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Subphenotypes of platelet count trajectories in sepsis from multi-center ICU data

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Although thrombocytopenia on admission to the ICU is associated with increased in-hospital mortality in septic patients, the role of longitudinally measured platelet counts, which are dynamically changing, is unclear. We aimed to identify patterns of dynamic platelet count trajectories and evaluate their association with outcomes and thrombocytopenia in septic patients. We tested the longitudinal platelet trajectory patterns of sepsis patients within the first four days of ICU admission in the MIMIC-IV database and their association with 28-day mortality, and independently validated our findings in the eICU-CRD database. Statistical methods used included multivariate regression, propensity score analysis, doubly robust estimation, gradient boosting model, and inverse probability weighting to ensure the robustness of our findings. A total of 22,866 septic patients were included in our study. The trajectory analysis categorizes patients into ascending (AS), stable (ST), or descending (DS) patterns. The risk of 28-day mortality was increased in the DS patients (OR = 2.464, 95%CI 1.895–3.203, $p < 0.001$) and ST patients (OR = 1.302, 95%CI 1.067–1.589, $p = 0.009$) compared to AS patients. The AS patients had lower ICU length of stay (2.36 vs. 4.32, $p < 0.001$) and 28-day maximum SOFA scores (5.00 vs. 6.00, $p < 0.001$) than the DS patients, but had more ventilator-free days within 28 days than the DS group (26.00 vs. 24.00, $p < 0.001$). The mediating effect of thrombocytopenia was significant ($p < 0.001$ for the average causal mediation effect (ACME)). Longitudinal platelet trajectory was associated with risk-adjusted 28-day mortality among patients with sepsis and was proportionally mediated through thrombocytopenia.

Keywords Platelet count trojectary, Sepsis, Inflammation, Immunity, Critical care, Prognosis

Sepsis is a life-threatening syndrome caused by a dysregulated host response to infection, affecting millions of people worldwide every year. Despite advances in critical care, sepsis remains a major cause of morbidity and mortality in intensive care units (ICUs). According to the latest epidemiological data, the global ICU mortality rate for sepsis ranges from 20 to 40%, depending on the region and patient population studied[1,2]. Moreover, sepsis survivors often experience long-term sequelae, such as impaired physical and cognitive function, and increased risk of death and readmission to the hospital[3,4]. Thus, there is an urgent need for reliable prognostic markers to guide early identification and management of sepsis patients at risk of poor outcomes. One potential marker is the platelet count, which has been shown to be associated with sepsis severity and mortality[5–7].

Platelets are known to be involved in clotting, inflammation, and immune responses, all of which have been linked to the prognosis of sepsis patients[8–10]. Dysregulation of the host's immune response can lead to platelet destruction and subsequent thrombocytopenia (platelet count $< 150 \times 10^9$/L)[11,12], further exacerbating the host's immune dysregulation[13]. Several biological mechanisms may contribute to this correlation. First, sepsis-induced inflammation and endothelial activation can lead to platelet activation and consumption, resulting in platelet sequestration and destruction. Additionally, sepsis can impair megakaryocyte function, which reduces platelet production and further exacerbates thrombocytopenia. Furthermore, platelets have multiple functions beyond hemostasis, including immune modulation, and their depletion may impair host defense against pathogens and exacerbate the inflammatory response[14,15].

Thrombocytopenia during ICU hospitalization has been identified as a potential prognostic indicator for sepsis patients[13,16,17], however, some studies have failed to find any such correlation and have even proposed conflicting views[18,19], Previous studies assessing the impact of thrombocytopenia on prognosis have generally evaluated platelet counts at specific time points related to ICU admission. However, these time points may not correspond to a homogeneous clinical situation[20]. It is worth noting that platelet counts are constantly changing

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and typically reach their nadir, indicating maximum coagulation dysfunction, around the fourth or fifth day of ICU admission[21,22]. In addition to the baseline platelet count at admission, the dynamic trajectory of platelets may also play a role in determining the prognosis of sepsis patients. However, to date, this relationship remains poorly understood and requires further investigation.

By leveraging large-scale databases, such as the eICU Collaborative Research Database (eICU-CRD) and the Medical Information Mart for Intensive Care IV (MIMIC-IV) database, we have applied unsupervised machine learning techniques to identify longitudinal patterns in platelet count trajectories in septic patients, and have evaluated their association with relevant clinical outcomes.

Methods and materials

Study design and data

We conducted an observational study using longitudinally collected and retrospectively stored high-resolution data from two databases, namely the Medical Information Mart for Intensive Care IV (MIMIC-IV) v1.0[23] and Philips eICU Collaborative Research Database (eICU-CRD) v2.0.[24] MIMIC-IV v1.0 contains 76,540 ICU stays of 53,150 patients admitted to the Beth Israel Deaconess Medical Center between 2008 and 2019. This information includes demographic data, laboratory test results, medications, vital signs, and radiology reports, which were obtained from digital electronic health records and hosted by the Laboratory for Computational Physiology at MIT. The eICU-CRD database v2.0 contains data on 200,859 ICU admissions of 139,367 patients admitted to 208 ICUs in 2014 and 2015. This database includes information on vital sign measurements, nursing care plans, disease severity measures, diagnostic and treatment information, and is maintained and de-identified by the Philips eICU Research Institute and MIT Laboratory for Computational Physiology. Additional concepts required for the two observational cohort studies were defined using the official code repository provided.

The use of the MIMIC database was granted approval by both the BIDMC institutional review board (2001-P-001699/15) and MIT (Approval ID: 10734458). Meanwhile, the eICU-CRD database, which lacked direct patient intervention, safety protocols, and de-identified all protected patients' health information, was exempt from institutional review board approval and in compliance with the privacy provisions of the Health Insurance Portability and Accountability Act (HIPAA), with a certification number of 1031219-2. As a result of the de-identification process, informed consent was waived. Furthermore, the study adhered to the STROBE guidelines for reporting observational studies[25].

Population selection criteria

Sepsis, a potentially fatal condition caused by an uncontrolled immune response to infection, has been defined as life-threatening organ dysfunction (Sepsis 3.0)[26]. To identify sepsis patients in the MIMIC-IV and eICU-CRD cohorts, the former relied on infection or suspected infection records and a significant increase in the Sequential Organ Failure Assessment score (SOFA) score of ≥ 2 points, while the latter used the Acute Physiology and Chronic Health Evaluation IV dataset[27]. The study excluded patients who were under 18 years old, had multiple ICU admissions, or lacked platelet count measurements within the first four days after ICU admission. Ultimately, the analysis included 15,839 and 7027 sepsis patients from the MIMIC-IV and eICU-CRD cohorts, respectively.

