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ORIGINAL RESEARCH

LC-MS-Based Serum Metabolomic Analysis Predicts the Risk of Tigecycline-Induced Coagulopathy in Critically Ill Patients

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Purpose: Tigecycline is widely used to treat multidrug-resistant infections. However, the high incidence of coagulopathy poses a significant clinical challenge. This observational study aimed to characterize the metabolomic profiles of critically ill patients receiving tigecycline and to identify potential metabolic traits to predict tigecycline-induced coagulopathy (TIC).

Patients and Methods: A total of 53 patients were enrolled and classified into TIC and non-TIC groups. Serum samples were collected at trough (Cmin), mid-dose (C1/2), and peak (Cmax) tigecycline concentrations. LC-MS–based untargeted metabolomics was applied to characterize metabolic profiles across these timepoints and to identify metabolites potentially predictive of TIC.

Results: By sequentially applying univariate analysis and multivariate LASSO-penalized Cox proportional hazards regression analysis, we identified 10, 10, and 9 metabolites at the Cmin, C1/2, and Cmax timepoints, respectively, as predictive markers of TIC. Importantly, patients with lower levels of lysophosphatidylcholines (LysoPCs) and lysophosphatidylethanolamines (LysoPEs) are more susceptible to coagulopathy following tigecycline therapy. In particular, receiver operating characteristic curve analysis of LysoPC (18:0), LysoPC (18:3), LysoPE (18:0), and LysoPE (18:4) measured at Cmin demonstrated an area under the curve close to 0.8, providing strong evidence for their potential as robust biomarkers for predicting TIC.

Conclusion: Our study indicated that metabolomics could be a valuable tool for predicting the risk of TIC and suggested that LysoPCs and LysoPEs might serve as hypothesis-generating candidates for future studies exploring potential therapeutic interventions.

Keywords: critically ill patients, metabolomics, tigecycline, coagulopathy, prediction

Introduction

The growing prevalence of multidrug-resistant (MDR) bacterial pathogens has become a serious global threat.[1] Therefore, the introduction of novel antibiotics with broad-spectrum activity against multidrug-resistant bacteria is urgently required. Tigecycline, a first-in-class tetracycline antibiotic, demonstrates potent activity against both aerobic and anaerobic Gram-positive and Gram-negative organisms, including methicillin-resistant *Staphylococcus aureus* (MRSA), extended-spectrum β-lactamase-producing *Escherichia coli*, penicillin-resistant *Streptococcus pneumoniae*, and vancomycin-resistant *Enterococcus faecalis*.[2,3] Approved by the US Food and Drug Administration in 2005 for the treatment of complicated skin and soft tissue infections, complicated intra-abdominal infections, and later for community-acquired pneumonia in 2009, tigecycline was introduced into the Chinese market in late 2011.[4] Nowadays, tigecycline is widely used in clinical practice.

As a broad-spectrum antibiotic, tigecycline demonstrates excellent antibacterial activity and mild adverse drug reactions (ADRs). Frequently reported ADRs include nausea, vomiting and diarrhoea.[5] However, with the widespread

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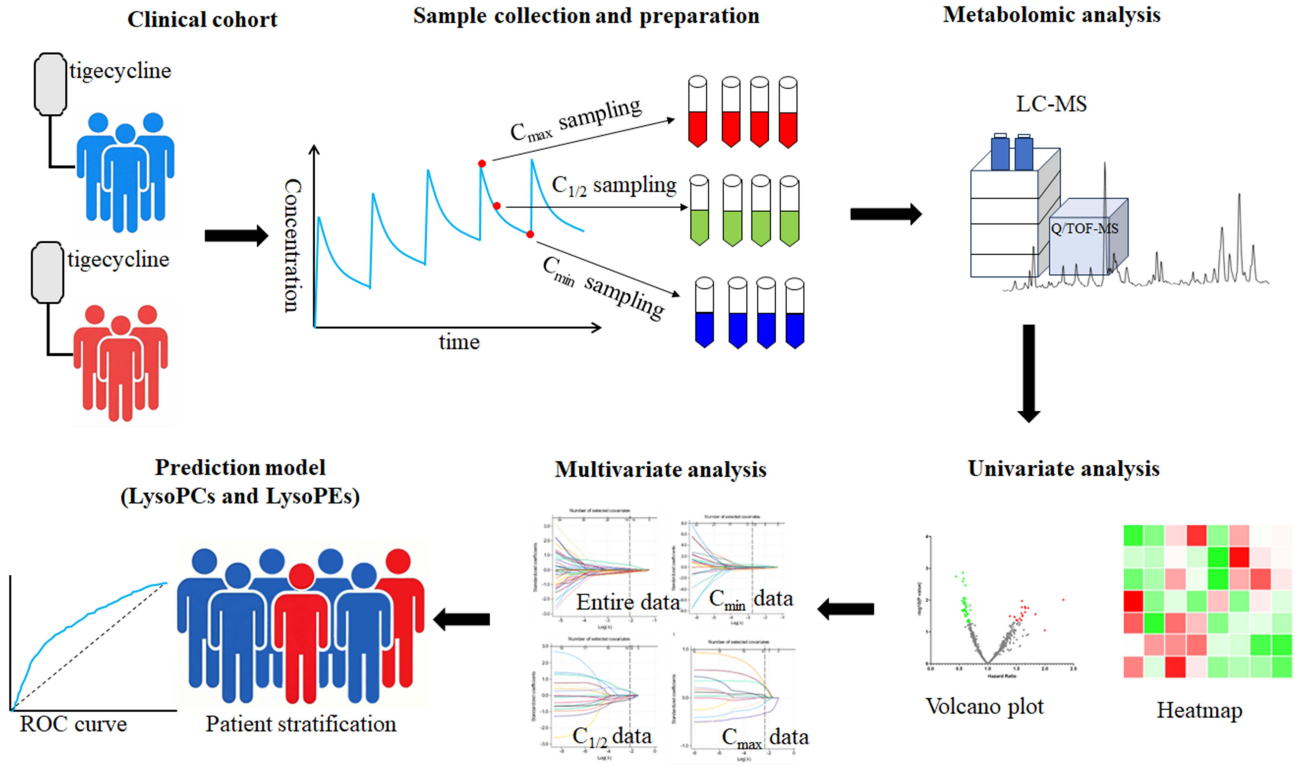
Graphical Abstract



clinical use of tigecycline, coagulopathy has begun to appear and has attracted the attention of physicians. The first case of tigecycline-induced coagulopathy (TIC) was reported by Herwig Pieringer et al in 2010, and then a series of case reports and clinical characteristics have been published.[6] Approximately 50% patients experienced TIC with increased prothrombin time (PT), activated partial thromboplastin time (aPTT) and thrombin time (TT), and/ or decreased fibrinogen (Fib).[7–10] Coagulopathy can aggravate bleeding complications, prolong ICU stays and increase mortality.[11] Among the patients who received tigecycline, about 10% suffered from gastrointestinal bleeding, mucocutaneous bleeding and/or airway bleeding.[7,12]

The significantly different responses of patients to tigecycline treatment depend on many factors such as lifestyle, genetic background and clinical factors. Case reports and retrospective studies describe the medical problems and clinical management of a single patient or group of individuals, which advances medical science and improves healthcare delivery. At this time, almost all published studies are case reports and retrospective studies aimed at revealing the multiple risk factors associated with TIC.[7–9,13–15] Reported risk factors include treatment dose and duration, baseline fibrinogen levels, and patient sex. However, the conclusions remain inconsistent and sometimes contradictory. Given the inherent limitations of case reports and retrospective studies, definitive causal relationships between these risk factors and the development of coagulopathy require further investigation through well-designed prospective studies.[16] Additionally, clinical indicators of TIC, that is, the levels of Fib, PT, aPTT, and TT, are insufficient to predict the risk, let alone the potential causative processes.

