflect the common pathophysiological process of EBSI: (1) Systemic LPS release activates the TLR4 pathway to trigger platelet apoptosis [23, 24]; (2) Immune thrombosis leads to intravascular platelet consumption [24, 25]. While this process clears pathogens, it also causes extensive microvascular occlusion and tissue damage, which is one of the core mechanisms of septic shock and organ failure; (3) Inflammatory factor storm (such as tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6)) inhibits megakaryocyte maturation [26, 27]. High levels of inflammatory factors directly mediate severe sepsis manifestations such as fever, hypotension, and capillary leakage, and their inhibition of bone marrow further weakens the body's compensatory capacity. Therefore, the degree of platelet count decreases, the rate of decrease, and the recovery time reflect the severity of the body's damage from infection. A large number of evidence-based medical evidence shows that thrombocytopenia in sepsis pa-

tients, especially severe reduction or rapidly progressive reduction, is an independent risk factor for organ dysfunction such as shock, acute respiratory distress syndrome (ARDS), acute kidney injury (AKI), disseminated intravascular coagulation (DIC), and death [28–31]. The sharp decrease in platelet count not only means an increased risk of hemostatic dysfunction but also marks the excessive activation of the inflammation-coagulation cascade, the aggravation of endothelial damage, and the exhaustion of bone marrow reserve function, which are direct manifestations of critical ill state and poor prognosis. Future studies need to combine strain virulence gene typing to further clarify how pathogen-specific mechanisms affect the amplitude and pattern of thrombocytopenia and ultimately affect the severity and outcome of the disease.

The animal experiment results in this study further support the clinical observation that EBSI can directly and rapidly trigger significant thrombocytopenia. Scanning electron microscopy observed platelet pseudopod extension, aggregation, and bacterial surface adhesion, showing the process of platelet activation and consumption in the early stage of infection. Our transcriptome analysis indicates that thrombocytopenia during infection is synergistically driven by multiple pathways. These pathways not only mediate the decrease in platelet count but also serve as critical molecular hubs connecting severe infections to poor clinical outcomes. Consequently, therapeutic intervention targeting these key pathways (such as TLR4/MyD88 or JNK/MAPK) could potentially ameliorate thrombocytopenia and improve survival rates in patients with severe ESBI.

This study still has some limitations. Firstly, single-center retrospective design may limit the extrapolation of conclusions, and the impact of ESBL-positive strains on thrombocytopenia was not analyzed. Secondly, standard strains were used in animal experiments, not including clinical drug-resistant strains. Based on the above findings, we put forward the following clinical practice suggestions: (1) Baseline platelet level at admission should be routinely assessed for CA-EBSI patients, as it is the cornerstone for predicting subsequent platelet dynamic change trajectory and potential disease severity risk. Intensive monitoring from D0 to D3 should be implemented for patients with moderate reduction at admission, aiming to early identify patients who may rapidly progress to high-risk status (severe thrombocytopenia); (2) Elderly patients (>70 years old) or patients requiring mechanical ventilation should use the nomogram model to screen disease progression, so as to timely strengthen hemodynamic monitoring, organ function support, and targeted anti-infection treatment, and improve the prognosis to the greatest extent.

5. Conclusions

Thrombocytopenia in CA-EBSI patients is characterized by early and rapid progression, and the baseline platelet level significantly affects the progression of the disease. Advanced age and mechanical ventilation are independent risk factors for severe reduction. Our findings indicate that thrombocytopenia may be driven by multiple factors and is mechanistically linked to platelet activation and consumption.

ABBREVIATIONS

CA-EBSI, community-acquired *Enterobacterales* bloodstream infections; BSI, bloodstream infection; MODS, multiple organ dysfunction syndrome; PLT, platelet count; SOFA, Sequential Organ Failure Assessment; APACHE II, Acute Physiology and Chronic Health Evaluation II; BMI, body mass index; WBC, white blood cell count; RBC, red blood cell count; Hb, hemoglobin; L, lymphocyte count; PLR, platelet to lymphocyte ratio; N%, neutrophil percentage; PCT, procalcitonin; CRP, C-reactive protein; Scr, serum creatinine; eGFR, estimated glomerular filtration rate; LDH, lactate dehydrogenase; Lac, lactate level; PT, prothrombin time; APTT, activated partial thromboplastin time; INR, international normalized ratio; ALB, albumin; CVC, central vein catheterization; MV, mechanical ventilation; CRRT, continuous renal replacement therapy; KP-BSI, *K. pneumoniae* bloodstream infection; EC-BSI, *E. coli* bloodstream infection; sTCP, the severe thrombocytopenia group; nTCP, the non-severe thrombocytopenia group; LASSO, least absolute shrinkage and selection operator; OR, odds ratio; CI, confidence interval; ROC, receiver operating characteristic curve; AUC, area under the curve; ARDS, acute respiratory distress syndrome; AKI, acute kidney injury; DIC, disseminated intravascular coagulation; *K. pneumonia*, *Klebsiella pneumonia*; *E. coli*, *Escherichia coli*; ICU, intensive care unit; COPD, chronic obstructive pulmonary disease; ATCC, American Type Culture Collection; MH, Mueller-Hinton; LB, Luria-Bertani; PBS, phosphate-buffered saline; CFU, colony forming unit; SPF, specific pathogen free; EDTA, ethylenediaminetetraacetic acid; IQR, interquartile range; MIMIC, Medical Information Mart for Intensive Care; LPS, lipopolysaccharide; TLR4/MyD88, toll-like receptor 4/myeloid differentiation primary response 88; JNK/MAPK, c-Jun N-terminal kinase/mitogen-activated protein kinase; CD, cluster of differentiation; TNF-α, tumor necrosis factor-α; IL-6, interleukin-6.

AVAILABILITY OF DATA AND MATERIALS

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

AUTHOR CONTRIBUTIONS

XXW and PHY—designed the research study. XXW and JN—performed the research. PHY, CZ and ZSH—provided help and advice. JN and CZ—analyzed the data. XXW, JN and LNC—wrote the manuscript. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The institutional review board of Sir Run Run Shaw Hospital approved the study protocol and waived from the need for a consent form (20240215-576). The institutional review board of Sir Run Run Shaw Hospital approved the animal study

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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