Variable extraction

The main exposure of the study is different platelet count trajectory patterns, requiring measurement of platelet count for four consecutive days after ICU admission, with the lowest value being selected if there are multiple measurements within a day. Covariate selection is based on the factors that have been identified in previous literature and guidelines as having an impact on the outcomes of sepsis patients, and factors that affect the platelet count of patients[28–30]. Baseline characteristics within the first 24 h of ICU admission are extracted using Structured Query Language (SQL), including age, gender, race (Caucasian, African American, Asian, Hispanic, Native American and Other/Unknown), and type of first ICU admission (Cardiac ICU, medical ICU, surgical ICU, Neuro ICU). Laboratory data include hemoglobin (g/dL), platelets (10^9/L), white blood cell count (10^9/L), creatinine (mg/dL), and glucose (mg/dL). Vital signs include heart rate (/min), respiratory rate (/min), temperature (°C), and systolic blood pressure (mmHg). Disease severity scores include SOFA (Sequential Organ Failure Assessment), APS-III (Acute Physiology Score III), APACHE-IV (Acute Physiology and Chronic Health Evaluation IV), and SAPS-II (Simplified Acute Physiology Score II). For variables with multiple measurements within 24 h, we select the measurement that best reflects the severity of the disease. Comorbidity include heart failure, peripheral vascular disease, cerebrovascular disease, chronic obstructive pulmonary disease, diabetes, kidney disease, malignancy, and severe liver disease, all obtained through ICD-9 diagnosis codes recorded prior to discharge. Life support within the first 24 h of ICU admission includes the use of mechanical ventilation and dialysis treatment.

Variables with missing proportions exceeding 10% were excluded from our study. We addressed missing data for covariates using multiple imputation by chained equations (R package, mice) and generated 5 datasets.[31] The results for each dataset were then pooled using Rubin's rule[32]. The details of the proportion of missing variables can be found in the supplementary material (Fig. S1).

Outcomes

The primary outcome of this study is the mortality rate within 28 days of admission to the ICU, measured through hospital and insurance database records. Secondary outcomes include number of ventilator-free days within 28 days (defined as the number of days from successful weaning to day 28; patients who died before weaning are considered to have no ventilator-free days), length of stay in the ICU, and maximum SOFA score within the first 28 days after ICU admission.

Statistical methods

Continuous variables that follow a normal distribution are described as mean and standard deviation (SD) and compared between different platelet count trajectory patterns using analysis of variance (ANOVA). Non-normally distributed continuous variables are summarized as median and interquartile range (IQR) and compared between different patterns using Wilcoxon rank-sum test or Kruskal–Wallis test. Variable normality was assessed using the Kolmogorov–Smirnov test. Categorical variables were described in terms of frequency (n) and proportion (%), and tested using chi-square or Fisher's exact tests.

The present study investigates the relationship between longitudinal platelet count dynamic trajectory patterns (LPTP) and outcomes in septic patients in the MIMIC-IV sepsis cohort, and independently validates this relationship in the eICU-CRD sepsis cohort. First, unsupervised machine learning methods were used to identify LPTP, this specific methods used included factor analysis to reduce dimensionality and clustering algorithms such as k-means to group or cluster data points based on their similarities[33]: (i) 24 summary measures that characterize LPTP were calculated (Supplementary Materials, Table S4); (ii) factor analysis of these 24 measurements was performed to identify the measurements that best describe the main characteristics of LPTP; (iii) based on the previously selected measurements, cluster analysis was performed on the LPTP of septic patients. The optimal number of trajectories was determined by the following three criteria: the observed overall R2, pseudo-F statistics, and the third cluster criterion (CCC) (Supplementary Materials, Table S5)[34], resulting in the identification of three LPTP: the Ascending, Stable, and Descending groups.

Second, the independent association of the three LPTP with patient outcomes was inferred using double robust estimation. Double robust estimation combines multivariate regression models with propensity score models to provide unbiased estimates of the association between exposure and outcome[35,36]. Tree-based regression models built iteratively were used to evaluate the propensity scores of patients' LPTP, we included the following variables in the model to calculate propensity scores: age, gender, ethnicity, first careunit, hemoglobin, creatinine, glucose, heart rate, respiratory rate, temperature, systolic pressure, SOFA score, peripheral vascular disease, cerebrovascular disease, chronic pulmonary disease, diabetes, chronic renal disease, malignant cancer, severe liver disease, mechanical ventilation, dialysis, platelet transfusion, antiplatelet, immunotherapy, hematologic diseases, and thrombotic diseases, thereby minimizing confounding variable imbalances between different platelet count trajectory pattern groups. As the number of iterations or regression trees added to the model increases, the model becomes more complex. Balance measures are required for model fitting, particularly the two balance measures used in the stopping rules: the absolute standardized mean difference (ASMD) and the Kolmogorov–Smirnov (KS) statistic. The estimated propensity score is used as a weight, and a weighted cohort is then generated using the inverse probability weighting (IPW) model.[36] A logistic regression was then applied to the weighted cohort in order to adjust for variables that were found to be unbalanced among the different platelet count trajectory patterns in the propensity score model, resulting in a double robust analysis. The variance inflation factor (VIF < 5.0) in collinear diagnosis (Supplementary Material, Table S3), as well as baseline variables that are considered clinically relevant or related to outcome in univariate relationships ($p < 0.10$), were adjusted as potential confounders in the multivariate logistic regression model. To compare secondary outcomes, patients were weighted according to estimated propensity scores for different platelet count trajectory patterns. Differences in observed absolute standardized difference (ASD) and statistical significance were then calculated using analysis of variance (ANOVA) for continuous outcomes and McNemar test for categorical outcomes. Bonferroni correction was applied for comparisons of secondary outcomes among the three platelet count trajectory patterns ($p < 0.0167$ considered statistically significant).

Third, causal mediation analysis were conducted to analyze, whether subsequent thrombocytopenia mediate the LPTP on outcomes of sepsis.