Metabolomics generates comprehensive profiles of endogenous metabolites in biological matrices, and these metabolites reflect the functional output of the genome and proteome, offering insights into cellular processes, metabolic pathways, and potential biomarkers for disease.[17] Coagulopathy is characterized by the unbalance between coagulation and fibrinolysis, which involved coagulation and complement related genes and proteins.[18] Thus, small-scaled changes in gene expression and protein abundance are amplified to produce obvious large-scale changes in endogenous metabolite level. During the last few decades, studies have linked metabolomic profiles to disease onset, drug action and mortality,

appreciating the blood metabolome as a direct reflection of human physiopathological state.[19–21] Therefore, the pretreatment plasma metabolome has the potential to reveal TIC risk. To the best of our knowledge, in this study, a liquid chromatography–quadrupole time-of-flight mass spectrometry-based metabolomic approach was employed to identify distinct metabolic signatures associated with tigecycline treatment response. Our results will pave the way for personalized therapeutic strategies for tigecycline treatment.

Materials and Methods

Study Participants

Critically ill patients aged ≥ 18 years who intravenously received tigecycline for > 72 h at the intensive care unit of Nanjing Drum Tower Hospital from January 1, 2022, to December 31, 2023, were enrolled. The two-year period was selected because plasma metabolite profiles are generally stable within individuals over such a timeframe, and this duration covers two full seasonal cycles, helping to mitigate potential seasonal variability.[22] The sample size was based on patient availability and included all patients meeting the inclusion criteria. Patients received a 100 mg loading dose and 50 mg twice a day thereafter, or a 200 mg loading dose and 100 mg twice a day thereafter, depending on the physicians' decisions. Patients with Fib levels lower than 2.0 g/L, missing clinical data, or bleeding complications before tigecycline treatment were excluded. This study was approved by the ethics committee of Nanjing Drum Tower Hospital (No. 2022–040-01) and registered at the China National Medical Research Registration and Filing Information System (No. MR-32-22-017478). Written informed consent was signed by all the study participants. The study was conducted according to the Declaration of Helsinki.

Data Collection and Definitions

Sociodemographic data, including age, sex, maintained tigecycline dose, therapy duration, infection site, and pathogenic microorganisms, were extracted from the hospital information system. Sequential Organ Failure Assessment (SOFA) scores and Acute Physiology and Chronic Health Evaluation (APACHE) II scores were used to assess illness severity. Since serum vitamin K levels and coagulation factor concentrations were not routinely measured, hematological and coagulation-related indices, such as hemoglobin (HGB), white blood cell count (WBC), platelet count (PLT), PT, aPTT, Fib and TT, which indirectly reflect the function of the intrinsic and extrinsic coagulation pathways, were recorded at baseline, during, and after tigecycline treatment.[23] Other clinical biochemical parameters of hepatic and renal functions were also collected. Baseline values were defined as the most recent laboratory results available within 24 hours prior to the initiation of tigecycline therapy, reflecting the patient's status at treatment onset.[24] TIC was defined by the following criteria: aPTT >60 s, PT > 17s, TT > 24s, or Fib < 2 g/L occurring after tigecycline therapy.[7,10] The patients were divided into two groups: TIC group and non-TIC group.

Sample Collection and Preparation

After steady-state concentrations were reached (ie at least six tigecycline administrations), peripheral venous blood samples were collected at three time points: immediately after the end of administration, 6h after administration, and before the next administration. All samples were divided into two parts: one part was used for drug concentration detection; another was centrifuged at 3000 rpm for 10 min at 4 °C to obtain serum and then stored at −80 °C for metabolomic analysis. Tigecycline concentrations, including peak concentration (Cmax), trough concentration (Cmin), and mid-dosing interval concentration (C1/2), were obtained from the hospital information system. The area under the time-concentration curve (AUC) was calculated and extrapolated from 0 to 24 h based on the linear trapezoidal rule using each concentration set.[9,25]

For metabolomic analysis, frozen serum samples were thawed in a refrigerator at 4 °C and mixed with ice-cold acetonitrile in a volume ratio of 1:4. Then the mixture was vortexed thoroughly for 5 min and centrifuged twice (14000 rpm, 4 °C, 10 min) to obtain supernatant for instrumental analysis.

LC-MS Based-Metabolomics

Metabolomic experiment was performed on ultra-performance liquid chromatography system (Exion LC AD System, AB SCIEX LLC., Redwood City, CA, USA) coupled with quadrupole time-of-flight mass spectrometer (TripleTOF® 5600+, AB SCIEX LLC., Redwood City, CA, USA). For chromatographic separation, each sample in a volume of 2 μL was introduced into an ACQUITY HSS T3 column (2.1 mm × 100 mm, 1.8 μm) (Waters, Milford, MA, USA) connected to an Acquity HSS T3 Vanguard pre-column (2.1 mm × 5 mm, 1.8 μm) (Waters, Milford, MA, USA). Mobile phases A and B were acetonitrile and 0.1% formic acid, respectively. Gradient elution was performed at a flow rate of 0.4 mL/min as follows: 0.0 −1.5 min 99%B; 1.5 −13 min, 99%-1%B; 13–16.5 min, 1%B, returned to initial conditions, and maintained for 3 min. For mass spectrometric data acquisition, the samples were delivered to a DuoSpray ion source through an electrospray ionization probe. The information-dependent acquisition mode was used to trigger tandem mass events. The ion spray voltages were 5500 V and −4500 V in the positive and negative modes, respectively. The source temperature was 550 °C. The pressure values for the nebulizer, heater, and curtain gases were set to 55, 55, and 35 psi, respectively. The collision energy was 35V with a collision energy spread of 15V. Additionally, an automated calibration delivery system was used to calibrate the mass spectrometer. To investigate the robustness and reproducibility of the analytical run, pooled serum quality samples (QCs) from the individuals involved in this study were injected ten times at the beginning of the batch analysis and periodically throughout the analytical batch.