Fourth, subgroup analysis is conducted on sepsis cohort from the MIMIC-IV and eICU-CRD databases to explore the consistency of the relationship between the LPTP and sepsis outcomes across subgroups defined by demographic and clinical characteristics such as age, gender, baseline platelet count, baseline SOFA score, comorbidities, platelet transfusion, and anti-platelet therapy through a multivariate (full-model) adjusted logistic regression analysis. The interaction between these factors and the LPTP is evaluated by comparing the differences between two models, the basic model and the model adjusted for the interaction term.

Finally, sensitivity analyses are performed to evaluate the robustness of the study's conclusions and the potential impact of different statistical models. These analyses include application of three different association inference models: a doubly robust model adjusting for all confounding variables, an IPW model based on propensity scores, and a doubly robust model adjusting for baseline imbalanced variables. The results of these models are reported and compared in terms of effect sizes and p-values, and further sensitivity analysis is conducted by excluding patients with platelet transfusion, antiplatelet treatment or patients with platelet organ dysfunction on admission (platelet count < 150 × 10^9/L). A two-tailed value of $p < 0.05$ was considered statistically significant. All statistics were performed using the R software (version 4.1.2, www.r-project.org).

Results

Platelet count trajectories patterns and patient characteristics

Through trajectory analysis, three distinct longitudinal patterns of platelet count were identified from MIMIC-IV sepsis cohort. These patterns were characterized as follows: (i) ascending (AS), where platelet count increases following admission to the ICU; (ii) stable (ST), where platelet count remains relatively consistent; and (iii) descending (DS), where platelet count rapidly decreases after ICU admission, as illustrated in Fig. 1A. Upon validation of the model trained on MIMIC-IV using the eICU-CRD dataset, similar trajectory patterns were observed, as depicted in Fig. 1B.

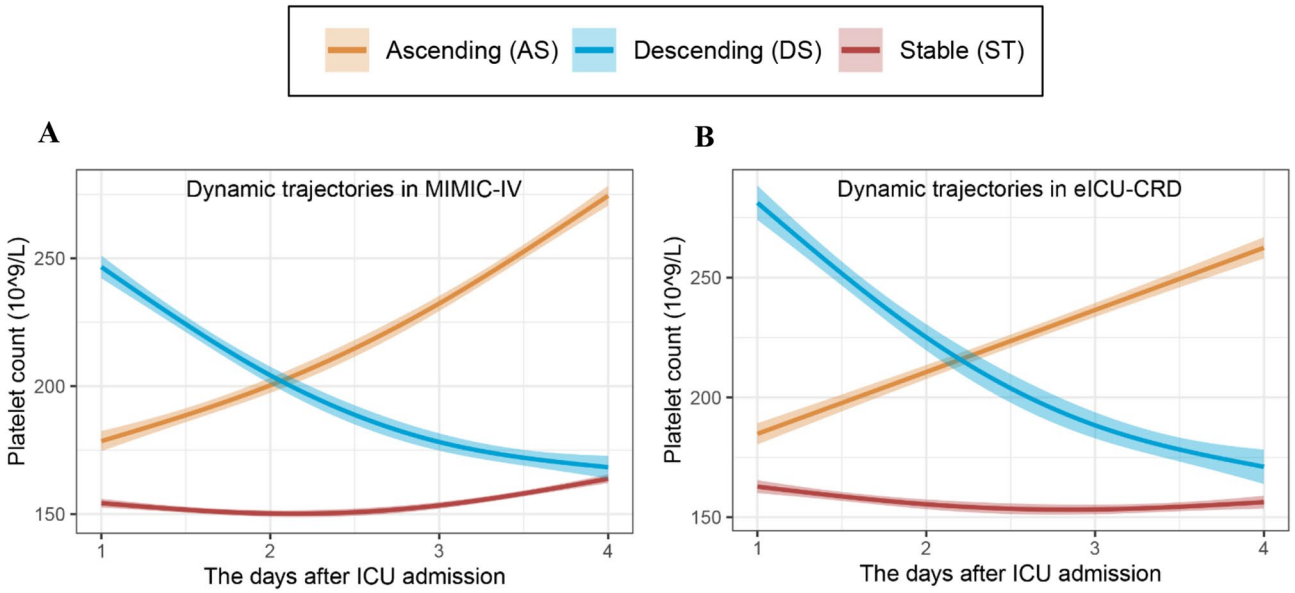


Fig. 1. Trajectory pattern plot of platelet count changes within the first four days of ICU admission in the MIMIC-IV (**A**) and eICU-CRD (**B**) databases.

The MIMIC-IV database encompasses a grand total of 382,278 admissions to the intensive care unit, of which an exclusive cohort of 32,408 individuals suffered from sepsis. Filtering through well-established exclusionary protocols, a select subgroup of 15,839 patients admitted between 2008 and 2019 was ultimately chosen for the purposes of analysis. On the other hand, the eICU-CRD database boasts 200,859 admissions to the ICU, which features a distinctive pool of 28,667 sepsis-afflicted patients. Filtering through exclusionary criteria, only 7027 patients admitted between 2014 and 2015 were deemed eligible for inclusion in the present study (Fig. 2).

Table 1 presents the baseline characteristics of the patients in the two sepsis cohorts. The age of the patients was similar, with a median age of 68.0 (IQR: 56.8–78.8) in the MIMIC-IV database and 67.0 (IQR: 56.0–79.0) in the eICU-CRD database. Upon admission to the ICU, the initial Sequential Organ Failure Assessment score was 6.0 (IQR: 4.0–9.0) for both cohorts. The 28-day mortality rate was 10.45% in the MIMIC-IV cohort and 14.99% in the eICU-CRD cohort. Information on the grouped baseline characteristics of the patients based on their trajectory patterns is provided in Table S1 (for MIMIC-IV) and Table S2 (for eICU-CRD). Variables that demonstrated significant differences across trajectory patterns, including demographic characteristics, laboratory test results, vital signs, disease severity scores, complications, initial support treatment upon ICU admission, and diseases affecting platelet counts, will be considered as potential confounding factors and included in subsequent models.