Statistical Analyses

The raw mass data were imported into MS-DIAL software for peak alignment, peak picking and peak identification. The resulting peak features with at least 80% presence in the analyzed samples and a relative standard deviation (RSD) of less than 20%, as calculated for the pooled quality control samples, were retained for further analysis. If any, missing values were imputed to half of the minimum value for the corresponding metabolites. Following normalization to the total spectral area and logarithmic transformation, unsupervised principal components analysis (PCA) was performed to assess the stability of the analytical system. Meanwhile, the peak features were unambiguously or putatively annotated according to the criteria proposed by the Metabolite Standard Initiative (MSI).[26] Metabolite identification was confirmed through a combination of methods. For metabolites with authentic standards available in our laboratory, identification was based on matching both accurate mass and retention time. For others, tandem mass spectrometry (MS/MS) fragmentation patterns were compared with reference spectra in public databases, such as the Human Metabolome Database (HMDB, http://www.hmdb.ca) and Metlin (http://metlin.scripps.edu).

To compare the baseline clinical data between the TIC and non-TIC groups, appropriate statistical tests were used, including the Student's *t*-test, Mann–Whitney *U*-test, chi-square test, or Fisher's exact test. To identify factors associated with the development of TIC, univariate Cox proportional hazards regression analysis was performed using baseline clinical data, tigecycline regimen, and metabolite levels. Owing to the exploratory nature of this study, multiple-testing corrections were omitted to preserve potential risk predictors.[27,28] Additionally, we employed Least Absolute Shrinkage and Selection Operator (LASSO)-penalized Cox proportional hazards regression analysis with ten-fold cross-validation to determine a risk signature for coagulopathy. All statistical analyses were performed using STATA MP 16.0 (StataCorp, TX, United States). Statistical significance was set at $p < 0.05$.

Results

General Patient Characteristics

In total, 53 critically ill patients treated with tigecycline were included in this study. The cohort was predominantly male (67.92%), with a mean age of 61.75 years (±18.09). Of these patients, 21 (39.62%) had pulmonary infections, and 24 (45.28%) had more than one infection. A total of 30.19% of the patients were infected with Acinetobacter baumannii, and 56.60% were infected with two or more pathogenic microorganisms. Baseline patient characteristics are presented in Table 1.

Tigecycline Therapy

As shown in Table 1, 20 (37.74%) patients received a standard dose (50 mg/12 h followed by a 100 mg loading dose) of tigecycline and thirty-three (62.26%) received a high dose (100 mg/12 h followed by a 200 mg loading dose). The

Table 1 Baseline Characteristics of Patients in This Study

Variable	Total (n=53)	TIC Group (n=24)	Non-TIC Control Group (n=29)	P-value
Females/ males, n (%)	17/36	10/14	7/22	0.176
Age, years, mean±SD	61.75±18.09	64.33±14.79	59.62±20.43	0.453
SOFA score, median (IQR)	7.0[4.5–9.5]	6.5[5.0–12.0]	7.0[4.0–9.5]	0.693
APACHE II score, mean±SD	22.53±9.28	24.38±9.39	21.00±9.07	0.291
Tigecycline therapy duration, day, median (IQR)	12.0[8.5–17.0]	14.0[8.0–18.0]	11.0[8.0–15.0]	0.188
Dose, 100/50 mg q12h	33/20	14/10	19/10	0.591
Combination therapy during Tigecycline therapy				
Cefoperazone/sulbactam	34 (64.15%)	16 (66.67%)	18 (62.07%)	0.728
Heparin	44 (83.02%)	20 (83.33%)	24 (82.76%)	0.956
NOAC	2 (3.77%)	1 (4.17%)	1 (3.45%)	0.891
Cyclooxygenase inhibitors	10 (18.87%)	5 (20.83%)	5 (17.24%)	0.739
P2Y12 receptor antagonists	5 (9.43%)	3 (12.50%)	2 (6.90%)	0.487
Infected site n (%)				0.563
Pneumonia infection	21 (39.62%)	7 (29.17%)	14 (48.28%)	
Skin and skin-structures infection	4 (7.55%)	2 (8.33%)	2 (6.90%)	
Intra-abdominal infection	4 (7.55%)	2 (8.33%)	2 (6.90%)	
Two or more than	24 (45.28%)	13 (54.17%)	11 (37.93%)	
Pathogenic microorganism (%)				0.447
Klebsiella pneumoniae	7 (13.21%)	2 (8.33%)	5 (17.24%)	
Acinetobacter baumannii	16 (30.19%)	9 (37.50%)	7 (24.14%)	
Two or more than	30 (56.60%)	13 (54.17%)	17 (58.62%)	
Baseline laboratory parameters				
WBC,×109/L, mean±SD	13.08±9.40	15.00±12.52	11.19±5.44	0.249
HGB, g/L, mean±SD	91.47±26.27	92.33±34.78	90.76±16.92	0.238
PLT, ×109/L, mean±SD	235.09±99.25	231.71±101.23	237.90±99.29	0.775
PT, s, mean±SD	12.22±1.11	12.43±1.31	12.05±0.90	0.283
aPTT, s, mean±SD2	27.77±3.15	28.00±2.86	27.57±3.41	0.721
TT, s, mean±SD	17.07±1.90	17.36±2.29	16.84±1.51	0.503
FIB, g/L, mean±SD	5.02±1.90	4.84±1.76	5.18±2.02	0.598
INR, mean±SD	1.15±0.33	1.15±0.25	1.15±0.39	0.431
ALT, U/L, mean±SD	25.57±16.16	22.15±12.83	28.41±18.20	0.189
AST, U/L, mean±SD	33.86±26.48	28.54±12.69	38.27±33.55	0.936

(*Continued*)

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Table 1 (Continued).

Variable	Total (n=53)	TIC Group (n=24)	Non-TIC Control Group (n=29)	P-value
TBil, μmol/L, mean±SD	9.88±5.81	11.04±6.00	8.93±5.57	0.150
TP, g/L, mean±SD	59.55±10.21	59.47±11.43	59.62±9.29	0.789
ALB, g/L, mean±SD	33.88±5.64	34.00±6.00	33.79±5.43	0.844
Cr, μmol/L, mean±SD	84.34±38.35	88.92±35.59	80.55±40.71	0.292
BUN, mmol/L, mean±SD	14.49±8.10	15.06±8.40	14.02±7.97	0.586

Abbreviations: TIC, tigecycline-induced coagulopathy; SOFA, Sequential Organ Failure Assessment; APACHE, Acute Physiology and Chronic Health Evaluation; SD, standard deviation; IQR, interquartile range; WBC, white blood cell count; HGB, hemoglobin; PLT, platelet count; PT, prothrombin time; aPTT, activated partial thromboplastin time; TT, thrombin time; FIB, fibrinogen; INR, international normalized ratio; ALT, alanine aminotransferase; AST, aspartate aminotransferase; TBil, total bilirubin; TP, total protein; ALB, albumin; Cr, creatinine; BUN, blood urea nitrogen; NOAC, novel oral anticoagulants.

median (IQR) duration tigecycline therapy was 12.0 days [8.5–17.0]. The initial steady-state concentrations (Cmax, C1/2, and Cmin) were 1.524±0.993 mg/L, 0.682±0.575 mg/L, and 0.494±0.434 mg/L, respectively. The average value of AUC0-24h calculated using the linear trapezoidal rule was 19.949 ±14.562 mg·h/L. Following tigecycline therapy, there was a significant prolongation of PT, aPTT, and TT and a significant reduction in Fib ($p<0.05$) (Figure S1). However, no significant changes in PLT were observed before and after treatment, and the slight decrease in PLT was considered negligible ($p > 0.05$) (Figure S1). In the comparison between patients receiving tigecycline 100 mg twice daily and those receiving 50 mg twice daily, no significant differences were observed in PT, aPTT, TT, Fib, INR, or PLT before and after tigecycline administration (Figure S2).