Primary outcome

Multivariable logistic regression analysis revealed a significantly higher 28-day mortality risk among patients with ST or DS trajectory patterns, with adjusted odds ratios (ORs) of 1.451 (95% CI 1.201–1.753) or 2.424 (95% CI 1.962–2.995) in MIMIC-IV and 1.326 (95% CI 1.080–1.627) or 2.519 (95% CI 1.950–3.255) in eICU-CRD (Table 2). Following inverse probability weighting (IPW), the baseline covariate imbalances between the AS trajectory pattern group and the ST or DS trajectory pattern group were substantially reduced (Fig. 3), while the associations remained robust (Table 2). Doubly robust estimation further confirmed the higher 28-day mortality risk among patients with ST or DS trajectory patterns (Table 2). Additionally, the association between the platelet count trajectory pattern and 28-day mortality rate remained significant in most subgroups of both MIMIC-IV and eICU-CRD, except for a small subset of subgroups with insufficient sample sizes (Fig. 4). After excluding patients who received platelet transfusion, antiplatelet therapy or patients with platelet organ dysfunction on admission (platelet count $< 150 \times 10^9$/L), the ST or DS trajectory pattern group still had a higher 28-day mortality risk than the AS trajectory pattern group (Table S6-S8, Figure. S2-S3).

Secondary outcomes with propensity score-weighted cohorts

The relationship between platelet count trajectory patterns and secondary outcomes was assessed to explain the potential reasons for the survival benefit of elevated platelet count trajectories. The AS trajectory pattern group had lower ICU hospitalization days (2.36 vs. 4.32, $p < 0.001$) and 28-day maximum SOFA scores (5.00 vs. 6.00, $p < 0.001$) than the DS trajectory pattern group, but had more ventilator-free days within 28 days than the DS group (26.00 vs. 24.00, $p < 0.001$) in the MIMIC-IV sepsis weighted cohort. Consistent findings were observed in the eICU-CRD sepsis weighted cohort as shown in Table 3. Nevertheless, no significant differences were found in these three secondary outcomes between the AS trajectory pattern group and the ST trajectory pattern group.

The relationship among platelet count trajectory pattern, thrombocytopenia occurring after ICU day 4, and 28-day mortality rate is shown in Fig. 5A. Thrombocytopenia mediated the relationship between platelet count

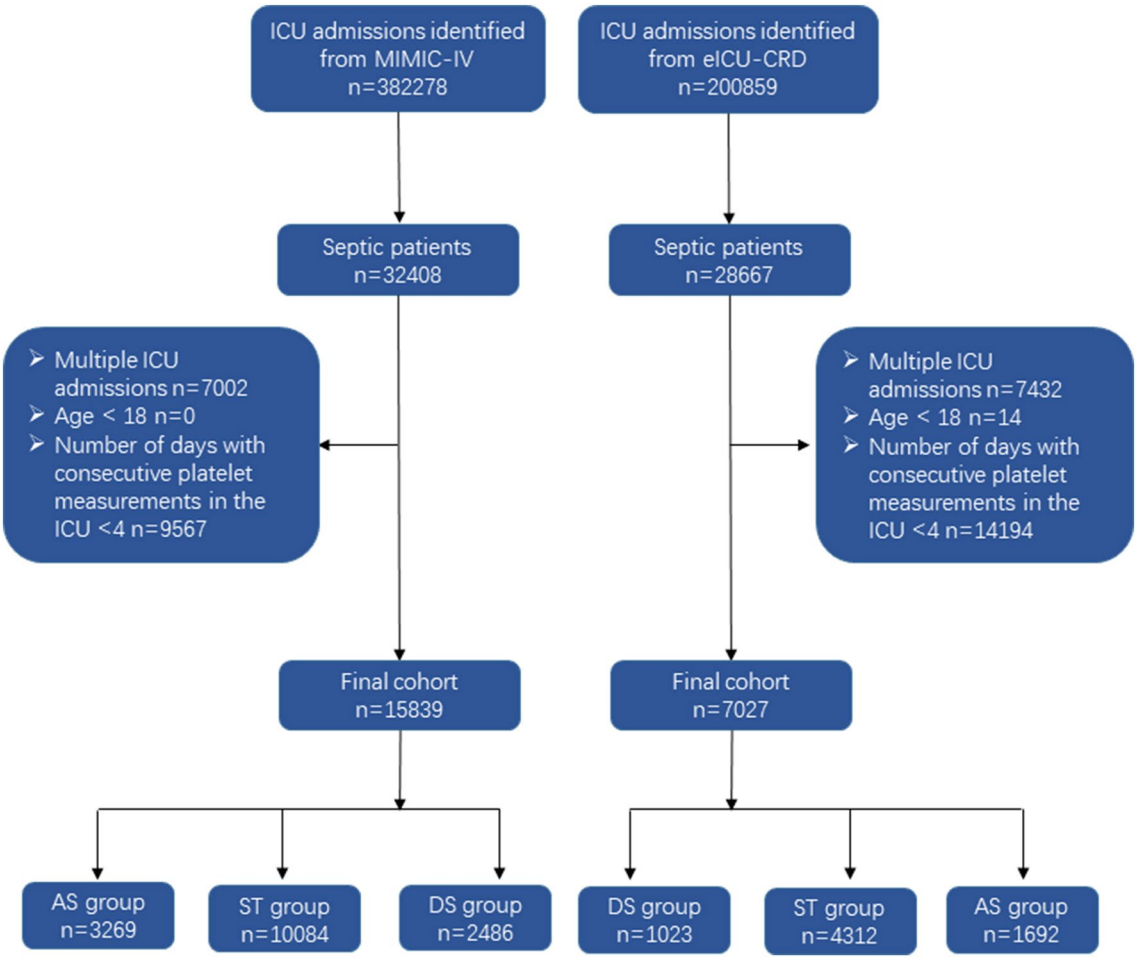


Fig. 2. Overview of the methods used for data extraction.

trajectory pattern and 28-day mortality rate in septic patients, with mediation percentages of 30.91% (95% CI 20.30–54.00, $p < 0.001$) in MIMIC-IV and 37.14% (95% CI 24.06–77.00, $p < 0.001$) in eICU-CRD (Fig. 5B).

Discussion

To our knowledge, this is the first study specifically evaluating platelet count trajectory patterns as a major prognostic factor in consecutive sepsis patients. Our study demonstrates that stable or decreasing platelet count trajectories at ICU admission are associated with significantly increased risk-adjusted 28-day mortality compared to elevated platelet count trajectories. Additionally, compared to elevated platelet count trajectories, decreasing platelet count trajectories are associated with shorter ventilator-free days within 28 days, longer ICU length of stay, and higher maximum SOFA scores. In terms of 28-day mortality, the mediating effect of thrombocytopenia on the relationship between platelet count trajectories and sepsis patients is significant.