After tigecycline therapy, 45.28% of the patients experienced coagulopathy. There was no significant difference in the incidence of coagulopathy between patients receiving tigecycline 100 mg twice daily and those receiving 50 mg twice daily (Table 1). The median time from tigecycline initiation to coagulopathy onset was 8.5 (IQR 7.0–11.5) days, similar to the timing identified by previous research.[8,29] Based on hematological and coagulation-related indices, the enrolled patients were divided into two groups: the non-TIC group (29 patients) and the TIC group (24 patients). As shown in Table 1, there was no significant difference between the two groups in terms of age, sex, SOFA score, APACHE II score, infection site, and pathogenic microorganisms ($p > 0.05$). Similarly, the baseline laboratory parameters, including PT, aPTT, TT, PLT, Fib, and INR, were comparable ($p > 0.05$) (Figure 1). The median duration of tigecycline therapy was 14.0 days (IQR 8.0–18.0) in the TIC group and 11.0 days (IQR 8.0–15.0) in the non-TIC group, showing no significant difference ($p > 0.05$). After tigecycline treatment, the levels of PT, aPTT, TT and INR in non-TIC group increased by 12.34%, 13.47%, 8.13% and 8.07%, respectively, meanwhile these four indices in TIC group increased by 34.78%, 65.41%, 19.25% and 23.87%, respectively. In addition, the Fib and PLT values decreased by 33.58% and 13.89%, respectively, in the non-TIC group and by 69.88% and 7.71%, respectively, in the TIC group. Notably, all coagulation indices, except PLT, exhibited significant changes over the course of tigecycline therapy in both TIC and non-TIC groups (Figure 1). PLT values were lower in the non-TIC group compared to the TIC group, but this difference was not statistically significant and may reflect random variability due to the small cohort size. Additionally, PT, aPTT, TT, and INR levels were significantly higher, whereas Fib levels were markedly lower in the TIC group than in the non-TIC group (Figure 1). As illustrated in Figure 1, the Cmax, C1/2, Cmin, and calculated AUC0-24 values were comparable between the non-TIC and TIC groups (1.430±1.021 mg/L vs 1.637 ±0.969 mg/L,0.688 ± 0.6612 mg/L vs 0.675 ± 0.539 mg/L, 0.493 ± 0.478 mg/L vs 0.495 ± 0.383 mg/L, 19.450 ± 15.692 mg·h/L vs 20.553 ± 13.378 mg·h/L, all $p>0.05$). Furthermore, During the 14-day post-discontinuation follow-up, key coagulation parameters (eg, aPTT, PT, TT, and Fib) of 70.2% TIC patients showed a trend toward normalization within 5 days after tigecycline discontinuation, which was consistent with previously published studies.[12,29]

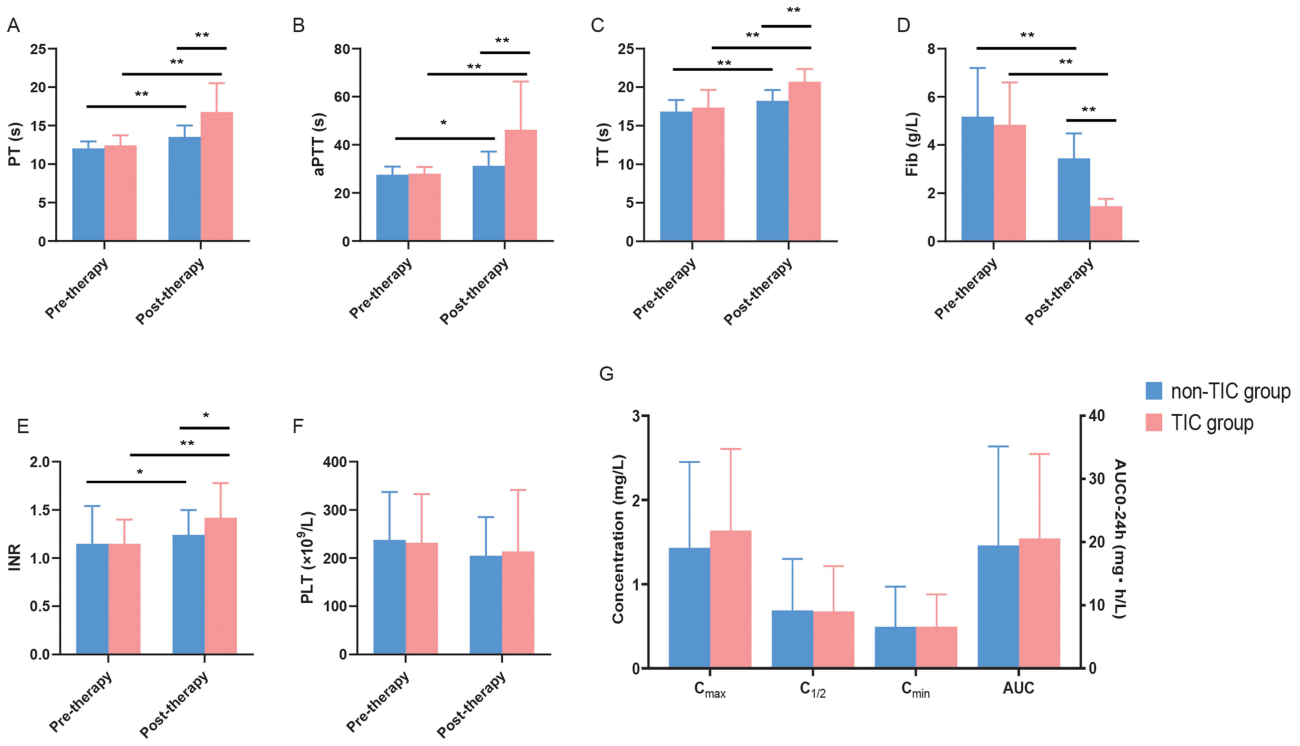

Figure 1 Comparison of PT (**A**), aPTT (**B**), TT (**C**), Fib (**D**), INR (**E**), and PLT (**F**) levels before and after treatment in the TIC and non-TIC groups, and comparison of Cmax, C1/2, Cmin, and AUC0-24 of tigecycline between the two groups (G). All values are expressed as the means+SD. *p < 0.05, **p < 0.01.
Abbreviations: TIC, tigecycline-induced coagulopathy; PT, prothrombin time; aPTT, activated partial thromboplastin time; TT, thrombin time; FIB, fibrinogen; INR, international normalized ratio; PLT, platelet count.