Longitudinal data on platelet count can offer more insights into disease progression compared to a single measurement at a specific time.[37,38] Trajectory analysis is a useful tool for revealing the hidden patterns in repeated measurements. Our study highlights that septic patients who did not exhibit thrombocytopenia upon ICU admission but showed a rapidly declining platelet count during their hospital stay may have a higher likelihood of a poor prognosis.

Among the three trajectory patterns, the descending pattern, characterized by a rapid decline in platelet count over time, is associated with a high poor prognosis risk. Conversely, septic patients with an elevated platelet count tend to have a favorable clinical outcome. Platelets play an important role in coagulation and hemostasis[39], and more specifically, they may be involved in the pathophysiology of DIC (disseminated intravascular coagulation) in conjunction with endothelial cell and leukocyte activation[14,15]. In septic patients, DIC is indeed a typical cause of low platelet counts. Therefore, the increased mortality in septic patients with a decreasing trajectory of platelet counts may be due to severe thrombotic complications and increased platelet consumption reflected by an increased frequency or severity of bleeding. In addition to their well-known role in hemostasis, platelets have emerged as important players in inflammation and immune regulation. In sepsis, platelet activation can be induced directly by inflammatory mediators or microbial components, leading to the initiation of the coagulation cascade[40,41] and subsequent microthrombus formation, ischemia, and multi-organ dysfunction[42–44]. However, activated platelets can also contribute to the maintenance of endothelial integrity

	MIMIC-IV (N = 15,839)	eICU-CRD (N = 7027)
Age (years)	68.0 (56.8–78.8)	67.0 (56.0–79.0)
Male (gender)	9231 (58.3%)	3640 (51.8%)
Ethnicity		
Caucasian	10,692 (67.5%)	5408 (77.5%)
African American	1289 (8.14%)	665 (9.54%)
Asian	442 (2.79%)	154 (2.21%)
Hispanic	502 (3.17%)	328 (4.70%)
Native American	36 (0.23%)	60 (0.86%)
Other/Unknown	2878 (18.2%)	359 (5.15%)
First ICU location		
Cardiac ICU	4906 (31.0%)	996 (14.2%)
Med-Surg ICU	3837 (24.2%)	4742 (67.5%)
MICU	2989 (18.9%)	860 (12.2%)
Neuro ICU	400 (2.53%)	142 (2.02%)
SICU	3707 (23.4%)	287 (4.08%)
Laboratory data		
Hemoglobin (g/dL)	9.60 (8.20–11.1)	10.1 (8.60–11.6)
Platelets (10^9/L)	153 (106–219)	171 (114–241)
WBC (10^9/L)	14.0 (10.1–19.1)	15.6 (10.6–22.0)
Creatinine (mg/dL)	1.20 (0.80–1.80)	1.50 (0.97–2.58)
Glucose (mg/dL)	145 (118–193)	165 (128–231)
Vital signs		
Heart rate (/min)	71.0 (61.0–82.0)	78.0 (67.0–91.0)
Respiratory rate (/min)	12.0 (10.0–15.0)	15.0 (12.0–18.0)
Temperature (°C)	36.4 (36.0–36.7)	36.4 (36.1–36.8)
SBP (mmHg)	88.0 (79.0–97.0)	85.0 (75.0–97.0)
Severity of illness		
SOFA	6.0 (4.0–9.0)	6.0 (4.0–9.0)
APS-III	50.0 (37.0–69.0)	54.0 (40.0–72.0)
APACHE-IV	–	69.0 (53.0–86.0)
SAPS-II	38.0 (31.0–48.0)	–
Comorbidities		
Heart failure	4787 (30.2%)	1375 (19.6%)
Peripheral vascular disease	1961 (12.4%)	361 (5.14%)
Cerebrovascular disease	2113 (13.3%)	851 (12.1%)
Chronic pulmonary disease	4233 (26.7%)	1328 (18.9%)
Diabetes	4935 (31.2%)	2289 (32.6%)
Renal disease	3577 (22.6%)	1380 (19.6%)
Malignant cancer	2173 (13.7%)	1261 (17.9%)
Severe liver disease	1251 (7.90%)	208 (2.96%)
Charlson comorbidity index	6.00 (4.00–8.00)	5.00 (3.00–6.00)
Support within the first 24 h		
Mechanical ventilation	7904 (49.9%)	2246 (32.0%)
Dialysis	773 (4.88%)	240 (3.45%)
Platelet treatment		
Platelet transfusion	2021 (12.8%)	276 (3.93%)
Antiplatelet treatment[1]	6034 (38.1%)	1548 (22.0%)
Immunotherapy	202 (1.28%)	22 (0.31%)
Hematologic diseases[2]	202 (1.28%)	9 (0.13%)
Thromboinflammatory diseases[3]	195 (1.23%)	20 (0.28%)
Thrombotic diseases[4]	1836 (11.6%)	399 (5.68%)

Table 1. Baseline characteristics of sepsis patients in MIMIC-IV and eICU-CRD. Data are represented as median (interquartile range) or n (%). *ICU* intensive care unit, *MICU* medical ICU, *SICU* surgical ICU, *SOFA* Sequential Organ Failure Assessment, *APS-III* Acute Physiology Score III, *APACHE-IV* Acute Physiology and Chronic Health Evaluation IV, *SAPS-II* Simplified Acute Physiology Score II, *WBC* white blood cell, *SBP* systolic pressure. [1]Including aspirin, clopidogrel, ticagrelor, tirofiban, cilostazol and dipyridamole. [2]Including sickle cell disease, thalassemia, vitamin B12 deficiency anemia, megaloblastic anemia, immunologic thrombocytopenic purpura, aplastic anemia. [3]Including disseminated intravascular coagulation, Behcet's disease, systemic lupus erythematosus, antiphospholipid syndrome, inflammatory bowel diseases. [4]Including ischemic heart disease, ischemic stroke, deep-vein thrombosis, and pulmonary embolism.