Serum Metabolic Traits

A total of 159 serum samples from 53 critically ill patients (three samples per patient) were analyzed to investigate the serum metabolic traits of the non-TIC and TIC groups. As can be seen in the PCA score plot for all analyzed samples (Figure S3), QC replicates clustered well, exhibiting the high reproducibility and robustness of the metabolomic approach. By applying MSI criteria, 170 metabolites were unambiguously or putatively annotated. To further investigate the associations between baseline clinical data, tigecycline regimen, metabolite levels, and the risk of coagulopathy, we conducted univariate Cox proportional hazards regression analysis. Both the baseline clinical data and tigecycline regimen showed no significant differences between the TIC and non-TIC groups. Additionally, with a threshold of $p < 0.05$, 24, 18, and 13 differential metabolites were identified from the metabolomic data at the Cmin, C1/2, and Cmax sampling points, respectively (Figure 2A). These metabolites significantly predicted the occurrence of coagulopathy in tigecycline-treated patients. Among these, 19 metabolites were positively correlated with the risk of coagulopathy and the remaining 36 were negatively correlated (Figure 2B). The relative quantification of these metabolites in the TIC and non-TIC groups is depicted as a heatmap (Figure 2C). Notably, glycerophospholipids, primarily lysophosphatidylcholines (LysoPCs) and lysophosphatidylethanolamines (LysoPEs), represent the predominant structural classes of differential metabolites. We also found that the levels of lysophosphatidylcholines (LysoPCs) and lysophosphatidylethanolamines (LysoPEs) were relatively lower in the TIC group and were negatively associated with the risk of coagulopathy.

After univariate data prefiltration, multivariate LASSO-penalized Cox proportional hazards regression analysis was performed. Metabonomic data from serum samples collected at different time points were analyzed either holistically or separately. To determine the optimal degree of regularization, 10-fold cross-validation was employed to select the parameter Lambda (λ) via the minimum criteria. Variables with nonzero coefficients were retained to construct the final model (Figure S4). In the LASSO-penalized Cox proportional hazards regression analysis incorporating metabolomic data from all samples, 14 metabolites from various sampling time points were selected (Figure 3A). For

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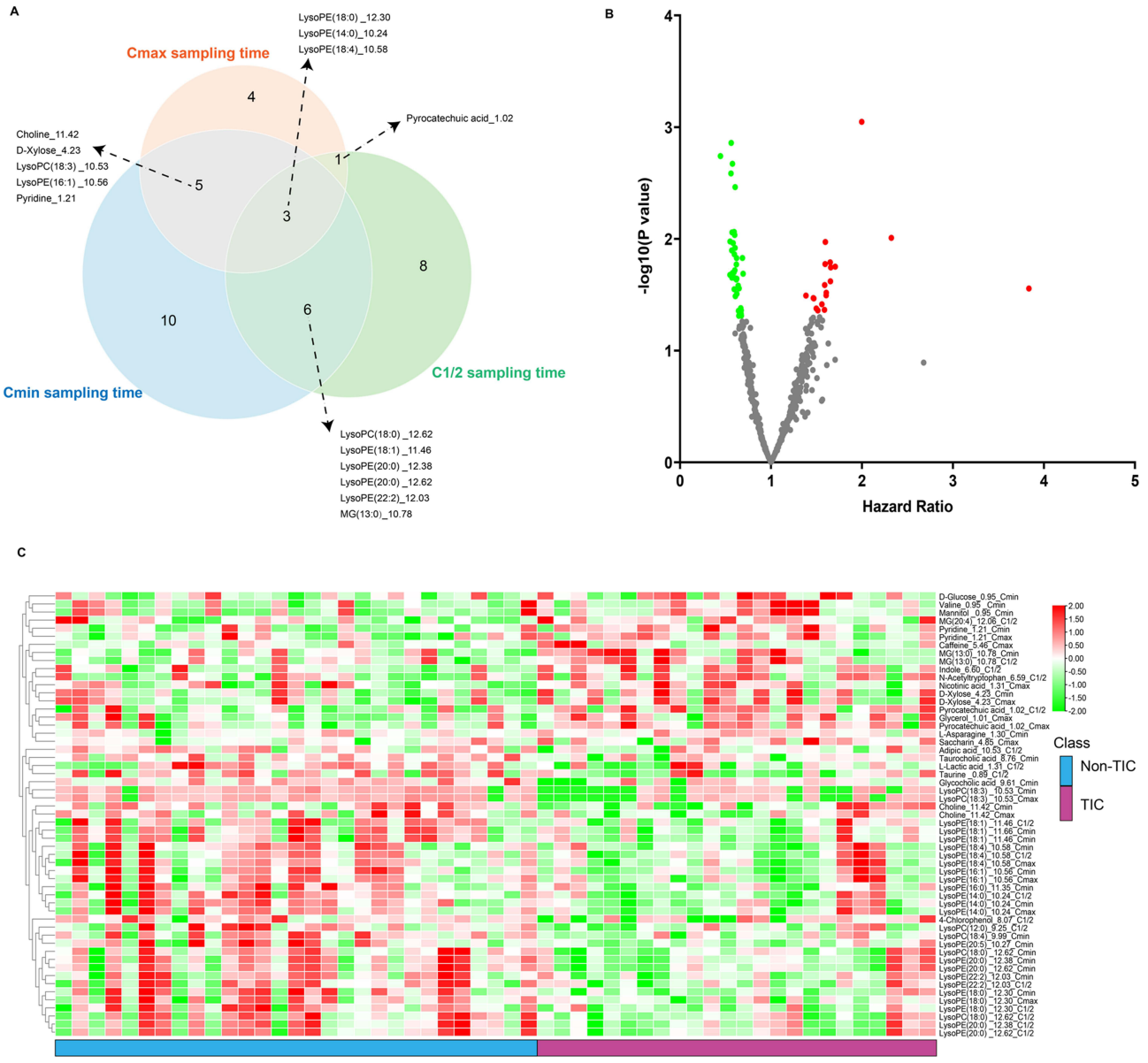

Figure 2 (**A**) A venn diagram illustrating the differential metabolites identified through metabolomic analysis of serum samples collected at Cmax, C1/2, and Cmin time points. (**B**) A volcano plot depicting the differential metabolites between the TIC and non-TIC groups. Red dots indicate metabolites identified as risk factors for coagulopathy, while green dots represent those associated with protective effects. (**C**) A heatmap displaying the changes in metabolite levels between the non-TIC and TIC groups. Each column represents a sample and each row represents a metabolite. Red and green colors indicate relatively high and low metabolite contents, respectively.
Abbreviations: TIC, tigecycline-induced coagulopathy.

metabolomic data derived from Cmin samples, 10 metabolites were identified, of which four were glycerophospholipids (Figure 3B). Similarly, For C1/2 samples and Cmax samples, 10 and 9 metabolites were screened out, respectively (Figure 3C and D). Time-dependent receiver operating characteristic (ROC) curves demonstrated strong predictive performance, with area under the curve (AUC) values of 0.882, 0.941, 0.911 and 0.934 at 3, 7, 14 and 21 days, respectively (Figure 4A). The AUCs for Cmin, C1/2, and Cmax demonstrated strong predictive performances, with each AUC exceeding 0.8. (Figure 4B–D). Furthermore, tigecycline-treated patients were stratified into high-risk and low-risk groups based on the predictive model established using metabolomic data collected at either individual or multiple sampling time points, which showed a statistically significant difference in Kaplan- Meier curves ($p<0.001$, Figure S5).

Integrating the findings from both univariate and multivariate analyses, LysoPCs and LysoPEs were identified as common differential metabolites between TIC and non-TIC groups at various sampling time points. In addition, lower

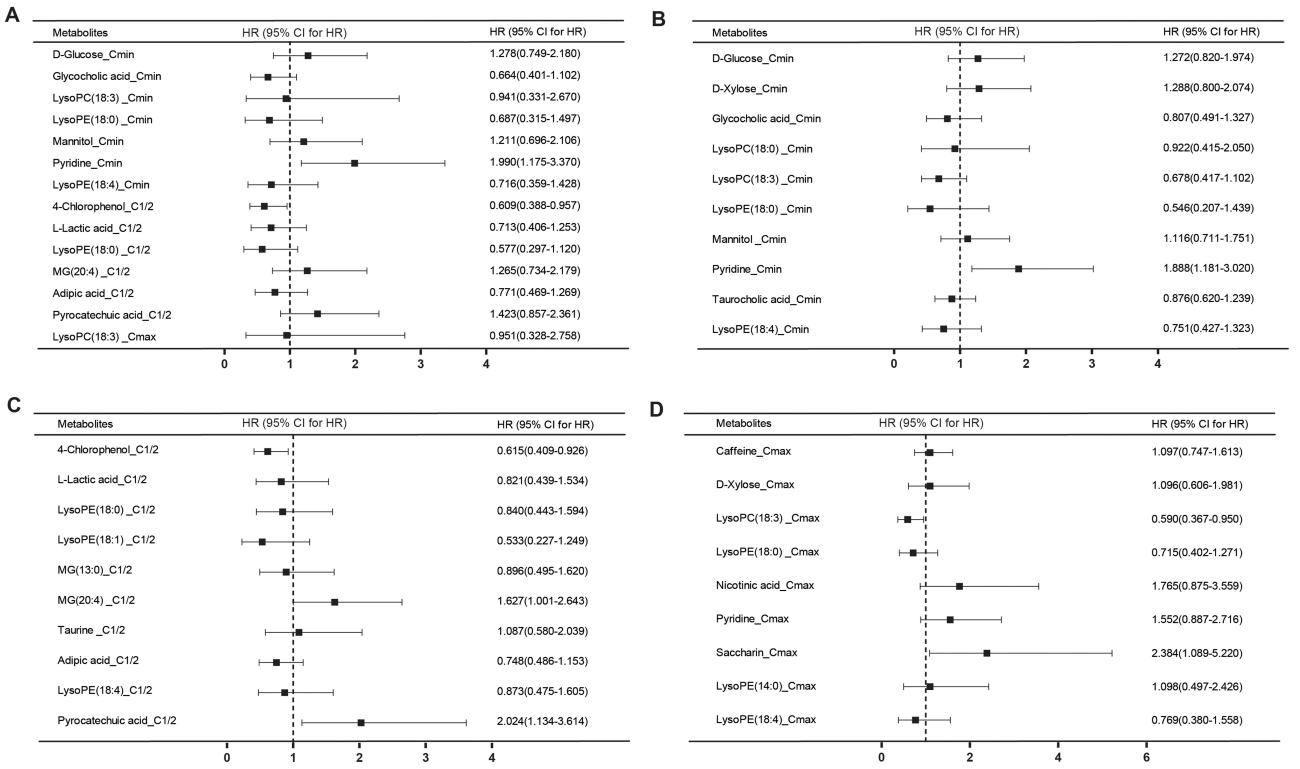

Figure 3 Forest plots showing hazard ratios derived from multivariate Cox proportional hazards regression analysis. (**A**) Analysis of the entire metabolomic dataset; (**B**) Analysis of metabolomic data from Cmin samples; (**C**) Analysis of metabolomic data from C1/2 samples; (**D**) Analysis of metabolomic data from Cmax samples.
Abbreviations: HR, hazard ratio; CI, confidence interval.

concentrations of these metabolites were associated with a higher risk of coagulopathy. We developed a risk prediction model using LysoPCs and LysoPEs, effectively stratifying tigecycline-treated patients into high-risk and low-risk groups (Figure 5A and B). The AUC values for the time-dependent ROC curves were very close to 0.8, suggesting that the model exhibited relatively good predictive performance (Figure 5C). Using the median risk score of all patients as the cutoff value, patients were stratified into high- and low-risk groups. The Kaplan-Meier survival curve revealed a significant difference in survival between these groups ($p < 0.001$; Figure 5D), with high-risk patients being more prone to developing TIC events.

Discussion

Tigecycline is a derivative of minocycline with structural modifications to evade acquired efflux pumps and ribosomal protection, which are the main genetic mechanisms of minocycline resistance. Since its launch for clinical use, tigecycline has been the last-resort choice for the treatment of complicated infections caused by both MDR Gram-positive and Gram-negative bacteria. However, with increasing clinical usage, adverse effects of tigecycline have emerged and become a growing clinical concern. Between January 2005 and December 2020, 223 TIC events (accounting for 14.70% of the total adverse events) were reported by the US Food and Drug Administration Adverse Event Reporting System.[30]

Consistent with previous research, our results demonstrated that tigecycline significantly prolonged PT, aPTT, and TT, and reduced Fib, with the most significant impact on Fib.[7,12,31,32] In comparison, no differences were found in the baseline levels of PT, aPTT, TT, and Fib between the TIC and non-TIC groups. Strikingly, after tigecycline therapy, significantly higher levels of PT, aPTT and TT and lower levels of Fib were observed in the TIC group than in the non-TIC group. Although it is generally true that prolonged and high-dose medication treatment may be detrimental, we noted no significant difference in the therapy regimen or in vivo exposure to tigecycline between the TIC and non-TIC groups.