Method	Cluster	MIMIC-IV			eICU-CRD		
		OR	95%CI	*p* value	OR	95%CI	*p* value
Multivariate	AS	Reference			Reference		
	ST	1.451	(1.201–1.753)	< 0.001	1.326	(1.080–1.627)	0.007
	DS	2.424	(1.962–2.995)	< 0.001	2.519	(1.950–3.255)	< 0.001
IPW	AS	Reference			Reference		
	ST	1.254	(1.041–1.511)	0.017	1.302	(1.062–1.598)	0.011
	DS	2.610	(2.049–3.325)	< 0.001	2.511	(1.872–3.367)	< 0.001
Doubly robust with all covariates	AS	Reference			Reference		
	ST	1.305	(1.069–1.592)	0.009	1.259	(1.016–1.560)	0.035
	DS	2.461	(1.892–3.201)	< 0.001	2.250	(1.638–3.089)	< 0.001
Doubly robust with unbalanced covariates	AS	Reference			Reference		
	ST	1.302	(1.067–1.589)	0.009	1.252	(1.015–1.546)	0.036
	DS	2.464	(1.895–3.203)	< 0.001	2.297	(1.655–3.188)	< 0.001

Table 2. Primary outcome analysis with four different models in the sepsis patient cohort of MIMIC-IV and eICU-CRD. AS, ST, and DS respectively denote Ascending, Stable, and Descending trajectory patterns group. IPW, Inverse probabilistic treatment weighting.

and reduce vascular permeability, which may facilitate their role in mediating the inflammatory process and host defense mechanisms[45,46], as well as participate in wound healing and vascular remodeling[47]. Moreover, platelets exhibit their own antimicrobial defense mechanisms, including aggregating around pathogens, binding and internalizing microorganisms[48,49], and secreting antimicrobial peptides and free radicals[50,51], thereby limiting the growth and dissemination of bacteria. A decreasing platelet count trajectory indicates a decline in platelet-mediated antimicrobial defense[39], whereas an elevated platelet count trajectory may reflect pathogen consumption and immune defense function recovery[52–54]. Nonetheless, it is important to note that abnormally elevated platelet counts may pose a risk of thrombotic diseases[55].

Thrombocytopenia and its association with outcomes in septic patients have been widely investigated[13,16,17,56]. Traditionally, platelet counts at specific time points related to ICU admission, such as the initial platelet count upon ICU admission, have been evaluated. However, these time points may not represent homogeneous clinical conditions[20]. The platelet count of septic patients admitted to the ICU changes continuously with the progression of the disease and only stabilizes on the fourth day[21]. Referring only to the initial platelet count may result in loss of valuable information, such as the relationship between platelet count trajectory and patient prognosis. Our study findings indicate that thrombocytopenia that occurs after the fourth day of ICU admission mediates the relationship between platelet count trajectory and 28-day mortality in septic patients. This suggests that the dynamic trajectory of platelet count may act as an early warning signal for subsequent thrombocytopenia and influence the mortality rate of septic patients. Approximately 30–37% of the effect of platelet count trajectory patterns on mortality was mediated by thrombocytopenia, as demonstrated by our mediation analysis, and the trajectory pattern can still offer considerable information through other potential mechanisms.

We investigated several hypotheses to explain the survival benefits associated with an elevated platelet count trajectory in patients, and compared the differences in ventilator-free days at day 28, ICU length of stay, and 28-day maximum SOFA scores among different platelet count trajectory patterns. Our results revealed that the group with an elevated platelet count trajectory had a longer ventilator-free days at day 28 and ICU stay, as compared to the group with a declining platelet count trajectory. Furthermore, the 28-day maximum SOFA scores in the elevated platelet count trajectory group was lower than that in the declining platelet count trajectory group. However, due to the limitations in sample size, we are unable to infer whether the relationship between platelet count trajectory and the risk of sepsis-related mortality is driven by differences in these three secondary outcomes.

Our study is subject to several limitations that are worth noting. Firstly, as a retrospective cohort study, there are inherent limitations in the study design. Secondly, we were unable to measure lactate, cytokine levels, and other inflammatory markers that could have helped elucidate the relationship between inflammation and platelet count. Thirdly, we made efforts to adjust for potential causes of thrombocytopenia and confounding factors that may affect platelet count, such as platelet transfusion, antiplatelet therapy and thrombotic complications, in multiple models. We recognize that our study may not have captured all potential thrombotic and thrombo-inflammatory events, and their impact on long-term outcomes remains uncertain. Our sensitivity analyses confirmed the robustness of our findings, although unknown confounding factors may still be present. Fourthly, the demographic characteristics of the two databases we used for our study are heterogeneous, which should be taken into account when interpreting our results. Finally, it's worth noting that the diagnosis of sepsis patients in the eICU-CRD database was based on the clinical record of APACHE-IV unit, rather than the sepsis 3.0 diagnostic criteria, which may have led to an underestimation of the number of confirmed sepsis patients.

Conclusion

Our findings indicate that platelet count trajectory patterns are associated with sepsis outcomes, with thrombocytopenia potentially mediating this effect.

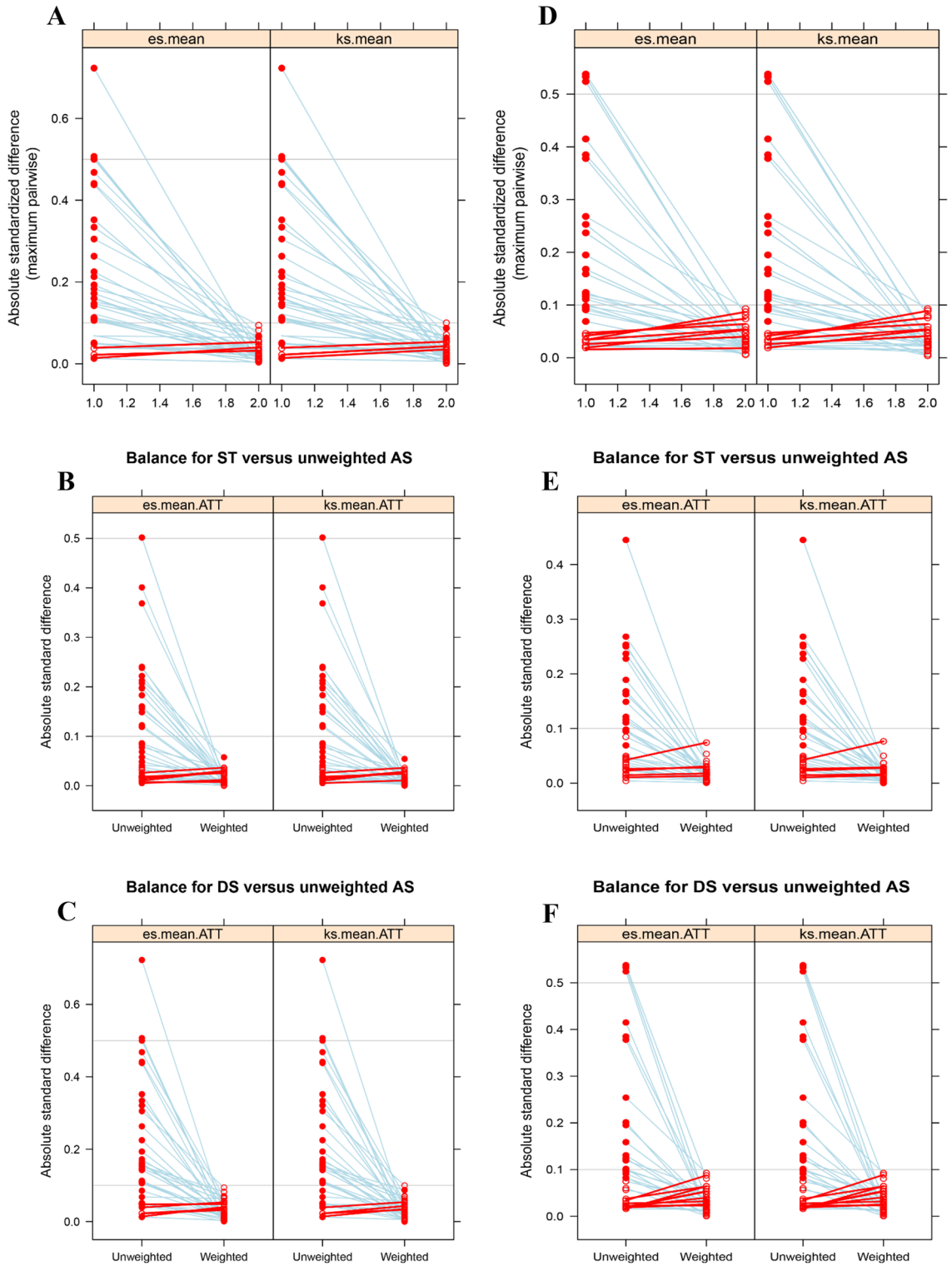


Fig. 3. Information on balance statistics. Comparison of the absolute standardized mean difference (ASMD) of pretreatment covariates between the weighted and unweighted treatment groups (**A**: MIMIC-IV, **D**: eICU-CRD). Comparison of the absolute standardized mean difference (ASMD) of pretreatment covariates between the weighted and unweighted treatment groups across different treatment groups (**B** and **C**: MIMIC-IV, **E** and **F**: eICU-CRD). es: Standardized effect size of pretreatment variables; ks: Kolmogorov–Smirnov *p* values for weighted pretreatment variables; AS, ST, and DS respectively denote Ascending, Stable, and Descending trajectory patterns.

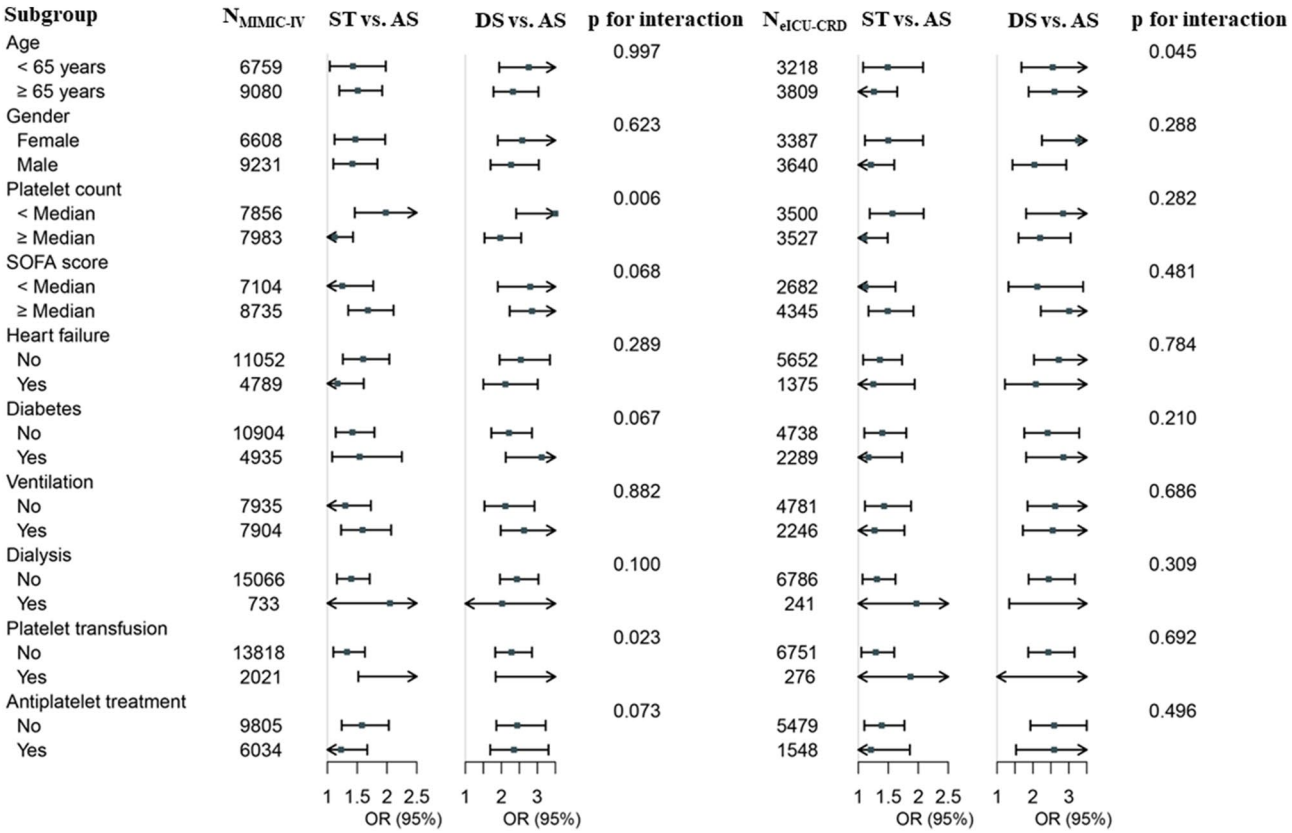
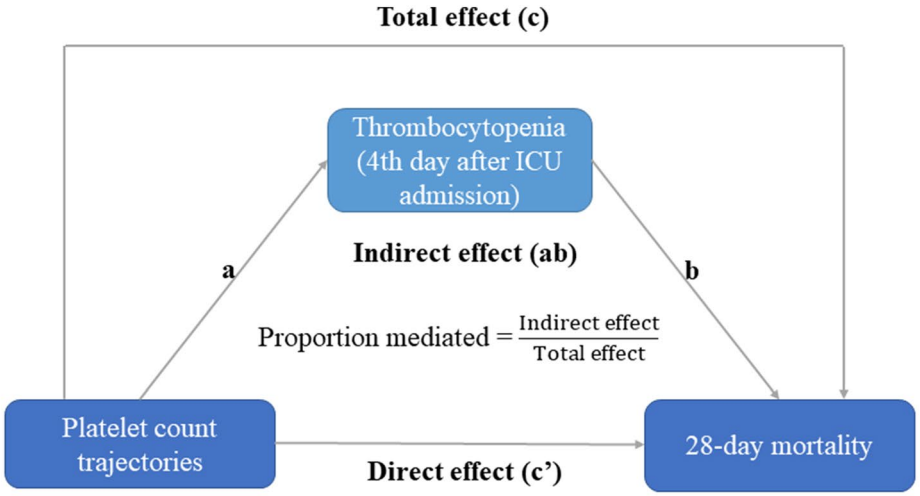