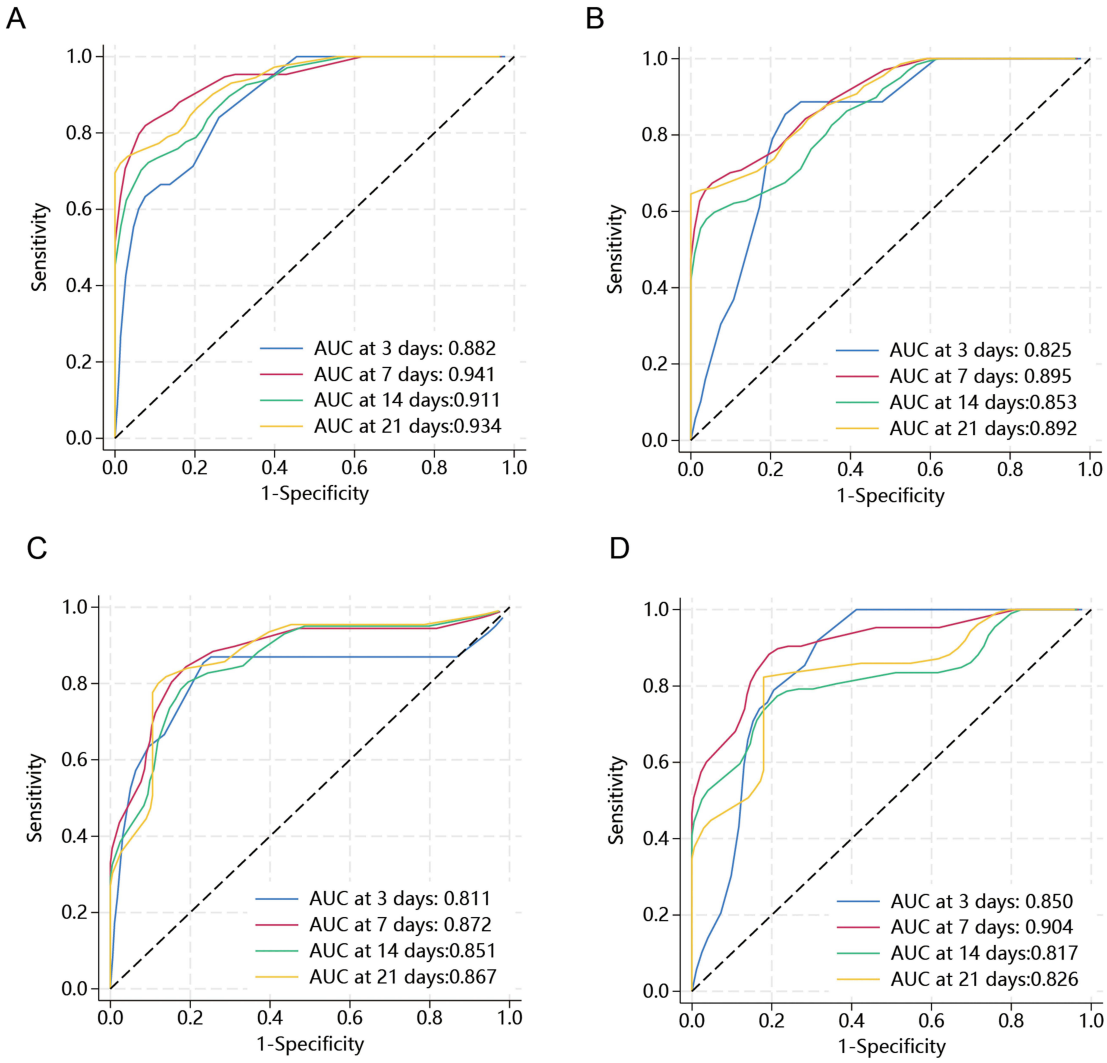

Figure 4 Time-dependent receiver operating characteristic curves generated based on metabolic features selected from the entire metabolomic analysis (**A**), as well as from the C_{min} (**B**), $C_{1/2}$ (**C**), and C_{max} (**D**) metabolomic analyses.
Abbreviations: AUC, area under the curve.

This suggests that the observed differences between the groups were likely due to patients' inherent characteristics rather than the effects of tigecycline treatment.

Metabolomics, the profiling of endogenous metabolites in biological matrices, is widely regarded as the endpoint of the "omics" cascade. Changes in the metabolome are the ultimate responses of an organism to genetic alterations, diseases, and environmental stimuli. Clinically, blood metabolites such as cholesterol are used as predictive indices for cardiovascular diseases. Therefore, metabolomics is a potential tool for precision medicine, which stratifies patients based on their risk of developing diseases or suffering from side effects. In this study, we characterized the baseline metabolomic profiles of critically ill patients treated with tigecycline and identified metabolic traits that could be used to predict TIC. Univariate analysis highlighted 55 discriminant metabolites, whereas the subsequent combination of multivariate LASSO-penalized Cox proportional hazards regression analysis, processing metabolomic data either holistically or separately for different sampling time points, restricted the discriminant metabolite list. Our results demonstrated that glycerophospholipids are negatively correlated with the occurrence of coagulation dysfunction.

Glycerophospholipids, the most abundant phospholipids in mammalian cell membranes, are critical for membrane organization and cellular functionality. Phosphatidylcholines (PCs) and phosphatidylethanolamines (PEs) make up more than 50% of the composition.[33] The enzymatic breakdown of these phospholipids generates LysoPCs and LysoPEs,