Fig. 4. Forest plot of subgroup analysis for 28-day survival of septic patients according to platelet count trajectory patterns in the MIMIC-IV and eICU-CRD databases.

Secondary outcomes	AS group	ST group	DS group	Effect size	*p* value
MIMIC-IV					
Ventilator-free days at day 28	26.00 (23.00–27.00)	26.00 (23.00–27.00)	24.00 (19.00–26.00)	0.294	< 0.001
ICU length of stay	2.36 (1.34–4.45)	2.804 (1.478–5.021)	4.32 (2.60–7.68)	0.263	< 0.001
Maximum SOFA score within day 28	5.00 (4.00–7.00)	5.00 (4.00–8.00)	6.00 (4.00–8.00)	0.169	< 0.001
eICU-CRD					
Ventilator-free days at day 28	26.00 (22.00–28.00)	26.00 (21.00–28.00)	24.00 (17.00–28.00)	0.226	< 0.001
ICU length of stay	2.79 (1.63–4.92)	3.29 (1.79–5.73)	4.75 (2.67–7.79)	0.248	< 0.001
Maximum SOFA score within day 28	5.00 (4.00–7.00)	5.00 (4.00–8.00)	7.00 (5.00–9.00)	0.221	< 0.001

Table 3. Secondary outcome analysis with inverse probability weighting cohorts. AS, ST, and DS respectively denote Ascending, Stable, and Descending trajectory patterns. *SOFA* sequential organ failure assessment, *ICU* intensive care unit.

A



B

	Total effect (c)		Direct effect (c')		Indirect effect (ab) via thrombocytopenia		Proportion mediated	
	β (95% CI)	P	β (95% CI)	P	β (95% CI)	P	% (95% CI)	P
MIMIC-IV	0.032 (0.022-0.040)	<0.001	0.022 (0.010-0.030)	<0.001	0.010 (0.008-0.014)	<0.001	30.91 (20.30-54.00)	<0.001
eICU-CRD	0.042 (0.022-0.060)	<0.001	0.026 (0.005-0.040)	<0.001	0.016 (0.011-0.020)	<0.001	37.14 (24.06-77.00)	<0.001

Fig. 5. Causal mediation analysis. The total effect of platelet count trajectory on 28-day mortality is represented by path c, whereas the direct effect of platelet count trajectory on 28-day mortality after controlling for the level of thrombocytopenia is represented by path c'. The effect of the platelet count trajectory on the level of thrombocytopenia is represented by path a; the effect of thrombocytopenia on 28-day mortality, after controlling for the platelet count trajectory, is represented by path b. The indirect effect of platelet count trajectory on 28-day mortality through thrombocytopenia can then be quantified as the product of path a and path b (**A**). Total, direct, and indirect association of platelet count trajectory with 28-day mortality mediated via thrombocytopenia (**B**).

Data availability

We would like to clarify that the data utilized in this study were sourced from the MIMIC-IV database (https://physionet.org/content/mimiciv) and the eICU-CRD (https://physionet.org/content/eicu-crd). MIMIC-IV and eICU-CRD are publicly available repositories containing de-identified electronic health records from critically ill patients. These databases provide valuable resources for conducting research in critical care medicine and were accessed in compliance with their respective usage agreements and data sharing policies.

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Author contributions
K.W. contributed to drafting the article. K.W. and W.F. contributed to the conception and design of the study. K.W. and D.L. contributed to the analysis and interpretation of data. W.F. contributed to reviewing the final manuscript.

Competing interests
The authors declare no competing interests.

Ethical approval
The MIMIC-IV database used in the present study was approved by the Institutional Review Boards (IRB) of the Beth Israel Deaconess Medical Center (No. 2001-P-001699/15). Study samples were collected from eICU-CRD and MIMIC-IV at PhysioNet (https://physionet.org/). Access to this database (Approval ID: 10734458) was granted to Dr. Shouqiang Zhu from the Graduate School of Air Force Medical University, who was responsible for data extraction.

Informed consent
We would like to clarify that the MIMIC-IV and eICU-CRD databases, utilized in this study, do not require informed consent as they contain de-identified patient information. All data accessed from these databases were anonymized and comply with relevant privacy regulations. This study was conducted in accordance with the principles outlined in the Declaration of Helsinki and the institutional guidelines for research involving human subjects. The need for informed consent was waived by the Institutional Review Board at the Beth Israel Deaconess Medical Center, as the data were de-identified and anonymized prior to use.

Additional information
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