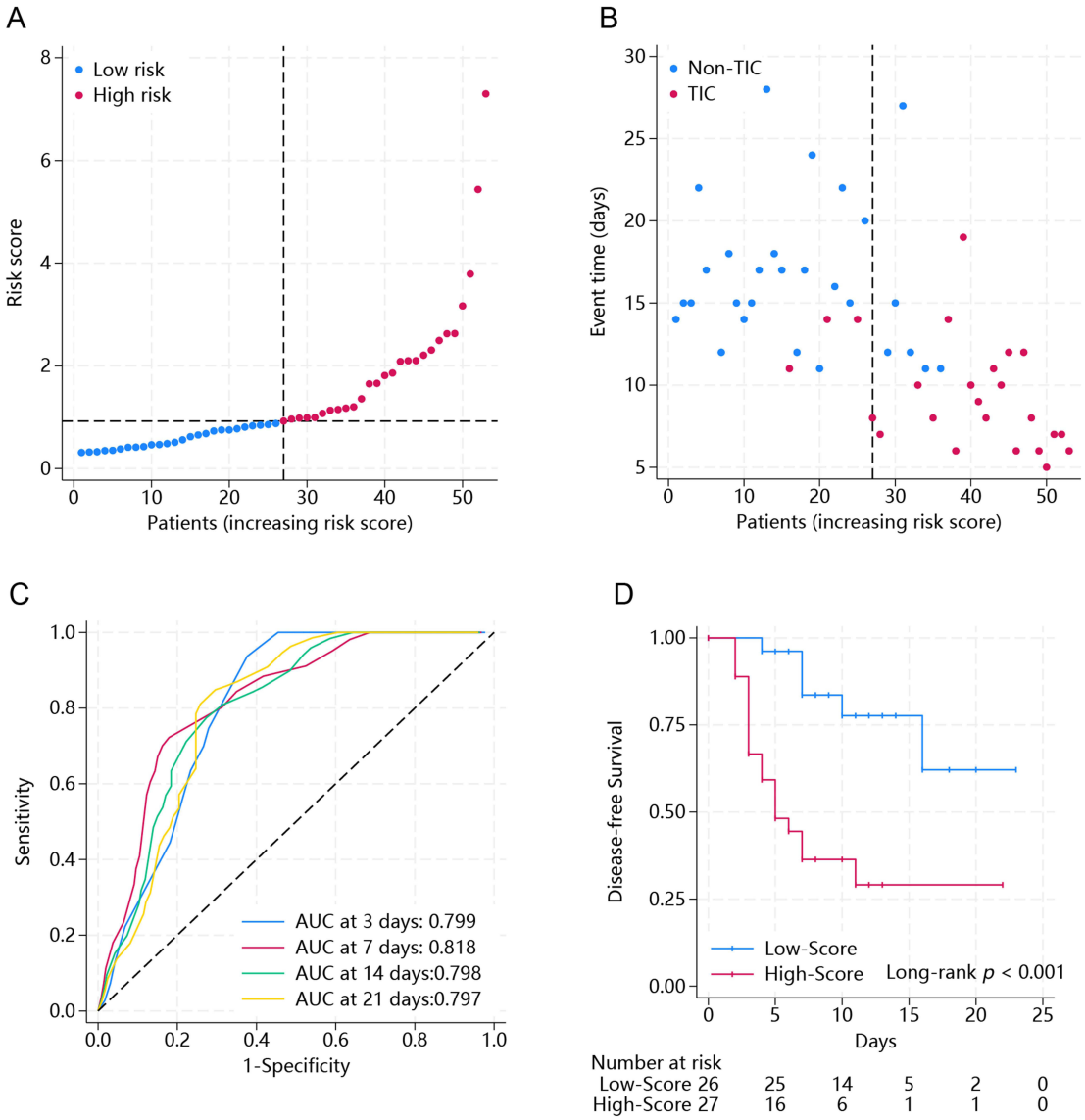

Figure 5 The LysoPCs and LysoPEs signature predicts tigecycline-induced coagulopathy. (**A**) The distribution of risk-score. (**B**) The distribution of patients' status. (**C**) Time-dependent receiver operating characteristic curves, and the AUC was assessed at 3, 7, 14 and 21 days. (**D**) Kaplan-Meier survival curve of high and low-risk patients.
Abbreviations: AUC, area under the curve.

which are critical signaling molecules that mediate cell-cell communication and regulate multiple cellular signaling pathways.[34] Accumulating evidence indicates that disturbances in LysoPCs and LysoPEs could serve as earlier predictive biomarkers or intervention targets for a variety of diseases, including pneumonia, diabetes, viral infections, thrombotic and hemostatic disorders.[35–40] Indeed, coagulation dysfunction is often accompanied by the disturbances of glycerophospholipids metabolism. Specifically, both circulating LysoPCs and LysoPEs have been reported to be reversibly associated with coagulation function indices.[41,42] In line with this, our study found that patients with low levels of LysoPC (18:0), LysoPC (18:3), LysoPE (14:0), LysoPE (18:0), LysoPE (18:1) and LysoPE (18:4) were more susceptible to developing coagulopathy after tigecycline therapy.

Although reduced LysoPC and LysoPE levels have been associated with unfavorable disease outcomes, the mechanisms by which they influence coagulation are multifaceted and intricate. They interact with various cell membranes, such as those of platelets and endothelial cells, and directly influence the activity of coagulation factors in plasma.[43,44] Additionally, LysoPCs and LysoPEs might interact with thrombin or fibrinogen, altering the kinetics of clot formation and then changing the TT and Fib value.[45,46] Moreover, they could affect the extrinsic and intrinsic coagulation

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pathways, primarily by targeting tissue factor VIIa, Xa and IXa.[47] In conjunction with ROC analysis of LysoPC (18:0), LysoPC (18:3), LysoPE (18:0) and LysoPE (18:4) measured at Cmin sampling time, our results suggested that these glycerophospholipids could serve as potential predictors for TIC.

Beyond glycerophospholipids, mannitol and pyridine levels observed in our study might also be correlated with the risk of coagulopathy. Clinical studies have shown that additional use of mannitol is associated with an increased risk of coagulation disorders. Notably, in neurosurgical patients receiving mannitol therapy for intracranial pressure management, the incidence of coagulation abnormalities is substantially higher than that in matched controls.[48] This is probably due to its strong inhibition of the fibrinogen/fibrin conversion axis during thrombus formation, as well as its concurrent impairment of platelet functionality.[49,50] In the context of the potential effects of pyridine on hemostatic processes, direct experimental evidence and clinical studies remain scarce. However, studies have indicated that pyridine derivatives, such as pyridine-based macrocycles, possess anticoagulant properties by selectively inhibiting coagulation Factor XIa.[51] Further molecular modeling has revealed that functionalization at the 3 - position of the pyridine ring and N-oxide formation could enhance its anticoagulant activity.[52]

The present study has several limitations. We analyzed baseline metabolomic profiles of patients treated with tigecycline to identify distinct metabolic signatures potentially associated with the risk of coagulopathy. Although the identified signatures, particularly glycerophospholipids, may represent novel predictors, this study should be regarded as hypothesis-generating. The findings require validation in larger, independent cohorts across multiple centers. Furthermore, targeted quantification of candidate metabolites is needed to assess their reproducibility and clinical utility. Despite spanning two full seasonal cycles, the two-year data collection period may not fully account for potential seasonal or epidemiological influences. At this stage, the results were not ready for clinical application without rigorous external validation.

Conclusion

In summary, we described the comprehensive metabolomic profiles of patients treated with tigecycline and identified the potential predictive metabolites for coagulopathy. Although patient demographics, baseline clinical data, and tigecycline regimen were not strong independent predictors of coagulopathy, several metabolites were found to be negatively or positively correlated with coagulopathy risk. Notably, the model incorporating LysoPC (18:0), LysoPC (18:3), LysoPE (18:0), and LysoPE (18:4) detected at the Cmin sampling time demonstrated strong predictive capability. Our findings provide a novel approach for stratifying tigecycline-treated patients based on their potential coagulopathy risk. While the identified metabolic signatures suggest possible intervention targets, these results are exploratory and require further validation. In particular, external validation in independent cohorts is essential before clinical application of this model can be considered.

Data Sharing Statement

The data are contained within the article or in the Supplementary Material. Additional information can be obtained from the corresponding author Min Wang upon reasonable request.

Ethics Approval and Informed Consent

This study was approved by the ethics committee of Nanjing Drum Tower Hospital (No. 2022-040-01) and registered at the China National Medical Research Registration and Filing Information System (No. MR-32-22-017478). Written informed consent was signed by all the study participants.

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Disclosure

The author(s) report no conflicts of interest in this work.

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